



# Confirmation of the presence of vaccine DNA in the Pfizer anti-COVID-19 vaccine

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# TITLE PAGE

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**Title: Confirmation of the presence of vaccine DNA in the Pfizer anti-COVID-19 vaccine**

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## ABSTRACT

The rapid production of messenger RNA (mRNA)-based vaccines has been chosen as the most suitable strategy to fight the COVID-19 pandemic. Three studies reported the presence of DNA in significant amounts in Pfizer mRNA vaccines. We aimed to confirm the presence of this residual DNA. Vaccine plasmid DNA quantification using the Qubit fluorometer on a vaccine vial showed it was 216 ng/dose on average and approximately 24 times greater, reaching 5,160 ng/dose on average, after treatment with Triton-X-100. In addition, we obtained by next-generation sequencing the sequence of the complete plasmid DNA vaccine matrix (7,824 base pairs) with high coverage (98.3%) and sequencing depths (mean, 4,181-4,389 reads), indicating the presence of the plasmid DNA in high copy number. These results calls for an assessment of the copy number and nature of DNA in mRNA vaccines at a larger scale and in multiple batches, notably regarding the putative risk of DNA integration after delivery into cells.

## TEXT

The SARS-CoV-2 pandemic led to the very fast (<1 year) development of vaccines (Krammer, 2020; Topol, 2021), and more than 13 billion doses have been administered worldwide (including more than 150 million in France) (<https://coronavirus.jhu.edu/map.html>). This was in line with the current framework of thinking that advocate accelerating vaccine development to counter the rapid spread of an emerging infectious disease (Cohen, 2016). The Pfizer-BioNTech COVID-19 mRNA vaccine (or BNT162 Vaccine-19) has been the most used. It is a suspension injected intramuscularly that contains a codon-optimized single-stranded, 5'-capped mRNA encoding a full-length, modified SARS-CoV-2 spike protein once released into target cells (Jackson et al, 2020; Krammer, 2020; Polack et al, 2020). To allow a massive production of the BNT162 Vaccine-19 mRNA, manufacturing included transfection into *Escherichia coli* of the modified spike gene sequence using a plasmid with a final size of 7,824 base pairs (bp) that notably contains an active bacterial origin of replication, an initiation factor of the SV40 virus initiation factor, and a gene of resistance to kanamycin (Rapporteur Rolling Review critical assessment report, 2020). After bacteria lysis, the plasmid DNA matrix was extracted and linearized before its transcription by a T7 RNA polymerase in presence of N-methyl pseudoUridine then its hydrolysis. The Pfizer mRNA vaccine contains in principle extremely minimal quantities of DNA. Nonetheless, at least three studies reported the presence of DNA in Pfizer mRNA vaccine doses in significant amounts (Speicher et al, 2023; McKernan et al, 2023; König and Kirchner, 2024; Hughes, 2024). In a situation such as this, we believed that it was necessary to confirm or deny the presence of DNA without necessarily wanting to draw conclusions about its presence.

We carried out DNA quantification using the Qubit fluorometer (Invitrogen, Carlsbad, CA, United States) on a vaccine vial (batch no. GJ7184) which showed it was 216 ng/dose on average. However, after treatment with Triton-X-100, DNA quantity was approximately 24 times greater, reaching 5,160 ng/dose on average. As the vaccine dose is reported to contain 30 µg of RNA this means that it contained in this test 17% of DNA.

In addition, next-generation sequencing (NGS) was performed as previously described (Colson et al, 2022) with the Illumina technology using the Nextera XT paired end strategy on MiSeq instruments (Illumina Inc., San Diego, CA, United States), following the manufacturer's recommendations. NGS reads were mapped on the vaccine plasmid sequence deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Sayers et al, 2023) with the Accession no. OR134577.1. Reads were processed using bwa-mem2 v.2.2.1 (<https://github.com/bwa-mem2/bwa-mem2>) (Jung et al, 2022) and Samtools v.1.17 (<https://www.htslib.org/>) (Li et al, 2009). Reads shorter than 200 nucleotides and covering less than 90% of the vaccine plasmid were removed using Samtools v.1.17. Freebayes v1.3.6 (<https://github.com/freebayes/freebayes>) (Garrison and Marth, 2012) and Bcftools v.1.17 (<https://samtools.github.io/bcftools/bcftools.html>) were used for identifying mutations and generating consensus. NGS in absence of prior reverse transcription step of two vaccine batches (FP8191 and FP9359), which generated more than 140,000 reads larger than 200 bases, made it possible to retrieve the complete sequences of the DNA plasmid (GenBank Accession No. PP544445 and PP544446) used to produce the vaccine RNA, with 7.824 base pairs with high sequencing depth (>4000 reads). Approximately between 63% and 97% of the sequenced DNA was from the vaccine. Moreover, we observed that DNase treatment (TURBO DNA-free kit, Invitrogen) of the nucleic acid extracts prevented the obtaining of NGS reads (only 2 and 1 reads, respectively, were mapped on the reference sequence).

Therefore, here we found by several approaches and in different batches of the Pfizer-BioNTech COVID-19 mRNA vaccine the abundant presence of DNA. In such vaccines, the maximal residual quantity of DNA not degraded during vaccine purification that are authorized by the European Medicines Agency and the Food and Drug Administration are 330 ng of DNA per mg of RNA, or 10 ng of DNA per dose (Klinman et al, 2010; Rapporteur Rolling Review critical assessment report, 2020). Our findings are in line with those of other (not peer reviewed) studies that reported the presence of DNA in Pfizer-BioNTech COVID-19 mRNA vaccine vials by qPCR and the obtaining of full-length vaccine plasmid by NGS (McKernan et al, 2023; Speicher et al, 2023). In the Speicher et al's study, residual DNA quantities, ranging between 1,896 and 3,720 ng per dose were estimated using fluorometer based measurements with Qubit, and vaccine spike targeting qPCR obtained cycle threshold values ranging between 18.0 and 23.8 at 1:10 dilution, and  $16.9 \pm 0.5$  on undiluted vials contents (Speicher et al, 2023).

As a matter of fact, these results of huge quantities of plasmid DNA sequences per vaccine dose notably raise issues regarding a putative risk of its integration in the human genome after its entry into cells due to their packaging into cationic lipids (Klinman et al, 2010). In DNA-based gene therapy, it was reported that a proportion of 10- 20% of cells are usually transfected and about 1-10% of the transiently transfected cells became stably transfected as a result of subsequent integration likely through crossover events during nuclear envelope membrane reformation at telophase (Haraguchi et al., 2002; Lim et al, 2023; Devaux and Camoin-Jau, 2024). Although the potential risk that DNA found in mRNA-based vaccines integrate and either induce expression of an oncogene or shut down expression of a tumor suppressor are extremely low (Wang et al, 2004; Klinman et al, 2010), this should deserve additional investigations.

Overall, this work confirms that a significant quantity of vaccine DNA, which is above recommended levels (Rapporteur Rolling Review critical assessment report, 2020; Klinman et al, 2010), was present in the tested vaccine batches. Previous findings warrant to be confirmed at a larger scale, which is technically very easy to perform on a large panel of vaccine vials from different batches in different laboratories worldwide. There may be variability from one batch to another, and this requires regular quality controls on the different batches to be consistent with the requirements of good laboratory practices, and to liberate DNA from lipid nanoparticles.

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## **Data Availability**

Sequences of the DNA plasmids recovered from Pfizer mRNA vaccine batches FP8191 and FP9359 are available from GenBank with Accession No. PP544445 and PP544446, respectively.

## **Conflicts of Interest**

DR declares grants or contracts and royalties or licenses from Hitachi High-Technologies Corporation, Tokyo, Japan. He is a scientific board member of Eurofins company, and a founder and shareholder of a microbial culture company (Culture Top), and two biotechnology companies (Techno-Jouvence, and Gene and Green TK). Funding sources played no role in the design and performance of the study, the collection, management, analysis and interpretation of the data, or the preparation, review and approval of the manuscript.

## **Author Contributions**

Conceptualization, methodology, formal analysis and interpretation, writing-original draft preparation, writing—review and editing: DR.

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#### **Ethics**

Not applicable.

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