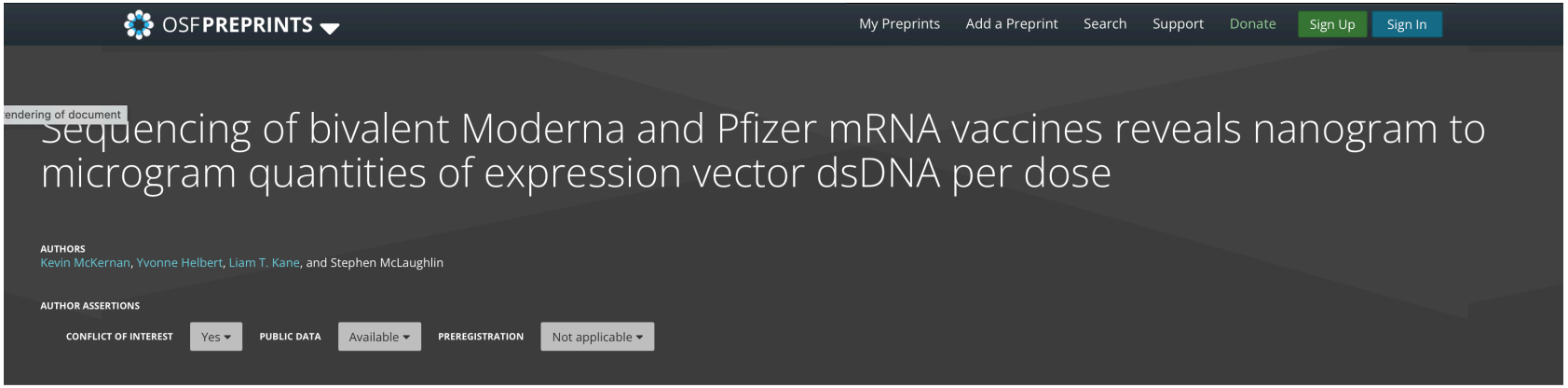
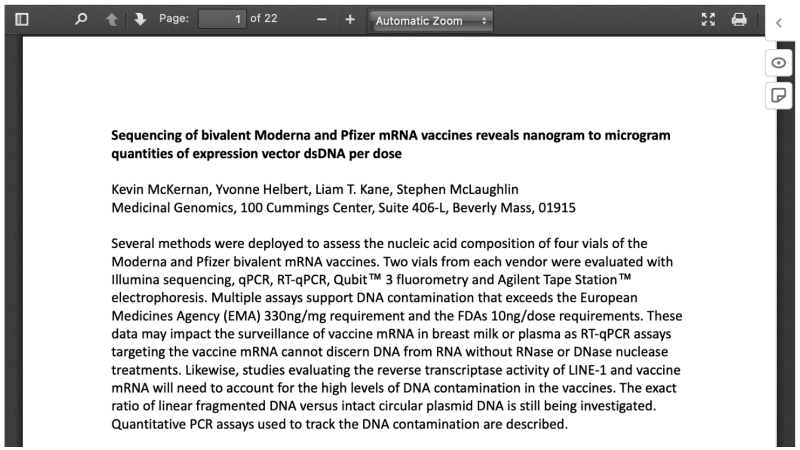
# Covid 19 “vaccines”. Where do we stand now?

Kevin McKernan,
CSO Medicinal Genomics

October 19th

# Illumina sequencing and RT-qPCR and qPCR





All sequence data is public and qPCR assays and sequence publicly available.

OSF PREPRINTS

My Preprints | Add a Preprint | Search | Support | Donate | Sign Up | Sign In

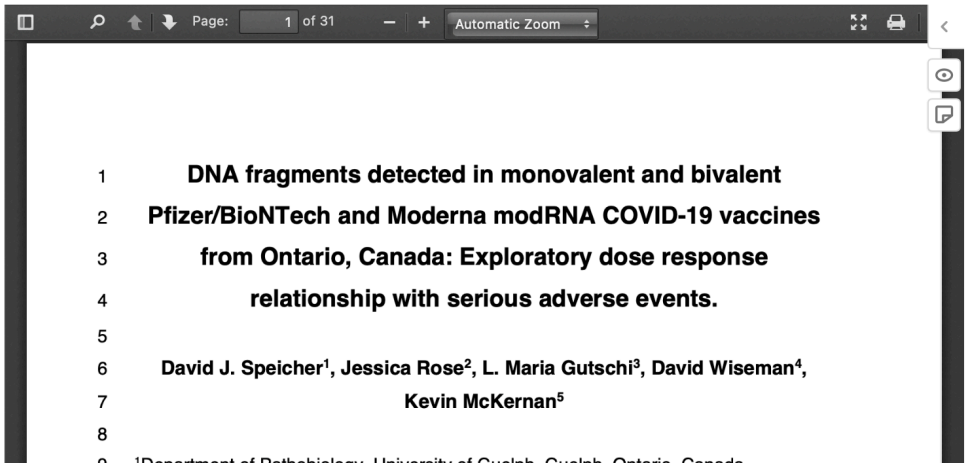
# DNA fragments detected in monovalent and bivalent Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines from Ontario, Canada: Exploratory dose response relationship with serious adverse events.


AUTHORS

David J Speicher, Jessica Rose, L. Maria Gutschi, David M Wiseman PhD, and Kevin McKernan


AUTHOR ASSERTIONS

CONFLICT OF INTEREST: Yes | PUBLIC DATA: Available | PREREGISTRATION: Not applicable



Page: 1 of 31 Automatic Zoom

1 **DNA fragments detected in monovalent and bivalent**
2 **Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines**
3 **from Ontario, Canada: Exploratory dose response**
4 **relationship with serious adverse events.**
5
6 **David J. Speicher[1], Jessica Rose[2], L. Maria Gutschi[3], David Wiseman[4],**
7 **Kevin McKernan[5]**
8

[1]Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada

Download preprint

Views: 206456 | Downloads: 19611

plaudit Be the first to endorse this work

## Abstract


Background: In vitro transcription (IVT) reactions used to generate nucleoside modified RNA (modRNA) for SARS-CoV-2 vaccines currently rely on an RNA polymerase transcribing from a DNA template. Production of modRNA used in the original Pfizer


Show more

## Supplemental Materials

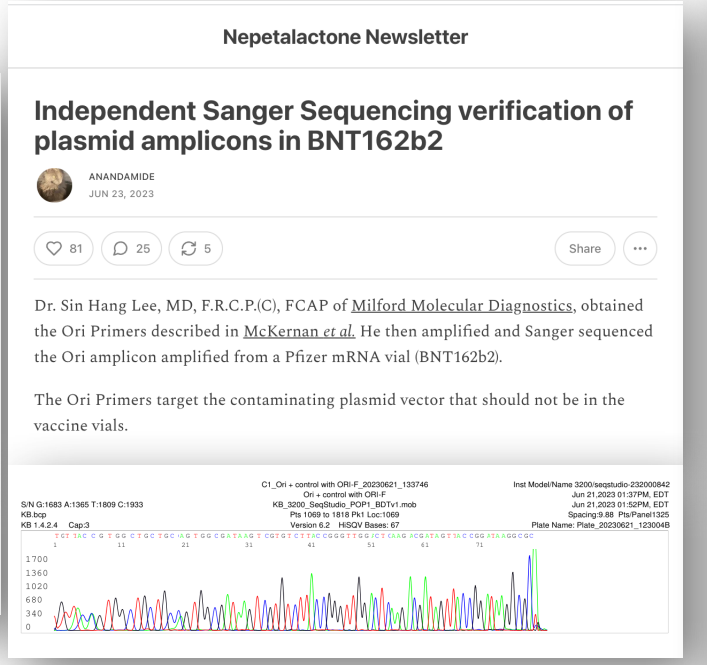
https://osf.io/xv3nz/

## preprint DOI

# International replication

## Dr. Phillip Buckhaults


## Dr. Sin Lee




## Dr. Brigitte Konig


## These agencies all Admit to it being there now

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA U.S. FOOD & DRUG ADMINISTRATION

Government of Canada

# Peer Reviewed

## Uses Fluorometry without RNase A



Open Access | Commentary

### Methodological Considerations Regarding the Quantification of DNA Impurities in the COVID-19 mRNA Vaccine Comirnaty®

by Brigitte König [1,2] and Jürgen O. Kirchner [3,*]

[1] Magdeburg Molecular Detections GmbH & Co. KG, 39104 Magdeburg, Germany
[2] Institute of Medical Microbiology and Virology, Faculty of Medicine, University of Leipzig, 04103 Leipzig, Germany
[3] Independent Researcher, 22307 Hamburg, Germany
* Author to whom correspondence should be addressed.

*Methods Protoc.* **2024**, *7*(3), 41; https://doi.org/10.3390/mps7030041

**Submission received: 12 March 2024 / Revised: 6 May 2024 / Accepted: 7 May 2024 / Published: 8 May 2024**

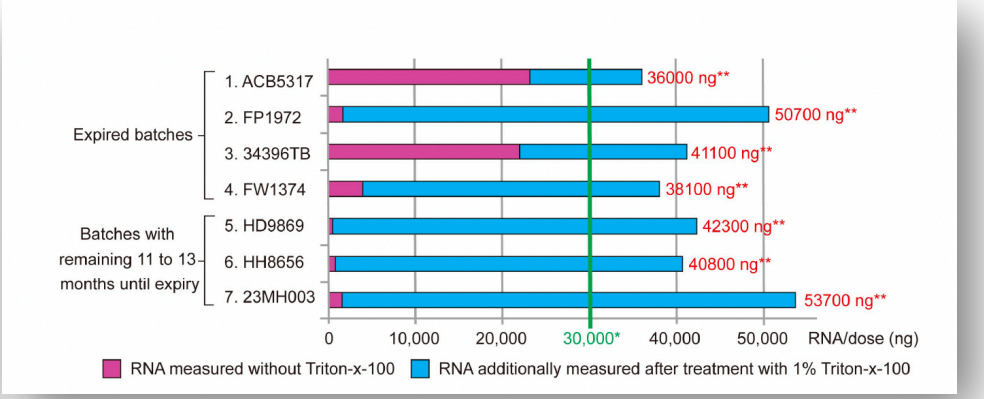
(This article belongs to the Section Biomedical Sciences and Physiology)



**Abstract**

DNA impurities can impact the safety of genetically engineered pharmaceuticals; thus, a specific limit value must be set for them during marketing authorisation. This particularly applies to mRNA vaccines, as large quantities of DNA templates are used for their production. Furthermore, when quantifying the total DNA content in the final product, we must observe that, in addition to the mRNA active ingredient, DNA impurities are also encased in lipid nanoparticles and are therefore difficult to quantify. In fact, the manufacturer of the mRNA vaccine Comirnaty (BioNTech/Pfizer) only measures DNA impurities in the active substance by means of a quantitative polymerase chain reaction (qPCR), whose DNA target sequence is less than just 1% of the originally added DNA template. This means that no direct DNA quantification takes place, and compliance with the limit value for DNA contamination is only estimated from the qPCR data using mathematical extrapolation methods. However, it is also possible to dissolve the lipid nanoparticles with a detergent to directly measure DNA contamination in the final product by using fluorescence spectroscopic methods. Experimental testing of this approach confirms that reliable values can be obtained in this way.

**Keywords:** mRNA vaccines; Comirnaty; DNA impurities; fluorescence spectroscopy; Qubit fluorometry




Many important points-
Pfizer is under quanting with qPCR.

Pfizer has RT-qPCR assays described in the EMA documents but doesn't use them for RNA quant?

DNA is not Quant'd at the same part of the process as the RNA?

We are no longer debating IF the vaccines are contaminated
Just how many **LOG** scales they are off

## Recent DNA Contamination paper

Konig et al

ANANDAMIDE
MAY 18, 2024

228 42 37 Share ···

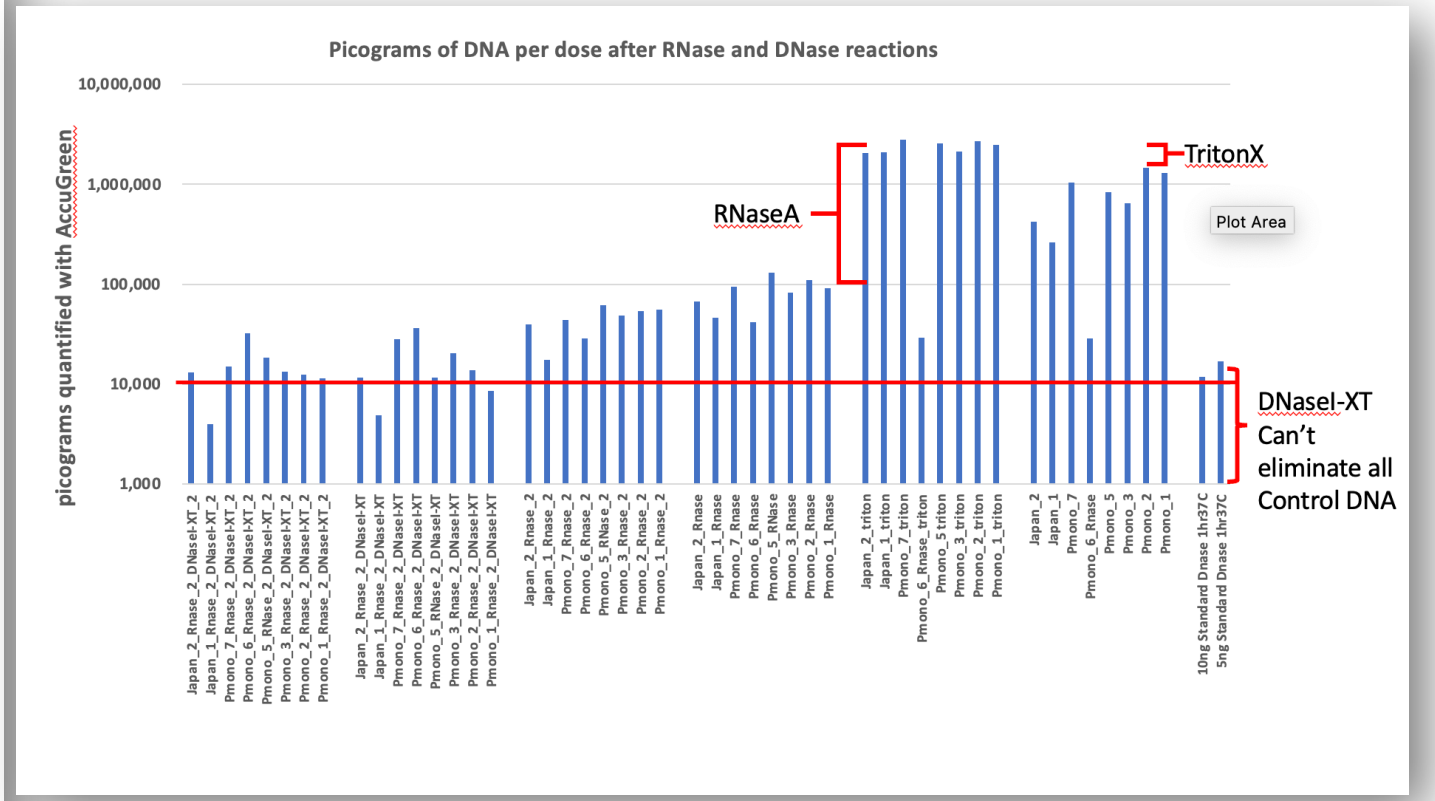
**Children's Health Defense**
@ChildrensHD

DNA Contamination in Pfizer COVID Vaccine Exceeded 500 Times Allowable Levels, Study Finds

"We are no longer debating whether the shots are contaminated. We're just debating whether they are 10-fold or 100-fold over the limit..."
@Kevin_McKernan

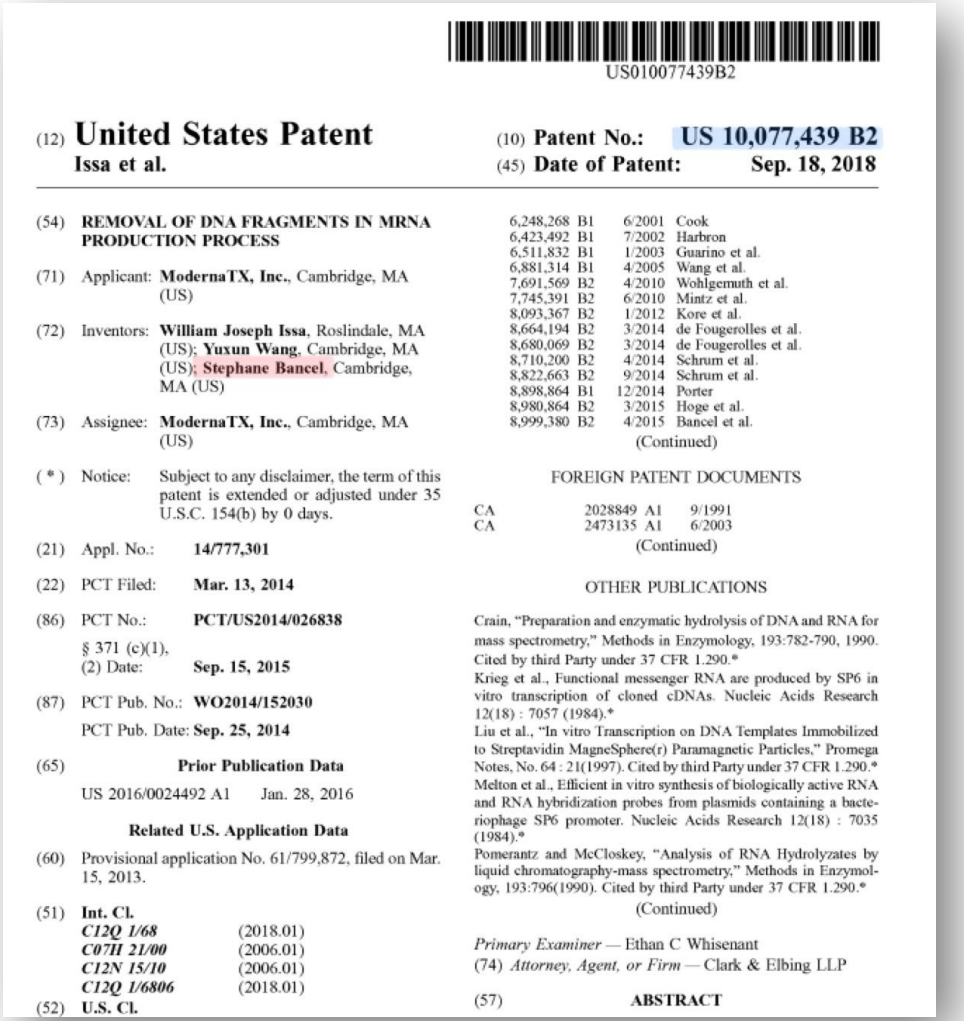

From childrenshealthdefense.org

5:02 PM · May 16, 2024 · **16.9K** Views



How did they miss by 100 fold?

Moderna Patents speak to the failure of qPCR to measure all DNA:
CEO Stephane Bancel is an inventor.



US010077439B2

(12) **United States Patent**
**Issa et al.**

(10) **Patent No.: US 10,077,439 B2**
(45) **Date of Patent: Sep. 18, 2018**

(54) **REMOVAL OF DNA FRAGMENTS IN MRNA PRODUCTION PROCESS**


(71) Applicant: **ModernaTX, Inc.**, Cambridge, MA (US)

(72) Inventors: **William Joseph Issa**, Roslindale, MA (US); **Yuxun Wang**, Cambridge, MA (US); **Stephane Bancel**, Cambridge, MA (US)

(73) Assignee: **ModernaTX, Inc.**, Cambridge, MA (US)



( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/777,301**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/026838**
§ 371 (c)(1),
(2) Date: **Sep. 15, 2015**

(87) PCT Pub. No.: **WO2014/152030**
PCT Pub. Date: **Sep. 25, 2014**

(65) **Prior Publication Data**

US 2016/0024492 A1 Jan. 28, 2016

**Related U.S. Application Data**

(60) Provisional application No. 61/799,872, filed on Mar. 15, 2013.

(51) **Int. Cl.**
***C12Q 1/68*** (2018.01)
***C07H 21/00*** (2006.01)
***C12N 15/10*** (2006.01)
***C12Q 1/6806*** (2018.01)

(52) **U.S. Cl.**



| | | | |
|---|---|---|---|
| 6,248,268 | B1 | 6/2001 | Cook |
| 6,423,492 | B1 | 7/2002 | Harbron |
| 6,511,832 | B1 | 1/2003 | Guarino et al. |
| 6,881,314 | B1 | 4/2005 | Wang et al. |
| 7,691,569 | B2 | 4/2010 | Wohlgemuth et al. |
| 7,745,391 | B2 | 6/2010 | Mintz et al. |
| 8,093,367 | B2 | 1/2012 | Kore et al. |
| 8,664,194 | B2 | 3/2014 | de Fougerolles et al. |
| 8,680,069 | B2 | 3/2014 | de Fougerolles et al. |
| 8,710,200 | B2 | 4/2014 | Schrum et al. |
| 8,822,663 | B2 | 9/2014 | Schrum et al. |
| 8,898,864 | B1 | 12/2014 | Porter |
| 8,980,864 | B2 | 3/2015 | Hoge et al. |
| 8,999,380 | B2 | 4/2015 | Bancel et al. |

(Continued)

FOREIGN PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| CA | 2028849 | A1 | 9/1991 |
| CA | 2473135 | A1 | 6/2003 |

(Continued)

OTHER PUBLICATIONS

Crain, "Preparation and enzymatic hydrolysis of DNA and RNA for mass spectrometry," Methods in Enzymology, 193:782-790, 1990. Cited by third Party under 37 CFR 1.290.*

Krieg et al., Functional messenger RNA are produced by SP6 in vitro transcription of cloned cDNAs. Nucleic Acids Research 12(18) : 7057 (1984).*

Liu et al., "In vitro Transcription on DNA Templates Immobilized to Streptavidin MagneSphere(r) Paramagnetic Particles," Promega Notes, No. 64 : 21(1997). Cited by third Party under 37 CFR 1.290.*

Melton et al., Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Research 12(18) : 7035 (1984).*

Pomerantz and McCloskey, "Analysis of RNA Hydrolyzates by liquid chromatography-mass spectrometry," Methods in Enzymology, 193:796(1990). Cited by third Party under 37 CFR 1.290.*

(Continued)



*Primary Examiner* — Ethan C Whisenant
(74) *Attorney, Agent, or Firm* — Clark & Elbing LLP


(57) **ABSTRACT**



extraction is based on the differential partitioning of DNA and RNA into organic and aqueous phases.

DNase I is an endonuclease that cleaves DNA by breaking phosphodiester bonds and produces smaller DNA fragments and/or di-, tri- and oligonucleotides which are subsequently removed by size-based separation methods. However, it is challenging to quantitatively determine the DNase I digestion efficiency and DNase I itself requires to be inactivated or removed in the subsequent process. Quantitative PCR is often applied to measure the residual DNA but it only detects the DNA molecules that contain both qPCR primers thus does not measure all other smaller DNA molecules that are partially digested. To overcome this challenge, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) approach can be used where a total nuclease digestion is performed on the RNA drug substance sample following the DNA removal step. The presence of individual residual deoxynucleotides is quantitatively assayed against deoxynucleotide standards using MS/MS and the abundance is reported.

# When the TGA is asked for its PCR protocol

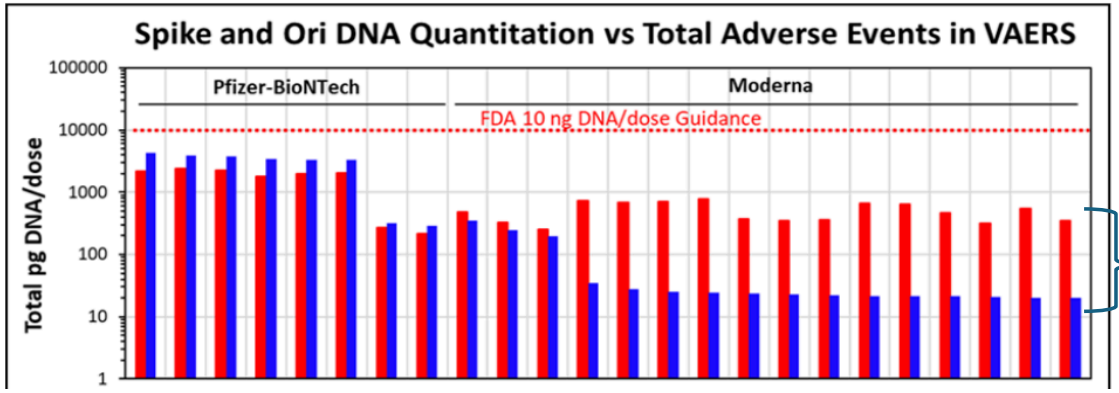

## al In-Process Control Method – Moderna SOP-1020

**wing is unmodified presentation of the original Moderna SOP-1020, which was provided mmercial in confidence arrangements and is not to be distributed beyond the HPRG.**

**.0 PURPOSE**

The purpose of this procedure is to detect and quantify residual plasmid DNA in mRNA Drug Substance (DS) or mRNA Product intermediate (MPI) using a real time quantitative PCR (qPCR) assay designed to amplify the kanamycin resistance gene in the plasmid.

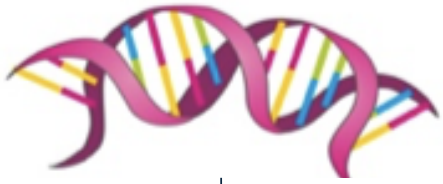




# Background- How did this happen?

## Process 1 used In Vitro Transcription (IVT)

### Synthetic DNA template



### IVT reaction

**Figure 3**

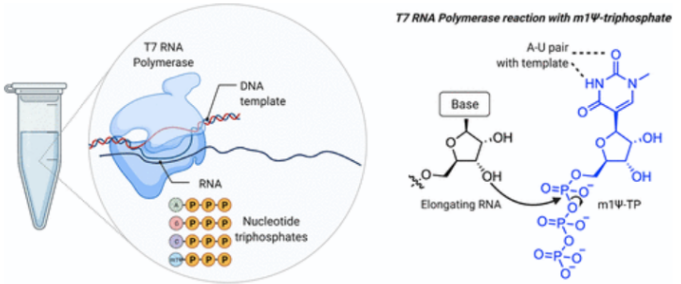
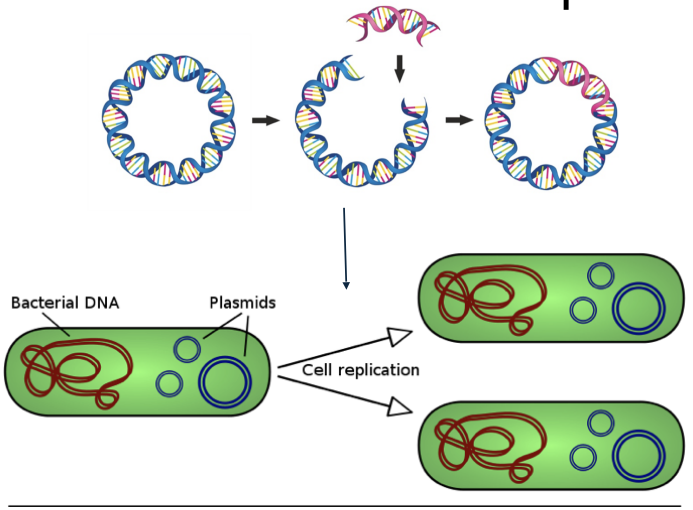



Figure 3. Production of m1Ψ mRNAs by *in vitro* transcription. Left: Components of *in vitro* transcription reaction. Right: Incorporation of m1Ψ-triphosphate into RNA is guided by m1Ψ's ability to form a canonical base pair with adenine of the DNA template in the T7 RNA polymerase active site.

Nance et al

## Process 2 used Plasmids in E.coli

### cloned DNA template




### IVT reaction

**Figure 3**

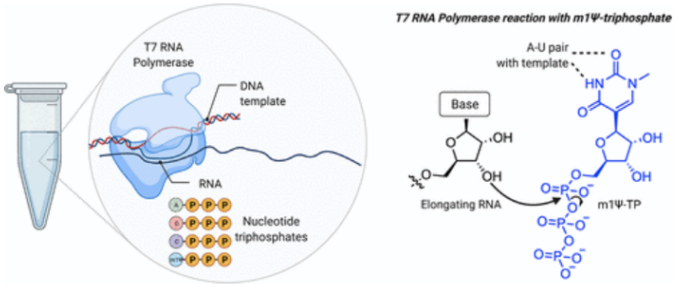
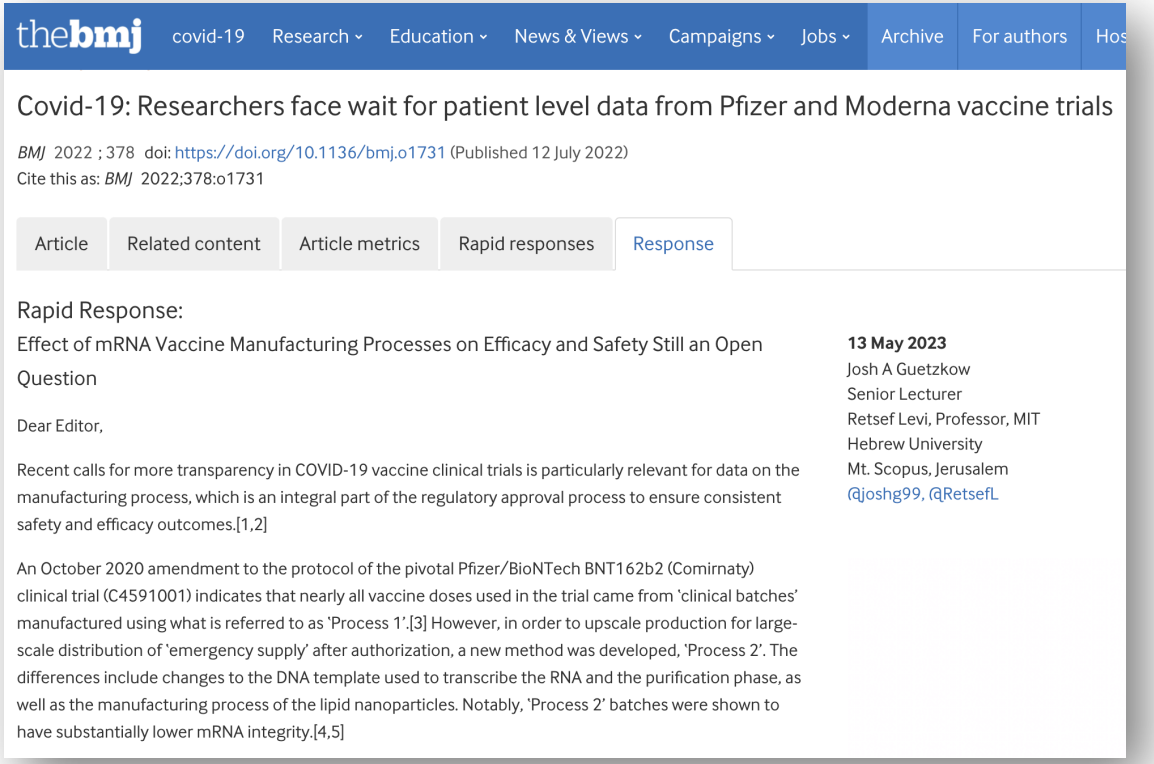



Figure 3. Production of m1Ψ mRNAs by *in vitro* transcription. Left: Components of *in vitro* transcription reaction. Right: Incorporation of m1Ψ-triphosphate into RNA is guided by m1Ψ's ability to form a canonical base pair with adenine of the DNA template in the T7 RNA polymerase active site.

Nance et a

# Process 1 (IVT) vs Process 2 (E.coli)



thebmj covid-19 Research Education News & Views Campaigns Jobs Archive For authors Hos

Covid-19: Researchers face wait for patient level data from Pfizer and Moderna vaccine trials

*BMJ* 2022 ; 378 doi: https://doi.org/10.1136/bmj.o1731 (Published 12 July 2022)
Cite this as: *BMJ* 2022;378:o1731

Article | Related content | Article metrics | Rapid responses | Response

Rapid Response:

Effect of mRNA Vaccine Manufacturing Processes on Efficacy and Safety Still an Open Question

Dear Editor,

Recent calls for more transparency in COVID-19 vaccine clinical trials is particularly relevant for data on the manufacturing process, which is an integral part of the regulatory approval process to ensure consistent safety and efficacy outcomes.[1,2]

An October 2020 amendment to the protocol of the pivotal Pfizer/BioNTech BNT162b2 (Comirnaty) clinical trial (C4591001) indicates that nearly all vaccine doses used in the trial came from 'clinical batches' manufactured using what is referred to as 'Process 1'.[3] However, in order to upscale production for large-scale distribution of 'emergency supply' after authorization, a new method was developed, 'Process 2'. The differences include changes to the DNA template used to transcribe the RNA and the purification phase, as well as the manufacturing process of the lipid nanoparticles. Notably, 'Process 2' batches were shown to have substantially lower mRNA integrity.[4,5]

**13 May 2023**
Josh A Guetzkow
Senior Lecturer
Retsef Levi, Professor, MIT
Hebrew University
Mt. Scopus, Jerusalem
@joshg99, @RetsefL

An October 2020 amendment to the protocol of the pivotal Pfizer/BioNTech BNT162b2 (Comirnaty) clinical trial (C4591001) indicates that nearly all vaccine doses used in the trial came from 'clinical batches' manufactured using what is referred to as 'Process 1'.[3] However, in order to upscale production for large-scale distribution of 'emergency supply' after authorization, a new method was developed, 'Process 2'. The differences include changes to the DNA template used to transcribe the RNA and the purification phase, as well as the manufacturing process of the lipid nanoparticles. Notably, 'Process 2' batches were shown to have substantially lower mRNA integrity.[4,5]

The protocol amendment states that "each lot of 'Process 2'-manufactured BNT162b2 would be administered to approximately 250 participants 16 to 55 years of age" with comparative immunogenicity and safety analyses conducted with 250 randomly selected 'Process 1' batch recipients. To the best of our knowledge, there is no publicly available report on this comparison of 'Process 1' versus 'Process 2' doses.

Two documents obtained through a Freedom of Information Act (FOIA) request[6] describe the vaccine batches and lots supplied to each of the trial sites through November 19, 2020[7] and March 17, 2021,[8] respectively. According to these documents, doses from 'Process 2' batch EE8493Z are listed at four trial sites prior to November 19, and four other sites are listed with 'Process 2' batch EJ0553Z in the updated document. Both batches were also part of the emergency supply for public distribution. The CDC's Vaccine Adverse Event Reporting System, known to be underreported,[9] lists 658 reports (169 serious, 2 deaths) for lot EE8493[10] and 491 reports (138 serious, 21 deaths) for lot EJ0553.[11]

Furthermore, additional 'Process 1' batch EE3813 doses with distinct Pfizer lot numbers were added to the later batch document[7] at over 70% of trial sites, potentially supplied at a later stage to enable vaccination of placebo patients with BNT162b2. The 6-month interim clinical study report[12] from the Comirnaty trial notes that "the IR for any AE and at least 1 related AE and severe AE for participants who originally received placebo and then received BNT162b2 are greater (205.4 per 100 PY, 189.5 per 100 PY, 6.0 per 100 PY) than the IRs (83.2 per 100 PY, 62.9 per 100 PY, 4.3 per 100 PY) for participants who originally were randomized to BNT162b2" (p222). It is unclear whether there is a connection between the lots administered to the crossover placebo subjects and the elevated rate of AE's.

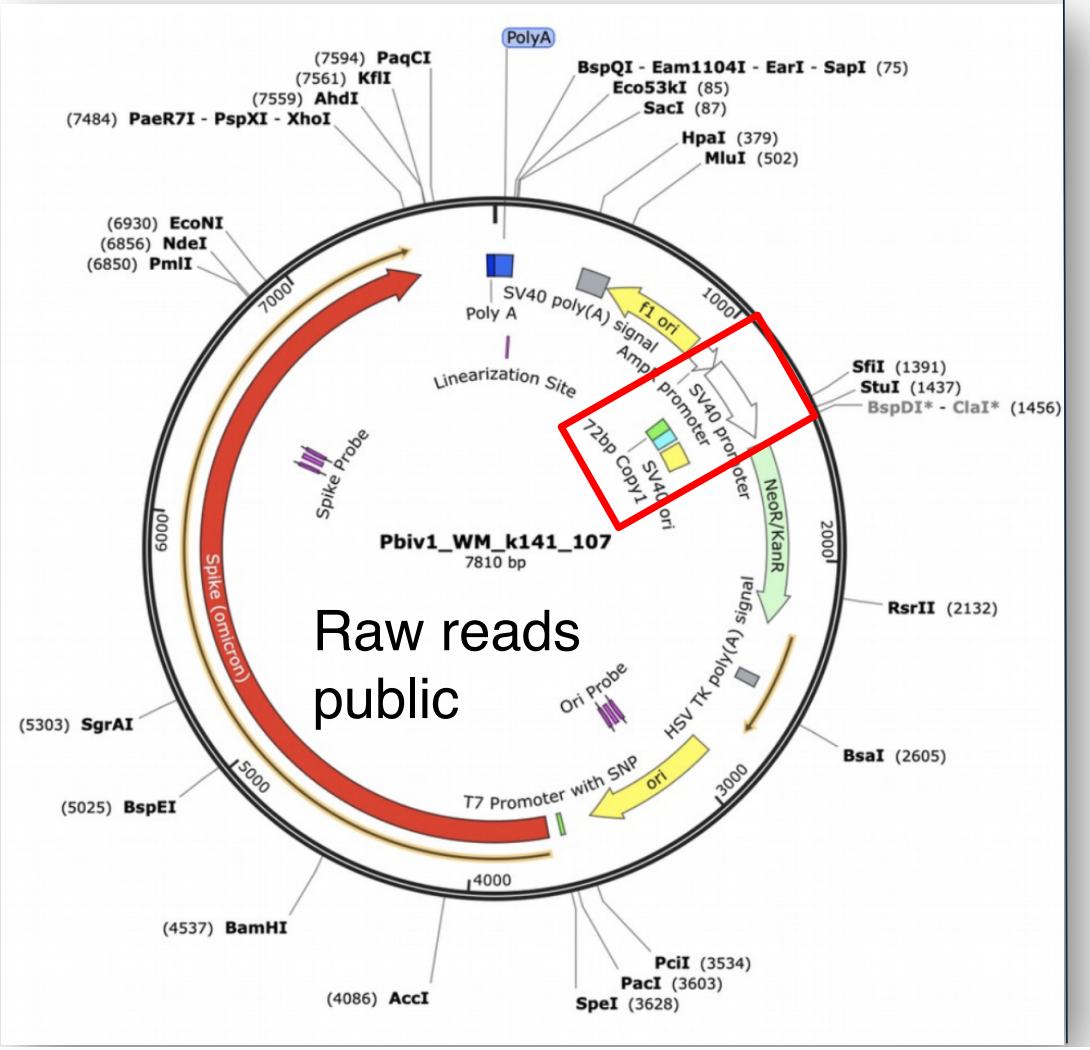
Finally, a recent study found significant variability in the rate of serious adverse events (SAEs) across 52 different lots of Comirnaty marketed in Denmark.[13] This finding underscores the importance of understanding better the potential impact of variability in the production process of COVID-19 mRNA vaccines on efficacy and safety.

Evidence from existing research and trial documents highlights the importance of publicly disclosing the analysis comparing reactogenicity and safety of process 1 and 2 batches as specified in the trial protocol, and more generally patient-level batch and lot data from the trial.

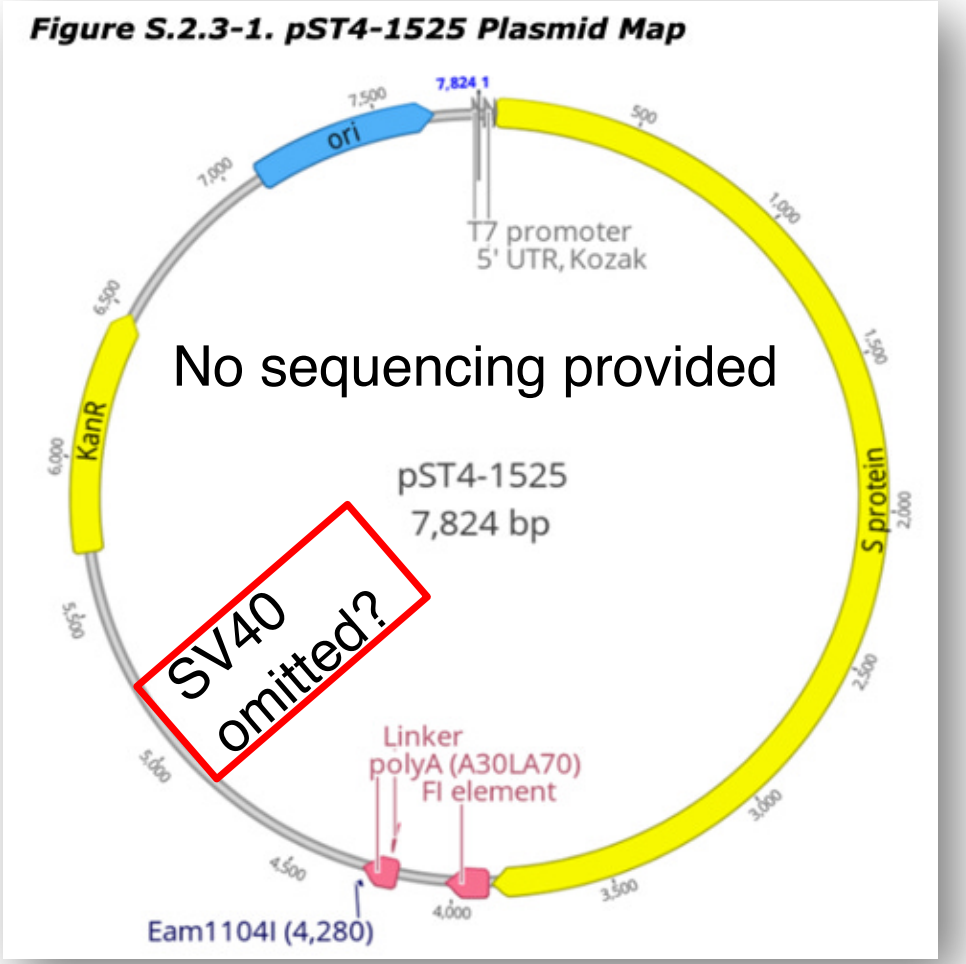
Josh Guetzkow
Retsef Levi

The trial was run on Process 1 lots
250 people received Process 2 lots (plasmids)
The world received Process 2 lots

# Independent Illumina sequencing




# What was disclosed to the EMA



**Initial Public Regulatory Response**


1)Yes, SV40 is there


2)Yes, Pfizer did **NOT** spell this out


3)DNA is too small in length to matter


4)DNA is too small in quantity to matter


5)DNA is non-functional

 Regulators are correct  Regulators are wrong

# SV40 Enhancers are used in Gene Therapy: Nuclear Targeting Sequences (NTS) Fact Checkers will not address this slide!

Dean Lab

Home

Projects

Publications

Lab Members

News

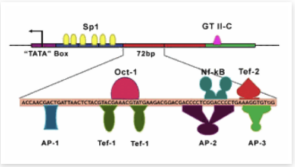
URMC / Labs / Dean Lab / Projects / Nuclear Targeting of Plasmids and Protein-DNA Complexes

Make a Gift

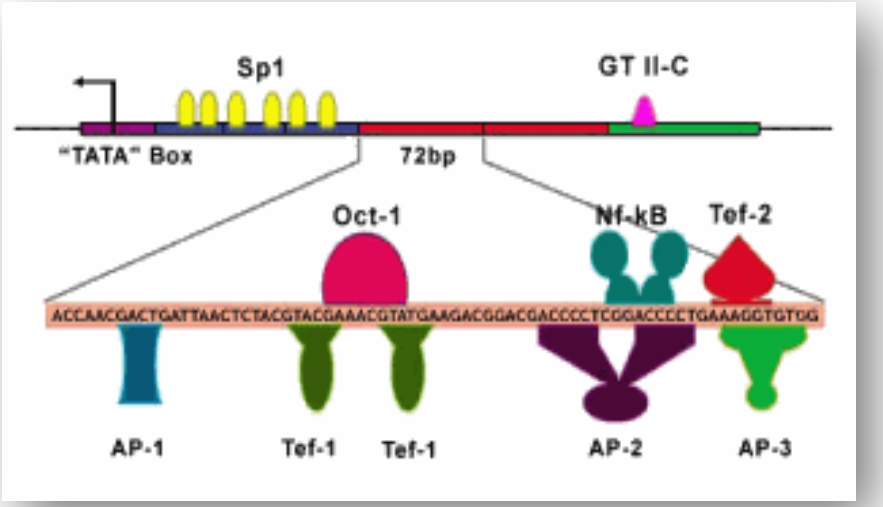
## Nuclear Targeting of Plasmids and Protein-DNA Complexes

My laboratory studies the mechanisms and applications of plasmid and DNA-binding protein nuclear localization. Our long term goals are to develop gene therapy approaches to the treatment of a variety of human diseases by focusing on the development of novel non-viral intra- and extracellular delivery methods. Our main emphasis is in the area of pulmonary gene delivery and function. Perhaps the major problem hindering gene therapy is the inefficiency of gene transfer to slowly and non-dividing cells. While many aspects of non-viral vector design are being addressed, one critical area that has not received adequate attention is the nuclear import of vector DNA. Clearly, without the translocation of plasmid DNA into the nucleus, no gene expression, or "gene therapy" can take place. My laboratory continues to identify and characterize novel DNA sequences to promote nuclear import of non-viral vectors, both in cultured cells and in vivo, as well as sequences that promote cytoplasmic and intranuclear trafficking.

Over the past 15 years, work from our laboratory has addressed the nuclear targeting and entry of plasmid DNA. Using cultured cells, we have shown that plasmids are able to enter the nuclei of cells in the absence of cell division and its accompanying nuclear envelope breakdown. Assays used to follow the movement of DNA include in situ hybridization, reporter gene expression, GFP-, YFP-, and RFP- tagged proteins, and live cell imaging of fluorescently-labeled plasmids and RNAs. As for all other macromolecular exchange between the cytoplasm and nucleus, DNA nuclear entry is mediated by the nuclear pore complex.




Organization of transcription factor binding sites on the SV40 enhancer.

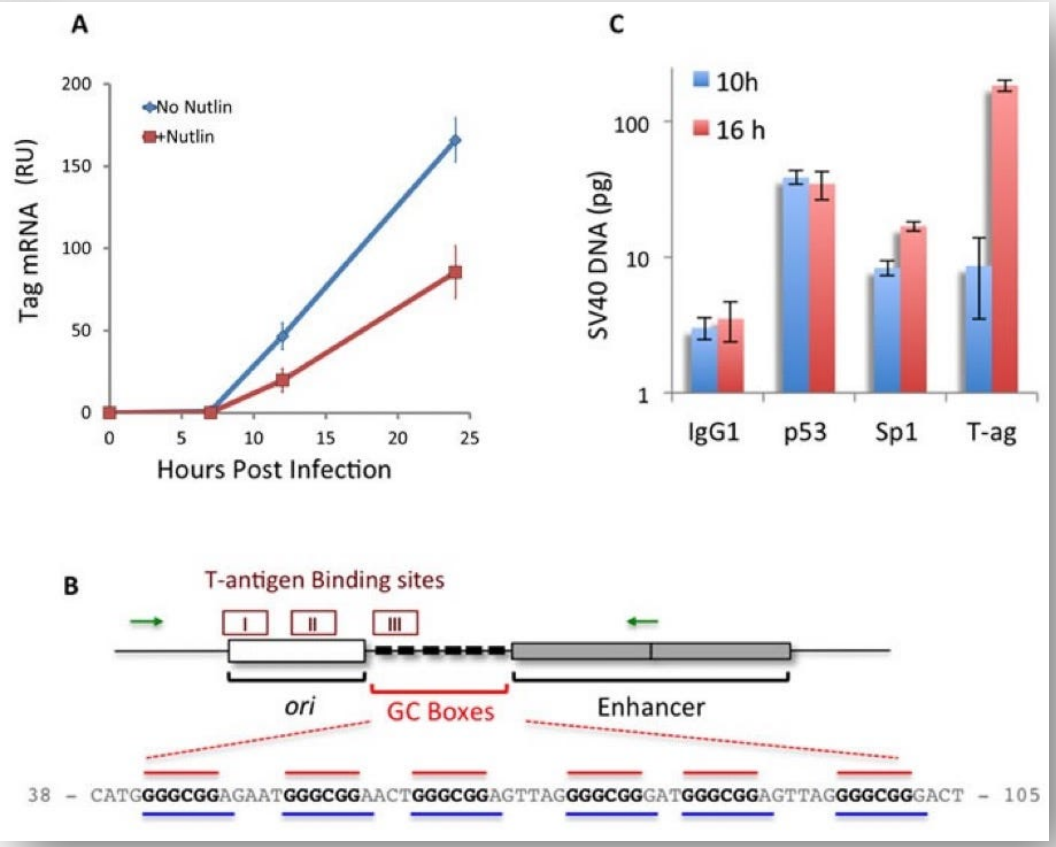


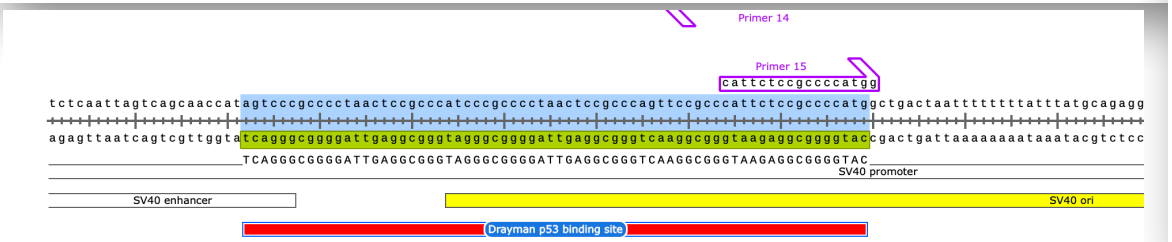
# SV40 Promoter Binds to p53 Tumor Suppressor gene

Figure 7

**p53 binds to the SV40 early promoter, correlating with a decrease in T-ag mRNA**

**A.** CV-1 cells, with or without 16 hours Nutlin3 pre-treatment, were infected with SV40 and the level of T-ag mRNA, represented as relative units, was measured by quantitative RT-PCR at the indicated time-points, with HPRT RNA as an internal standard. Note that the T-ag protein is seen at 9 hours post infection (Figure S4). The results shown are mean ± S.E. of 5 independent experiments. For the statistical analysis, we compared the area under the curves and found that it was significantly lower in Nutlin3 treated cells compared to untreated cells (680±50 AU vs. 1400±142 AU, respectively. *p*-value = 0.004). **B.** Diagram of the regulatory region of the SV40 genome presenting the *ori* - origin of replication, the GC-boxes and the Enhancer, composed of duplicated 72 bp. The 3 T-ag binding sites are shown on top, and DNA sequence of the GC-boxes with the overlapping Sp1 (red) and p53 (Blue) binding sites below (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). The green arrows designate the location of the PCR primers used in the ChIP experiments. **C.** Binding of Sp1, p53 and T-ag to SV40 DNA *in vivo* was determined by ChIP at the indicated time points. DNA recovered from the immune precipitate was quantified by PCR with SV40 DNA as an internal standard. Results are mean ± S.E. of 3 independent experiments.




# FDA guidelines were derived from Cell Substrate gDNA

10pg of gDNA was the limit pre-NCVIA

10ng of gDNA = 1,000 copies of the human genome

10ng of 200bp DNA = ~50 Billion copies

Many more active DNA ends
(3'Hydroxyls and 5' Phosphates)

equals $6 \times 10^5$ pg, or 600 ng. Thus, if the amount of residual cell-substrate DNA in a product is 10 ng, then the safety factor with respect to an infectious event for cellular DNA containing an infectious viral genome is 600 ng ÷ 10 ng, or 60. If the cell contains more than a single viral genome, then this safety factor would be reduced accordingly. As stated above, safety factors of $\geq 10^7$ have been considered appropriate with respect to cell-substrate DNA, and thus, a safety factor of 60 or lower would be insufficient. To obtain a safety factor in the $\geq 10^7$ range, either the level of cell-substrate DNA would need to be lowered below 10 ng, or the biological activity of the DNA would need to be reduced by nuclease digestion or chemical inactivation. Assuming that only one copy of the retroviral DNA was present, then the amount of residual cell-substrate DNA would need to be 10 fg or lower. However, if there were 100 copies of the infectious viral genome, the amount of DNA would need to be reduced to 100 ag. Reducing residual cell-substrate DNA to these levels, even with the hardiest of viral vaccines, would likely be impractical and difficult to document. Therefore, with certain cell substrates, additional treatments of the DNA might be recommended.

nature › scientific reports › articles › article

Article | Open Access | Published: 26 April 2023

## High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection

Samuel Lim, R. Rogers Yocum, Pamela A. Silver & Jeffrey C. Way

*Scientific Reports* **13**, Article number: 6835 (2023) | Cite this article

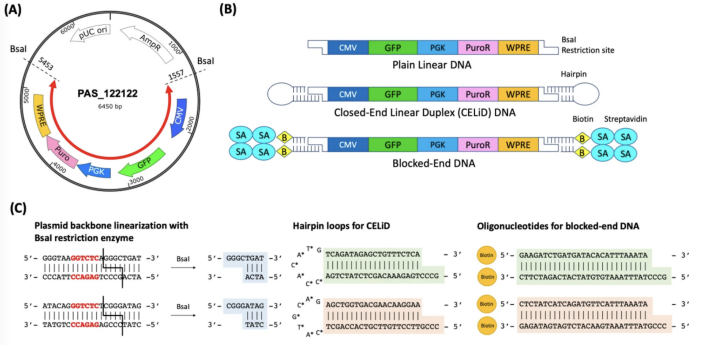
**5153** Accesses | **199** Altmetric | Metrics

### Abstract

In gene therapy, potential integration of therapeutic transgene into host cell genomes is a serious risk that can lead to insertional mutagenesis and tumorigenesis. Viral vectors are often used as the gene delivery vehicle, but they are prone to undergoing integration events. More recently, non-viral delivery of linear DNAs having modified geometry such as closed-end linear duplex DNA (CELiD) have shown promise as an alternative, due to prolonged transgene expression and less cytotoxicity. However, whether modified-end linear DNAs can also provide a safe, non-integrating gene transfer remains unanswered. Herein, we compare the genomic integration frequency upon transfection of cells with expression vectors in the forms of circular plasmid, unmodified linear DNA, CELiDs with thioester loops, and Streptavidin-conjugated blocked-end linear DNA. All of the forms of linear DNA resulted in a high fraction of the cells being stably transfected—between 10 and 20% of the initially transfected cells. These results indicate that blocking the ends of linear DNA is insufficient to prevent integration.

**Figure 1**

From: High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection




Design of DNA constructs used in this study. **(A)** Circular plasmid used as the common backbone for constructing various end-modified linear DNAs. The plasmid consisted of two constitutive expression cassettes for the GFP reporter and puromycin resistant selection marker, in addition to the WPRE element, flanked by the two *BsaI* restriction sites. The red arrow indicates a portion of the plasmid corresponding to the linear DNAs. **(B)** Structure of the linear DNAs. The end regions of the CELiD consisted of closed hairpin loop structures. The ends of blocked-end DNA contained biotin-labeled oligonucleotides, which were further non-covalently bound to streptavidin tetramers. **(C)** Detailed sequences of the sticky ends created by the plasmid backbone linearization by the *BsaI* restriction enzyme, as well as the hairpin loops and oligonucleotides complementary to each end. Starred bases indicate positions of phosphorothioate linkages on the 5′ side.

# Moderna Patent speaks to the risk of insertional mutagenesis from DNA

(12) **United States Patent**
**de Fougerolles et al.**

(10) **Patent No.: US 10,898,574 B2**
(45) **Date of Patent: *Jan. 26, 2021**

(54) **DELIVERY AND FORMULATION OF ENGINEERED NUCLEIC ACIDS**

(71) Applicant: **ModernaTX, Inc.**, Cambridge, MA (US)

(72) Inventors: **Antonin de Fougerolles**, Waterloo (BE); **Sayda M. Elbashir**, Cambridge, MA (US)

(73) Assignee: **ModernaTX, Inc.**, Cambridge, MA (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/927,730**

(22) Filed: **Mar. 21, 2018**

(65) **Prior Publication Data**

US 2019/0060458 A1 Feb. 28, 2019

**Related U.S. Application Data**

(60) Continuation of application No. 15/379,284, filed on Dec. 14, 2016, now Pat. No. 9,950,068, which is a division of application No. 14/337,513, filed on Jul. 22, 2014, now Pat. No. 9,533,047, which is a continuation of application No. 13/897,362, filed on May 18, 2013, now abandoned, which is a continuation of application No. 13/437,034, filed on Apr. 2, 2012, now Pat. No. 8,710,200.

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| 5,034,506 | A | 7/1991 | Summerton et al. |
| 5,426,180 | A | 6/1995 | Kool |
| 5,489,677 | A | 2/1996 | Sanghvi et al. |
| 5,512,439 | A | 4/1996 | Hornes et al. |
| 5,591,722 | A | 1/1997 | Montgomery et al. |
| 5,637,459 | A | 6/1997 | Burke et al. |
| 5,639,873 | A | 6/1997 | Barascut et al. |
| 5,641,400 | A | 6/1997 | Kaltenbach et al. |
| 5,789,578 | A | 8/1998 | Burton et al. |
| 5,808,039 | A | 9/1998 | Reddy et al. |
| 5,989,911 | A | 11/1999 | Fournier et al. |
| 6,022,715 | A | 2/2000 | Merenkova et al. |
| 6,022,737 | A | 2/2000 | Niven et al. |
| 6,248,268 | B1 | 6/2001 | Cook |
| 6,303,378 | B1 | 10/2001 | Bridenbaugh et al. |
| 6,423,492 | B1 | 7/2002 | Harbron |
| 6,511,832 | B1 | 1/2003 | Guarino et al. |
| 6,521,411 | B2 | 2/2003 | Hecker et al. |
| 7,691,569 | B2 | 4/2010 | Wohlgemuth et al. |
| 8,075,780 | B2 | 12/2011 | Pearce |
| 8,093,367 | B2 | 1/2012 | Kore et al. |
| 8,664,194 | B2 | 3/2014 | de Fougerolles et al. |
| 8,680,069 | B2 | 3/2014 | de Fougerolles et al. |
| 8,691,750 | B2 | 4/2014 | Constien et al. |
| 8,710,200 | B2 | 4/2014 | Schrum et al. |
| 8,716,465 | B2 | 5/2014 | Rossi et al. |
| 8,802,438 | B2 | 8/2014 | Rossi et al. |
| 8,822,663 | B2 | 9/2014 | Schrum et al. |

(Continued)

FOREIGN PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| CA | 2028849 | A1 | 9/1991 |
| CA | 2473135 | A1 | 6/2003 |

(Continued)

35

## BACKGROUND OF THE INVENTION

There are multiple problems with prior methodologies of delivering pharmaceutical compositions in order to achieve effective protein expression both for therapeutics and bio- 40
processing applications. For example, introduced DNA can integrate into host cell genomic DNA at some frequency, resulting in alterations and/or damage to the host cell genomic DNA. Alternatively, the heterologous deoxyribo- 45
nucleic acid (DNA) introduced into a cell can be inherited by daughter cells (whether or not the heterologous DNA has integrated into the chromosome) or by offspring.

In addition, there are multiple steps which must occur after delivery but before the encoded protein is made which can effect protein expression. Once inside the cell, DNA 50

# modRNA is found in Heart Tissue 30 days after vax (Krauson)
# modRNA is found in Lymph Nodes 60 days after vax (Roltgen)

npj | vaccines

Search Log in

Content About Publish

articles > article

Download PDF

Article | Open Access |
Published: 27 September 2023

## Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients

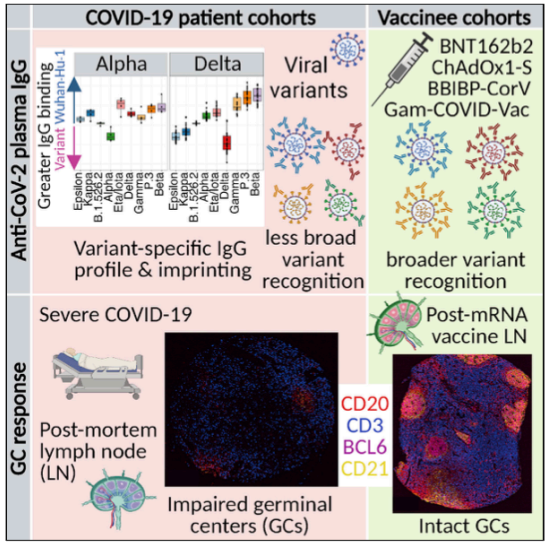
Aram J. Krauson, Faye Victoria C. Casimero, ... James R. Stone + Show authors

Cell

Article

## Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination

### Graphical abstract




### Authors

Katharina Röltgen, Sandra C.A. Nielsen, Oscar Silva, ..., Benjamin A. Pinsky, Kari C. Nadeau, Scott D. Boyd

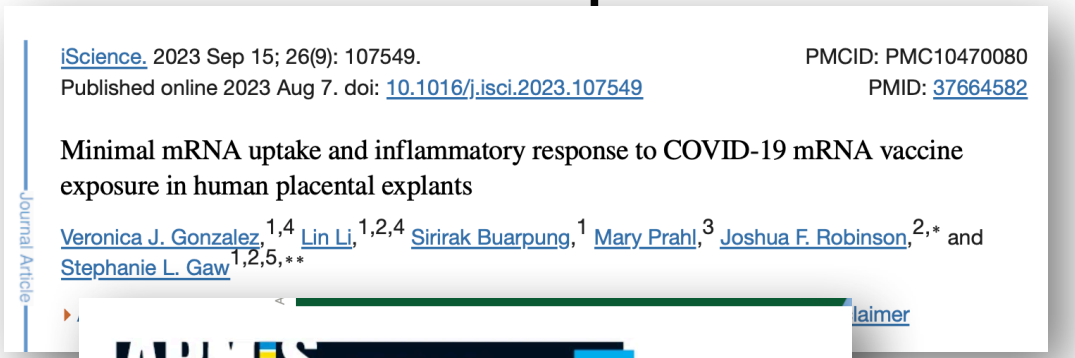
### Correspondence

publications_scott_boyd@stanford.edu

### In brief

Human antibody responses to SARS-CoV-2 differ between vaccination and infection, with mRNA vaccination inducing more productive lymph node GC responses and several vaccine types stimulating IgG antibodies capable of recognizing a broader range of viral variants.

# Spike nucleic acid persistence

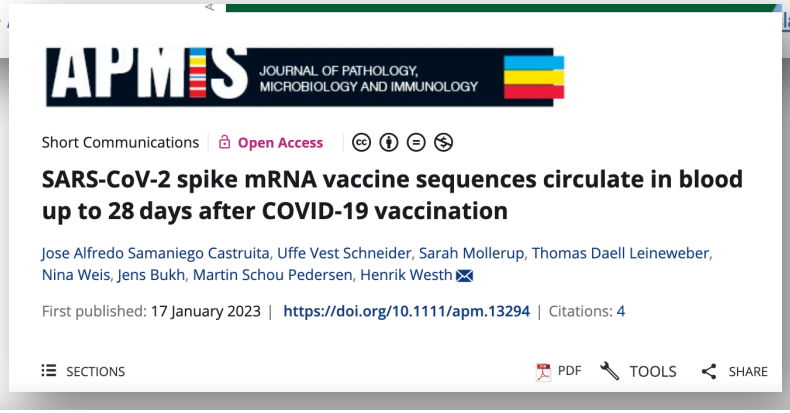


iScience. 2023 Sep 15; 26(9): 107549.
Published online 2023 Aug 7. doi: 10.1016/j.isci.2023.107549
PMCID: PMC10470080
PMID: 37664582

Minimal mRNA uptake and inflammatory response to COVID-19 mRNA vaccine exposure in human placental explants

Veronica J. Gonzalez,[1,4] Lin Li,[1,2,4] Sirirak Buarpung,[1] Mary Prahl,[3] Joshua F. Robinson,[2,*] and Stephanie L. Gaw[1,2,5,**]

Found in Placenta 2-10 days after (Gonzalez



APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

Short Communications | Open Access

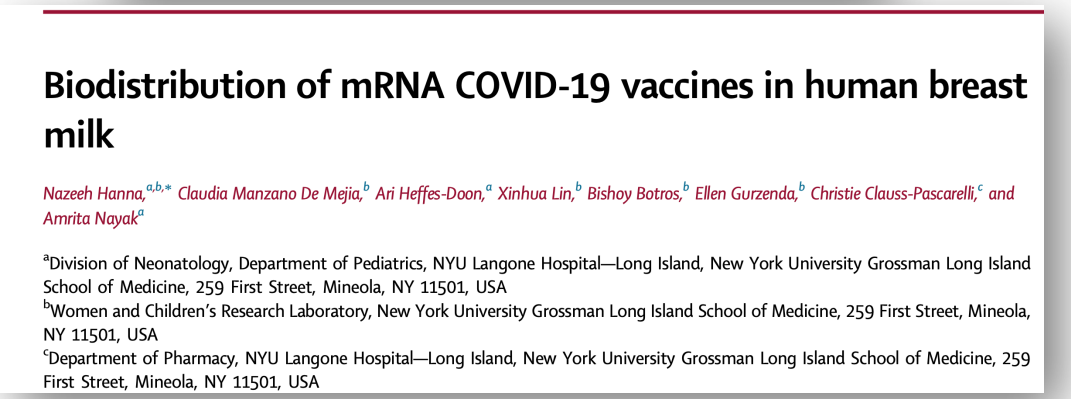
**SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination**

Jose Alfredo Samaniego Castruita, Uffe Vest Schneider, Sarah Mollerup, Thomas Daell Leineweber, Nina Weis, Jens Bukh, Martin Schou Pedersen, Henrik Westh

First published: 17 January 2023 | https://doi.org/10.1111/apm.13294 | Citations: 4

SECTIONS PDF TOOLS SHARE

Found in Plasma 28 days later (Castruita)



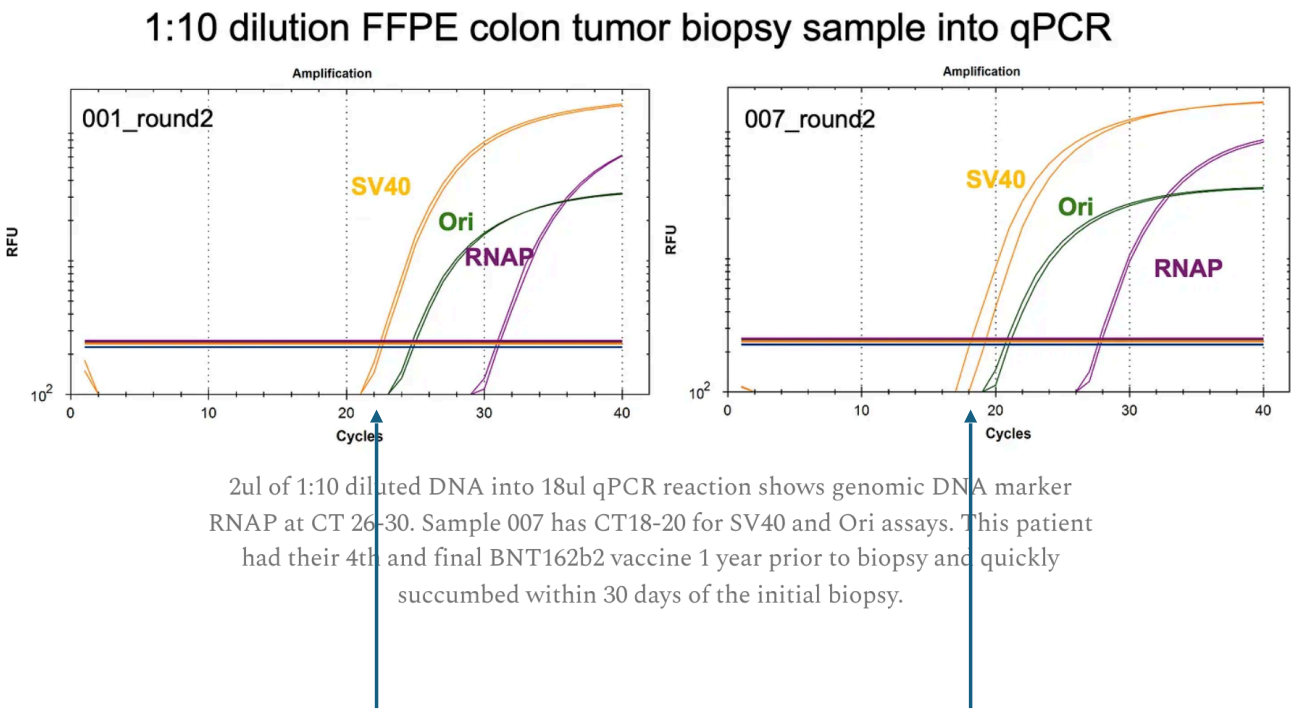
**Biodistribution of mRNA COVID-19 vaccines in human breast milk**

*Nazeeh Hanna,[a,b,*] Claudia Manzano De Mejia,[b] Ari Heffes-Doon,[a] Xinhua Lin,[b] Bishoy Botros,[b] Ellen Gurzenda,[b] Christie Clauss-Pascarelli,[c] and Amrita Nayak[a]*

[a]Division of Neonatology, Department of Pediatrics, NYU Langone Hospital—Long Island, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA
[b]Women and Children's Research Laboratory, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA
[c]Department of Pharmacy, NYU Langone Hospital—Long Island, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA

Found in breast milk 5 days later (Hanna)

# Colon Cancer Biopsy 1 year after vaccination




2ul of 1:10 diluted DNA into 18ul qPCR reaction shows genomic DNA marker RNAP at CT 26-30. Sample 007 has CT18-20 for SV40 and Ori assays. This patient had their 4th and final BNT162b2 vaccine 1 year prior to biopsy and quickly succumbed within 30 days of the initial biopsy.

64,000 Fold dilution

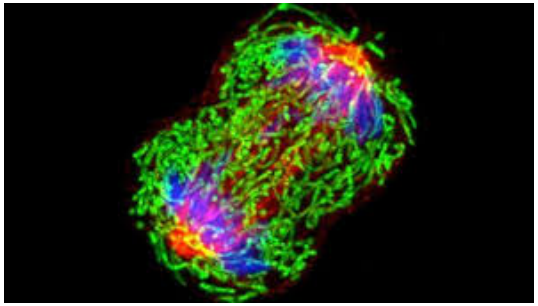
300ul – 1.2ml injection (4 jabs)

87,000 ml Body Volume

~64,000X dilution upon injection

These measurements are higher than what we see in some fresh vaccine vials.
Not what you would expect from a 64,000 fold dilution upon injection.
Unless the mammalian origins of replication are replicating

# Cancer?



**We are always Cancering. When the mutagenesis outpaces the immune system, you begin to notice it.**

**<u>The 3 hit hypothesis</u>**

**1)Increased mutagenesis with dsDNA contamination**

**2)Chronic insult to the Innate immune system from modRNA vax.**

**N1-methyl-pseudouridine, Lymphocytopenia, neutropenia, IgG4**

**3)Inhibition of p53 and BRCA1 (guardians of the genome)**

**Zhang and Wafik El-Diery, Jiang et al**

**4)Estrogen Receptor (Maarten Fornerod)**

**5)IgG4 class switch**

**6)Immune privileged cells**

# Cytosolic DNA can cause Cancer via cGAS-STING

## The cytosolic DNA-sensing cGAS-STING pathway in cancer

**John Kwon**[1], **Samuel F. Bakhoum**[1,2]

[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

[2]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

### Abstract

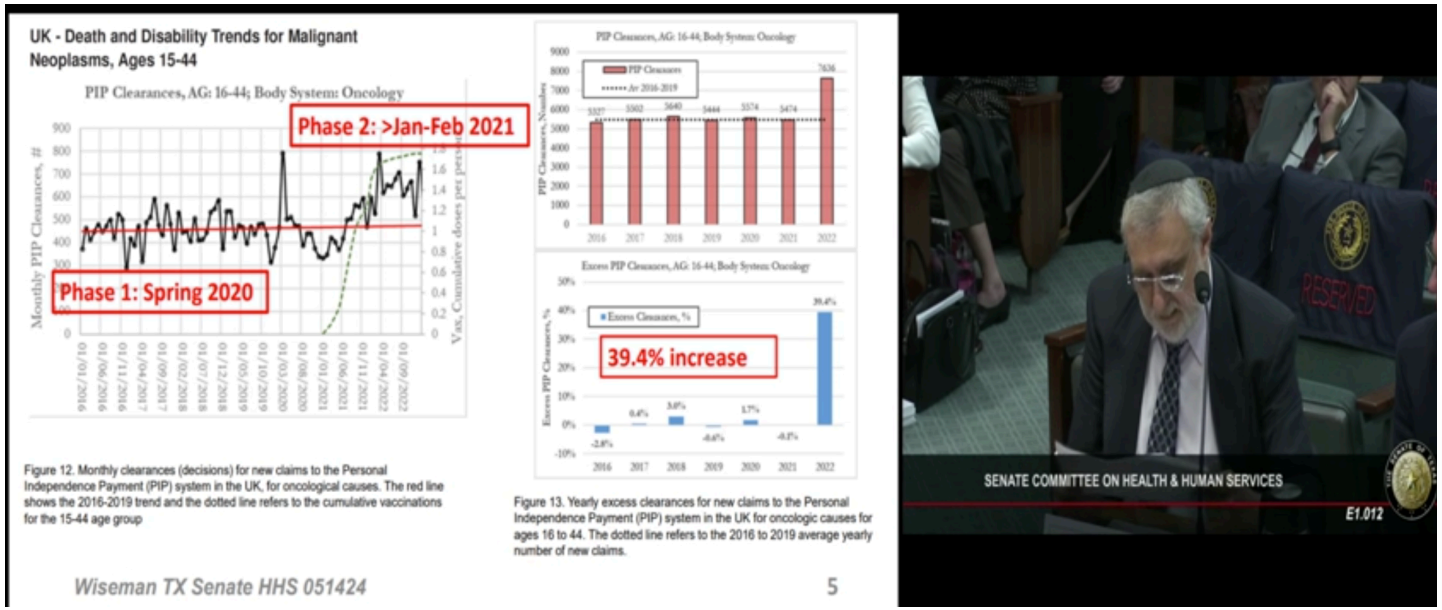
The recognition of DNA as an immune stimulatory molecule is an evolutionarily conserved mechanism to initiate rapid innate immune response against microbial pathogens. Recently, the cGAS-STING pathway has been discovered as an important DNA sensing machinery in innate immunity and viral defense. Recent advances have now expanded the roles of cGAS-STING to cancer. Highly aggressive, unstable tumors have evolved to co-opt this program to drive tumorigenic behaviors. In this review, we will discuss the link between the cGAS-STING DNA sensing pathway with anti-tumor immunity as well as cancer progression, genomic instability, the tumor microenvironment, and pharmacologic strategies for cancer therapy.

### Statement of significance

The cGAS-STING pathway is an evolutionary conserved defense mechanism against viral infections. Given its role in activating immune surveillance, it has been assumed that this pathway primarily functions as a tumor suppressor. Yet, mounting evidence now suggests that depending on the context, cGAS-STING signaling can also have tumor and metastasis-promoting functions and its chronic activation can paradoxically induce an immune suppressive tumor microenvironment.

---

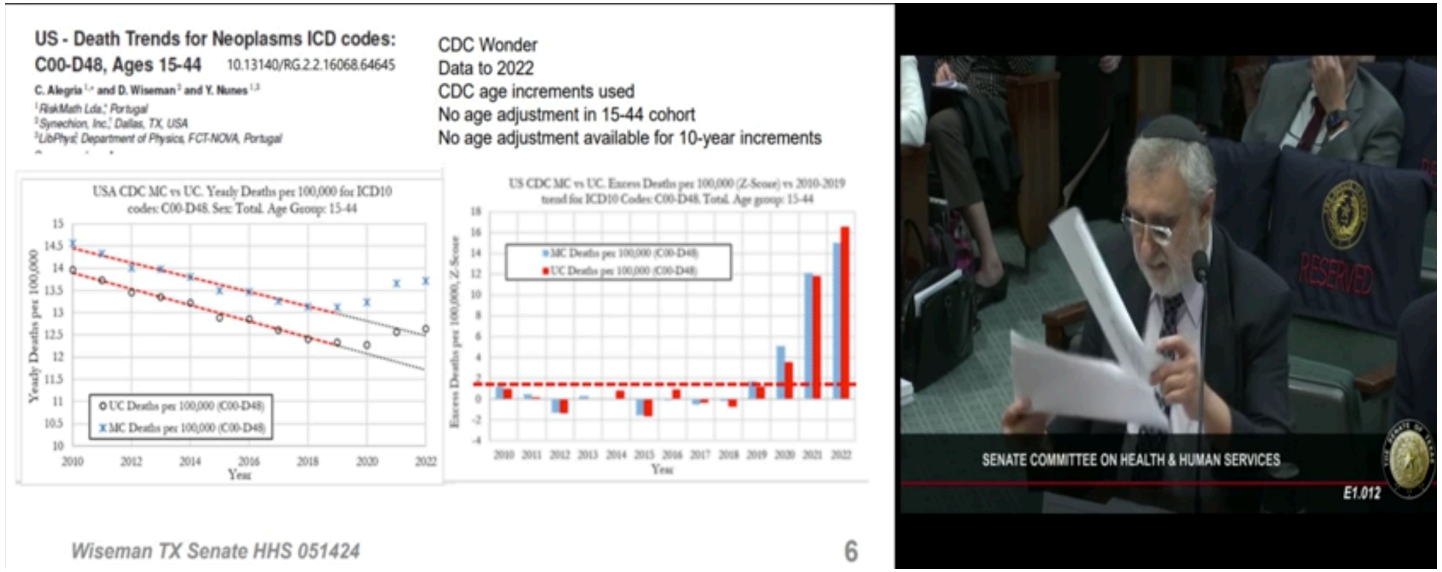
- Its not there!
- Its too little to matter!
- Fine, Its there but it doesn't get into the cell
- Its harmless in the Cytosol
- It will never get into the nucleus





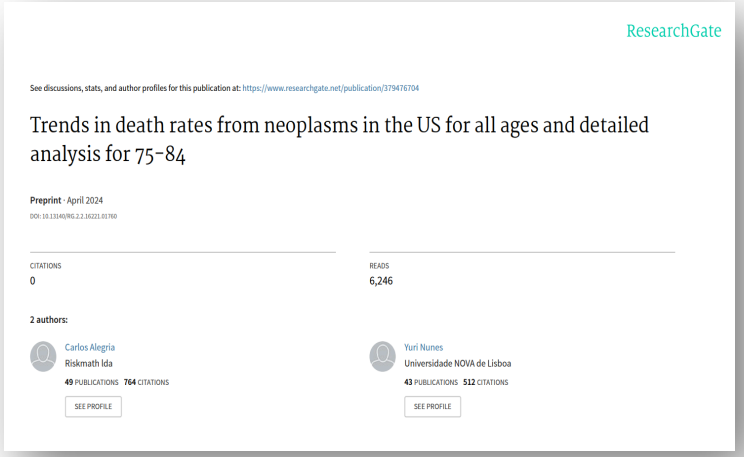


Dr. David Wiseman
Texas Senate 2024









# Pfizer did not specifically highlight the SV40 sequence

Annotation software by default highlights these sequences.
Someone had to intentionally delete them!!!

EPOCH HEALTH

LOG IN SUBSCRIBE

COVID VACCINES

## European Regulator Confirms Pfizer Did Not Highlight DNA Sequence in COVID-19 Vaccine

*Regulator claims fragments left by sequence are at acceptable levels.*

"'While the full DNA sequence of the plasmid starting material was provided in the initial marketing authorization application for Comirnaty, the applicant did not specifically highlight the SV40 sequence,' the European Medicines Agency (EMA) told The Epoch Times in an email.

The email came after Health Canada told The Epoch Times it expects sponsors to identify sequences such as the Simian Virus 40 (SV40) DNA enhancer but that Pfizer did not.

The EMA said parts of the SV40 sequence are 'commonly present in plasmids used for manufacturing of biological active substances,' but neither authorities nor Pfizer have been able to say why the sequence was made part of the Pfizer shot.

# The Health Canada, FDA and the EMA: Intent to Deceive

> **"Health Canada expects sponsors to identify any biologically functional DNA sequences within a plasmid (such as an SV40 enhancer) at the time of submission..Although the full DNA sequence of the Pfizer plasmid was provided at the time of initial filing, the sponsor did not specifically identify the SV40 sequence."**

FDA: "The omitted regions are not material to plasmid manufacturing"

You cannot manufacture plasmid DNA without the Promoter for Selection genes

## Regulators, Moderna Admit Risks

As claimed by Dr. Malone, multiple health agencies have admitted to DNA contamination in the mRNA COVID-19 vaccines.In an email to The Epoch Times last month, Health Canada confirmed the presence of the SV40 DNA sequence in the Pfizer vaccine, which the company failed to disclose previously.

In another email to The Epoch Times, the European Medicines Agency also confirmed that the Pfizer vaccine contains the SV40 sequence, which the company's partner BioNTech did not highlight in its application.

It isn't clear whether the presence of the SV40 sequence in the plasmid DNA of mRNA vaccines was identified by Pfizer when the company applied for approval from the FDA.

Dr. Malone pointed out that Moderna "clearly acknowledges" the risks of genotoxicity associated with the DNA delivered via its vaccines.

Both the FDA and WHO guidelines demand all Open Reading Frames and promoters like the SV40 elements must be disclosed to regulators.

The regulators are rewriting their own guidelines on the fly to accommodate for this

cdn.who.int

In addition, the identity, source, isolation and sequence of the gene encoding the antigen(s); a description of the steps involved in the construction of the entire plasmid; a detailed functional map of the plasmid; information on the source and function of component parts of the plasmid known to have biological activities, such as origins of replication, viral/eukaryotic promoters and other expression signals and genes encoding selection markers, should be provided. A clear rationale should be provided for the use of specific regions of DNA, such as the promoter or a gene encoding a selection marker and special attention should be given to the nature of a selection marker.

No detailed report for the studies initiated to enhance the robustness of the DNase digestion step is provided. However, the MAH shows data indicating that the likely root cause for the [redacted] residual DNA is [redacted] It is also confirmed that activity testing on incoming enzymes will be implemented by the end of second quarter 2021 as requested in Recommendation 3.

guidelines-for-assuring-the-quality-and-non-clinic...

or a biological function be either inactivated by genetic manipulation to remove any undesirable activity, or justified. Further, although the relevance at this stage may not be understood, as part of characterization, a DNA sequence homology check of the plasmid with the international databases (e.g. the National Center for Biotechnology Information, National Institute for Health, USA, and/or other international nucleotide databases) should be performed to investigate the presence of unintended sequences of biological significance such as those encoding cellular growth functions or alternative and unanticipated reading frames.

The identity of the plasmid after transfection into the bacterial cell to be used for production should be confirmed in addition to the phenotype of the cell.

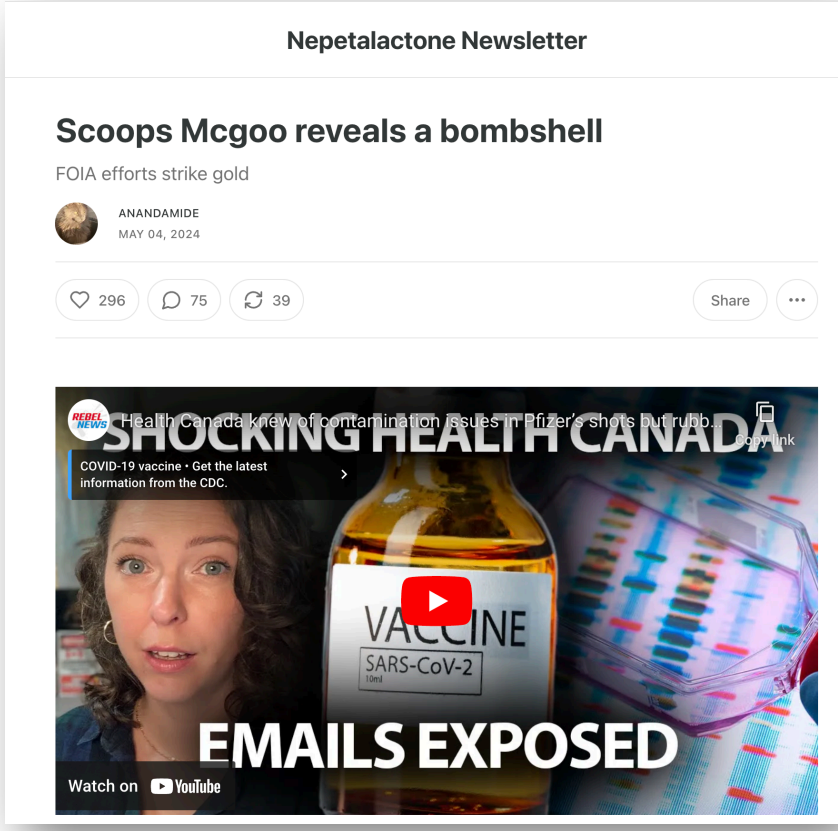
**A. Product Manufacture**

You should describe in the manufacturing summary all components used during manufacture as well as those present in the final product. You should provide detailed descriptions of the plasmid construction, including the source and diagrams of all plasmids used, and all intermediate recombinant DNA cloning procedures. You should provide the DNA sequence of the entire plasmid present in the Master Cell Bank (MCB) along with an annotated sequence identifying all open reading frames including any unexpected open reading frames and/or other sequence elements. During intermediate steps in the production process, various methods can be used for identity testing, including restriction enzyme mapping and polymerase chain reaction (PCR). However, complete sequencing of the bulk plasmid vaccine is prefered.

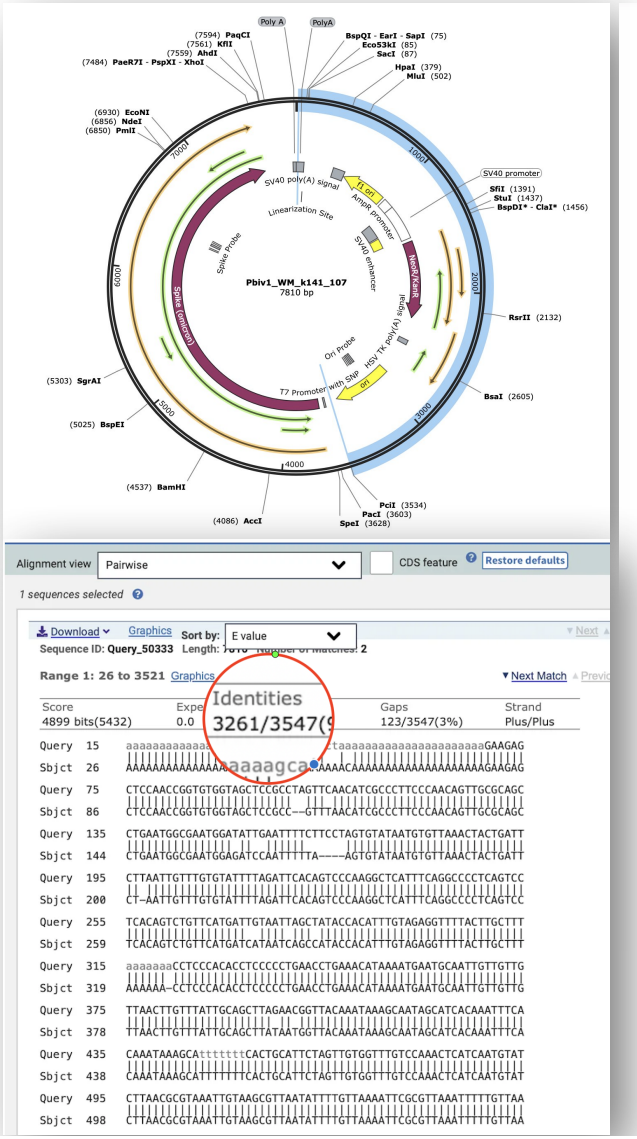
The description of the manufacturing process should be sufficiently detailed to enable an assessment of the safety of the product. If lots produced for preclinical safety studies are

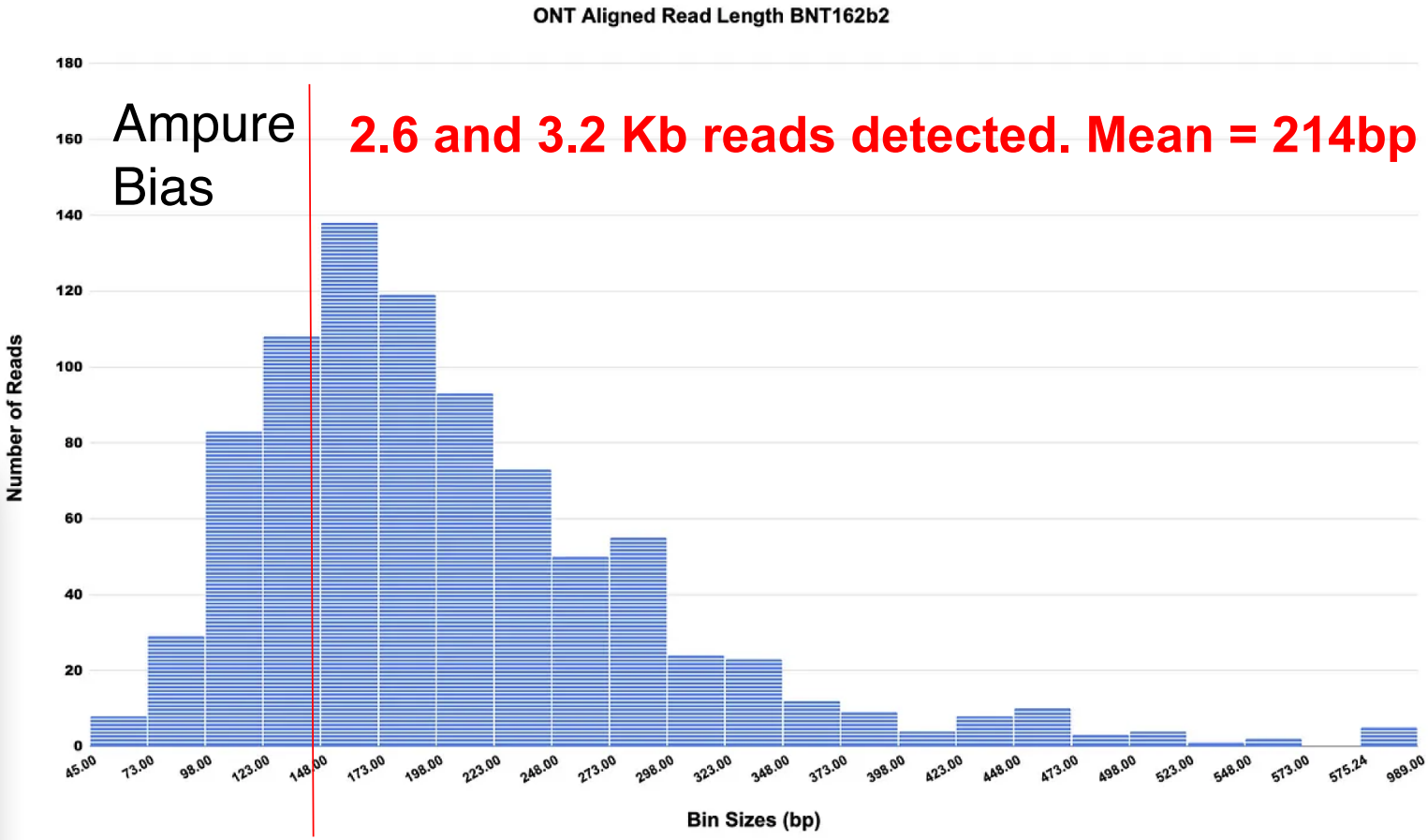
# Private Regulatory Response

1)Yes, SV40 is in the Pfizer vaccine
2)Yes, Pfizer did **NOT** spell this out
3)Pfizer should make a DNA fragment length assay
(we told the public you measured this)
4)Pfizer- we don't have one. It will take time.
5)HC- Please remove this DNA
(We told the public its harmless)



# Too Little-Too small: qPCR under estimates the total DNA contamination




Oxford Nanopore Sequencing

# Regulators public messaging is not congruent with their internal emails

- Pfizer doesn’t even have an assay that can measures DNA fragment length
- Regulators are telling the public the DNA is of no consequence while asking Pfizer to remove it
- They are claiming DNA is tested for while EMA leaks show Pfizer is not measuring the DNA and RNA with the same tools or at the final product stage.