OVERRIDING PUBLIC INTEREST REPORT

CONCERNING

UNDECLARED SV40 SEQUENCES IN COMIRNATY'S MANUFACTURING PLASMID

SUBMITTED WITHIN THE FRAMEWORK OF THE

TRANSPARENCY4SAFETY INITIATIVE (T4SI)



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Abbreviations and Units

Abbreviation	Definition
AmpR	Ampicillin resistance
ATMP	Advanced therapy medicinal product
BLAST	Basic Local Alignment Search Tool
BWP	Biologics Working Party
CAT	Committee for Advanced Therapies
cDNA	Complementary DNA
cGAS	Cyclic GMP–AMP synthase
CHMP	Committee for Medicinal Products for Human Use
CJEU	Court of Justice of the European Union
COVID-19	Coronavirus disease 2019
CTD	Common Technical Document
CTP	Cytidine triphosphate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DP	Drug product
DS	Drug substance
dsDNA	Double-stranded DNA
EC	European Community
eCTD	Electronic Common Technical Document
EMA	European Medicines Agency
EU	European Union
FDA	United States Food and Drug Administration
FOI	Freedom of information
FOIA	Freedom of Information Act
HC	Health Canada
IAS	Issue Analysis Summary
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Abbreviation	Definition
KanR	Kanamycin resistance
LIDAR	Name of *McKernan*'s fluorescence detection method for qPCR
LNP	Lipid nanoparticle
MAA	Marketing authorisation application
MHRA	Medicines and Healthcare products Regulatory Agency
MIT	Massachusetts Institute of Technology
modRNA	Nucleoside-modified messenger RNA
MP	Member of Parliament
mRNA	Messenger RNA
MW	Molecular weight
NCBI	National Center for Biotechnology Information
NeoR	Neomycin resistance
OC	Outstanding concern
OIA	Official Information Act (New Zealand)
OIC	Office of the Information Commissioner of Canada
OPIR	Overriding Public Interest Report
P1 / P2	Process 1 / Process 2
PCR	Polymerase chain reaction
PDF	Portable Document Format
pDNA	Plasmid DNA
PEI	Paul-Ehrlich-Institut
PPQ	Process performance qualification
PRAC	Pharmacovigilance Risk Assessment Committee
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
RSI	Request for supplementary information
S/MAR	Scaffold/matrix attachment region
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SCC	Supreme Court of Canada

Abbreviation	Definition
STING	Stimulator of interferon genes
SV40	Simian virus 40
T4SI	Transparency4Safety Initiative
TFEU	Treaty on the Functioning of the European Union
TGA	Therapeutic Goods Administration
TLR9	Toll-like receptor 9
TRON	Translational Oncology at the University Medical Center Mainz
TRS	Technical Report Series
UK	United Kingdom
US	United States
VRBPAC	Vaccines and Related Biological Products Advisory Committee
WHO	World Health Organization

Units

Unit	Meaning
bp	Base pair
kb	Kilobase
mg	Milligram
mL	Millilitre
ng	Nanogram
pg	Picogram
µg	Microgram
µL	Microlitre

Executive Summary

This Overriding Public Interest Report consolidates independent laboratory findings, scientific literature, and manufacturer and regulatory records concerning SV40-derived sequences in residual plasmid DNA associated with BioNTech/Pfizer's Comirnaty. It compiles evidence of an overriding public interest in the full, unredacted disclosure of documents held by the European Medicines Agency (EMA). Disclosure is necessary to enable independent examination of potential health risks, the adequacy of BioNTech/Pfizer's representations, the EMA's regulatory assessment, and resulting legal responsibility.

BioNTech/Pfizer has confirmed that its manufacturing plasmid contains SV40-derived regulatory elements, including a promoter, enhancer, origin of replication, and polyadenylation signal. These elements were not specifically identified in the marketing-authorisation dossier. The report examines whether this omission impaired regulatory scrutiny of the plasmid's functional properties and residual presence in the finished product.
The report identifies deficiencies in the available safety assessment, including validation of DNA-removal processes; use of target-specific qPCR assays as a proxy for total residual DNA; reliance on drug-substance testing rather than finished-product exposure assessment; and application of general residual-DNA thresholds without adequate consideration of the impurity profile, fragment characteristics, and lipid-nanoparticle delivery system. It also examines whether the transition from clinical Process 1 to commercial Process 2 was adequately supported by comparability evidence after the planned clinical comparison was discontinued.

The scientific assessment addresses mechanisms that require product-specific investigation: lipid-nanoparticle-mediated cellular delivery, nuclear trafficking, persistence, unintended gene expression, genomic integration, and insertional genotoxicity.
The available evidence does not substantiate the categorical safety assurances given, particularly regarding plasmid-derived DNA protected and delivered by lipid nanoparticles. The occurrence, frequency, and clinical significance of these mechanisms remain unresolved.

The overriding public-interest exception under Article 4(2) of Regulation (EC) No 1049/2001 must be invoked to protect public health and uphold the precautionary principle, including the European Commission's duty to act and suspend the marketing authorisation. This requires transparent access to all documents concerning the regulatory handling of the SV40 sequences.

1 SV40 Elements in BioNTech/Pfizer's Confidential Gene Therapy Plasmid

This Overriding Public Interest Report (OPIR), prepared under the Transparency4Safety Initiative (T4SI), critically examines the safety claims advanced by BioNTech/Pfizer concerning undeclared SV40 sequence elements in the manufacturing plasmid used for Comirnaty and the subsequent regulatory response of the European Medicines Agency (EMA). These elements were first identified by Kevin McKernan in 2023. Two regulators have described their status in their own terms. The EMA's Biologics Working Party records that, while the full plasmid sequence was provided in the initial marketing authorisation application, "the applicant did not specifically highlight the SV40 sequence, as it was considered to be a non-functional part of the plasmid" (BWP, Position on Residual DNA in Comirnaty, p. 6), and an Australian evaluator recorded that the plasmid "also contains a SV40 promoter and f1 ori region (not shown in plasmid map presented by Sponsor but found in BLAST and reported in *Speicher* & *McKernan* papers)" (TGA, Residual DNA and SV40 Correspondence, p. 155). The report further assesses the scientific basis of the asserted safety claims in light of the available scientific and regulatory knowledge. It should be read alongside the OPIR on Residual DNA, which has already been submitted to the EMA.

This report raises serious concerns about BioNTech/Pfizer's alleged use of SV40 sequences from its gene therapy programme that were not specifically highlighted in the dossier and the adequacy of EMA's assessment of the production plasmid and residual DNA. It challenges whether regulatory control and oversight of novel mRNA technologies have been sufficiently rigorous and independent from undue influence by pharmaceutical companies.

The record further shows that BioNTech/Pfizer was permitted to submit safety information irrespective of its accuracy. The EMA and other medicine regulatory authorities appear to accept information that is demonstrably false, contrary to regulatory guidance, well-established safety concerns, and the respective state of scientific knowledge. This report also documents publicly available communications within and between drug regulators, including the European Medicines Agency (EMA), Health Canada (HC), and UK regulators, obtained through access-to-documents requests in different countries. These communications provide evidence that the EMA neither conducted an independent investigation nor expressed any discernible intention to withdraw the marketing authorisation, despite BioNTech/Pfizer's failure to specifically highlight the SV40 elements in its dossier.

Full transparency of the Common Technical Document (CTD) is therefore essential to expose the erroneous conclusions and decisions of the EMA's Biologics Working Party (BWP) and its Committee for Medicinal Products for Human Use (CHMP). This evidence calls for an

independent investigation into the completeness of the marketing authorisation application, regulatory due diligence, and potential liability: Was the material plasmid information withheld, and why was the omission not detected? Did subsequent findings justify the suspension or revocation of marketing authorisation, sequence removal, or investigation of possible fraud? What legal and scientific grounds supported the variation procedure, and which independently verified data substantiated its approval?

These questions arise from findings that were first reported outside the regulatory system. Kevin McKernan, an independent US researcher, first identified the SV40 sequences, which had not been specifically highlighted in the dossier, in plasmid DNA recovered from BioNTech/Pfizer vaccine vials and presented his results in an analysis published on his research blog on Substack on 16 February 2023, entitled "Deep sequencing of the Moderna and Pfizer bivalent vaccines…" (*McKernan*, 2023, Deep Sequencing of the Moderna and Pfizer Bivalent Vaccines). A detailed preprint was published on 10 April 2023 describing the SV40 promoter/enhancer region and providing access to the sequencing data (*McKernan* et al., 2023, preprint, doi: 10.31219/osf.io/b9t7m). The assembled 7,810-bp sequence was subsequently deposited in GenBank under accession number OR134577.1, submitted on 13 June 2023 and recorded on 20 June 2023 (NCBI GenBank).

This OPIR concludes that the safety assessments submitted by BioNTech/Pfizer and endorsed by the EMA are fundamentally flawed. The failure to apply established regulatory and scientific knowledge concerning plasmid DNA, incomplete characterization of residual DNA and insufficiently relevant supporting literature leave the asserted absence of risk unsubstantiated. A purported exemption from the regulatory requirements applicable to gene therapy medicinal products cannot justify departure from substance-specific safety standards, particularly given BioNTech/Pfizer's expertise and development history in gene therapy.

BioNTech/Pfizer bears responsibility for complete, accurate and scientifically substantiated submissions; the EMA bears responsibility for their critical assessment. The report characterizes these failures as serious misconduct warranting independent investigation into breaches of their respective obligations and any resulting liability. This is particularly urgent because the product remains authorised while the contamination with excessive residual DNA, including SV40 sequence elements, identified in this report remains unremedied.

This OPIR therefore calls for immediate, complete and unredacted disclosure of all relevant scientific and regulatory documentation. Only full transparency can clarify the evidence, establish responsibility and restore confidence in medicines safety, including prophylactic

vaccines. This case exemplifies an overriding public interest in disclosure that must prevail over claims of commercial confidentiality by BioNTech/Pfizer.

2 SV40 Sequences and Confirmation by BioNTech

A detailed overview of the independent studies that first identified the SV40 sequences alongside residual plasmid DNA levels exceeding the applicable thresholds and their findings is provided in the *Overriding Public Interest Report on Residual DNA*, which should be read in conjunction with the present report.

2.1 Independent Researcher Kevin McKernan Coincidentally Identifies Undeclared SV40 Elements

On 16 February 2023, Kevin *McKernan*, the founder of Medicinal Genomics and a former research and development team leader at the Whitehead Institute/MIT Human Genome Project, publicly reported that the residual DNA levels in Moderna and BioNTech/Pfizer COVID-19 modRNA vaccines exceeded established thresholds. He also identified SV40 sequences in the BioNTech/Pfizer vaccine that were absent from the plasmid map in the Common Technical Document in his Substack newsletter, *Nepetalactone Newsletter*, under the pseudonym "Anandamide" (Medicinal Genomics, Kevin *McKernan*—Biographical Profile; *McKernan*, 2023, Deep Sequencing of the Moderna and Pfizer Bivalent Vaccines).

He explained that, coincidentally, he sequenced these in February 2023 while employing vaccine samples as RNA controls to troubleshoot an unrelated sequencing experiment focused on cannabis pathogens, rather than investigating vaccine impurities (FDA, VRBPAC Meeting Transcript, p. 115; Barnett, 2023, Interview with Kevin *McKernan* (3 October)).

More specifically, *McKernan* found sequences corresponding to the spike protein-coding region, aminoglycoside resistance markers, bacterial origins of replication, and T7 promoters (*McKernan*, 2023, Deep Sequencing of the Moderna and Pfizer Bivalent Vaccines). The assembled 7,810-bp sequence was subsequently deposited in GenBank under accession OR134577.1, submitted on 13 June 2023, and recorded on 20 June 2023 (NCBI GenBank).

His annotation of the reconstructed BioNTech/Pfizer plasmid identified a kanamycin/neomycin-resistance gene, an SV40 promoter/enhancer region with an SV40 origin of replication, and an SV40 polyadenylation signal. Additional annotated elements included an f1 bacteriophage origin of replication and an AmpR promoter; kanamycin and neomycin resistance were attributed to the same resistance gene (*McKernan*, 2023, Intent to Deceive (23 October)).

These findings bear directly on the safety of the product, so other researchers began independent investigations of residual DNA in mRNA vaccines (Barnett, 2023, Interview with Kevin *McKernan* (3 October)). On 12 September 2023, cancer geneticist Phillip Buckhaults reported detecting plasmid DNA in two Pfizer vaccine batches before the South Carolina Senate Medical Affairs Committee and called for investigations into possible genomic integration and a subsequent theoretical cancer risk (Buckhaults, 2023, Presentation to the South Carolina Senate, pp. 1, 3 and 7; South Carolina Senate, Hearing Record (12 September 2023)).

The independent studies and their findings are summarized in the *Overriding Public Interest Report on Residual DNA* by Behrendt and Steger (2026).

Particularly relevant are the methodological critiques of *Kaiser* et al. and *Achs* et al., whose studies reported residual DNA levels within regulatory limits. *Kirchner* et al. (2025, preprint, doi: 10.20944/preprints202411.1912.v5) questioned Kaiser et al.'s interpretation of the measurements and whether DNA losses during extraction had been adequately assessed. *McKernan* et al. (2026, doi: 10.18632/oncotarget.28913) argued that the methods used by *Achs* et al. could underestimate residual DNA because of problems with quantification, fragment detection, and sample preparation, limiting the study's reliability for regulatory assessment.

In 2026, *McKernan* introduced LIDAR, a multiplex qPCR detection chemistry that combines multicolour fluorescence with high-resolution melting analysis, which was subsequently applied to different plasmid targets and amplicon lengths to investigate how DNA fragmentation affects quantitative measurements (*McKernan*, 2026, LIDAR (18 June); *McKernan*, 2026, A Novel qPCR Detection Chemistry (18 July)).

2.2 Independent Testing of Vaccine Vials by Dr David J. *Speicher*

Following *McKernan*'s discovery, Dr David J Speicher, a Canadian virologist, personally tested 41 COVID-19 modRNA vaccine vials from Canada, Australia and Ireland for residual plasmid DNA and the SV40 promoter-enhancer, using methods developed by Kevin *McKernan*. Each study measured DNA directly in the vial contents, rather than relying on the manufacturer's release testing of the drug substance. The Canadian and Australian vials were measured by two independent methods, total DNA by Qubit fluorometry after RNase A digestion and specific plasmid targets by quantitative PCR (qPCR); the Irish vials were measured by qPCR alone. The SV40 promoter/enhancer-ori sequence was detected in every BioNTech/Pfizer vial tested and in no Moderna vial. The method, sample condition and stated limits of each study

are set out below, together with the published study on which the contrary regulatory position rests.

2.2.1 Ontario Vials: *Speicher*, Rose and *McKernan* (Autoimmunity, 2025)

Dr Speicher quantified residual DNA in 32 vials representing 16 unique lots obtained in Ontario, Canada: 10 BioNTech/Pfizer vials from 6 lots and 22 Moderna vials from 10 lots. Total DNA was measured by Qubit fluorometry and specific plasmid targets by qPCR, applying methods developed by *McKernan*, while Rose performed the analysis of adverse event reports (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517). The vials were held in pharmacy storage at +2 to +8 °C, transported in insulated containers with frozen gel packs, and placed in the laboratory refrigerator within five hours of receipt (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Methods). qPCR was performed on vials diluted 1:10 and added directly to the master mix, without extraction.

By qPCR, specific DNA of multiple plasmid targets ranged from 0.22 to 7.28 ng per dose for BioNTech/Pfizer and from 0.01 to 0.78 ng per dose for Moderna (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Abstract). The SV40 promoter/enhancer-ori sequence was detected in all BioNTech/Pfizer vials and in none of the Moderna vials, at 0.25 to 23.72 ng per dose. All Moderna vials were within the 10 ng per dose limit on every target, while two of the six BioNTech/Pfizer lots, comprising three vials, exceeded that limit for the SV40 promoter/enhancer-ori: lot FM7380 at 16.06 ng per dose and lot FN7934 at 23.72 and 18.10 ng per dose (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Table 3).

Measured by fluorometry coupled with RNase A digestion, total DNA in every product exceeded the 10 ng per dose guideline of the FDA and the WHO by 36- to 627-fold, at 371 to 1,548 ng per dose for BioNTech/Pfizer and 1,130 to 6,280 ng per dose for Moderna (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Conclusion and Results). These data correspond to 1.23×10^8 to 1.60×10^{11} plasmid DNA fragments per dose encapsulated in lipid nanoparticles (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Abstract). The divergence between the two methods shows that target-specific qPCR substantially underestimates total residual DNA, the equivalence examined in Section 4.2.2.

Oxford Nanopore sequencing of 865 reads from a BioNTech/Pfizer paediatric monovalent vial, lot FL8095, found mean and maximum DNA fragment lengths of 214 bp and 3.5 kb (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517, Figure 6). That vial was sequenced separately and is not one of the 32 vials quantified above. The authors state the limits of the work in their own terms, urging that it be "replicated under forensic conditions" (*Speicher* et

al., 2025, doi: 10.1080/08916934.2025.2551517). The published study advanced against these findings is *Achs* et al., which is addressed in Section 2.2.2.

2.2.2 Methodological Critique of *Achs* et al. (*McKernan*, *Speicher* and Rose, Oncotarget, 2026)

Achs et al. is the study on which regulators rely for the proposition that residual DNA in Comirnaty sits within approved limits. The authors analysed 9 batches of Comirnaty and 6 batches of Spikevax by four approaches, namely qPCR, fluorometry, capillary electrophoresis and short-read sequencing, and concluded that residual DNA in all 15 batches is below approved limits (*Achs* et al., 2025, doi: 10.1038/s41541-025-01304-9). *McKernan*, *Speicher* and Rose answered that study in Oncotarget, identifying methodological choices that bias the result toward underestimation of residual DNA and concluding that, collectively, these flaws render the study's conclusions invalid for regulatory assessment of residual DNA contamination (*McKernan* et al., 2026, doi: 10.18632/oncotarget.28913, Abstract). The figures the authors of the study themselves report do not carry their conclusion as far as it has been taken.

The highest average copy number *Achs* et al. report is 9.8×10^8 copies of the kanamycin-resistance target per dose, which the authors convert to 8 ng of DNA per dose against a regulatory limit of 10 ng (*Achs* et al., 2025, doi: 10.1038/s41541-025-01304-9, Discussion). That conversion credits every detected copy with the mass of a complete plasmid, so it is a plasmid-equivalent ceiling rather than a measured mass, and it is the most generous accounting their data permit. Their best case therefore stands at four-fifths of the limit before any adjustment is applied. The conversion equation defines MW as "the molecular weight of the fragment in grams per mole (based on its length in base pairs)", yet neither the plasmid length nor its molecular weight is stated anywhere in the paper, so the quantity compared with the regulatory limit is not defined in the publication that reports it.

Two adjustments are required within that reading, and neither is applied. The authors' own short-read sequencing gives median residual-DNA fragment lengths of 154 bp for Comirnaty and 144 bp for Spikevax, which their Discussion rounds to 150 bp (*Achs* et al., 2025, doi: 10.1038/s41541-025-01304-9, Results and Discussion). For random fragmentation at a median length L, the probability that an amplicon of length a survives intact is P = e^(−a ÷ L); for the 63 bp kanamycin amplicon at a 150 bp median this gives 65.7 per cent, so the detected copies understate the plasmid-derived molecules present by a factor of 1.52, and applying that correction to the authors' own 8 ng figure gives 12.2 ng per dose, which exceeds the limit. Their spike assays detect products of 228 to 233 bp, longer than the median fragment they themselves measure; at the same median the correction factor is 4.73 and the corrected

figure is 37.8 ng per dose. The sequence of greatest biological interest is therefore the one their assay design is least able to detect, and the shortfall is quantifiable rather than speculative.

Three features of the method compound the effect, each stated by the authors. Residual DNA is released from the lipid nanoparticles by heating at 95 °C for ten minutes before isolation, a treatment that fragments DNA further; by comparison, *Speicher* et al. used 95 °C for eight minutes before fluorometric quantification, and *McKernan* et al. subsequently reduced the heat treatment to one minute. Recovery is reported only in aggregate, at 60 to 80 per cent for phenol-chloroform extraction and 90 to 95 per cent for the column method, with no breakdown by fragment size, so preferential loss of the shortest fragments is neither excluded nor measured. The capillary-electrophoresis kit is described in the methods as quantifying from 50 pg/µL per sample, while 10 ng in a 300 µL dose is 33.3 pg/µL, so a non-compliant sample can be reported as undetectable by that method. *Achs* et al. is one of the three publications on which regulatory safety claims rest, as examined in Section 4.2.3.

The conclusion these data support is narrow and is stated as such: the authors' own measurements place residual DNA at or above the regulatory limit once their own fragment-size data are applied to their own copy numbers, and the analytical methods chosen cannot exclude higher values.

2.2.3 Australian Vials Tested for Fidge v. Pfizer Australia Pty Ltd (2024)

Dr *Speicher* served as consulting expert in *Fidge v. Pfizer Australia Pty Limited and Moderna Australia Pty Limited* before the Federal Court of Australia, and swore an affidavit on 13 September 2024 exhibiting his report of 9 September 2024 (*Speicher*, 2024, *Affidavit*, pp. 1–3 and Annex DS-4). Three vials were tested: BioNTech/Pfizer lot FN0565, adult monovalent; BioNTech/Pfizer lot FR4268, child monovalent; and Moderna lot 2100695, child and adult monovalent. Both BioNTech/Pfizer vials were full and unopened, while the Moderna vial had no intact flip-off cap, appeared to have been punctured and was at half volume (*Speicher*, 2024, *Affidavit*, Annex DS-3, p. 31; *Vaccine Vial Testing Report*, p. 3). All three vials were past their printed expiry dates of June 2022, August 2022 and 25 June 2022 on each of the testing dates of 28 May, 10 June, 5 July and 11 July 2024.

The vials were received at 12:17 on 14 May 2024, and the documented chain of evidence runs from 08:07 to 12:17 on that day, a period of four hours and ten minutes. The vials were shipped on 15 kg of dry ice; no dry ice remained on arrival, the contents were cool to the touch, and the temperature of the package was not recorded. The opening of the package

was video recorded, the vials were photographed, and they were stored at 2 to 8 °C (*Speicher*, 2024, *Affidavit*, Annex DS-3, pp. 31–33; *Vaccine Vial Testing Report*, p. 3).

Measured by fluorometry with RNase A digestion, total DNA in all three vials exceeded the Therapeutic Goods Administration (TGA) guidance of 10 ng per dose, ranging from 78 ng per dose in BioNTech/Pfizer lot FR4268 to 1,460 ng per dose in the Moderna vial, a 7.8- to 146-fold exceedance. The spike and ori targets were detected in all three vials by qPCR, and the SV40 promoter/enhancer-ori sequence was detected in both BioNTech/Pfizer vials and in neither run of the Moderna vial (*Speicher*, 2024, *Vaccine Vial Testing Report*, pp. 1, 6–10). The qPCR mass conversions printed in the report of 9 September 2024 are not reproduced here. The direction of these results is consistent with the Ontario results set out in Section 2.2.1.

The TGA's response to this evidence has not been obtained in a primary form. As reported in the Australian press in September 2024, a TGA spokesperson described the results as "not reliable", citing the absence of method validation, three vials of unknown provenance, one vial having been opened, the expiry of all three vials, a chain of evidence covering four hours and the absence of a temperature log (Barnett, 2024, *DNA Contamination in Australian mRNA Covid Shots*). The affidavit itself records the opened vial, the expiry of all three vials, the four-hour Canadian custody record and the absence of a temperature log (*Speicher*, 2024, *Affidavit*, Annexes DS-3 and DS-4). Expiry and storage would be expected to reduce, not increase, the amount of DNA measured in a vial, and the TGA's own published statement on residual DNA makes no mention of the SV40 promoter or enhancer and no mention of lipid nanoparticles (TGA, Statement on Residual DNA).

Documents released by the TGA under Freedom of Information request 25-0058 record its own evaluator's finding that the plasmid "contains a SV40 promoter and f1 ori region (not shown in plasmid map presented by Sponsor but found in BLAST and reported in *Speicher* & *McKernan* papers)" (TGA, Residual DNA and SV40 Correspondence, p. 155). The same entry records that "[t]he SV40 enhancer region can promote nuclear transport of DNA", qualified in the same bullet by the assessment that the risk is low given the low level of DNA and the low probability of integration events, and that "[t]he TGA does not hold any evidence that the residual DNA is encapsulated in lipid nanoparticles" (TGA, Residual DNA and SV40 Correspondence, p. 155). The agency's internal risk note of 10 October 2024 opens with the words "Assumption that the 10 ng/dose and 200 bp fragments is met in the vaccine" (TGA, Residual DNA and SV40 Correspondence, p. 154), so the assessment proceeds from an assumption of compliance rather than from measurement. The only stated ground for setting the independent evidence of exceeding thresholds of residual DNA aside was that it "has not been published in a peer-reviewed journal" (TGA, Residual DNA and SV40 Correspondence, pp. 153–154).

2.2.4 Irish Vials

In late 2024, Dr William Ralph of The Ballagh Health Centre, Wexford, Ireland, instructed Dr *Speicher* to test vials of COVID-19 modRNA vaccine for residual plasmid DNA, confirmed by letter of 12 November 2024. Six BioNTech/Pfizer Comirnaty vials from four lots were tested: lot FK0723, adult monovalent, expiry 30 June 2022; lot GD6803, adult bivalent Wuhan/BA.1, expiry 30 April 2023; lot HH1302, adult monovalent XBB.1.5, two vials, expiry 31 January 2025; and lot LK8837, adult monovalent JN.1, two vials, expiry 30 November 2025. Four of the six vials, being the XBB.1.5 and JN.1 boosters, were within their printed expiry at the time of testing. The vials were tested by qPCR alone; fluorometry and sequencing were not performed, for reasons of reagent availability and the work being unfunded (*Speicher*, 2025, Pfizer Comirnaty JN.1 Booster, "Vaccine Vials Tested", Table 1 and "Methods").

Two limits attach to these results and are stated here rather than left to be found. The chain of custody of the Irish vials was not documented and cold storage was not maintained during shipping, as Dr *Speicher* recorded publicly on 19 March 2025; the vials were refrigerated before and after shipping (*Speicher*, 2025, Pfizer Comirnaty JN.1 Booster, "Vaccine Vials Tested"). The dates of receipt and of the qPCR run are carried in Dr *Speicher*'s laboratory record and should be stated on its face when the signed laboratory report is filed. No fluorometry was performed, so no total-DNA figure is available for the Irish vials, and the values below are target-specific qPCR measurements of the kind that Section 4.2.2 shows to underestimate total residual DNA.

The spike target exceeded the 10 ng per dose limit in all six vials, at 40.99 to 88.76 ng per dose. The ori target ranged from 5.59 to 37.77 ng per dose and exceeded the limit in five of the six vials. The SV40 promoter/enhancer-ori sequence was detected in all six vials, including both JN.1 boosters, at 9.13 to 52.15 ng per dose, exceeding the limit in five of the six; the single vial below the limit on the ori and SV40 targets is one of the two XBB.1.5 vials of lot HH1302 (*Speicher*, 2025, Pfizer Comirnaty JN.1 Booster, "Results", qPCR results table). Detection of the SV40 promoter-enhancer in boosters supplied in 2024 indicates that the element was not removed from the manufacturing plasmid after it was identified in 2023.

One feature of the SV40 assay bears on how these figures are read. In silico analysis of the deposited plasmid sequence OR134577.1 shows that the reverse primer and the probe each bind twice, in the two tandem 72 bp SV40 enhancer repeats, so the assay generates two probe-positive products of 80 bp and 152 bp rather than the single product described in the methods. The conversion basis in the published figures is the 72 bp amplicon mass (*Speicher*, 2025, Pfizer Comirnaty JN.1 Booster, "Methods"). Independent sequence analysis of the deposited plasmid shows that all three assay targets are single-copy, so copies per dose are plasmid-molecule equivalents at each target; the SV40 amplicon, however, behaves as

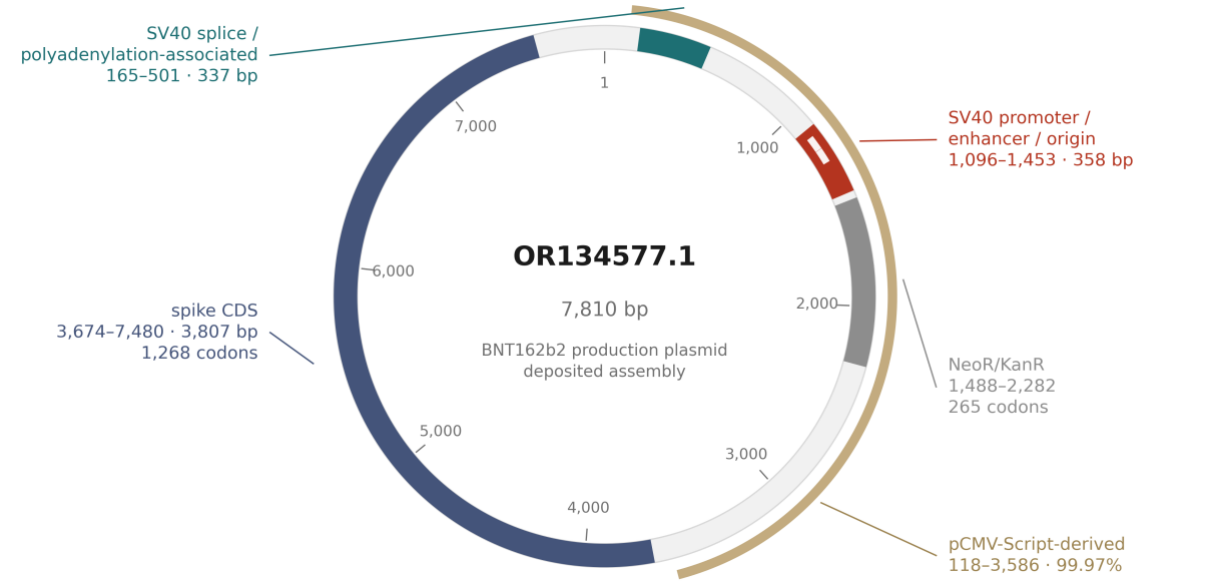
approximately twice the stated 72 bp in both the Irish and the Australian tables (145.7 and 144.0 bp fitted respectively), consistent with the two tandem 72 bp enhancer repeats the primer-probe set spans. The printed SV40 nanogram values are therefore amplicon-equivalent masses at the assay product, not whole-plasmid masses, and understate whole-plasmid mass by roughly the ratio of the amplicon to the 7,810 bp plasmid. The spike and ori exceedances are unaffected by this question. The 72 bp figure is the regulator's own definition of the element rather than an arbitrary choice. That definition, its provenance in Wildeman (1988, doi: 10.1139/o88-067) and the two occurrences in OR134577.1 are set out in Section 2.3 (Health Canada, SV40 Issue Analysis Summary and Correspondence; sequence comparison performed for this report). The probe and the reverse primer of the assay described here lie wholly within that 72-mer, so the assay quantifies the same element the regulator investigated.

The Irish results have not been published in a peer-reviewed journal. They were presented at An Injection of Truth, Calgary, on 3 March 2025, reported by Dr *Speicher* on 19 March 2025 (*Speicher*, 2025, Pfizer Comirnaty JN.1 Booster, "Related Presentations"), and form part of a draft manuscript dated 19 March 2026 covering the Australian and Irish vials. The figures above are as published by Dr *Speicher*; the signed laboratory report from the testing laboratory should be filed with this report before the figures are relied on in regulatory proceedings.

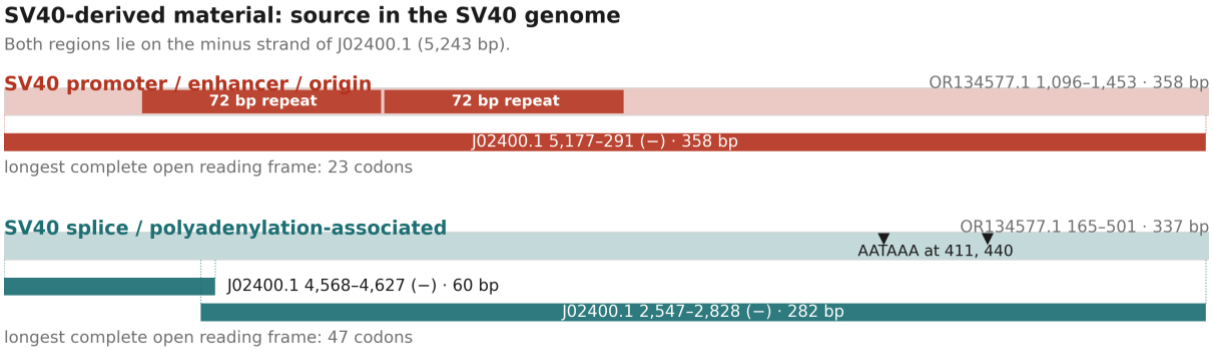
2.3 SV40 Elements in the Publicly Deposited Plasmid Sequence

The publicly deposited 7,810-bp sequence OR134577.1, assembled by *McKernan* et al. from the BioNTech/Pfizer bivalent vaccine material, contains two SV40-derived regions totalling 695 bp, approximately 8.9% of the assembled sequence. The deposited assembly is 7,810 bp, whereas the Agency's own rolling review records the construct as 7,824 bp (EMA, Comirnaty Rolling Review—Quality Aspects (19 November 2020), p. 23); the 14 bp difference is consistent with an assembly reconstructed from sequencing reads rather than taken from the manufacturer's own sequence file, and is itself a reason to require disclosure of that file.

To establish their identity, direct comparisons were made with the SV40 reference genome, accounting for strand orientation and the reference genome's circular structure (GenBank, OR134577.1 and J02400.1; sequence comparison performed for this report).



A



B

Figure 1: SV40-derived regions of the BioNTech/Pfizer Comirnaty production plasmid. (A) Circular map of GenBank OR134577.1 (7,810 bp). (B) Source of the SV40-derived material in the SV40 genome, GenBank J02400.1. All coordinates were derived from the deposited sequences for this report by exact-match seeding with ungapped bidirectional extension on both strands, the reference being treated as circular; the spike and NeoR/KanR coding sequences were located by open-reading-frame detection, independently of any prior annotation. Red marks the SV40 promoter/enhancer/origin region (1,096–1,453; 358 bp), which carries the two tandem 72 bp enhancer repeats at 1,137 and 1,209. Teal marks the SV40 splice/polyadenylation-associated region (165–501; 337 bp), a composite of two separated SV40 intervals that share five bases at their junction. Together these total 695 bp, 8.9 per cent of the assembled sequence. Neither region retains the capacity to encode T-antigen: the longest complete open reading frame is 23 codons in the regulatory region and 47 codons in the splice/polyadenylation region, against 708 and 174 amino acids for SV40 large T- and small t-antigen respectively. The gold arc marks the pCMV-Script-derived span (118–3,586), which matches GenBank AF028239.1 810–4,278 across 3,469 bp with a single

mismatch, 99.97 per cent identity. Sequence analysis performed for this report; the analysis script is on file.

SV40-derived region	Position in OR134577.1	Length	Identification
Splice/polyadenylation-associated region	165–501	337 bp	Composite region matching two portions of the SV40 genome, located within the parental vector's SV40 polyadenylation cassette.
Promoter/enhancer/origin region	1096–1453	358 bp	Continuous SV40 regulatory region immediately upstream of the neomycin/kanamycin-resistance gene

(GenBank, OR134577.1, J02400.1 and AF028239.1; Agilent, pCMV-Script Vector, pp. 2–3).

The SV40 promoter/enhancer-origin region ends 34 bases before the NeoR/KanR coding sequence. In the parental pCMV-Script vector, the SV40 early promoter drives the expression of the resistance gene for G418 selection in mammalian cells. Pfizer likewise explained to HC that the backbone contains elements for mammalian and bacterial use, while the SV40-dependent mammalian selection function is not used during plasmid production in E. coli (GenBank, OR134577.1, FEATURES; Agilent, pCMV-Script Vector, p. 2; Health Canada, SV40 Correspondence, p. 21). The coordinates and 8.9 per cent figure refer specifically to the deposited assemblies. Within the promoter/enhancer-origin region the SV40 enhancer is present as two tandem 72 bp repeats. HC's own investigation used a 72 bp enhancer query sequence attributed to Wildeman (1988), and that sequence occurs exactly twice in OR134577.1, at positions 1137 and 1209; it is the only sequence of that length duplicated anywhere on the plasmid (Health Canada, SV40 Issue Analysis Summary and Correspondence; sequence comparison performed for this report). That department's own alignment reached the same result in its own words, recording that both plasmids "contain a tandem double repeat of the SV40 enhancer" and, on further analysis, "the full SV40 promoter, which is composed of a double tandem repeat of the enhancer sequence and other regulatory sequences" (Health Canada, SV40 Issue Analysis Summary and Correspondence). Regulatory records refer to "the SV40 enhancer" in the singular, although the deposited sequence carries two copies of it.

2.4 Confirmation by BioNTech/Pfizer of SV40 Elements in the Plasmid

The public release of these sequences and of residual DNA levels above the applicable limits triggered immediate interest from media and fact-checking organizations. In turn, this situation required the authorities to act against BioNTech/Pfizer while reassuring the public that no safety concerns exist. Official releases verify that the regulators consciously kept the safety issues out of public communications.

BioNTech/Pfizer subsequently confirmed to the regulators that the sequences published by *McKernan* et al. correctly describe the four SV40 regulatory elements in the production plasmid. This is documented in the assessment of the European Medicines Agency's Biologics Working Party (BWP), which cites BioNTech's answers directly:

"BioNTech/Pfizer can confirm that *McKernan* et. al. correctly describe that SV40 promoter, SV40 enhancer, SV40 origin, and SV40 polyA signal elements are present in the DNA plasmid, which is a starting material in the manufacturing process of BNT162b2 / Comirnaty Drug Substance." (BWP, Position on Residual DNA in Comirnaty).

The later Pfizer Residual DNA Characterization Report INX100594280, available in releases by both HC and the EMA, confirmed that "All Comirnaty DS batches were confirmed positive for SV40 sequence elements." (Pfizer, Residual DNA Characterization Report, p. 2).

3 Chronology and BioNTech/Pfizer's Safety Allegations

3.1 Chronology of Events and Available Official Releases

The released disclosures from Canada, the European Union, the United Kingdom, New Zealand, and Australia document an international response in which regulators coordinated both their enquiries and public explanations, while BioNTech/Pfizer advanced a consistent defence of the SV40-derived sequences in its production plasmid that allegedly was taken from their earlier gene therapy research programme, as will be discussed further down.

Across the jurisdictions examined, BioNTech/Pfizer presented the same core safety claims: compliance with residual DNA limits, absence of the complete virus or SV40 oncogenes, limited biological activity of the remaining fragments, and no identified safety signal. The troubling feature is the distance between the confidence of these assurances and the unresolved questions, internal disagreements, and reluctance to press the manufacturer, as visible in the correspondence. Annex I identifies the public disclosures by various regulators (Health Canada, SV40 Correspondence, pp. 2–4, 21–26 and 270–271; BWP, Position on Residual DNA in Comirnaty, pp. 2–7; FDA, Letter to Joseph Ladapo, pp. 1–2; MHRA, Residual

DNA and SV40 Safety Response, response of 28 March 2024; TGA, Residual DNA and SV40 Correspondence, Document 30).

The Canadian disclosures date the start of the SV40 issue with a media enquiry from Matthew Horwood on 17 July 2023, referring to the findings of Kevin McKernan and Phillip Buckhaults. It prompted internal examination of the plasmid and preparation of an Issue Analysis Summary. The draft circulated on 27 July 2023 stated: "It was not known or expected that these plasmids would contain the SV40 enhancer."

The available documents provide evidence that BioNTech/Pfizer did not declare the full plasmid sequence in the marketing authorisation application, because the SV40 elements were not included in the plasmid map. This distinction is important because information present in a dossier was not recognized as requiring explicit regulatory attention until external scrutiny brought it forward. *McKernan* had already raised this issue during the US Food and Drug Administration (FDA) advisory committee's public hearing on 15 June 2023 (Health Canada, SV40 Correspondence, pp. 131–134; Health Canada, SV40 Issue Analysis Summary and Correspondence, draft circulated 27 July 2023; BWP, Position on Residual DNA in Comirnaty, p. 6; FDA, VRBPAC Meeting Transcript, pp. 114–116).

HC's Quality Clarifax of 4 August 2023 requested a justification for the SV40 elements, updated plasmid annotations, and characterization of DNA fragment sizes and residual intact circular plasmids. Pfizer's response of 9 August 2023 described the safety risk as minimal, but acknowledged that the requested characterization data "are not readily available and will require time to generate." It also pointed out that these data had not previously been requested across global markets. The commitment to provide those data by 1 December 2023 was therefore accompanied by reassurance given before the measurements existed. On 22 August 2023, HC requested an assessment of bacterial replication capability (Health Canada, SV40 Correspondence, pp. 6–7 and 21–26).

International coordination activities are directly evidenced by a HC–EMA meeting invitation for 17 August 2023 and correspondence arranging discussion at the EMA–FDA–HC cluster meeting on 24 August 2023. On 23 August 2023, a HC official, Dean Smith, explained that it would be useful "to be on the same page on this topic between agencies". He envisaged collective steps to encourage Pfizer to remedy the situation before a possible autumn 2024 campaign and explicitly expressed concern about an adverse effect on public acceptance of vaccination. On this record the agencies gave precedence to vaccine acceptance over the safety question (Health Canada, SV40 Correspondence, pp. 161–162 and 427–430).

The exchanges after Pfizer's reply expose the limits of the willingness of the responsible HC officers to act without international backing. On 25 August 2023, Tong Wu wrote: "As expected, Pfizer didn't commit." Smith replied: "It will still require FDA and EMA support, but that may still be achievable." Four days later, Wu advised that "we should not tell Pfizer our interaction with EMA and FDA on this," adding, in reference to the other authorities, "especially they do not seem to care much at this moment." His next sentence was equally revealing: "However, we can not say nothing!" These are unusually candid expressions of regulatory frustration and caution. They support the criticism of institutional reluctance to confront the manufacturer independently; however, attributing that reluctance to pharmaceutical lobbying would require further evidence (Health Canada, SV40 Correspondence, pp. 2–4; spelling retained; Health Canada, SV40 Correspondence and Quality Review, replacement p. 150).

The less-redacted Canadian quality review reveals HC's position, stating that it "strongly recommends the removal of unnecessary DNA elements," and confirms that its reviewers state: "The review team disagrees with this justification." Pfizer agreed to evaluate the request without committing to removal. This was accepted by the regulators, and Pfizer was not obliged to remove the SV40 sequences (Health Canada, SV40 Correspondence and Quality Review, replacement p. 193; Health Canada, SV40 Correspondence, pp. 3 and 16–20; Pfizer, Update on Plasmid-Backbone Modification, pp. 55–56).

The same quality review closes by stating HC's intended course: "Health Canada will continue to work with our international partners, seeking harmonized regulatory approach to remove unnecessary DNA elements from the plasmid used in vaccine production" (Health Canada, SV40 Correspondence and Quality Review, Quality Evaluation Executive Summary, internal p. 4; replacement p. 193). The internal correspondence of 25 August 2023 records the same intention in operational terms, a departmental official writing that removal "will still require FDA and EMA support, but that may still be achievable", and that the manufacturer's negative reply "supports our need to coordinate with our international partners" (Health Canada, SV40 Correspondence and Quality Review, replacement p. 150). One regulator therefore sought a harmonized international approach to removing the SV40 elements, and recorded that it could not proceed without EMA support.

No document released to date explains EMA's approach. The Canadian legislature investigated the SV40 issue in *Order Paper Question Q-1406*, asked on 17 June 2026 and answered on 21 September 2026, which asked at part (j) "what communications or coordination have occurred between HC and other regulators on residual deoxyribonucleic acid and its potential impacts", naming the FDA, the EMA, the MHRA, the TGA and any others. HC's reply addresses none of the eleven parts of the question. It states that deoxyribonucleic

acid is a common impurity in biologic drugs, that the SV40 promoter/enhancer sequence was found to be a residual fragment, that "the fragment is inactive, has no functional role, and was measured to be consistently below the limit required by the Department and other international regulators", and that "to date, over 3 billion doses of COMIRNATY have been administered world-wide, including over 69 million doses in Canada" with no safety signals related to residual DNA detected (House of Commons of Canada, Reply to Order Paper Question Q-1406 (2026)). Records responsive to part (j) exist on the department's own documents, being the internal correspondence of 25 August 2023 and the closing commitment of the quality review quoted above, and they were not produced.

HC's investigation was also narrowed at the outset to exclude any potential health hazard. Circulating the draft 'Issue Analysis Summary' on 27 July 2023, the officials wrote: "here's a draft IAS to document our investigation into the SV40 enhancer. I've left out any analysis into impact on human health as that may require something a bit more in-depth. I've framed it more regulatory along with negative public perception of SV40 sequences" (Health Canada, SV40 Issue Analysis Summary and Correspondence, email of 27 July 2023; released 16 July 2026).

The summary was subsequently abandoned. The reply to *Order Paper Question Q-2670* of 19 June 2024 states that an Issue Analysis Summary "was initially considered to document the process, but was later deemed to be unnecessary as the residual DNA content in the final product was consistently below the limit of 10 ng/human dose" (House of Commons of Canada, Reply to Order Paper Question Q-2670 (2024)). The ground given for abandoning the analysis is the same specification whose adequacy is in dispute.

HC's negligent handling of questions about potential harm from SV40 sequences in the vaccine is also evident from *Order Paper Question Q-1363*, asked by Dean Allison MP on 16 June 2026 and answered on 21 September 2026: "what scientific evidence, studies, or data is HC relying on to determine that any SV40 fragments present are inactive or non-functional?"

The reply states that the sequence "was found to be a residual DNA fragment in the Pfizer BioNTech COVID-19 vaccine", that "the presence of a fragment of the SV40 sequence is not the same as the presence of the whole virus itself", and that "the fragment is inactive, has no functional role, and was measured to be consistently below the limit required by HC and other international regulators" (House of Commons of Canada, Reply to Order Paper Question Q-1363 (2026)). No study, dataset, internal assessment or other reference is cited to substantiate the conclusion that these sequences pose no risk to human health. This disregard for the protection of human health is particularly grave given the documented acknowledgement by the author of the 'Issue Analysis Summary' that he deliberately omitted

any analysis of their potential health effects while reassuring that the sequences have no functional role.

The same four-sentence formula appears, almost verbatim, in the reply to *Q-990* of 8 May 2026, which asked how the plasmid DNA template is manufactured, linearized and quality-controlled, described the regulatory framework instead, and concluded that "if a product has been authorized by HC, it has been determined to be compliant with the relevant regulations" (House of Commons of Canada, Reply to Order Paper Question Q-990 (2026)), and in the reply to Q-1406 tabled four months later.

In an email of 13 October 2023, an EMA correspondent wrote: "An aligned position would help navigating the communication challenge." HC reported that Pfizer had emphasized that Canada was the only agency raising these issues and welcomed EMA's involvement. On 31 October, the Biologics Working Party (BWP) of the European Medicines Agency (EMA) formally documented that the "Lines To Take" issued by the EMA were already being employed by national competent authorities to address public enquiries. This constitutes evidence of coordinated conduct by the EMA and other regulators, suggesting that their actions were undertaken not in good faith to protect public safety, but rather to favour BioNTech/Pfizer and suppress information instead of acting proactively to safeguard public health (Health Canada, SV40 Correspondence, pp. 270–271; BWP, Position on Residual DNA in Comirnaty, p. 2).

BioNTech/Pfizer confirmed in the BWP document the existence of the SV40 promoter, enhancer, origin, and polyadenylation signal in the production plasmid, while it explained that the elements had been "deemed not relevant and thus not annotated" because they were unused in the manufacturing process. The manufacturer invoked purification and validated batch testing, the absence of the complete virus and oncogenes, theoretical estimates of small residual quantities, and the absence of reported mutation or vaccine-induced cancer.

Despite EMA's regulatory knowledge about DNA plasmids, it concluded that SV40 sequences "do not pose a risk for patient safety." The BWP accepted the central reassurance, while requesting a dossier update, risk assessment, and further characterization. Confirmatory testing of the finished product was described as something that "could be considered." Public reassurance and further evidence generation were therefore proceeding simultaneously (BWP, Position on Residual DNA in Comirnaty, pp. 3–7).

The document also records commitments whose results do not appear in the released record. It lists further analytical evaluation of residual DNA at drug substance level, covering the size distribution of residual DNA fragments and residual intact circular plasmid, an "[a]ssessment

of the presence of SV40 sequences in residual DNA", and an assessment of the plasmid's transformation capability in bacteria, each with a planned completion date of 1 December 2023 (BWP, Position on Residual DNA in Comirnaty, p. 5). No results of those analyses appear among the documents released under access-to-documents request ASK-289849. The same position records the manufacturer's reason for testing the active substance rather than the finished product, namely that analysis of finished product "requires additional sample manipulation, including extraction of residual DNA from the LNP, which may impact the ability to sensitively detect and characterize residual DNA", and that "[a]ny residual DNA present in the DS is presumed to be present in the DP" (BWP, Position on Residual DNA in Comirnaty, p. 5). The regulatory position therefore rests on a presumption about the finished product, and on avoiding the extraction step that independent testing applies.

The reasons why the independent findings were set aside are also recorded in the BWP document. The BWP stated: "[t]he methods used in the publication mentioned above and their demonstration of suitability for the intended purpose (i.e. validation) have not been assessed here, as peer-review of this publication is beyond the scope of the current comments". Its criticism instead rested on the vials being of "unknown provenance" and sent "anonymously in the mail without cold packs" (BWP, Position on Residual DNA in Comirnaty, p. 5). Despite the potential health hazard, the committee expressly declined to assess the methods applied and available in the publications, as set out in Section 2.2.3.

A similar approach is documented from the United States. On 14 December 2023, the FDA described genomic incorporation as "quite implausible" and invoked in its defence its manufacturing controls, animal studies, and surveillance data. In March 2024, the UK Medicines and Healthcare products Regulatory Agency (MHRA) characterized the SV40-derived fragment as "inactive" and having "no functional role." Its earlier FOI response had nevertheless stated that, for fragmented DNA and the SV40 enhancer, it had not tested Comirnaty at vial level and had no current plans to do so. These records distinguish the authority of a public assurance from the extent of the particular independent testing disclosed in support of it (FDA, Letter to Joseph Ladapo, pp. 1–2; MHRA, Fragmented DNA and SV40 Testing, p. 1; MHRA, Residual DNA and SV40 Safety Response, response of 28 March 2024).

The EMA requested that BioNTech/Pfizer update the plasmid documentation, instead of revoking the marketing authorisation for the non-declaration. In response, BioNTech submitted variation II/0202 on 15 December 2023 to the EMA and the procedure was initiated on 27 December 2023. Pfizer's residual DNA risk assessment was internally approved on 14 December. The Pfizer Residual DNA Characterization Report, INX100594280, was accepted by the Committee for Medicinal Products for Human Use (CHMP), which approved the dossier update and considered that the additional characterization did not require revision of the risk

assessment. Removal of unused plasmid elements was assigned to subsequent lifecycle management, "outside the framework of this procedure." The identical Pfizer Residual DNA Characterization Report, INX100594280, was also provided to HC and is included in the Canadian disclosure, but with a different level of redactions; the available releases do not independently establish that the separately submitted Canadian and European risk-assessment versions are identical (Pfizer, Response to EMA Request for Supplementary Information, pp. 1–2; Pfizer, Residual DNA Characterization Report, approval record; CHMP, Comirnaty Variation Assessment II/0202, pp. 2 and 4–5; Pfizer, Residual DNA Risk Assessment, Health Canada, Pfizer Residual DNA Submissions, pp. 4–11; Pfizer, Residual DNA Characterization Report (Health Canada release), pp. 28–54).

The residual DNA measure method as a process related impurity for Comirnaty is a plasmid-targeted qPCR performed by BioNTech/Pfizer alone, which cannot measure host-cell DNA, so that gap remains open on the regulator's own record.

In an update internally approved on 22 February 2024 BioNTech/Pfizer said it "would potentially be able to discuss preliminary outcome by approximately the end of 2024". A request pursued in August 2023 has thus become a possible discussion of preliminary results after more than a year. The disclosed update contains no commitment to complete the removal by that date (Pfizer, Update on Plasmid-Backbone Modification, pp. 55–56).

Australian records verify both the international circulation of arguments and the need to distinguish independent laboratories from independent methods of research. The Therapeutic Goods Administration (TGA), in its October 2024 internal discussion, referred to the FDA and MHRA positions; its replies repeated the language of 'small, inactive SV40-derived fragments'. The TGA conducted residual DNA testing and reported that results within specifications of 10 ng/dose. However, the disclosed Pfizer protocol states that most of the method is "copied verbatim from an in-process control method," with specified adaptations. During preparation of the public statement, an internal email explained its purpose: "we can refer any future inquiries regarding this subject directly to this statement." (TGA, Residual DNA qPCR Protocols, Document 1, p. 1; TGA, Residual DNA and SV40 Correspondence, Document 30; TGA, Drafts and Correspondence on the Residual DNA Statement, p. 26; TGA, Summary Report on Residual DNA and Endotoxin Testing, laboratory report).

The TGA's published statement on residual DNA asserts that "the TGA has also independently tested 27 batches of COVID-19 mRNA vaccines by qPCR" and that the vaccines met the required limits for residual DNA. However, neither the results of that testing, nor the method used, nor its validation has been released by the TGA. The methods used for the independent investigations are public, whereas the TGA, as well as other regulators, keeps its test methods

confidential, as discussed in Section 6.2. The same published statement makes no mention of the SV40 promoter or enhancer, and no mention of lipid nanoparticles.

The disclosures of New Zealand add to the impression that medicines regulators are more willing to shield pharmaceutical companies from public scrutiny than to hold them accountable. On 1 February 2024 Medsafe withheld information regarding the submission and annotation of the plasmid sequence on confidentiality grounds. For the specifically requested internal discussions concerning manufacturer batch testing and confidence in quantitative polymerase chain reaction (qPCR), it stated that "this information does not exist." Nevertheless, the response repeated the assurances concerning DNase treatment, internationally agreed limits, validated qPCR, and the absence of SV40 proteins. The disclosed record therefore contains a safety explanation without the particular internal deliberations sought by the requester (Medsafe, Residual DNA and SV40 Response, p. 1).

Disclosure disputes are part of this chronology. In HC (Re), 2026 OIC 43, the Information Commissioner found that certain information had been withheld without satisfying the claimed exemptions and ordered its release. The decision records that Pfizer opposed the disclosure while also upholding protection for other proprietary information. Subsequent Canadian releases in July and September 2026 exposed passages that were previously obscured by redactions. Public access to regulators' own reasoning therefore depended, in part, on sustained challenges to confidentiality claims (Information Commissioner of Canada, Health Canada (Re), 2026 OIC 43, paras. 3–6, 15–17, 25–31 and order; Health Canada, SV40 Issue Analysis Summary and Correspondence, release of 16 July 2026; Health Canada, SV40 Correspondence and Quality Review, replacement pages released 8 September 2026).

Taken together, these records raise serious concerns regarding the independence and transparency of precautionary oversight. Manufacturer assurances became a shared regulatory explanation, while additional characterization was still being requested, reviewers disputed the justification for retaining unnecessary elements, and removal remained outside the completed variation procedure. The documented reluctance to press Pfizer without international support deserves scrutiny. The fragmented disclosures point to a grave scandal: the medicines law has been hollowed out, and drug safety has been traded away by the very authorities responsible for protecting it. Rather than prioritizing the safety of human use and established scientific evidence, the process appears to have relied on unilateral assessments that departed from these standards (Health Canada, SV40 Correspondence, pp. 2–4; BWP, Position on Residual DNA in Comirnaty, pp. 2 and 5–7; CHMP, Comirnaty Variation Assessment II/0202, p. 5; Pfizer, Update on Plasmid-Backbone Modification, pp. 55–56).

3.2 BioNTech/Pfizer's Safety Allegations Analysed in Detail

Given the broader significance of the SV40 sequences that were not specifically highlighted in the dossier, this section examines BioNTech's assertion that residual-DNA SV40 sequences pose no safety risk to vaccine recipients, an assertion apparently accepted by the EMA despite the available evidence and contrary to regulatory knowledge. This analysis seeks to establish that neither the manufacturer nor the EMA treated safety considerations with sufficient seriousness or concern for human health.

In its October 2023 responses to the EMA, available in the BWP assessment document, BioNTech/Pfizer explained both its conclusion that the SV40 elements did not raise safety concerns and the reasons why these elements had not been explicitly identified in the original plasmid documentation.

These statements provide the basis for examining the EMA's scrutiny of the manufacturer's arguments regarding the protection of human health and the application of the precautionary principle. BioNTech argued:

"More importantly, **the SV40 regulatory region is neither relevant for plasmid production in E. coli nor for the production of mRNA,** since only prokaryotic elements of the plasmid backbone are utilised for these purposes. The elements of the SV40 regulatory region are used to drive neomycin/kanamycin resistance in mammalian cells. This function is not used during plasmid production in E. coli. Furthermore, it should be noted that the **SV40 promoter/enhancer is neither an oncogene nor an infectious virus, which are the primary safety concerns for residual DNA**. Since the SV40 promoter/enhancer DNA does not contain known oncogenes, infectious agents, or regions that could lead to functional transcripts, the DNA does not present any specific safety concerns." (BioNTech/Pfizer, statement of 12 October 2023, reproduced in BWP, Position on Residual DNA in Comirnaty, pp. 3–4; emphasis added).

"With respect to the claim that "DNA contamination (…) exceeds the European Medicines Agency (EMA) 330 ng/mg requirement and the FDAs 10 ng/dose requirements": The manufacturing process of Comirnaty includes multiple steps designed to purify the mRNA molecules from the DNA template. These manufacturing steps have been extensively validated, and relevant data have been provided for evaluation at the time of MAA and in subsequent related variations. Furthermore, each batch is routinely controlled using a method described in the dossier and appropriately validated" (BioNTech/Pfizer, additional information of 24 October 2023, reproduced in BWP, Position on Residual DNA in Comirnaty, p. 4).

BioNTech/Pfizer's strategy becomes clearer upon examining the arguments presented in the residual DNA risk assessment available in HC's disclosure. This document is presumably identical to the version submitted by BioNTech to the EMA and therefore enables a critical assessment of BioNTech/Pfizer's scientific reasoning regarding whether residual DNA, including sequence elements such as the SV40 promoter, could pose safety risks to vaccine recipients.

The reasoning runs in parallel. First, Pfizer stated that the residual DNA quantification "assay is a widely recommended standard for residual DNA testing […] and approved by the regulatory authorities worldwide. The assay used in Comirnaty testing is highly sensitive, enabling detection and quantification of trace amounts of DNA." (Pfizer, Residual DNA Risk Assessment).

Second, it stated that all commercial drug substance batches met the WHO safety threshold of 10 ng/dose, which "indicates that the Comirnaty manufacturing process is robust, with appropriate control strategies in place ensuring consistent low levels of residual DNA." (Pfizer, Residual DNA Risk Assessment).

Third, Pfizer stated that the "plasmid DNA used in Comirnaty production does not contain genes associated with causing cancer (oncogenes) and is not replication competent in mammalian cells […], that is, it cannot be amplified in the human body. Therefore, it is not considered to pose a safety risk." (Pfizer, Residual DNA Risk Assessment).

Regarding the "likelihood of integration," Pfizer asserts that "[u]pon intramuscular administration of the vaccine, residual DNA present outside of the LNP, would be phagocytosed by endogenous inflammatory cells as common for extracellular DNA […]. Residual DNA encapsulated within the LNP would be delivered to the cytoplasm with the vaccine mRNA. In the cytoplasm, residual DNA is not transcriptionally active and is rapidly degraded (with a half-life of 50–90 mins) […]. Moreover, its transport to the nucleus and potential genomic integration are impeded by multiple biological mechanisms […]. **Therefore, the likelihood of any residual DNA integrating into the human genome is regarded as negligible.** Decades of clinical experience with DNA-based vaccines, which use plasmid DNA for the final vaccine product, show no evidence supporting concerns about chromosomal integration of DNA […]. Moreover**, extensive research** has shown that plasmids or plasmid-derived DNA do not persist in the body, even when administered at high doses via various routes […]. The low (ng) amount of residual DNA can be further contextualized by considering that individuals are continuously exposed to extrinsic viral and bacterial DNA via the gut (e.g.: through the microbiome) as well as through mucosal surfaces." (Pfizer, Residual DNA Risk Assessment; emphasis added).

With respect to residual DNA, Pfizer concludes that "[t]here is no scientific evidence that the presence of residual DNA in Comirnaty, which is below the internationally recommended threshold levels, poses a safety risk to vaccinees." (Pfizer, Residual DNA Risk Assessment).

Pfizer went on to analyse the potential health risks for vaccine recipients associated with the SV40 sequences by stating, "The sequence elements in the residual DNA, including the SV40 promoter, do not facilitate chromosomal integration, nor do they contribute to the persistence or replication of the plasmid in the human body […]. No SV40 proteins are encoded or present in the vaccine. Importantly, the SV40 sequence elements are not oncogenes and do not cause cancer […]. As described above, […] residual DNA is expected to degrade rapidly and has a very low likelihood of reaching the nucleus. In the unlikely event that the SV40 promoter/enhancer and the [redacted information] elements would reach the nucleus intact and transient expression of the resistance gene occurs, this would have no biological effect on the vaccinee. Therefore, these sequence elements do not pose any safety risk to the vaccinee." (Pfizer, Residual DNA Risk Assessment).

4 Inadequate Safety Assessment of Residual Plasmid DNA and SV40 Elements

Assessing the safety of SV40 sequences in the production plasmid requires a broader examination of residual plasmid DNA. Issues addressed in the OPIR on Residual DNA are therefore revisited where relevant to the assessment of SV40 elements. The report identifies the inappropriate assessments of regulatory thresholds, deficiencies in analytical methods, and unsupported safety conclusions advanced by BioNTech/Pfizer and accepted by the EMA. It examines contrary scientific evidence and the regulatory requirements for characterizing residual plasmid DNA in the context of the manufacturing process and delivery system (T4SI, OPIR on Residual DNA, 2026).

The following sections examine the general implications of residual DNA, the specific functions of SV40 elements and the potential for genomic integration, before addressing the implications for regulatory assessment, plasmid provenance and disclosure.

4.1 Inadequate Manufacturing Controls and DNase I Validation

Both BioNTech/Pfizer and regulatory authorities recognize that residual DNA is a process-related impurity. Its potential biological activity depends on its quantity, molecular structure, sequence content, and formulation. The assessment must distinguish naked DNA from

plasmid-derived DNA, whose protection and cellular delivery are facilitated by lipid nanoparticles (LNPs).

4.1.1 Inadequately Substantiated Comparability Between Process 1 and Process 2

The EMA assessed the transition from clinical Process 1 to commercial Process 2 as a manufacturing comparability issue. The change involved increased scale, replacement of the PCR-derived template with linearized plasmid DNA, and modified purification. The EMA also recorded differences in RNA species. The question is whether the comparability assessment justified the extrapolation of the clinical findings to the commercial product, including its changed residual DNA impurity profile (EMA, Comirnaty Public Assessment Report, pp. 18–20 and 32–34).

Describing the Process 2 template as linearized plasmid DNA identifies its form at the start of in vitro transcription. The preceding manufacturing stage involves the amplification of circular plasmids in bacteria. This distinction is relevant to the possible persistence of intact circular molecules and the control strategy for residual plasmid DNA. Wang et al. illustrated the circular plasmid stage and discussed the detection of replication-competent plasmid DNA in the context of vaccine quality control (Wang et al., 2024, doi: 10.64336/001c.127890, Figure 1).

Bacterial amplification also introduces a compositional difference from PCR amplification. Depending on the production strain, bacterial Dam and Dcm methylation can modify adenine and cytosine residues in the plasmid template. *McKernan* et al. identify the potential biological relevance of these bacterial methylation patterns as an additional variable in the Process 1 / Process 2 comparison. Assessment of their contribution to innate immune recognition requires characterization of the actual residual molecules and appropriate functional evidence (*McKernan* et al., 2026, doi: 10.18632/oncotarget.28913).

The SV40 elements not specifically highlighted in the original dossier make the scope of the P1/P2 comparability assessment directly relevant. Comparability of selected RNA attributes does not, by itself, establish comparability of plasmid-derived impurities. The published testing panel in Pfizer's post-authorisation PPQ comparability report includes RNA and lipid nanoparticle attributes but lists no tests for residual DNA or SV40 sequences (Pfizer, PPQ Comparability Report, 26 March 2021, pp. 4–7). The MHRA subsequently confirmed that the planned exploratory clinical comparison of P1 and P2 safety and immunogenicity was removed through protocol amendment 20 in September 2022, citing the extensive use of

vaccines manufactured via Process 2 (MHRA, P1/P2 Comparability Response (IR 23/510), 21 September 2023, p. 5).

Disclosure must therefore include the comparability protocol, acceptance criteria, residual DNA results and scientific justification for extrapolating clinical findings from P1 to P2. The PPQ report and the decision to remove the planned clinical comparison must be examined together to establish which evidence supported comparability and whether that evidence adequately addressed plasmid-derived impurities. This request is supported by the comparability principles in ICH Q5E, which call for evaluation of changes in impurity profiles and recognize that routine specification testing alone is generally insufficient to assess manufacturing changes (ICH Q5E, 2004, sections 2.2.2–2.2.3).

ICH Q5E calls for the assessment of changes in impurity profiles, characterization of new impurities where possible, and additional non-clinical or clinical evidence where safety implications remain unresolved. Routine specification compliance alone is generally insufficient to establish comparability. These criteria make the changed plasmid-derived impurity profile an integral part of the P1/P2 assessment (ICH Q5E, sections 2.2.2–2.2.3, pp. 8–9).

4.1.2 Inefficient DNase I Digestion of RNA:DNA Hybrids

The EMA and other regulatory agencies based their safety conclusions on the approved manufacturing controls, validations and risk assessment presented by BioNTech/Pfizer, as documented in Pfizer's Residual DNA Risk Assessment, the BWP position, and CHMP variation assessment. The BWP described the purification steps as "extensively validated"; the CHMP subsequently accepted the additional characterization without requiring revision of the risk assessment (Pfizer, Residual DNA Risk Assessment, internal pp. 3–4; BWP, Position on Residual DNA in Comirnaty, p. 5; CHMP, Comirnaty Variation Assessment II/0202, pp. 4–5).

One of these officially accepted methods is DNase I digestion, which does not degrade all DNA structures with equal efficiency. Sutton et al. demonstrated that RNA:DNA hybrid substrates were resistant to DNase I digestion. *Lenk* et al., including BioNTech authors, describe DNase I activity against these hybrids as "at least 100-fold below that for dsDNA". Demonstrated digestion of double-stranded plasmid DNA therefore cannot establish equivalent clearance of RNA-associated DNA (Sutton et al., 1997, doi: 10.1042/bj3210481, pp. 481–486; Lenk et al., 2024, doi: 10.3389/fmolb.2024.1426129, section on RNA:DNA hybrids).

The manufacturer's technical discussion of DNase I also describes substrate-dependent differences in digestion efficiency. These limitations are relevant to RNA-associated DNA

generated during in vitro transcription. The use of DNase I therefore requires experimental demonstration of clearance under the actual process conditions, including the structures most resistant to digestion (Thermo Fisher Scientific, DNase I Demystified; Sutton et al., 1997, doi: 10.1042/bj3210481; Lenk et al., 2024, doi: 10.3389/fmolb.2024.1426129).

Speicher et al. and *McKernan* et al. discuss inefficient digestion of RNA:DNA hybrids and differences between spike and backbone targets. RNA-associated DNA is particularly relevant in the transcribed spike region. The reported 10–100-fold differences between spike-encoding and kanamycin-encoding sequences make the selection of an assay target a substantive part of the clearance assessment. A kanamycin-region assay requires validation against the possible enrichment of protected spike-region DNA (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517; *McKernan* et al., 2026, doi: 10.71189/JIM/2026/V02N01A04).

The development of DNase I-XT for improved removal of residual DNA associated with RNA during in vitro transcription further illustrates the significance of enzyme selection and substrate structure. The relevant manufacturing records must establish which enzyme preparation, reaction conditions and analytical targets were used. Disclosure of the historical primer sets is also necessary to resolve the criticism that an available spike assay was replaced by a kanamycin-region assay (*Lenk* et al., 2024, doi: 10.3389/fmolb.2024.1426129; *McKernan* et al., 2026, doi: 10.71189/JIM/2026/V02N01A04; CTD 3.2.S.4.2 and 3.2.S.4.3).

BioNTech/Pfizer's own patent also documents sensitivity to manufacturing conditions: "Residual DNA was increased with higher CTP starting concentration". The proposed explanation is reduced DNase I activity resulting from lower free magnesium concentrations. These experiments establish a relevant process dependency (Pfizer/BioNTech, US20230183769A1, Example 4, Figure 16).

Moderna's manufacturing patent expressly recognizes the safety relevance of template removal: "The DNA template used in the mRNA manufacturing process must be removed to ensure the efficacy of therapeutics and safety, because residual DNA in drug products may induce activation of the innate response and has the potential to be oncogenic in patient populations." It also states that qPCR detects DNA containing both primer targets and "does not measure all other smaller DNA molecules that are partially digested." The patent describes total nuclease digestion followed by liquid chromatography–tandem mass spectrometry to quantify residual DNA. These passages directly identify the relationship between incomplete digestion, incomplete analytical detection, and impurity control (Moderna, US10077439B2, "cDNA Template Removal and Clean-Up" and "mRNA Characterization").

The available Comirnaty CTD files disclose a linearized plasmid calibration standard, whereas the primer sequences and underlying validation results remain undisclosed. The Mainz/Rentschler process-validation summary reports successful validation but redacts the relevant process parameters and acceptance criteria. Read alongside the Characterization Report and supplementary response, these documents leave independent reviewers unable to establish how fragmentation, resistant DNA structures, and analytical recovery were addressed in this study. The decisive evidence is the demonstrated relationship between DNase digestion, subsequent physical removal, and the total residual DNA represented by the reported assay result (BioNTech/Pfizer, qPCR Analytical Procedures (CTD 3.2.S.4.2), pp. 2–4; BioNTech/Pfizer, qPCR Validation Overview (CTD 3.2.S.4.3), p. 1; BioNTech/Pfizer, Process Validation Summary—Mainz/Rentschler (CTD 3.2.S.2.5), pp. 2–4; Pfizer, Residual DNA Characterization Report; Pfizer, Response to EMA Request for Supplementary Information).

In regulatory terms, ICH Q11 calls for the experimental demonstration of process-related impurity removal and evidence that small-scale validation models represent the commercial manufacturing process. DNase I assessment therefore requires clearance data covering the relevant DNA structures and actual process conditions (ICH Q11, section 7.2, pp. 12–13).

4.1.3 Unresolved DNase I Validation under Recommendation 7

At authorisation, the EMA considered the DNase digestion robustness insufficiently demonstrated and required further results under Recommendation 7 (EMA, Comirnaty Public Assessment Report, p. 17).

The subsequent 2022 assessment specifically addressed the applicant's omission of inconclusive small-scale results concerning the relationship between DNase I activity and measured residual DNA, together with its recommendation against process adjustment. EMA stated: "This is not found acceptable, and data should be provided to support that no change is needed." Recommendation 7 remained partly fulfilled at that stage (EMA, Comirnaty Variation Assessment IB/0106/G, p. 16).

ICH Q11 defines process validation through documented, reproducible performance within established operating parameters and places supporting biological process validation data in the CTD 3.2.S.2.5. A conclusion that Recommendation 7 was fulfilled requires a corresponding experimental record (ICH Q11, section 7.1, p. 12).

4.1.4 Incomplete Plasmid Characterization Despite Regulatory Requirements

The BWP required an update describing the origin of all genetic elements in the plasmid starting material, an adequate risk assessment, and the results of additional characterization of the product. The CHMP subsequently accepted the dossier update and concluded that the additional data required no revision of the risk assessment (BWP, Position on Residual DNA in Comirnaty, pp. 6–7; CHMP, Comirnaty Variation Assessment II/0202, pp. 4–5).

Any regulatory assessment must connect the complete plasmid description with validated impurity measurements and the biological properties of the finished product, particularly where BioNTech/Pfizer omitted elements from the plasmid description. This omission normally results in revocation of the marketing authorisation. Rather, the CHMP's variation decision left the highly important questions unresolved.

From a regulatory perspective, the history of the manufacturing plasmid, its sequence composition, and functional annotation are essential for evaluating templates and controlling their residual impurities. ICH Q5B addresses expression-construct components, including replication origins, promoters, enhancers, and selection markers, within its scope for recombinant DNA-derived protein products. The EMA's gene therapy guideline expressly addresses plasmids used to generate RNA and calls for the description of the backbone, transgene, selection gene, and other regulatory sequences, together with the full nucleotide sequence. These provisions identify the scientific information needed to characterize such constructs (ICH Q5B, "Analysis of the Expression Construct"; EMA, Gene Therapy Medicinal Products Guideline (2018), p. 10).

The ATMP quality provisions expressly address documentation of process-related impurities and quantification of different plasmid forms throughout shelf life (Directive 2001/83/EC, Annex I, Part IV, as amended by Directive 2009/120/EC, sections 3.2.2(c)–(d)).

4.2 Inadequate DNA Quantification and Unsupported Safety Thresholds

4.2.1 Inadequacy of Drug Substance Testing as a Complete Exposure Assessment

BioNTech/Pfizer's explanation, reproduced by the BWP, stated that "Any residual DNA present in the DS is presumed to be present in the DP." The proposed initial investigations nevertheless favoured drug substance testing because extraction from lipid nanoparticles

could impair analytical sensitivity (BWP, Position on Residual DNA in Comirnaty, pp. 2 and 5; Pfizer, Residual DNA Risk Assessment, internal p. 3; Health Canada, Pfizer Residual DNA Submissions, p. 6).

Drug substance measurements address the amount of DNA present before formulation. Recipients encounter the finished drug product, in which encapsulation may alter nuclease protection and intracellular delivery of the mRNA. Establishing equivalence between these measurements requires evidence of recovery, the encapsulated fraction, and the effects of the finished product matrix.

The Residual DNA Characterization Report of January 2024 subsequently included the assessment of residual DNA quantitation in drug product samples and estimation of drug product DNA content. Its stated scope also covered fragment sizes, SV40 sequences, intact circular plasmids, and bacterial replication capability. The disclosed purpose and redacted drug product section provide no basis for treating intracellular exposure as directly measured. The experimental procedures and results are needed to determine which exposure questions were actually resolved (Pfizer, Residual DNA Characterization Report (Health Canada release), internal pp. 4–5; Health Canada package pp. 31–32).

ICH Q6B permits reliance on drug substance impurity testing, where the finished product has the same qualitative and quantitative impurity profiles. This calls for analytical procedures that address excipient interference. Both points support the demand for demonstrated equivalence and recovery in the LNP-containing matrix (ICH Q6B, section 4.2.3, p. 12).

4.2.2 Incorrect Equivalence Between Target-Specific qPCR and Total Residual DNA

Pfizer's Residual DNA Risk Assessment states that its assay "includes the entire plasmid and additional elements such as the SV40 promoter/enhancer sequences." The manufacturer's explanation reproduced by the Biologics Working Party (BWP) similarly concludes that "the reported residual DNA template result encompasses all DNA that may be present in the mRNA drug substance", based on its ability to detect linear and circular plasmids. The BWP accepted the method as appropriately validated, and the Committee for Medicinal Products for Human Use (CHMP) subsequently relied on the compliant residual DNA results from 236 batches (Pfizer, Residual DNA Risk Assessment, internal p. 4; Health Canada, Pfizer Residual DNA Submissions, p. 7; BWP, Position on Residual DNA in Comirnaty, pp. 5–6; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

The disclosed CTD procedure specifies fluorescence-based qPCR of diluted drug substance samples using target-specific primers and calibration against a linearized plasmid. It expressly states: "A qualified lot of linearized plasmid standard is used to generate the standard curve." The DNA concentration is calculated from the cycle threshold value of the sample relative to the curve, with adjustment for dilution. This is a target-dependent estimate of DNA concentration, but it does not directly measure every residual DNA fragment (BioNTech/Pfizer, qPCR Analytical Procedures (CTD 3.2.S.4.2), 18 September 2024, pp. 2–3 and 5).

The associated validation overview, approved 25 August 2023, identifies test method TM100010388, co-validation report VAL100146017, validation reports MVR-20-0007-qPCR and MVR-21-0006-V01-qPCR, and transfer report RPT-140496. It declares conformity with ICH Q2(R1), but provides references to the underlying experimental reports rather than their results. The later procedural version therefore cannot automatically be assumed to reproduce every assay configuration used for the historical batches (BioNTech/Pfizer, qPCR Validation Overview (CTD 3.2.S.4.3), p. 1, Table 3.2.S.4.3-1).

The mismatch between the linearized calibration standard and the fragmented DNA being measured matters here. A break within an amplification target prevents that molecule from contributing the expected signal, although its DNA remains in the sample. Linearization of a plasmid does not reproduce a heterogeneous population of nuclease-generated fragments. Accordingly, equivalent DNA masses can yield different qPCR results when their target integrity differs. Sedlackova et al. experimentally demonstrated fragmentation-dependent underestimation and recommended "equally fragmented control DNA." Moderna's manufacturing patent likewise acknowledges that qPCR "does not measure all other smaller DNA molecules that are partially digested" (Sedlackova et al., 2013, doi: 10.1186/1480-9222-15-5, Results, Figure 3 and Conclusions; US10077439B2, description, "mRNA Characterization").

The disclosed procedure leaves the primer sequences and the relevant reagent details undisclosed. Substantial operating parameters, acceptance thresholds, and calculation details were also withheld. The release therefore does not permit independent determination of the amplicon length and location or verification of how its signal represents residual sequences elsewhere in the plasmid. Its visible acceptance checks concern standard amplification, calibration performance, controls, and replicate consistency. Such checks can establish satisfactory performance for the selected target while leaving fragmentation-dependent underestimation unresolved (BioNTech/Pfizer, qPCR Analytical Procedures (CTD 3.2.S.4.2), pp. 2–5, Tables 3.2.S.4.2-2 to -4).

Kämmerer, Schulz, and Steger addressed sample preparation through lipid-nanoparticle disruption with 1% Triton-X-100 and RNase A treatment before fluorometric quantification. They reported 32.7–43.4 ng DNA per clinical dose in four BNT162b2 lots and detected SV40 promoter/enhancer sequences using PCR. Their quantitative results concerned fluorometry, whereas PCR combined with sequence analysis established sequence detection. This distinction is important when comparing these findings with manufacturer qPCR results, as lipid disruption, RNA interference, recovery, and calibration must be evaluated for each method and sample matrix (Kämmerer et al., 2024, BioNTech RNA-Based COVID-19 Injections, "DNA and RNA Quantification", Results and Figure 3).

BioNTech/Pfizer has to demonstrate that its validated method accurately quantifies the residual DNA population actually present, including the effects of fragmentation and sample composition. ICH Q2(R1), expressly invoked by the manufacturer, requires evaluation of relevant analytical characteristics, including accuracy, specificity, precision and quantitation limits. A satisfactory calibration curve or consistent batch results cannot substitute for that demonstration (ICH Q2(R1), Part I, "Types of Analytical Procedures to be Validated", and Part II, sections 1 and 4).

Independent assessment therefore requires TM100010388 and its relevant historical versions, the four identified validation and transfer reports, primer and amplicon information, calibration standard characterization, fragmentation and matrix recovery experiments, and the underlying batch calculations. These records would establish whether the claim to encompass all residual DNA is experimentally verified. The disclosed summaries alone do not provide a complete demonstration.

McKernan's LIDAR method extends this methodological discussion by combining multiplex fluorescence detection with high-resolution melting analysis. He applied different plasmid targets and amplicon lengths, including approximately 50–300 bp, to investigate fragmentation-dependent differences in quantification. Conventional qPCR can also use short amplicons; the decisive limitation is the requirement for an intact amplification target. The proposed method therefore warrants evaluation for fragment-size-dependent recovery, calibration and quantification in the vaccine matrix (*McKernan*, 2026, A Novel qPCR Detection Chemistry; *McKernan*, LIDAR qPCR Demonstration).

Related methodological criticisms were presented by Paul Cullen, Brigitte König, Brigitte Röhrig, Jens Schwachtje, Henrieke Stahl and Henrik Ullrich in a two-part analysis published in *Cicero*. The authors examined residual-DNA quantification, the interpretation of the regulatory DNA limit and the adequacy of regulatory safety assessments (Cullen et al., 2024,

DNA-Verunreinigungen—Part 1 (30 January); Cullen et al., 2024, DNA-Verunreinigungen—Part 2 (31 January)).

In regulatory terms, ICH Q2(R1) specifies the accuracy assessment of impurity-spiked samples or an appropriate independent procedure and the reporting of recovery across an analytical range. For the claimed measurement of total residual DNA, the validation must therefore address the fragmented population and complete analytical procedure (ICH Q2(R1), Part II, sections 4.2–4.3, p. 11).

4.2.3 Inadequate Methodological Basis of Three Publications Supporting Regulatory Safety Claims

Three subsequent publications have been invoked to support the position advanced by BioNTech/Pfizer and regulatory authorities that residual DNA levels in the products examined fall below the applicable regulatory threshold: Kaiser et al. (2025, doi: 10.1016/j.vaccine.2025.127022), *Achs* et al. (2025, doi: 10.1038/s41541-025-01304-9), and Nitta et al. (2026, doi: 10.1016/j.vaccine.2026.129080). It is important to explicitly identify their methodological limitations. Relevant methodological limitations are discussed in Section 2.

Kaiser et al. and *Achs* et al. challenged earlier reports on residual DNA. Both studies employed phenol/chloroform extraction and ethanol precipitation in their respective analytical workflows. These procedures may preferentially reduce the recovery of short DNA fragments and may therefore bias the estimates of total residual DNA downward. This concern was also recognized in the Overriding Public Interest Report Concerning Residual DNA in Comirnaty and Spikevax, which identified potential fragment-size-dependent losses during phenol/chloroform extraction, ethanol precipitation, silica-based purification, and magnetic bead cleanup (T4SI, OPIR on Residual DNA, 2026, pp. 7–8).

Kaiser et al. qualifies its own conclusion by stating: "While we do not see evidence for DNA content 534-fold higher than allowed by the regulatory authorities, we see a DNA content below and in some analyses 1.8-fold above the accepted quantities. We cannot explain this discrepancy in all certainty and we want to point out that our data are the result of experiments that cover the main biases but very likely not all biases that impact DNA quantification in mRNA vaccines" (*Kaiser* et al., 2025, doi: 10.1016/j.vaccine.2025.127022, Discussion). The study therefore reports exceedance of the limit in some analyses, by up to 1.8-fold, and states that it cannot fully account for the discrepancy with independent measurements. Its sample comprised four vials of Comirnaty and two of Spikevax (Kaiser et al., 2025, doi: 10.1016/j.vaccine.2025.127022, Methods), and after RNase treatment and

ethanol precipitation it reported "DNA impurities ranging from ~35 ng (Comirnaty) and ~ 55 ng (Spikevax) per vaccination dose", which is 3.5- and 5.5-fold the 10 ng per dose limit (*Kaiser* et al., 2025, doi: 10.1016/j.vaccine.2025.127022, Results, section 3.3).

The DNA measured in that study varies with the quantity of RNase applied. The authors record that "samples with high content of DNA (e.g. mean of 12.6 ng) were samples where 10 µg RNase was used, while samples with lower DNA content were pre-treated with 20 µg of RNase" (Kaiser et al., 2025, doi: 10.1016/j.vaccine.2025.127022, Results, section 3.3). The lowest reported values therefore arise from the most aggressive pre-treatment. Kämmerer et al., whose measurements the study was advanced against, identify the additional ethanol precipitation step as the material methodological difference, noting that it "is known to inefficiently precipitate short DNA sequences representing the majority of reminiscent DNA after DNase digestion during the manufacturing process" (Kämmerer et al., 2024, BioNTech RNA-Based COVID-19 Injections, Discussion). Both laboratories disrupted the lipid nanoparticles with Triton X-100 and both applied RNase, so the precipitation step is the variable that distinguishes them.

Achs et al. likewise reports figures that do not reach the conclusion drawn from them. The highest average copy number reported is 9.8×10^8 copies of the kanamycin-resistance target per dose, which the authors convert to 8 ng per dose against a 10 ng limit on a plasmid-equivalent basis (*Achs* et al., 2025, doi: 10.1038/s41541-025-01304-9, Discussion). Applying the authors' own median fragment length of 150 bp to their 63 bp kanamycin amplicon gives a survival probability of 65.7 per cent and a corrected figure of 12.2 ng per dose, while applying it to their 228 to 233 bp spike amplicons gives 21.2 per cent and 37.8 ng per dose. The arithmetic and its sources are set out in Section 2.2.2.

"Without demonstrating size-independent recovery, the overall percentages could mask preferential loss of smaller fragments, further biasing results." These criticisms concern that published study and cannot establish the performance of Pfizer's undisclosed assay without its validation data (*McKernan* et al., 2026, doi: 10.18632/oncotarget.28913, section "Lack of size-dependent recovery validation").

These criticisms challenge whether aggregate recovery estimates and target-specific measurements represent the complete residual DNA population. Preferential loss of short fragments, unequal amplicon lengths and inadequately characterized RNA interference can each affect the reported concentration. Unresolved bias in these measurements substantially weakens their use as evidence that residual DNA has been comprehensively characterized and shown to remain below the relevant threshold. The analyses of Kaiser et al., *Achs* et al. and Nitta et al. therefore require substantive consideration in the regulatory evaluation

(*McKernan* et al., 2026, doi: 10.18632/oncotarget.28913; *McKernan*, 2026, preprint, doi: 10.5281/zenodo.22801568; T4SI, OPIR on Residual DNA, 2026, pp. 7–8).

ICH Q2(R1) identifies specificity in the sample matrix and experimentally demonstrated accuracy as essential analytical characteristics. Applying these criteria to the cited publications reveals unresolved extraction losses, RNA interference, and target-dependent recovery as substantive deficiencies in their use as evidence of threshold compliance (ICH Q2(R1), Part I, section 2, p. 5; Part II, sections 1.2 and 4.2–4.3, pp. 9–11).

4.2.4 Unsafe 10 ng WHO-Threshold and Fragment Length Correlation

Pfizer described the WHO acceptance criteria as "considered safe for human use in biological products, including mRNA vaccines." The BWP relied on the approved residual DNA specification, and the CHMP accepted the overall safety conclusion at levels below that specification (Pfizer, Residual DNA Risk Assessment, internal p. 3; Health Canada, Pfizer Residual DNA Submissions, p. 6; BWP, Position on Residual DNA in Comirnaty, pp. 6–7; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

WHO Technical Report Series No. 978, Annex 3, explains that the potential risk depends on the size, quantity, and biological activity of residual DNA, together with its removal or inactivation during manufacturing. The numerical limit of 10 ng per parenteral dose does not account for "the size of the DNA fragments and any potentially inactivating steps in the manufacturing process." Acceptable limits should therefore be agreed upon a product-specific basis, considering the cell substrate, intended use, and manufacturing process (WHO, TRS 978, Annex 3, pp. 103–106; T4SI, OPIR on Residual DNA, 2026).

The scope of the cited WHO recommendations concerns animal cell substrates and expressly excludes products manufactured using microbial cells. The application of this framework to bacterial plasmid DNA in an LNP-formulated modRNA product requires a separate scientific justification. Treating the numerical benchmark as sufficient evidence of safety omits the formulation and biological properties on which the assessment depends (WHO, TRS 978, Annex 3, pp. 89 and 103–106; T4SI, OPIR on Residual DNA, 2026).

A regulator has stated the provenance of the specification in its own words. HC's quality review records that "[t]he current approved specification for residual DNA template (<10ng/dose) is in line with WHO recommendation concerning residual DNA fragments derived from continuous cell line (as worst case)" (Health Canada, SV40 Correspondence and Quality Review, Quality Evaluation Executive Summary, internal p. 4; replacement p. 193). The limit applied to a bacterial plasmid impurity in a lipid nanoparticle formulation is

therefore acknowledged by the regulator to derive from guidance addressed to continuous animal cell substrates, which is the extrapolation this section examines. The same page records the manufacturer's commitment to supply data on the size of DNA fragments and on residual plasmid capable of replication in bacterial cells by 1 December 2023, the date by which the BWP's Comirnaty position separately committed to four further analyses. No results of either set appear in the released records of either agency.

DNA quantity and fragment characteristics must be assessed together. DNA mass alone leaves the number of molecules and free ends unspecified. For an equal mass of double-stranded DNA, 200 bp fragments comprise 40 times as many molecules as 8,000 bp fragments. Linear fragments also provide free ends that can participate in cellular DNA repair and ligation processes. *McKernan* argued that this increase in potential integration substrates undermines the assurances based solely on DNA mass. Establishing the resulting integration probability requires evidence addressing uptake, nuclear access, fragment structure, and cellular repair processes (Sheng-Fowler et al., 2009, doi: 10.1016/j.biologicals.2009.02.015; *McKernan*, 2026, The 10ng Limited Hangout).

The mean fragment length also conceals the upper end of the distribution. *Speicher* et al. reported a mean of 214 bp and a maximum of 3.5 kb in sequencing of one vaccine vial. Short fragments can retain regulatory activity, whereas longer fragments may preserve larger functional units. The relevant assessment requires the complete size distribution, absolute quantities, and retained sequence functions, supported by validated recovery and quantification across fragment sizes (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517; WHO, TRS 978, Annex 3, pp. 103–106).

ICH Q6B links impurity acceptance criteria to the manufacturing process and the materials examined in non-clinical and clinical studies. This evidentiary requirement reinforces the need to justify the DNA limit against the actual impurity profile and formulation (ICH Q6B, sections 2.1.4 and 3, pp. 6 and 9–10).

4.3 Unsupported Assumptions about Residual DNA Delivery and Clearance

4.3.1 Incorrect Equivalence Between Environmental DNA Exposure and LNP-Mediated Delivery

Pfizer acknowledged that "Residual DNA encapsulated within the LNP would be delivered to the cytoplasm with the vaccine mRNA". Nevertheless, its risk assessment compared residual

DNA exposure with continuous exposure to bacterial and viral DNA through the gut and mucosal surfaces. The BWP and CHMP accepted the overall safety conclusion (Pfizer, Residual DNA Risk Assessment, internal pp. 3–4; Health Canada, Pfizer Residual DNA Submissions, pp. 6–7; BWP, Position on Residual DNA in Comirnaty, pp. 6–7; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

That comparison provides no quantitative equivalence between environmental exposure and intramuscular administration in a formulation designed to facilitate cellular delivery. *Speicher* et al. estimated that 70–90% of the DNA in the samples examined was LNP-associated or protected, with 10–30% free. Kämmerer et al. likewise addressed the importance of disrupting LNPs before DNA measurement. These findings make formulation, nuclease protection and intracellular availability essential components of the exposure assessment (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517; Kämmerer et al., 2024, BioNTech RNA-Based COVID-19 Injections).

The delivery literature establishes that formulation and administration conditions affect cellular uptake, gene expression, and biodistribution. Phua et al. compared naked and nanoparticle-formulated mRNA, while Di et al. showed that particle size and delivery route influence LNP distribution and transgene expression. Maurisse et al. compared methods of introducing DNA into primary and transformed mammalian cells. Zhu et al. demonstrated LNP delivery of plasmid DNA and transgene expression in culture and in vivo, including prolonged expression in animal models. These studies support assessment of the delivery context; however, their experimental systems do not supply a universal quantitative multiplier for the uptake or integration of residual DNA in Comirnaty (*Phua* et al., 2013, doi: 10.1016/j.jconrel.2012.12.029; *Di* et al., 2022, doi: 10.1007/s11095-022-03166-5; *Maurisse* et al., 2010, doi: 10.1186/1472-6750-10-9; *Zhu* et al., 2022, doi: 10.1038/s41467-022-31993-y).

Foreign DNA entering cells may activate cytosolic cGAS–STING and endosomal TLR9 signalling, with type I interferon production and inflammatory cytokine responses. The cited literature describes relationships between persistent DNA sensing, inflammation, cellular stress and tissue injury. Ndeupen et al. additionally investigated the inflammatory properties of the LNP component. These mechanisms require separate assessment of the contributions of the carrier and its nucleic acid cargo (*Motwani* et al., 2019, doi: 10.1038/s41576-019-0151-1; *Kwon and Bakhoum, 2020*, doi: 10.1158/2159-8290.CD-19-0761; *Ndeupen* et al., 2021, doi: 10.1016/j.isci.2021.103479; *Decout* et al., 2021, doi: 10.1038/s41577-021-00524-z).

King et al. demonstrated a role for DNA sensing and type-I interferon signalling in the inflammatory response to myocardial infarction. This provides mechanistic evidence relevant to cardiac inflammation and injury; its application to vaccine-associated myocarditis requires

investigation of the actual exposure and disease mechanism (*King* et al., 2017, doi: 10.1038/nm.4428).

Sheng-Fowler et al. identified infectivity and oncogenicity as the principal biological activities of concern for residual cell-substrate DNA in viral vaccines. The experimental literature on DNA uptake, recombination, and biological activity also includes Folger et al., Smithies et al., García-Olmo et al., and Mittra et al. These studies establish reasons to examine the fate and activity of foreign DNA after cellular entry. Their relevance to a particular vaccine depends on the DNA species, delivery conditions and exposure achieved (Sheng-Fowler et al., 2009, doi: 10.1016/j.biologicals.2009.02.015; *Folger* et al., 1982, doi: 10.1128/mcb.2.11.1372-1387.1982; Smithies et al., 1985, doi: 10.1038/317230a0; *García-Olmo* et al., 2010, doi: 10.1158/0008-5472.CAN-09-3513; *Mittra* et al., 2015, doi: 10.1007/s12038-015-9508-6).

A product-specific assessment must therefore address total DNA quantity, fragment structure, biological activity, manufacturing clearance, and LNP-mediated cellular delivery. Classifying DNA as a process-related impurity does not resolve these questions.

ICH S12 specifies a test article representative of the clinical formulation and an administration route reflecting clinical use. These design criteria support the direct investigation of LNP-associated DNA exposure (ICH S12, sections 4.2 and 4.5, pp. 3–4).

4.3.2 Unsupported Extrapolation of DNA Degradation and Persistence Data

Pfizer stated that residual DNA "is rapidly degraded (with a half-life of 50–90 mins)." This numerical assertion supports its subsequent conclusions concerning nuclear exposure, persistence, and integration (Pfizer, Residual DNA Risk Assessment, internal p. 3 and references 11–12 on internal p. 6; Health Canada, Pfizer Residual DNA Submissions, pp. 6 and 9).

The underlying experiment by Lechardeur et al. measured degradation of plasmid DNA microinjected into the cytoplasm of HeLa and COS cells. Crucially, the same publication reports inhibition of degradation "following the encapsulation of DNA in phospholipid vesicles". The cited half-life depends on the molecular form, carrier protection, release kinetics and recipient cell type (Lechardeur et al., 1999, doi: 10.1038/sj.gt.3300867, p. 482).

LNP protection is integral to the interpretation of this half-life. The formulation is intended to protect nucleic acids during delivery, and *Speicher* et al.'s estimates indicate substantial protection of residual DNA in the samples examined. A degradation estimate derived from microinjected DNA therefore requires justification before it is applied to DNA protected by the vaccine formulation. The relevant endpoints include release from LNPs, intracellular

persistence and elimination from exposed tissues (*Speicher* et al., 2025, doi: 10.1080/08916934.2025.2551517; Lechardeur et al., 1999, doi: 10.1038/sj.gt.3300867).

Pfizer asserted that "plasmids or plasmid-derived DNA do not persist in the body, even when administered at high doses via various routes," citing three clinical DNA-vaccine publications (Pfizer, Residual DNA Risk Assessment, internal p. 4 and references 20–22 on internal p. 7; Health Canada, Pfizer Residual DNA Submissions, pp. 7 and 10).

The cited studies evaluated influenza, Zika and SARS-CoV-2 DNA vaccines, principally through clinical safety, immunogenicity and efficacy endpoints. Such outcomes do not directly measure clearance of residual DNA from Comirnaty or establish the absence of persistent DNA in particular tissues. The formulations, constructs and administration conditions also differ (Ledgerwood et al., 2011, doi: 10.1016/S1473-3099(11)70240-7, pp. 916–924; Gaudinski et al., 2018, doi: 10.1016/S0140-6736(17)33105-7, pp. 552–562; Khobragade et al., 2022, doi: 10.1016/S0140-6736(22)00151-9, Methods and Outcomes, pp. 1313–1321).

ICH S12 treats distribution, persistence, and clearance as measured biodistribution endpoints and calls for sampling that captures their time course. Extrapolation from another construct requires justification (ICH S12, sections 2, 4.6, and 5.7, pp. 2, 4, and 7).

4.4 Unsupported Dismissal of the Biological Activities of SV40 Elements

BioNTech/Pfizer confirmed that the production plasmid contains an SV40 promoter, enhancer, origin of replication, and polyadenylation signal. Its explanation distinguished their role in mammalian selection from their lack of a role in bacterial plasmid production and mRNA manufacture. The assessment of residual DNA must address the functions these elements may retain after delivery to mammalian cells (BWP, Position on Residual DNA in Comirnaty, pp. 3–4 and 6; Pfizer, Residual DNA Risk Assessment, internal pp. 4–5; Health Canada, Pfizer Residual DNA Submissions, pp. 7–8).

4.4.1 Unjustified Inference of Safety from a Small SV40 Fraction

Pfizer described SV40 sequences as "a small fraction of the total residual DNA." The BWP requested SV40-specific investigation; the CHMP subsequently stated: "The SV40-derived sequences comprise a small fraction of the total residual DNA of Comirnaty and are included in the overall risk assessment" (BWP, Position on Residual DNA in Comirnaty, pp. 4–6; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

Relative abundance establishes the proportion measured, whereas absolute exposure and retained biological function require separate evidence. The proportion in the starting plasmid also cannot establish the proportion remaining after digestion and purification without data on differential degradation and recovery (Pfizer, Residual DNA Risk Assessment, internal p. 4; Health Canada, Pfizer Residual DNA Submissions, p. 7; BWP, Position on Residual DNA in Comirnaty, pp. 4–6; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

Short DNA fragments can retain regulatory activity even when they lack a complete protein-coding sequence. The SV40 enhancer contains tandem 72 bp repeats, and experimental studies attribute nuclear targeting activity to short sequences within this regulatory region. Other retained elements may include promoters, replication origins, hypermutation-targeting sequences and short open reading frames. Their activity depends on sequence integrity and molecular context (Dean et al., 1999, doi: 10.1006/excr.1999.4716; *McKernan* et al., 2026, doi: 10.18632/oncotarget.28913; *McKernan*, 2026, A Novel qPCR Detection Chemistry).

A correct assessment requires absolute SV40 quantities, the full fragment-size distribution, and identification of retained functional sequences, with validated detection and recovery across relevant fragment sizes. WHO expressly recognizes method-dependent results for small fragments. Redactions in Pfizer's Characterization Report limit independent verification of these endpoints, and the LNP formulation adds the exposure considerations addressed in Section 4.3 (WHO, TRS 978, Annex 3, p. 106; Pfizer, Residual DNA Characterization Report, internal pp. 4–5).

ICH Q6B calls for the characterization of impurities and, where possible, the evaluation of their biological activities. The relative abundance consequently leaves the functional assessment unresolved (ICH Q6B, section 2.1.4, p. 6).

4.4.2 Incorrect Reliance on Nuclear Barriers and Post-mitotic Muscle Cells

Pfizer stated that nuclear transport and potential integration are "impeded by multiple biological mechanisms". The BWP emphasized poor nuclear delivery by lipid particles and the predominantly post-mitotic state of muscle cells; the CHMP subsequently accepted a negligible integration likelihood (Pfizer, Residual DNA Risk Assessment, internal pp. 3–4; Health Canada, Pfizer Residual DNA Submissions, pp. 6–7; BWP, Position on Residual DNA in Comirnaty, p. 6; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

The BWP's reasoning is recorded in its own words: "while lipid particles have been demonstrated to promote the uptake of complexed mRNA molecules into the cytoplasm of targeted cells, they have usually a poor capacity to facilitate further transport of the

complexed molecules into the cell nucleus even in the case of highly mitotic cells. In this case, the vaccine is delivered into the muscle, where mostly post-mitotic cells reside. Therefore, the possibility that the low levels of residual impurities could alter DNA in vaccinated individuals seems highly unlikely" (BWP, Position on Residual DNA in Comirnaty, p. 6). The post-mitotic character of muscle tissue is advanced there as a ground for reassurance. In the nuclear-import literature it is the condition under which the SV40 enhancer is reported to matter, since the constructs were imported in growth-arrested and non-dividing cells.

An Australian regulatory evaluator reached the opposite conclusion on the same point. Documents released under Therapeutic Goods Administration Freedom of Information request 25-0058 record the finding that "[t]he SV40 enhancer region can promote nuclear transport of DNA", qualified in the same entry by the assessment that the risk is low given the low level of DNA and the low probability of integration events, and that the origin of replication "is not used for replication" (TGA, Residual DNA and SV40 Correspondence, p. 155). The same entry records that "[t]he TGA does not hold any evidence that the residual DNA is encapsulated in lipid nanoparticles. However, if it were, there is a low safety concern with the levels present as there is a sufficient safety margin" (TGA, Residual DNA and SV40 Correspondence, p. 155). The premise that residual DNA is not delivered within lipid nanoparticles is therefore unevidenced on that regulator's own record, and the nuclease-protection and fragment-count measurements bearing on it are set out in Sections 2.2.1 and 4.3.1.

The cited literature thus describes a sequence-dependent mechanism that can overcome the nuclear barrier without cell division. Dean's experimental work provides primary evidence for SV40 sequence-dependent nuclear import (Bai et al., 2017, doi: 10.1042/BSR20160616, PDF p. 7; Dean, 1997, doi: 10.1006/excr.1996.3427, pp. 293–302).

Dean et al. demonstrated that the 72 bp SV40 enhancer repeats facilitated nuclear import of experimental plasmid constructs. Before cell division, cytoplasmic injection of as few as 3–10 enhancer-containing plasmid copies per cell produced significant reporter expression. This result establishes nuclear delivery and expression at low copy number in that experimental system. Vacik et al., Dean et al. and Sattar et al. further discuss sequence-dependent nuclear targeting and its application to gene delivery (Dean et al., 1999, doi: 10.1006/excr.1999.4716; Vacik et al., 1999, doi: 10.1038/sj.gt.3300924; Dean et al., 2005, doi: 10.1038/sj.gt.3302534; Sattar et al., 2023, doi: 10.3389/fmicb.2023.1073789).

The emphasis on predominantly post-mitotic muscle cells also requires examination of the cells actually exposed. Sahin et al. describe dendritic cells as important targets of mRNA-based immunization, as does BioNTech's account of its mRNA platforms. *Di* et al. demonstrated

systemic distribution to other tissues after local LNP administration in an experimental model. An assessment confined to the properties of muscle cells therefore leaves exposure of antigen-presenting and other cells unresolved (*Sahin* et al., 2014, doi: 10.1038/nrd4278; BioNTech, mRNA Platforms; *Di* et al., 2022, doi: 10.1007/s11095-022-03166-5).

A product-specific assessment must determine whether residual fragments retain the relevant nuclear targeting sequences and reach susceptible cells in sufficient quantities. Nuclear entry and genomic integration are distinct endpoints that require appropriate experimental evidence. The nuclear import literature directly challenges the categorical reliance on the nuclear barrier or the absence of cell division as a sufficient safety argument. The ATMP integration-study provision requires scientific justification for omission, identifying lack of nuclear entry as one possible justification (Directive 2001/83/EC, Annex I, Part IV, section 4.2.3(g)). The SV40 nuclear-import evidence directly concerns that premise.

4.4.3 Unsupported Dismissal of Functional Promoters and Unintended Gene Expression

Pfizer considered the hypothetical entry of intact promoter/enhancer and resistance gene elements into the nucleus and stated that transient resistance gene expression "would have no biological effect to the vaccinee" (Pfizer, Residual DNA Risk Assessment, internal p. 5; Health Canada, Pfizer Residual DNA Submissions, p. 8).

This assertion extends beyond the argument that nuclear entry is unlikely. It assumes that, even if entry and expression occurred, their consequences would be biologically inconsequential. However, the passage identifies no expression threshold, affected cell population, duration, or experimental endpoint supporting this conclusion.

The BWP records that SV40 elements drive neomycin/kanamycin resistance in mammalian cells. Their function therefore depends on the cellular context. A conclusion that the effect would be clinically insignificant requires an exposure and consequence analysis. The absence of a role in bacterial manufacturing does not provide this analysis (BWP, Position on Residual DNA in Comirnaty, pp. 3–4 and 6).

The bacterial ColE1/pMB1 origin warrants consideration. Lemp et al. demonstrated a TATA-containing cryptic promoter within the pMB1/pUC origin that generates transcripts in mammalian cells. Readthrough into adjacent sequences and splicing can produce misleading results in reporter assays. The authors reported that pMB1/pUC origins predominated among the plasmids in the database they examined and identified homology with ColE1 and p15A origins, supporting the investigation of related backbones for unintended transcriptional

activity (Lemp et al., 2012, doi: 10.1093/nar/gks451; Microbe Notes, ColE1 Plasmid Overview).

Cryptic promoter activity refers to transcription initiated from a sequence whose promoter function is unrecognized or unintended in the construct under study. In chromosomal settings, chromatin organization and epigenetic regulation influence access to such sites. The plasmid-origin activity demonstrated by Lemp et al. provides a direct basis for examining unintended transcripts from the backbone introduced into mammalian cells (Lemp et al., 2012, doi: 10.1093/nar/gks451).

McKernan's discussion of cryptic promoters records literature searches dated 8 October 2025 and 6 December 2025 that identified the Lemp publication. *McKernan* subsequently reported Oxford Nanopore reads containing the entire spike open reading frame together with the ColE1 backbone and proposed that full-length spike expression from transfected DNA was hypothetically possible (*McKernan*, 2025, Cryptic Promoters; *McKernan*, 2026, A Novel qPCR Detection Chemistry).

The regulatory question is whether residual molecules preserve a transcriptionally competent arrangement and reach mammalian nuclei. Depending on the retained construct, potential transcripts could involve spike sequences, resistance genes, or other backbone regions. Assessing these possibilities requires sequence-resolved characterization, transcriptional analysis, and evaluation of biological consequences. The absence of an intended role for an element during bacterial manufacture leaves its possible activity after cellular delivery unresolved.

The ATMP toxicology provisions expressly require evaluation of the in vivo effects of unintended expression products (Directive 2001/83/EC, Annex I, Part IV, section 4.2.3(a)). This supports examination of transcriptionally active residual constructs.

4.4.4 Unsupported Exclusion of Plasmid Replication and Cellular Persistence

Pfizer stated that the plasmid "is not replication competent in mammalian cells". In its supplementary response, it invoked the requirement for large T-antigen in SV40-origin-dependent replication and referred back to its risk assessment. New supporting documentation for that particular answer was listed as "None". The CHMP subsequently accepted the assessment concerning cellular accumulation (Pfizer, Residual DNA Risk Assessment, internal p. 3; Health Canada, Pfizer Residual DNA Submissions, p. 6; Pfizer, Response to EMA Request for Supplementary Information, p. 2; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

Cellular persistence can occur in the absence of autonomous replication. Accumulation under repeated exposure depends on the relationship between delivery and elimination, whereas bacterial transformation assays address a different biological system. The large T-antigen argument concerns SV40-origin-dependent replication; the broader conclusion concerning cellular accumulation also depends on the clearance and integration assumptions in Pfizer's risk assessment.

McKernan questioned whether the combination of SV40, f1, and ColE1 origins permits persistence or replication in mammalian cells without large T-antigen. The literature cited in this discussion comprises different experimental systems: Madzak et al. investigated SV40-based shuttle vectors; Piechaczek et al. developed a vector combining the SV40 origin with chromosomal scaffold/matrix attachment regions (S/MARs); and Giannakopoulos et al. examined the functional contribution of S/MARs to episomal vectors. These studies make vector architecture and host-cell conditions central to the analysis. They require assessment of which supporting elements are present in Comirnaty's plasmid before replication competence can be inferred (*McKernan*, 2024, SV40 Origin of Replication in Mammalian Cells; Madzak et al., 1989, doi: 10.1016/0022-2836(89)90221-0; Piechaczek et al., 1999, doi: 10.1093/nar/27.2.426; Giannakopoulos et al., 2009, doi: 10.1016/j.jmb.2009.02.043).
The adequacy of the evidence already submitted is therefore decisive. The supplementary response's entry "None" for new supporting documentation leaves the assessment dependent on the earlier material and its ability to address the particular plasmid, delivery formulation, and exposed cells (Pfizer, Response to EMA Request for Supplementary Information, p. 2; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

ICH S12 recognizes episomal DNA as the basis for persistence and addresses sampling after repeated administration. The absence of autonomous replication leaves a separate persistence endpoint unresolved (ICH S12, section 4.6, p. 4, and Glossary, "Persistence", p. 10).

4.4.5 Incomplete Assessment of Genomic Integration and Carcinogenic Mechanisms

Pfizer stated: "No SV40 proteins are encoded or present in the vaccine. Importantly, the SV40 sequence elements are not oncogenes and do not cause cancer". The BWP reported no scientific evidence that the residual fragments could act as insertional mutagens; the CHMP reproduced the categorical cancer statement (Pfizer, Residual DNA Risk Assessment, internal p. 5; Health Canada, Pfizer Residual DNA Submissions, p. 8; BWP, Position on Residual DNA in Comirnaty, pp. 6–7; CHMP, Comirnaty Variation Assessment II/0202, p. 5).

The absence of viral oncogenes is insufficient to exclude insertional genotoxicity, a risk established through clinical gene therapy experience. Hacein-Bey-Abina et al. documented uncontrolled clonal T-cell proliferation following integration of a therapeutic retroviral vector near the human LMO2 proto-oncogene, causing its abnormal expression. The relevant mechanism was activation of a cellular proto-oncogene by vector insertion. These findings demonstrate why the absence of an encoded viral oncoprotein cannot substantiate a general exclusion of carcinogenic mechanisms (Hacein-Bey-Abina et al., 2003, doi: 10.1126/science.1088547).

The WHO expressly recognizes insertional mutagenesis through "inactivating a tumour suppressor gene or activating a proto-oncogene," while considering this pathway less likely because it requires integration at a relevant genomic site. A residual DNA assessment must therefore address the likelihood of exposure, nuclear entry, and consequential integration. The conclusion that these elements "do not cause cancer" because they are "not oncogenes" overlooks this separate mechanism (WHO, TRS 978, Annex 3, p. 104).

Experimental integration evidence also requires consideration of the vector and delivery system used. Strayer et al. documented integration and durable transgene expression with recombinant SV40-derived gene therapy vectors lacking large T-antigen. Lim et al. demonstrated high spontaneous integration rates for end-modified linear DNA after mammalian cell transfection. These findings establish mechanisms relevant to the assessment of foreign DNA and its molecular form. Quantifying their relevance to vaccine-derived fragments requires product-specific exposure and integration data (Strayer et al., 2002, doi: 10.1006/mthe.2002.0657; Lim et al., 2023, doi: 10.1038/s41598-023-33862-0).

Subsequent research by Šenigl et al. identified SV40 enhancer-associated targeting of somatic hypermutation in experimental cellular systems, further demonstrating that regulatory sequences require assessment beyond their protein-coding capacity (Šenigl et al., 2024, doi: 10.1016/j.tvr.2024.200293).

Drayman et al. demonstrated binding of p53 to the SV40 regulatory region and repression of viral T-antigen expression. This establishes interaction between a host tumour suppressor and the viral regulatory DNA. The further hypothesis that residual promoter fragments sequester enough p53 to impair tumour suppression requires a separate exposure and functional analysis. Such an effect cannot be inferred solely from DNA binding (Drayman et al., 2016, doi: 10.18632/oncotarget.10769).

Safety assessment must address regulatory sequence activity, the probability and genomic location of integration, and the consequences for cellular gene regulation. The absence of an encoded viral oncoprotein leaves these mechanisms unassessed unless the supporting evidence addresses them directly.

The ATMP framework separately specifies genotoxicity, carcinogenicity and integration investigations, with product-appropriate tumourigenicity models (Directive 2001/83/EC, Annex I, Part IV, sections 4.2.3(d)–(g)). These distinct endpoints require evidence beyond the absence of viral oncogenes.

4.4.6 Unresolved Experimental Evidence of Persistence and Putative Integration

BioNTech/Pfizer's conclusions concerning negligible integration, lack of persistence, and absence of replication provide the context for the subsequent experimental reports. The CHMP accepted the overall assessment in February 2024. Later findings require evaluation against the particular mechanisms and endpoints underlying that conclusion (Pfizer, Residual DNA Risk Assessment, internal pp. 3–5; Health Canada, Pfizer Residual DNA Submissions, pp. 6–8; CHMP, Comirnaty Variation Assessment II/0202, p. 5).
In 2024, *McKernan* reported analyses of human ovarian cancer cells (OVCAR3) exposed to Comirnaty and maintained through two serial passages. He detected SV40 promoter/enhancer, origin and spike targets, and interpreted sequencing reads as putative integration events involving spike DNA and chromosomes 9 and 12, including the FAIM2 locus. He also interpreted persistence through passage as evidence relevant to plasmid maintenance or replication (*McKernan*, 2024, Vaccine-Targeted qPCR of Cancer Cell Lines; *McKernan*, 2024, Plasmid DNA Replication in BNT162b2).

These reports raise concrete questions for independent replication. Confirmation requires validation of the DNA junctions and exclusion of contamination and library-construction artefacts, followed by investigation in primary cells and relevant in vivo systems. The reported observations concern a transformed cell line; establishing their implications for vaccine recipients requires evidence under the relevant exposure conditions.

McKernan also reported SV40 and origin-related qPCR signals in a colon cancer biopsy obtained approximately one year after the patient's fourth Comirnaty injection. He compared these signals with a human genomic control and interpreted the relatively high abundance as evidence consistent with integration or episomal replication. The report describes subsequent sequencing intended to distinguish those possibilities. The observation and the proposed explanation require separate confirmation because qPCR abundance alone cannot

determine whether the detected DNA is integrated, episomal or derived from another source (*McKernan*, 2024, SV40 Origin of Replication in Mammalian Cells).

McKernan has additionally reported plasmid-related sequences in publicly deposited RNA-sequencing datasets associated with *Lee* et al., *Knabl* et al., *Ryan* et al., *Odak* et al., and *Krawczyk* et al. *Chakraborty's* preprint reanalysed the *Ryan* dataset. (*McKernan*, 2025, DNA contamination entered into the Congressional record, 14 September; *Lee* et al., 2022, doi: 10.1016/j.celrep.2022.110680; Knabl et al., 2022, doi: 10.1093/rheumatology/keac281; *Ryan* et al., 2023, doi: 10.1016/j.xcrm.2023.100971; *Chakraborty*, 2024, preprint, doi: 10.31219/osf.io/hzyn3; Odak et al., 2024, doi: 10.1016/j.ebiom.2023.104947; *Krawczyk* et al., 2025, doi: 10.1038/s41586-025-08842-1).

ICH S12 calls for tissue-specific spike-and-recovery experiments and documentation of assay sensitivity and reproducibility. These requirements define concrete verification steps for reported tissue and cell culture findings (ICH S12, section 5.1, p. 5).

4.4.7 Incorrect Inference of Genomic Safety from Clinical Experience

Pfizer's October communication stated that "no signs of DNA mutation or COVID-19 vaccine-induced cancer have been reported to date." Its December assessment relied on more than one billion administered doses and the established clinical safety profile to conclude that residual DNA posed no safety risk (BWP, Position on Residual DNA in Comirnaty, p. 3; Pfizer, Residual DNA Risk Assessment, internal p. 5; Health Canada, Pfizer Residual DNA Submissions, p. 8).

The strength of this inference depends on whether surveillance can detect and attribute the particular outcome. The cited passages provide no defined sensitivity for genomic alterations, no residual DNA exposure classification, and no latency analysis for the proposed mechanisms. Routine adverse event reporting does not directly measure somatic integration or mutation.

Kuperwasser and El-Deiry reviewed 69 publications reporting malignancies temporally associated with SARS-CoV-2 infection and/or COVID-19 vaccination, and discussed mechanisms involving spike biology, immune dysregulation and residual plasmid DNA. The review identifies observations and hypotheses requiring studies designed to assess causation and quantify risk (Kuperwasser and El-Deiry, 2026, doi: 10.18632/oncotarget.28824).

Extensive clinical use provides relevant safety evidence whose interpretation depends on the outcomes measured and the sensitivity of surveillance. A categorical exclusion of genomic

effects requires evidence capable of detecting the proposed alterations, their latency, and their relationship to exposure. The manufacturer's conclusion exceeds what routine adverse-event reporting can establish about somatic integration or mutation. A regulator repeated the same inference three years later, stating in its reply to Order Paper Question Q-1406 of 21 September 2026 that "to date, over 3 billion doses of COMIRNATY have been administered world-wide, including over 69 million doses in Canada" and that no safety signals related to residual DNA have been detected (House of Commons of Canada, Reply to Order Paper Question Q-1406 (2026)). The absence of a detected signal supports that conclusion only so far as the surveillance relied on is capable of detecting the outcome in question, and no such capability is identified in that reply either.

The ATMP provisions require a long-term follow-up strategy and specifically identify neoplastic proliferation arising from insertional mutagenicity as a safety endpoint (Directive 2001/83/EC, Annex I, Part IV, sections 5.1.7 and 5.2.3(d)).

4.5 Inadequate Disclosure of Manufacturing Plasmid and Deliberate Omission of Functional Elements

4.5.1 Inadequate Disclosure of the Plasmid's Gene Therapy History

BioNTech/Pfizer explained that SV40 elements were unnecessary for bacterial plasmid production and mRNA manufacture. The BWP accepted their lack of a manufacturing function while recording their role in mammalian selection. The plasmid's development history is therefore directly relevant to why these elements were retained and how their functions were evaluated (Health Canada, SV40 Correspondence, p. 21; BWP, Position on Residual DNA in Comirnaty, pp. 3–4 and 6).

Publications that specifically describe the development and manufacture of BNT162b2 expressly acknowledge the use of expertise from Pfizer's gene therapy programme.

Thorn et al. stated: "At Pfizer, we utilized prior plasmid DNA (pDNA) manufacturing technology expertize from Pfizer's Gene Therapy Program." Lewis et al. likewise attributed the rapid development and production of Comirnaty partly to "prior experience in manufacturing critical starting raw material (plasmid DNA) from Pfizer's Gene Therapy Program" (Thorn et al., 2022, doi: 10.1016/j.copbio.2022.102803; Lewis et al., 2023, doi: 10.1016/j.xphs.2022.09.014).

The patent record provides a complementary account of the vector's development. BioNTech's Coronavirus vaccine patent application describes the manufacture of BNT162b2 in Example 11: the spike-coding DNA was cloned into "a starting plasmid vector with

backbone sequence elements for improved RNA stability and translational efficiency." The passage expressly cites Holtkamp et al. (2006, doi: 10.1182/blood-2006-04-015024) and Orlandini von Niessen et al. (2019, doi: 10.1016/j.ymthe.2018.12.011), connecting BNT162b2's design to earlier RNA platform work. It identifies the T7 promoter, untranslated regions, and interrupted A30LA70 poly(A) sequence among the relevant elements (WO2021213924A1, Example 11, "Manufacturing of in vitro transcribed RNA").

Two BioNTech/TRON patent disclosures describing pST1 constructs, based on *Holtkamp* et al. (2006, doi: 10.1182/blood-2006-04-015024), explicitly state: "The plasmid backbone was derived from pCMV-Script" (WO2016113395A1, Example 12; US10669322B2, Example 1). The manufacturer's documentation for this parental vector identifies an SV40 promoter at nucleotides 1807–2145 and an SV40 polyadenylation signal at nucleotides 811–1194. It also explains that the SV40 early promoter drives neomycin resistance expression for selection in mammalian cells (Agilent, pCMV-Script Vector, pp. 2–3). Pfizer's subsequent explanation to HC is consistent with that design: the production plasmid backbone contains elements for bacterial and mammalian use, while the SV40-dependent mammalian selection function is not used during plasmid production in E. coli (Health Canada, SV40 Correspondence, p. 21; BWP, Position on Residual DNA in Comirnaty, pp. 3–4 and 6).

The pST4 platform was also documented before the pandemic in a BioNTech application describing pST4 backbones for cytokine-mRNA production (WO2019154985A1, Example 1). The publicly available rolling review assessment report of 19 November 2020 subsequently identified Comirnaty's production plasmid as pST4-1525, comprising 7,824 bp. Its reproduced map and functional element table do not identify SV40 elements. The accompanying assessment expressly states that "the source and generation of the pST4-1525 plasmid used remain to be documented" (EMA, Comirnaty Rolling Review—Quality Aspects (19 November 2020), PDF pp. 23–24; EMA, Comirnaty Rolling Review—Overview and List of Questions (19 November 2020), p. 14).

Comparison of the deposited sequences provides additional evidence of a pCMV-Script-derived backbone: nucleotides 118–3586 of OR134577.1 match nucleotides 810–4278 of AF028239.1 across a continuous, ungapped 3,469-bp region, with 3,468 identical bases (99.97% identity). The matched SV40 promoter and polyadenylation regions, f1 origin, and neomycin/kanamycin resistance coding sequence retain complete sequence identity. Together with BioNTech/TRON's explicit description of pST1 backbones as derived from pCMV-Script, this supports a shared backbone and conservation of its functional elements. Establishing identity of the complete production plasmid requires comparison with the complete manufacturer's reference sequence (GenBank AF028239.1 and OR134577.1; Agilent, pCMV-Script Vector, p. 3; WO2016113395A1, Example 12).

These records document the use of Pfizer's gene therapy manufacturing expertise and connect BNT162b2's vector design to an established RNA platform, whose pST1 backbones were derived from an SV40-containing mammalian expression vector. The relevant implication is the retention of elements with documented mammalian functions in a production backbone subsequently used for the vaccine. This provenance strengthens the requirement for complete functional annotation and assessment of any residual molecules reaching the finished product.

ICH Q5B calls for the construction history, source, and function of component sequences, a detailed map, and the complete annotated plasmid sequence. This documentary standard directly supports the disclosure of the parental backbone and retained mammalian regulatory elements (ICH Q5B, section III.A, pp. 2–3).

4.5.2 Evidence of Deliberate Omission by BioNTech/Pfizer

Both EMA Rapporteur reports dated 19 November 2020 identified missing documentation concerning the history of Comirnaty's production plasmid. The overview stated: "However, details regarding the bacterial strain and the source and generation of the pST4-1525 plasmid used remain to be documented." It also considered the manufacturing and control information to be insufficient for a proper assessment (EMA, Comirnaty Rolling Review—Overview and List of Questions (19 November 2020), p. 14). The detailed quality assessment recorded the same deficiency as an outstanding concern: "Neither the source and generation of the pST4-1525 plasmid nor details on the specific E. coli strain used are documented (OC)" (EMA, Comirnaty Rolling Review—Quality Aspects (19 November 2020), PDF p. 31). The plasmid map and functional element table reproduced in that assessment did not identify SV40 elements (EMA, Comirnaty Rolling Review—Quality Aspects (19 November 2020), PDF p. 24).

Pfizer's response to HC's quality clarification request of 4 August 2023 explained that the backbone contained elements for bacterial and mammalian use, with the SV40 regulatory region supporting antibiotic resistance expression in mammalian cells. In the explanation reproduced by the BWP, the elements were considered irrelevant to the manufacturing process and consequently omitted from the functional annotation. The BWP also records that the complete plasmid nucleotide sequence was supplied in the original application (Health Canada, SV40 Correspondence, p. 21; BWP, Position on Residual DNA in Comirnaty, pp. 3–4 and 6).

Two regulatory records state the position in the authorities' own words. The BWP recorded that "[w]hile the full DNA sequence of the plasmid starting material was provided in the initial marketing authorisation application for Comirnaty, the applicant did not specifically highlight the SV40 sequence, as it was considered to be a non-functional part of the plasmid. They have since clarified this information in response to questions raised by EMA" (BWP, Position on Residual DNA in Comirnaty, p. 6). The same page records the manufacturer's confirmation that "SV40 promoter, SV40 Enhancer, SV40 Origin, and SV40 polyA signal elements are present in the DNA plasmid", and, in the adjacent sentence, that "[t]he elements of the SV40 regulatory region are used to drive neomycin/kanamycin resistance in mammalian cells" (BWP, Position on Residual DNA in Comirnaty, p. 6). A sequence characterized on that page as non-functional is described on the same page as driving resistance expression in mammalian cells.

An Australian evaluator recorded the same omission independently. The released assessment states that the plasmid "also contains a SV40 promoter and f1 ori region (not shown in plasmid map presented by Sponsor but found in BLAST and reported in *Speicher* & *McKernan* papers)", and that the plasmid "was based on a pCMV-TAG vector (I used BLAST analysis of sequence from Mod3 as not all info was placed in the plasmid map provided by the Sponsor)" (TGA, Residual DNA and SV40 Correspondence, p. 155). The evaluator interrogated the sequence directly to establish what the plasmid contained, the information not being in the map supplied. The BWP, for its part, concluded that the marketing authorisation holder "should submit a variation to update the dossier with details regarding the origin of all genetic elements present in the plasmid starting material used for the manufacturing of Comirnaty" (BWP, Position on Residual DNA in Comirnaty, p. 7). A regulator does not require a dossier to be completed unless it was incomplete.

Interestingly, HC's quality review for Comirnaty Omicron XBB.1.5, under the heading "SUMMARY OF KEY QUALITY ISSUES — Unnecessary regulatory elements present on plasmid", records that the "review team disagrees with this justification. Due to the fact that some plasmid-derived DNA fragments remain in the final product as a process-related impurity, the plasmid should not include unnecessary DNA regulatory elements. At a minimum, these regulatory elements should have been disclosed initially" (Health Canada, SV40 Correspondence and Quality Review, Quality Evaluation Executive Summary, internal p. 4; replacement p. 193). That is a regulator's finding, recorded in a quality review, that disclosure was owed and was not made. The same page records that HC's position was conveyed to the sponsor by follow-up Clarifax of 24 August 2023, and that "the Sponsor only agrees to further evaluate Health Canada's request".

That HC's department's own investigation records the finding in blunter terms still. Its Issue Analysis Summary, released on 16 July 2026 following an order of the Information Commissioner, states under the heading "Issue": "The SV40 promoter is present on all plasmids used to manufacture all lots of COMIRNATY and COMIRNATY Original & Omicron BA.5/BA.5 [sic] released onto the Canadian market and was not previously disclosed by Pfizer" (Health Canada, SV40 Issue Analysis Summary and Correspondence). The same document records that "Pfizer was required to provide a full annotation of all functional components of each plasmid", and that the original plasmid map did not contain one. A regulator therefore found, in its own investigation, that the element was present in every lot released onto its market and had not been disclosed by the manufacturer, and required the same annotation remedy as the European authority imposed in the same period. That sentence was withheld as third-party commercial information until its release was ordered, and reached the public on 16 July 2026, almost three years after it was written.

The same review states the grounds on which that department did not then treat the presence of the SV40 promoter as a concern, and they are recorded here so that its position can be weighed in full. Those grounds were that the plasmid is linearized and further digested during drug substance manufacture, that the approved specification of less than 10 ng per dose is in line with WHO recommendations, that the sponsor had committed to provide additional data on fragment size and on residual plasmid capable of replication in bacterial cells by 1 December 2023, and that there was "no peer-reviewed scientific literature suggesting that the SV40 promoter itself or the other non-functional elements pose a risk to human health" (Health Canada, SV40 Correspondence and Quality Review, Quality Evaluation Executive Summary, internal p. 4; replacement p. 193). That last statement was written in 2023. The peer-reviewed literature has developed since, and the department's position has not been revisited on the public record.

A further record of the same department is in tension with that review and has not been released. Order Paper Question Q-1406 refers to regulatory correspondence of 30 August 2023 issued under New Drug Submission Control No. 276302, in which it is stated that certain elements "may be needed to maintain the structural integrity of the plasmid", and asks which elements, what is meant by structural integrity, and what evidence supports the statement (House of Commons of Canada, Reply to Order Paper Question Q-1406 (2026)). One document of that department records that the elements are unnecessary and should be removed; another states that they may be needed. The reply addresses neither statement, and the correspondence itself has not been released.

The statement referred to the initial and subsequent marketing authorisation applications. Read with the manufacturer's explanation that the elements were deemed irrelevant, this

contemporaneous account supports an inference of deliberate omission from the functional description (Health Canada, SV40 Correspondence, p. 162; reproduced in Wiseman and Gutschi, section 7.2.3, p. 37).

These releases require further investigation into why the SV40 elements were not expressly identified, how the outstanding questions concerning the plasmid's origin were resolved, and whether the omission affected the regulatory safety assessment. The manufacturer's explanations and the authorities' subsequent decisions are legally relevant from an administrative and potential criminal law perspective.

Article 16 of Regulation (EC) No 726/2004 requires the marketing authorisation holder to account for scientific and technical progress and supply new information capable of affecting the authorisation. The identification of previously unannotated elements requires an assessment of their significance for product quality, impurity controls, and safety. Annotation alone cannot establish the adequacy of the original assessment or resolve the residual DNA questions (Regulation (EC) No 726/2004, Article 16; CHMP, Comirnaty Variation Assessment II/0202, pp. 4–5).

5 Legal Implications and the Obligation to Act

5.1 Protection of Human Health and the Precautionary Principle

The findings documented in this report engage the responsibility of the competent Union authorities to protect human health. The rights to life and physical integrity, the requirement to ensure a high level of human health protection, and the right to good administration govern the exercise of their regulatory powers (Charter of Fundamental Rights, Articles 2, 3, 35, and 41; TFEU, Article 168(1)).

The precautionary principle requires that potential risks be addressed before their consequences are established with certainty. Scientific uncertainty must be evaluated in a reasoned risk assessment and cannot be treated as affirmative evidence of safety. In *Pfizer Animal Health v Council,* the General Court recognized that protective measures may be adopted despite uncertainty concerning the existence and extent of risks, provided that the assessment rests on scientific grounds rather than conjecture (Case T-13/99, EU:T:2002:209, paras. 139–148).

This OPIR submits that the documented deficiencies in plasmid disclosure, residual DNA characterization, and the scientific basis of the manufacturer's assurances require urgent

regulatory reassessment. Its demand for precautionary action rests on the specific discrepancies identified in the preceding sections and their relevance to a product administered prophylactically to a large population. Protection of health must take precedence over the commercial interest in maintaining an authorisation without resolving material questions concerning its evidential basis.

The report calls upon the Commission to exercise its powers under Article 20 of Regulation (EC) No 726/2004 and to suspend the authorisation pending an adequate reassessment. The EMA must place the relevant evidence before the Commission and provide a reasoned scientific opinion addressing the concerns individually. The position advanced here is that continued reliance on the contested assurances, without the examination required by the precautionary principle, fails to discharge the authorities' protective responsibilities.

5.2 Diligent and Impartial Examination

The EMA's scientific discretion is accompanied by procedural guarantees. The competent institution must carefully and impartially examine the relevant aspects of the case and provide sufficient reasons for its conclusions. These requirements are particularly important when the assessment involves complex scientific questions and information supplied predominantly by the authorisation holder (Charter of Fundamental Rights, Article 41; CJEU, Technische Universität München, C-269/90, EU:C:1991:438, para. 14).

The report's allegations of defective administration concern the substance and chronology of the assessment: public assurances were coordinated while additional characterization remained outstanding; broad conclusions were drawn from evidence addressing narrower biological or analytical questions; and the subsequent variation accepted the risk assessment without requiring its revision. The regulatory record must establish how the additional evidence resolves each outstanding question and supports the conclusions adopted.

International coordination does not discharge EMA's own duty of examination. Agreement between authorities cannot substitute for the assessment of the underlying methods, data, and literature. Nor can preserving public confidence in vaccination determine the scientific conclusion. Public confidence depends on the demonstrable independence and reliability of the assessment.

The report therefore maintains that the deficiencies identified engage the principles of good administration, impartiality, and the duty to state reasons. Independent examination must establish their extent, their effect on the variation decision, and the measures required to remedy them.

5.3 Manufacturer Disclosure and Pharmacovigilance Obligations

5.3.1 Incomplete Plasmid Annotation in Light of Established and Subsequent Regulatory Standards

The regulatory standards available before the original conditional marketing authorisation, together with subsequent mRNA-specific guidance, reinforce the criticism of the incomplete plasmid annotation and insufficiently substantiated safety assessments.

ICH Q5B, adopted in 1995, established comprehensive documentation of expression constructs as a regulatory expectation: the manufacturer should describe the source and function of the constituent elements, including replication origins, antibiotic-resistance genes, promoters and enhancers, and provide a detailed component map and complete annotated plasmid sequence. Within the field of recombinant protein production, this demonstrates that plasmid characterization has already extended to the complete production construct (ICH Q5B, section III.A, printed p. 2).

More specifically, **EMA's 2018 gene-therapy guideline expressly addressed plasmids used to generate RNA**. It called for descriptions of the plasmid backbone, transgene, selection gene, and other regulatory sequences, the full nucleotide sequence, and justification for the inclusion of all genetic elements (EMA, Gene Therapy Medicinal Products Guideline (2018), section 4.1.2, pp. 9–10).

The WHO guidance of 29 November 2021 subsequently addressed plasmid-derived templates specifically in mRNA vaccine manufacturing. It called for cell-bank controls, demonstration of genetic stability, sequencing-based identification, and confirmation of both antigen-coding and regulatory sequences (WHO, Regulatory Considerations for mRNA Vaccines (2021), section 6.3.1.1, pp. 23–24).

EMA's March 2025 draft mRNA guideline states the annotation standard explicitly: the entire DNA template sequence should be provided, including annotation and justification of "all functional and non-functional elements". It additionally calls for template characterization, release testing, justified specifications, and relevant stability information. Its residual-DNA provisions specify orthogonal analytical methods and fragment-size analysis to demonstrate the effectiveness of enzymatic digestion and purification (EMA, Draft Guideline on Quality Aspects of mRNA Vaccines (2025), sections 4.2–4.3, pp. 4 and 6).

Of interest is also the European Pharmacopoeia framework, which follows a comprehensive approach. The published 2024 draft of chapter 5.40 requires detailed plasmid documentation, including its source, means of isolation, complete nucleotide sequence, and the source and function of component elements; it also addresses the plasmid backbone and testing of different plasmid forms (*Pharmeuropa* 36.2, April 2024, draft chapter 5.40, section 2.2, p. 50). Chapter 5.40 subsequently entered edition 12.1 alongside chapters 5.36 and 5.39, covering mRNA–LNP vaccines and mRNA substances, effective from 1 January 2026 (European Pharmacopoeia, 12th edition, Issue 12.1, chapter 5.40, DNA templates for the preparation of mRNA substances; implementation date: 1 January 2026).

Taken together, these standards oblige the manufacturer to declare every element of the plasmid, whether or not it is functional in bacterial production. BioNTech/Pfizer's explanation fails to address the requirement for comprehensive documentation and assessment of the complete construct. Subsequent standards also strengthen the basis for continued regulatory scrutiny: Article 16 requires marketing authorisation holders to incorporate scientific and technical progress, seek necessary changes to manufacturing and control methods, and communicate relevant new information. These continuing obligations encompass authorised variant updates and reinforce the need to reassess unresolved deficiencies in plasmid characterization and residual-DNA control (Regulation (EC) No 726/2004, Articles 6(1), 16, and 17).

6 Overriding Public Interest in Disclosure

6.1 Specific Grounds for Disclosure

This report substantiates an overriding public interest in independently examining the evidential basis and lawfulness of Comirnaty's marketing authorisation, its authorised commercial manufacturing process, and variation II/0202. The deficiencies identified concern residual-DNA quantification, DNase clearance, plasmid annotation, P1/P2 comparability, and the assessment of persistence, nuclear entry, and biological activity of residual DNA, including SV40 elements.

Disclosure would enable researchers independent of the marketing authorisation holder to verify the underlying methods, data, and regulatory reasoning against the scientific evidence and regulatory standards examined above. The application thus identifies specific circumstances establishing the public interest in disclosure, as required by the Court of Justice (CJEU, Strack v Commission, C-127/13 P, EU:C:2014:2250, para. 128).

The public interest advanced is the detection and correction of deficiencies affecting the protection of vaccine recipients and the lawful exercise of regulatory powers. Its significance derives from the continuing manufacture and administration of the authorised product and

the Union's obligation to ensure a high level of human health protection (TFEU, Article 168(1); Charter of Fundamental Rights, Article 35).

6.2 Documents Requested

The request encompasses the following records held by EMA, including their annexes, underlying data, and correspondence:

- **Plasmid documentation:** original and revised CTD sections; complete annotated maps and sequences; source and generation records; and explanations for the initial non-annotation and subsequent amendments.
- **EMA's/PEI's control sequencing of the plasmid map**
- **Analytical evidence:** the complete risk assessment submitted in sequence 0598, report INX100594280, and supporting records; analytical procedures and validation reports; extraction and recovery data; DNA-mass calculations; and the data underlying the assessment of 236 batches.
- **Manufacturing and comparability:** P1/P2 comparability assessments; evaluation of the changed impurity profile; records concerning the discontinued clinical comparison; and the complete Recommendation 7 file, including the grounds for closure.
- **Regulatory deliberations:** supplementary material relating to the BWP and CHMP decisions and all manufacturer responses; including meeting records; interagency correspondence; and records concerning the preparation, approval, and circulation of the "Lines to Take."
- **Subsequent measures:** evaluation of the removal of unused plasmid elements, the manufacturer's investigation and results, subsequent assessments, and resulting amendments to manufacturing or control requirements.
- **Confirmatory testing:** the results, analytical procedures and validation records of any confirmatory or independent testing of residual DNA in finished Comirnaty product carried out by or for EMA, an Official Medicines Control Laboratory or a national competent authority, including the testing of 27 batches asserted by the Therapeutic Goods Administration and the spot-testing records released by that agency in fully redacted form; together with the results of the four analyses committed to in the BWP position on residual DNA in Comirnaty for completion by 1 December 2023, namely the size distribution of residual DNA fragments, residual intact circular plasmid, the assessment of the presence of SV40 sequences in residual DNA, and the assessment of plasmid transformation capability in bacteria.
- **International coordination:** records of any exchange between EMA and HC, the FDA or other authorities concerning the removal of unnecessary DNA elements from the

Comirnaty manufacturing plasmid, including any response to HC's stated intention to seek a harmonized regulatory approach to that removal; and the data supplied by the manufacturer under its commitment of 1 December 2023 on the size of residual DNA fragments and on residual plasmid capable of replication in bacterial cells.

6.3 Exceptions, Balancing of Interests and Partial Access

Regulation (EC) No 1049/2001 applies to documents held by the EMA through Article 73 of Regulation (EC) No 726/2004. Articles 4(2) and 4(3) require disclosure where an overriding public interest prevails over the protected interest. The authors submit that the specific interest substantiated here outweighs the commercial confidentiality and deliberative interests invoked to withhold the evidence underpinning the contested safety assessment (Regulation (EC) No 726/2004, Article 73; Regulation (EC) No 1049/2001, Article 4(2)–(3)).

The Canadian proceedings concerning HC's *Issue Analysis Summary* provide a concrete example of why anticipated public criticism cannot, by itself, justify withholding regulatory records. In her final report of 8 May 2026, the Information Commissioner of Canada recorded Pfizer's concern that disclosure could mislead the public and cast doubt on the scientific integrity of its product design and manufacturing processes. Rejecting that justification and referring to *Merck Frosst Canada Ltd. v. Canada (Health)*, 2012 SCC 3, she stated: "A refusal to disclose for fear of public misunderstanding would undermine the fundamental purpose of the Act." She also distinguished HC's own assessments from information supplied by Pfizer and found that the claimed exemptions had not been established for certain passages. The complaint was upheld and disclosure of specified passages was ordered. The case illustrates the need to substantiate confidentiality claims by reference to the applicable legal requirements and the particular information withheld (Information Commissioner of Canada, *Health Canada (Re)*, 2026 OIC 43, 8 May 2026, paras. 31–38, 66–72 and Orders).

7 Obligation to Act and Reservation of Judicial Remedies

On the grounds developed in this report, **EMA is formally called upon to recommend to the European Commission the immediate precautionary suspension of Comirnaty's marketing authorisation and urgent temporary measures to halt further supply and use under Article 20 of Regulation (EC) No 726/2004.**

EMA is further called upon to disclose the identified records in full and unredacted form, obtain the outstanding evidence from the marketing authorisation holder, and arrange an

independent scientific reassessment of the commercial product. **The suspension should remain in place until the identified safety concerns have been resolved and an independent reassessment has established compliance with the applicable quality and safety requirements and a favourable benefit–risk balance.** That reassessment must address dossier accuracy, manufacturing controls and the obligation to incorporate scientific and technical progress (Regulation (EC) No 726/2004, Articles 16, 17 and 20).

The Executive Director of EMA is further called upon to transmit this report, and the requests for regulatory action without delay to the European Commission and the competent national authorities, and to refer the matters raised to the CHMP, CAT, PRAC, and BWP as well as to the PEI for examination within their respective mandates.

The Executive Director is requested to ensure coordinated consideration of the identified issues and a reasoned response specifying the assessments undertaken and the measures proposed (Regulation (EC) No 726/2004, Articles 5(2)–(3), 56, 57(1) and 64(2)(d)).

The CHMP is specifically called upon to request the CAT's specialist advice concerning residual plasmid DNA, gene-delivery mechanisms, and the associated quality and safety requirements examined in this report (Regulation (EC) No 1394/2007, Articles 21(2) and 23(c)). The EMA is requested to confirm the dates of transmission, recipients, and procedural references assigned.

The referral to the Commission shall expressly include the request to initiate the procedure under Article 20 and, following the requisite consultation of EMA, adopt a precautionary temporary suspension under Article 20(3), pending resolution of the substantiated safety questions. Any refusal must address the evidence presented and state the factual and legal grounds supporting continued authorisation (Regulation (EC) No 726/2004, Article 20(1)–(3); Charter of Fundamental Rights, Article 41(2)(c)).

This submission constitutes a **formal invitation to act under Article 265 TFEU** concerning the regulatory measures specified above. Each addressee is called upon to define its position within two months of receipt. In the absence of such a position, the authors reserve proceedings for failure to act, subject to the applicable requirements of standing, admissibility, and time limits (TFEU, Article 265).

The authors further reserve the judicial review of any express or implied confirmatory refusal of access and applications for suspension or other necessary interim measures in admissible proceedings. The separate timeframes governing document access procedures remain applicable (Regulation (EC) No 1049/2001, Articles 7–8; TFEU, Articles 263, 278 and 279).

Annex: Document Register

This register identifies the disclosure packages, manufacturer documents and related official records cited in the report. The same short document names are used throughout; official document and request numbers are retained here for identification. Dates distinguish adoption, manufacturer approval, submission and public release. Page references refer to the released PDFs unless marked "internal"; for the Pfizer risk assessment and characterization report, both internal document pages and HC package pages are specified where available. Where a complete document is not linked, the entry identifies the release page or extracts. The 2026 HC replacement pages disclose more of the same underlying records. Public statements and parliamentary answers are identified as such. Documentary cut-off: 5 October 2026.

European Union and EMA

Document and date	Contents and significance
BWP, Position on Residual DNA in Comirnaty *Adopted 31 October 2023*	EMA/CHMP/BWP/61303/2023 on the cover; EMA/CHMP/BWP/482571/2023 in the footers. Seven-page primary document: manufacturer statements of 12 October and 24 October 2023, BWP assessment, use of shared Lines To Take, and recommendation for EMA–HC–FDA liaison. The linked page documents release provenance and reproduces extracts; it does not provide the complete PDF.
Pfizer, Response to EMA Request for Supplementary Information *Internally approved 29 January 2024; submitted 30 January 2024*	Manufacturer answers on residual-DNA characterization and possible amplification without SV40 T-antigen; identifies INX100594280 and the earlier risk assessment in eCTD sequence 0598. It refers to a request dated 19 January 2024; the CHMP timetable records the formal RSI on 25 January.

Document and date	Contents and significance
Pfizer, Residual DNA Characterization Report *Internally approved 25 January 2024*	INX100594280. Manufacturer analytical report, including residual DNA and SV40-related characterization. This is the same report, not a second independent study, as also disclosed in the HC release of the Residual DNA Characterization Report with different redactions.
CHMP, Comirnaty Variation Assessment II/0202 *Opinion 29 February 2024*	EMA/CHMP/21199/2024; EMEA/H/C/005735/II/0202. Records the procedure, assessment, and recommendation to approve the dossier update; places possible removal of unused elements outside the procedure (pp. 2, 4–6).
EMA, Comirnaty Public Assessment Report 19 February 2021; Corr.2, 20 October 2023	EMA/707383/2020 Corr.2; EMEA/H/C/005735/0000. Public assessment report, including the DNase digestion observations and Recommendation 7.
EMA, Comirnaty Variation Assessment IB/0106/G 31 March 2022	EMA/CHMP/50784/2022; EMEA/H/C/005735/IB/0106/G. Type IB variation report cited in relation to Recommendation 7. Publicly hosted copy of the official record.
BioNTech/Pfizer, qPCR Analytical Procedures (CTD 3.2.S.4.2) Internally approved 18 September 2024	BNT162b2, Analytical Procedures – Quantitative Polymerase Chain Reaction (qPCR) [Omicron]. Five-page procedure; approval identifier 090177e1a1a7fd21. Describes the method cited in Section 4.2.2.
BioNTech/Pfizer, qPCR Validation Overview (CTD 3.2.S.4.3) Internally approved 25 August 2023	BNT162b2, Validation of Analytical Procedures – Quantitative Polymerase Chain Reaction (qPCR). One-page overview; approval identifier 090177e19e67698d. Identifies validation and transfer reports in Table 3.2.S.4.3-1.

Document and date	Contents and significance
BioNTech/Pfizer, Process Validation Summary—Mainz/Rentschler (CTD 3.2.S.2.5) Internally approved 25 August 2023	BNT162b2, Process Validation and/or Evaluation – Manufacturing Process [BNT Mainz and Rentschler]. Eight pages; approval identifier 090177e19e67b08d. Contains the manufacturing-process validation summary cited on pp. 2–4.

Canada and Health Canada

Document and date	Contents and significance
Health Canada, SV40 Correspondence A-2023-001013. Release package dated 9 August 2024	584-page package. Media enquiry and internal review (pp. 131–134), August Clarifaxes and Pfizer responses (pp. 5–7, 21–26), internal reluctance and coordination (pp. 2–4, 161–162, 270–271, 427–441), and quality review summary (pp. 16–20). It provides a broad correspondence record beyond the overlapping pages in the less-redacted SV40 Correspondence and Quality Review.
Health Canada, SV40 Issue Analysis Summary and Correspondence A-2024-000366. Less-redacted release 16 July 2026	Draft SV40 Issue Analysis Summary and accompanying email circulated on 27 July 2023. The release page provides the replacement package and the Information Commissioner's order. These are fuller disclosures of earlier records, not a new 2026 safety assessment.
Health Canada, SV40 Correspondence and Quality Review A-2023-000979. Replacement pages released 8 September 2026	Less-redacted replacement p. 150: emails of 25 August 2023. Replacement p. 193: Quality Evaluation Executive Summary (BGTD-FT-0723 V1.2, internal p. 4 of 5), including the recommendation to remove unnecessary elements and disagreement with the manufacturer's justification. The linked release provides both replacement pages and the accompanying commissioner's order.

Document and date	Contents and significance
Health Canada, Pfizer Residual DNA Submissions A-2024-000097. Release package dated 22 August 2024	A 56-page package containing six manufacturer documents listed below. The reported approval dates are internal manufacturer approvals, not the dates on which HC approved or received the documents.
Pfizer, Response to Health Canada Post-Decision Letter (December 2023) *Internally approved 19 December 2023*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 1–3. Reproduces the request for fragment-size and circular-plasmid characterization by 1 December 2023 and lists the risk assessment and updated plasmid documentation.
Pfizer, Residual DNA Risk Assessment *Internally approved 14 December 2023*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 4–11. Eight-page manufacturer's risk assessment. Its identity with the European version cited in eCTD sequence 0598 should not be assumed solely based on parallel submission histories.
Pfizer, Source, History and Generation of Plasmids *Internally approved 14 December 2023*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 12–23. Source, History, and Generation of Plasmids for the Omicron XBB.1.5 variant; updated description of plasmid elements.
Pfizer, Response to Health Canada Post-Decision Letter (January 2024) *Internally approved 26 January 2024*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 24–27. Additional response to the letter of 20 October 2023; lists a new technical report and refers back to the previously submitted risk assessment.

Document and date	Contents and significance
Pfizer, Residual DNA Characterization Report (Health Canada release) *Internally approved 25 January 2024*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 28–53; approval record, p. 54. The Canadian disclosure of the report is also listed under the EMA release of the Residual DNA Characterization Report, with different redactions. Cross-disclosure permits the comparison of visible passages.
Pfizer, Update on Plasmid-Backbone Modification *Internally approved 22 February 2024*	Health Canada, Pfizer Residual DNA Submissions (A-2024-000097), pp. 55–56. Reproduces HC's 30 August 2023 follow-up and reports an ongoing evaluation, with possible discussion of preliminary results around the end of 2024.
Information Commissioner of Canada, Health Canada (Re), 2026 OIC 43 *Decision 8 May 2026*	Information Commissioner file 5824-02216, concerning A-2024-000366. Orders disclosure of specified material whose withholding was not justified and retains protection for other information. Official adjudicative record explaining the later reduction of redactions.
House of Commons of Canada, Reply to Order Paper Question Q-990 Reply tabled 8 May 2026	Official parliamentary question and government-response record. 45th Parliament, 1st session.
House of Commons of Canada, Reply to Order Paper Question Q-1363 Reply tabled 21 September 2026	Official parliamentary question and government-response record. 45th Parliament, 1st session.
House of Commons of Canada, Reply to Order Paper Question Q-1406 Reply tabled 21 September 2026	Official parliamentary question and government-response record. 45th Parliament, 1st session.
House of Commons of Canada, Reply to Order Paper Question Q-2670 Reply tabled 19 June 2024	Official parliamentary question and government-response record. 44th Parliament, 1st session.

United Kingdom and MHRA

Document and date	Contents and significance
MHRA, SV40 Correspondence and Batch Testing FOI 23/743. Response 3 November 2023	Reports no official SV40-specific letters, emails, or correspondence within the request's scope and no testing outsourced to EMA; also describes general batch-release controls (pp. 1–2).
MHRA, Fragmented DNA and SV40 Testing FOI 23/796. Response 9 November 2023	States that MHRA had not tested Comirnaty at the vial level for fragmented DNA and the SV40 enhancer, and had no current intention to do so; distinguishes those tests from other independent batch tests. Date established by the correspondence record.
MHRA, SV40 Sequences and Quality Controls FOI 23/851. Undated response to request of 5 November 2023	Acknowledges the SV40-derived sequence material and distinguishes it from the sequence encoding the large T antigen; reiterates manufacturing and quality controls.
MHRA, SV40 Testing Response FOI 23/940. Response 2 January 2024	Reiterates that the MHRA had not tested mRNA vaccines for SV40 and had no current intention to perform such testing; continues the November position.
MHRA, Residual DNA and SV40 Safety Response FOI 24/212. Response 28 March 2024	Acknowledges residual SV40 promoter/enhancer fragments and potential LNP encapsulation; states MHRA's views on inactivity, cellular transfection, and genomic integration. It supplies an explanatory position, not batch-specific analytical results.
MHRA, P1/P2 Comparability Response (IR 23/510) 21 September 2023	Internal review of FOI 23/510. Page 5 addresses removal of the exploratory clinical Process 1/Process 2 comparison through protocol amendment 20 in September 2022.

Australia and TGA

Document and date	Contents and significance
TGA, Comirnaty Batch-Testing Records FOI 4558. Public release 4 October 2023	Redacted Comirnaty batch-testing records were obtained following a request that included residual DNA results. The related original decision and redaction reasons are disclosed separately in TGA, Decision on Comirnaty Batch-Testing Records.
TGA, Residual DNA qPCR Protocols FOI 5286. Public release 19 September 2024	Pfizer and Moderna residual-DNA qPCR procedures, Bio-PCR-Method-16 and -15, active from 17 October 2023. Explicitly documents the use of manufacturer in-process methods and laboratory adaptations.
TGA, Spikevax Laboratory Testing Records FOI 5199. Public release 4 October 2024	Spikevax laboratory testing records. Relevant to the broader testing and batch-control framework, not a Comirnaty-specific SV40 finding.
TGA, Residual DNA and SV40 Correspondence FOI 25-0058. Public release 17 December 2024	Correspondence supporting the October 2024 webpage and social-media communications. Document 30 includes a discussion of residual DNA and SV40, as well as references to FDA and MHRA positions.
TGA, Moderna qPCR Procedure and Reagent Records FOI 25-0069. Public release 24 December 2024	Moderna SOP-1020 and records concerning purchases of qPCR primers and probes; supplements the methods disclosed in TGA, Residual DNA qPCR Protocols.
TGA, Drafts and Correspondence on the Residual DNA Statement FOI 25-0070. Public release 24 December 2024	Drafts and emails tracing the preparation of the October 2024 public statement; p. 26 records the aim of directing future enquiries to that statement.

Document and date	Contents and significance
TGA, Decision on Comirnaty Batch-Testing Records FOI 27-3464. Public release 11 August 2026; underlying decision 29 September 2023	Original decision concerning FOI 4558, including the grounds for redaction. This adds the disclosure decision, rather than new laboratory evidence.
TGA, Batch Release and Parliamentary Responses FOI 27-3438. Public release 19 August 2026	Batch-release information and parliamentary answers. The TGA states that the supplied plasmid sequence did not specifically label the SV40 promoter and that it identified the promoter by sequence alignment in October 2023 (pp. 3–4).
TGA, Statement on Residual DNA *18 October 2024; public communication*	The published position was developed through correspondence disclosed in TGA, Residual DNA and SV40 Correspondence, and TGA, Drafts and Correspondence on the Residual DNA Statement. This includes connecting internal preparation with the eventual public message.
TGA, Summary Report on Residual DNA and Endotoxin Testing *Published 7 November 2024; updated 5 August 2025*	Public laboratory report describing TGA testing and results within the specifications. It documents that TGA performed the testing, while TGA, Residual DNA qPCR Protocols identifies the origins of the methods used.
Pfizer, PPQ Comparability Report 26 March 2021	BNT162b2 (PF-07302048) Comparability Report for PPQ Drug Product Lots, INX100451158. Released by the TGA under FOI 3659, document 04; testing panel and comparability information on pp. 4–7.

New Zealand and Medsafe

Document and date	Contents and significance
Medsafe, Residual DNA and SV40 Response OIA H2023034032. Response 1 February 2024	Information on plasmid-sequence submission and SV40 annotation is withheld under confidentiality provisions. States that the specifically requested internal discussions about manufacturer batch testing and qPCR confidence do not exist; supplies a general regulatory explanation (p. 1).

United States and FDA public records

Document and date	Contents and significance
FDA, VRBPAC Meeting Transcript *Hearing 15 June 2023*	*McKernan*'s public presentation concerning residual plasmid DNA and SV40-derived sequences (pp. 114–116). Establishes public presentation to an FDA advisory committee; not a committee endorsement or a FOIA response.
FDA, Letter to Joseph Ladapo *14 December 2023*	FDA's written response on residual DNA, SV40-related concerns, LNPs, and genotoxicity. Public correspondence, later referenced in other regulators' records; not identified here as a FOIA release.

Germany and official parliamentary records

Document and date	Contents and significance
German Bundestag, Government Answer on SV40 (20/9409) *Government answer 13 November 2023*	Answer to question 88, citing PEI, confirms the SV40 sequence in the manufacturing plasmid and sets out the government's assessment. Printed p. 66, PDF p. 72. Parliamentary answer, not an FOI release.

Document and date	Contents and significance
German Bundestag, Government Response on Residual DNA (20/9697) *Government response transmitted 30 November 2023; printed 12 December 2023*	Further government answers on residual DNA, applicable limits, and regulatory controls. Complements the Government Answer on SV40 (20/9409) and records the position communicated to Parliament.

Canadian document packages contain overlapping correspondence. They should be read together, with the less-redacted replacement pages taking precedence for those passages. The EMA and HC releases of the Residual DNA Characterization Report disclose the same manufacturer report. Publicly hosted copies retain their official document identifiers; the hosting site is not treated as the author of the underlying official record.