Plasmid derived dsDNA contamination in mRNA vaccines

ICS V
Feb 23, 2024

Kevin McKernan,
CSO Medicinal Genomics

Conflicts

We do not participate in any C19 related business
We manufacture qPCR kits for the agricultural genomics space
We authored 2 PrePrints on dsDNA contamination in mRNA vaccines

Illumina sequencing and RT-qPCR and qPCR

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Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Edit preprint

AUTHORS
Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin

AUTHOR ASSERTIONS

Conflict of Interest: Yes ▾ Public Data: Available ▾ Preregistration: Not applicable ▾

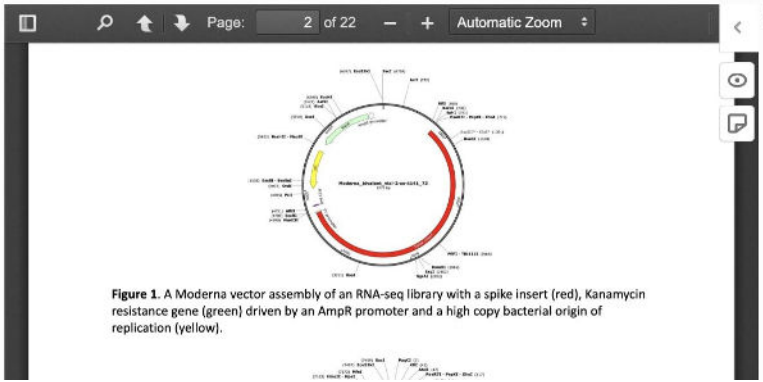

Figure 1. A Moderna vector assembly of an RNA-seq library with a spike insert (red), Kanamycin resistance gene (green) driven by an AmpR promoter and a high copy bacterial origin of replication (yellow).

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plaudit Iain B.H. Wilson has endorsed this work.

Abstract

Several methods were deployed to assess the nucleic acid composition of four vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA

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

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

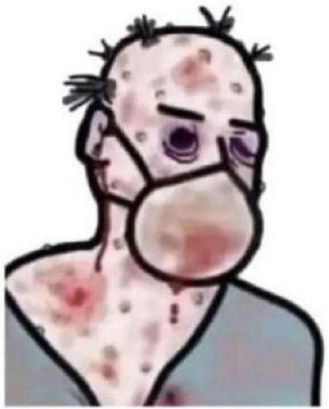
AUTHORS

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

AUTHOR ASSERTIONS

Conflict of Interest: Yes ▾ Public Data: Available ▾ Preregistration: Not applicable ▾

Fact Checker Chorus: “***It’s not peer reviewed!***”



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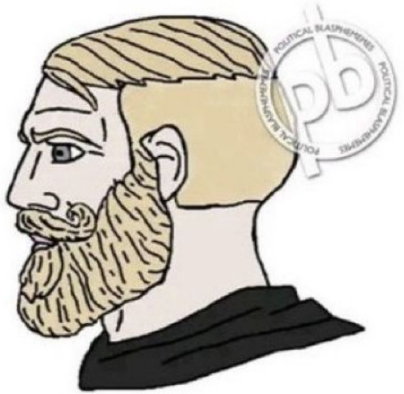
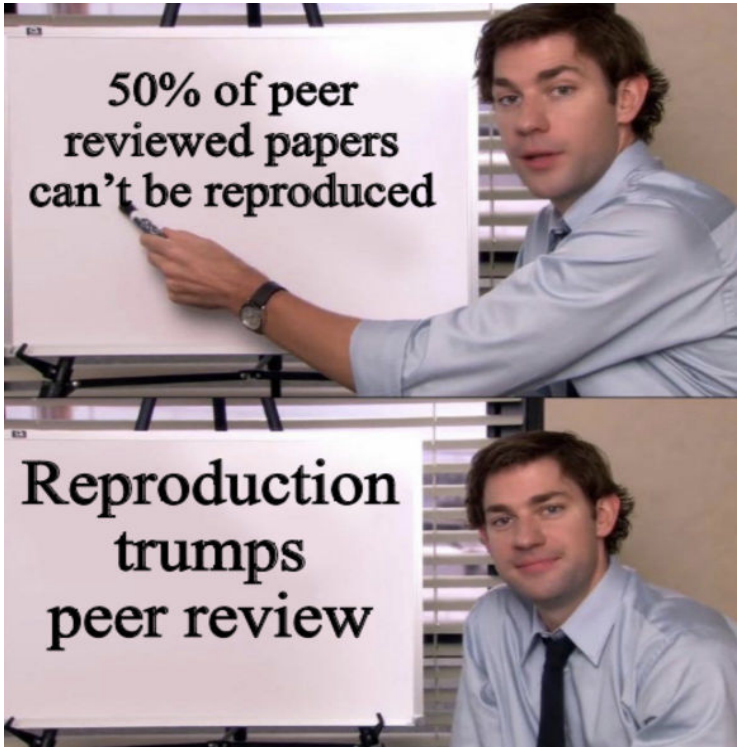


WHY? YOU GOT JABBED IN A PARKING LOT BY A STRANGER WITH A DRUG YOU KNEW NOTHING ABOUT JUST TO GET A FREE DONUT.

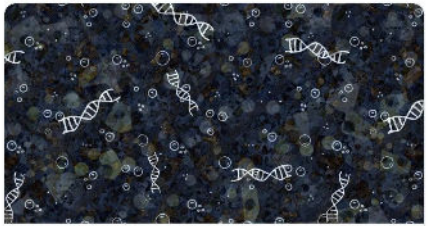


Reproduction

Translated from Japanese by Google

Professor Hiroshi Arakawa——Now, this time, I reanalyzed Dr. McKernan's raw data and partially reproduced the process.

On Finding Contaminating Vector DNA Sequences: Dr. McKernan's Raw Data Reanalysis



note.com
混入ベクターDNA配列の見つけ方について: McKernan博士の生データ再解析...
引き続き「RNAココナワクチンにDNAが混入している？」という疑惑についての記事になります。McKernan博士の当初の目的はファイザーとモデルナのワ...

6:48 AM · Apr 24, 2023 · **8,290** Views

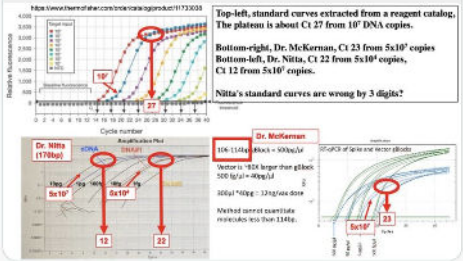
13 Retweets **2** Quotes **42** Likes **7** Bookmarks

藤川賢治 (FUJIKAWA Kenji) @ 医療統計情報通... @hudi... · Jun 2

(English is in the image)
左上、試薬カタログから抜き出した標準曲線で、
DNAコピー 10^7個 の場合プラトー(停滞)は Ct 27程度。
右下、McKernan氏 5*10^7 で Ct 23
左下、新田氏 5*10^4 で Ct 22、5*10^7 で Ct 12

新田氏 @takenitta 標準曲線は、桁を3桁間違ってると見てる。

これだと分る？ twitter.com/hudikaha/statu...



Fred Stalder @sudokuvariante · May 27

FYI: @Kevin_McKernan .
@raoult_didier said they also found DNA contamination in the mRNA vaccines.

> **dan** @danfrem17 · May 26
> Pr Didier Raoult ! Dans quelle cellule ça va ! On a pas de recul sur 5 - 10 ans plus tard ... 🧠😇
> Dr Jérôme MARTY : On n'a jamais eu autant de recul sur un vaccin que sur celui-là ! On a plus de 1 milliard de gens qui ont été vaccinés, ... , on a des milliers d'années de RECUL 🐭
> Show this thread

Willem Engel
@dancalegria

It is not misinformation, My labteam is one of the groups that have found plasmid DNA through de novo deep sequencing. See @Kevin_McKernan his blogs about the discovery. We already knew about in some cases nearly 50% genetic impurities hence the lowered standard at EMA. shitloads

8:08 AM · May 30, 2023 · **24.4K** Views

815X variance across 10 lots EMA

Residual DNA Template

Residual DNA template is a process-related impurity in BNT162b2 drug substance that is tested for at time of BNT162b2 drug substance release. Residual DNA is measured using a quantitative PCR (qPCR) test. Release test results for BNT162b2 drug substance, 1 nonclinical toxicology batch, 4 process 1 clinical/clinical inventory batches and 5 process 2 batches, are shown in Table S.4.5-9.

Table S.4.5-9. BNT162b2 Drug Substance Release Data for Residual DNA Template

Batch	Residual DNA Template (ng DNA/mgRNA)
RNA-RF200321-06	815.3[a]
R427-P020.2-DS	198
R438-P020.2-DS	<200
R443-P020.2-DS	3
R445-P020.2-DS	1
20Y513C101	17
20Y513C201	29
20Y513C301	10
20Y513C401	23
20Y513C501	211

a. Incorrect DNase I stock solution used, leading to higher values of residual DNA template

The specification for residual DNA template was based on the WHO recommendation of not more than 10 ng DNA/dose. Based on these considerations, and assuming a maximum dose of 30µg, the commercial acceptance criterion at release is ≤330 ng DNA/mg RNA.

International replication

Dr. Phillip Buckhaults

Dr. Sin Lee

Nepetalactone Newsletter

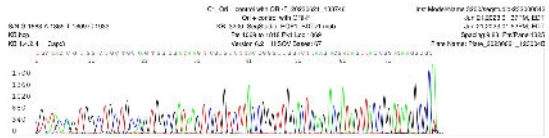
Independent Sanger Sequencing verification of plasmid amplicons in BNT162b2

ANANDAMIDE
JUN 23, 2023

81 25 5 Share

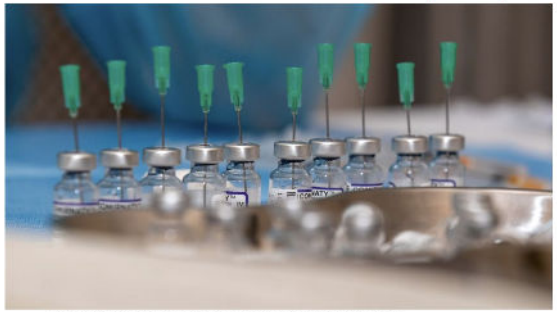
Dr. Sin Hang Lee, MD, F.R.C.P.(C), FCAP of Milford Molecular Diagnostics, obtained the Ori Primers described in McKernan *et al.* He then amplified and Sanger sequenced the Ori amplicon amplified from a Pfizer mRNA vial (BNT162b2).

The Ori Primers target the contaminating plasmid vector that should not be in the vaccine vials.



Dr. Brigitte Konig

Cicero
MAGAZIN FÜR POLITISCHE KULTUR



Verunreinigung in Corona-Impfstoffen

„Was passiert, wenn die DNA in den Zellkern eindringt?“

In Chargen des Impfstoffs Comirnaty wurde wiederholt Fremd-DNA entdeckt. Wissenschaftler machen sich Sorgen. Doch das Bundesgesundheitsministerium wiegelt ab. „Cicero“ sprach mit der Mikrobiologin und Immunologin Brigitte König, die den Impfstoff untersucht hat.

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

FDA U.S. FOOD & DRUG ADMINISTRATION

Government of Canada

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

Process 1 used In Vitro Transcription (IVT)

Synthetic DNA template

IVT reaction

Figure 3

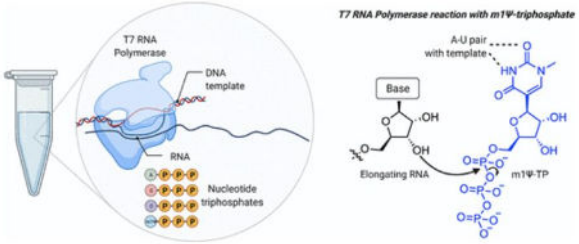
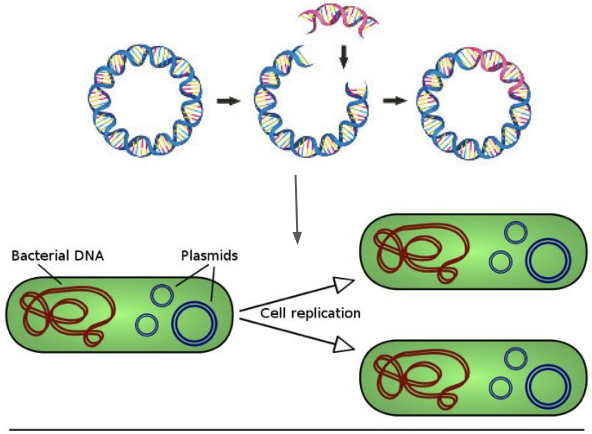


Figure 3. Production of m1Ψ mRNAs by *in vitro* transcription. Left: Components of *in vitro* transcription reaction. Right: Incorporation of m1Ψ-triphosphate into RNA is guided by m1Ψ's ability to form a canonical base pair with adenine of the DNA template in the T7 RNA polymerase active site. Nance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

Process 2 used Plasmids in E.coli

cloned DNA template



IVT reaction

Figure 3

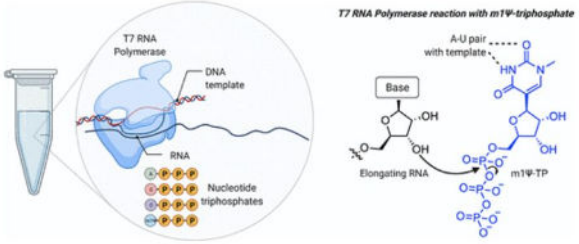
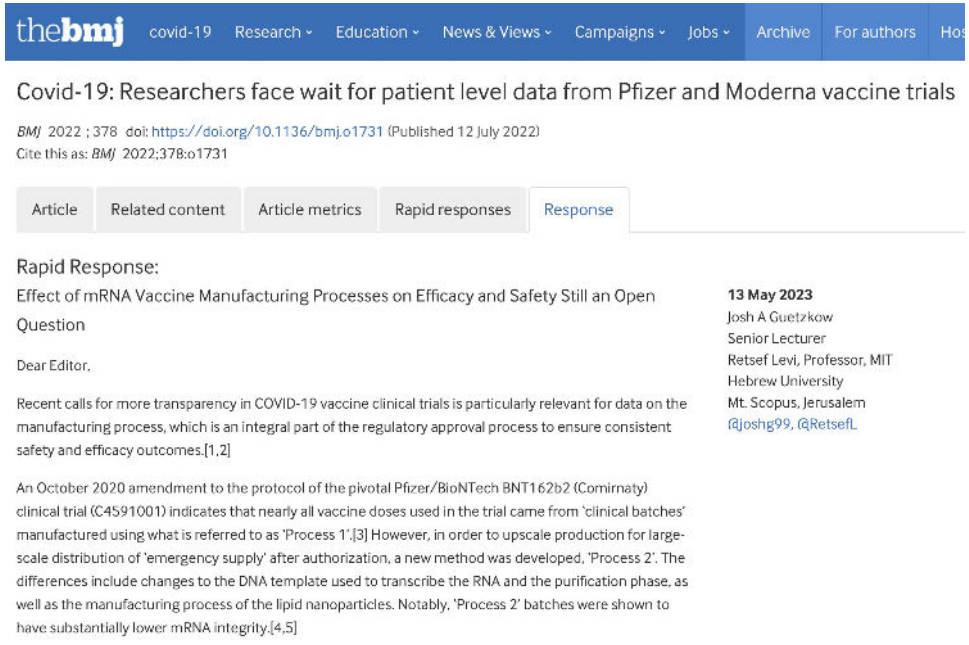


Figure 3. Production of m1Ψ mRNAs by *in vitro* transcription. Left: Components of *in vitro* transcription reaction. Right: Incorporation of m1Ψ-triphosphate into RNA is guided by m1Ψ's ability to form a canonical base pair with adenine of the DNA template in the T7 RNA polymerase active site. Nance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

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



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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

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Rapid Response:

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

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

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]

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

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]

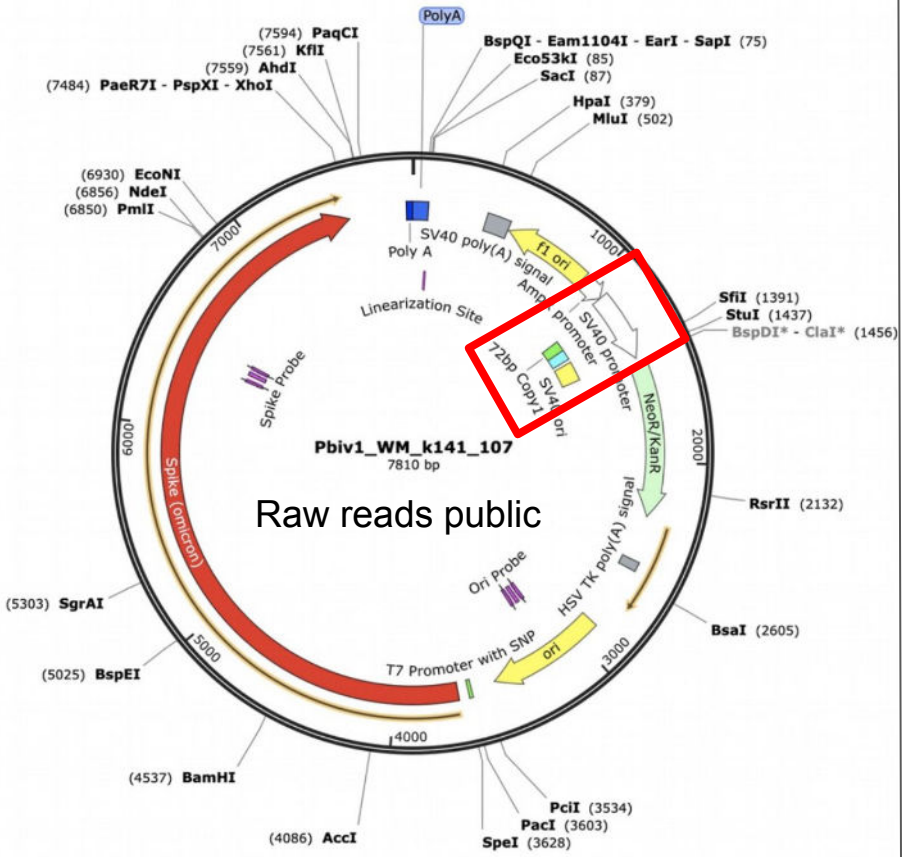
Furthermore, additional 'Process 1' batch EE3813 doses with distinct Pfizer lot numbers were added to the later batch document[7] at over 70% of trial sites, potentially supplied at a later stage to enable vaccination of placebo patients with BNT162b2. The 6-month interim clinical study report[12] from 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.

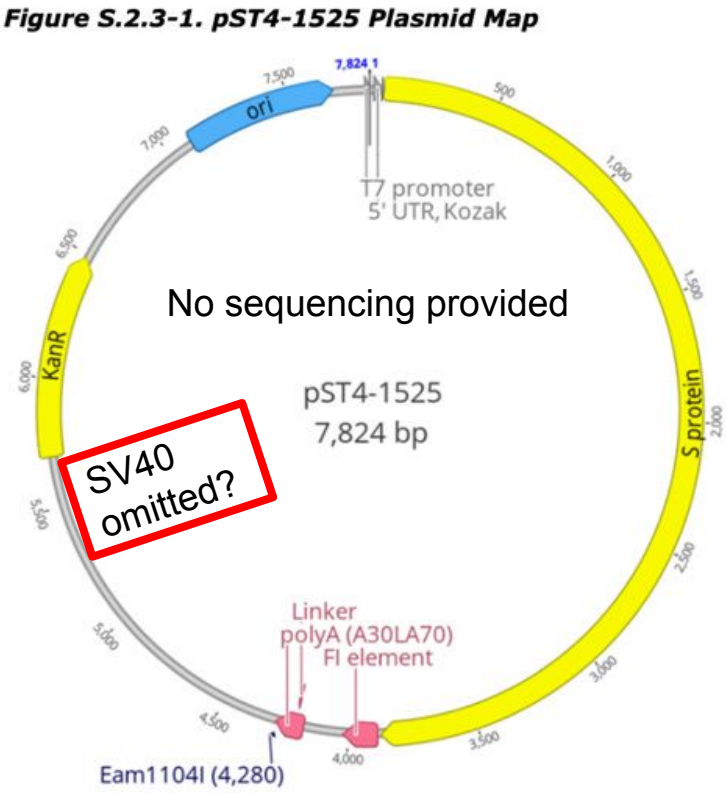
Evidence from existing research and trial documents highlights the importance of publicly disclosing the analysis comparing reactogenicity and safety of process 1 and 2 batches as specified in the trial protocol, and more generally patient-level batch and lot data from the trial.

Josh Guetzkow
Retsef Levi

Independent Illumina sequencing



What was disclosed to the EMA



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

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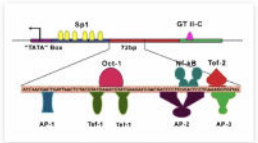
URMC / Labs / Dean Lab / Projects / Nuclear Targeting of Plasmids and Protein-DNA Complexes

Make a Gift

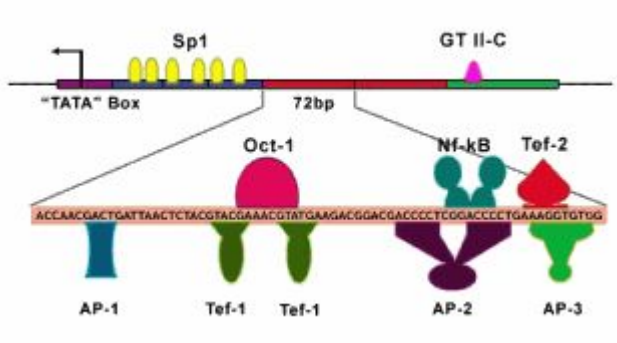
Nuclear Targeting of Plasmids and Protein-DNA Complexes

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.

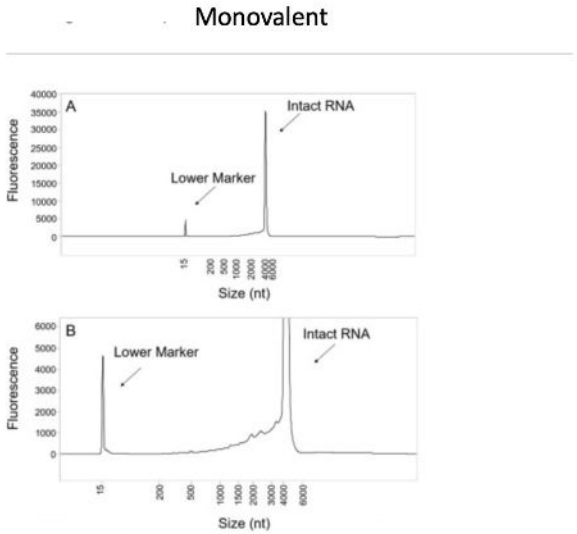


Organization of transcription factor binding sites on the SV40 enhancer.



Limitations- Expired lots
Fact Checkers will not address this slide!

The RNA integrity on arrival can be confirmed



Patel et al electrophoresis of the BNT162b2 mRNA vaccine.

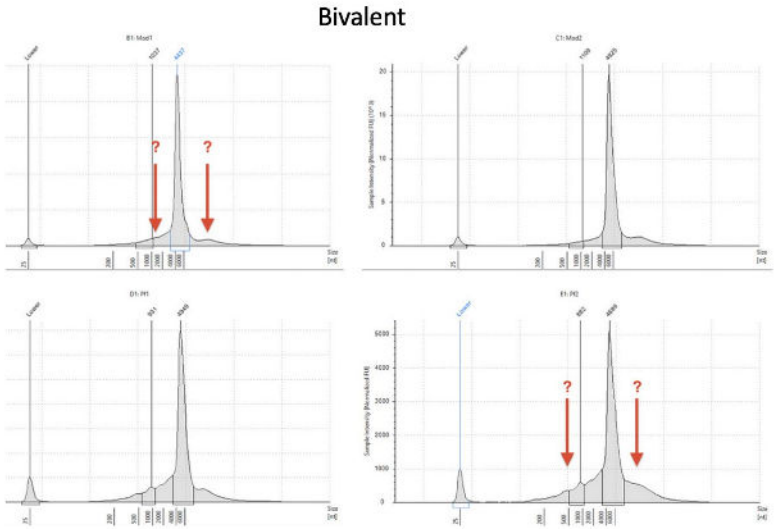


Figure 1. Agilent Tape Station Electrophoresis of the Bivalent vaccines. Moderna mRNA-1273.214 (Top) and the Pfizer bivalent vaccine (Bottom).

Expired lots were injected into patients

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

(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
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8,999,380	B2	4/2015	Bancel et al.

(Continued)

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(Continued)

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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.

How to Game the Regulators

Use two different yard sticks

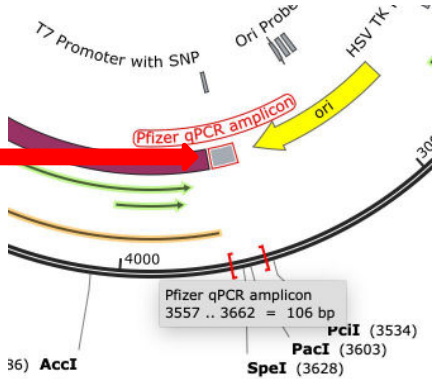
EMA regulations are a Ratio of RNA:DNA of 3030:1

Inflate RNA with RiboGreen

Deflate DNA with qPCR

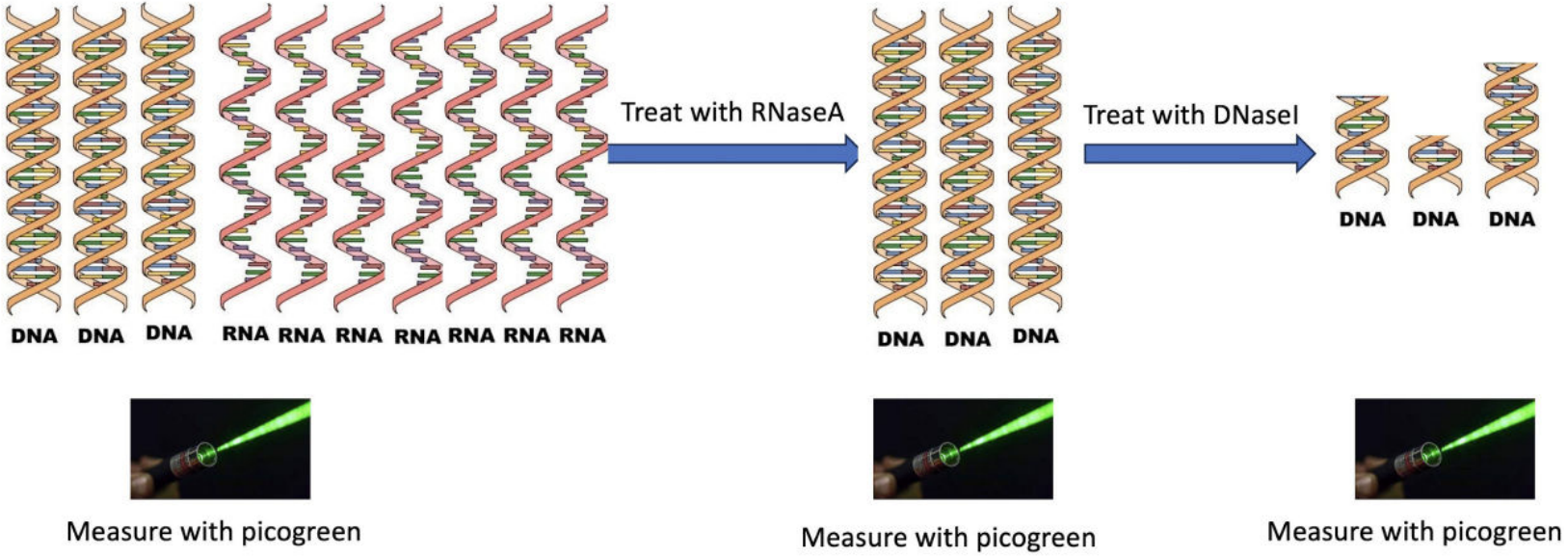
RT-qPCR measures RNA?
qPCR measures DNA?

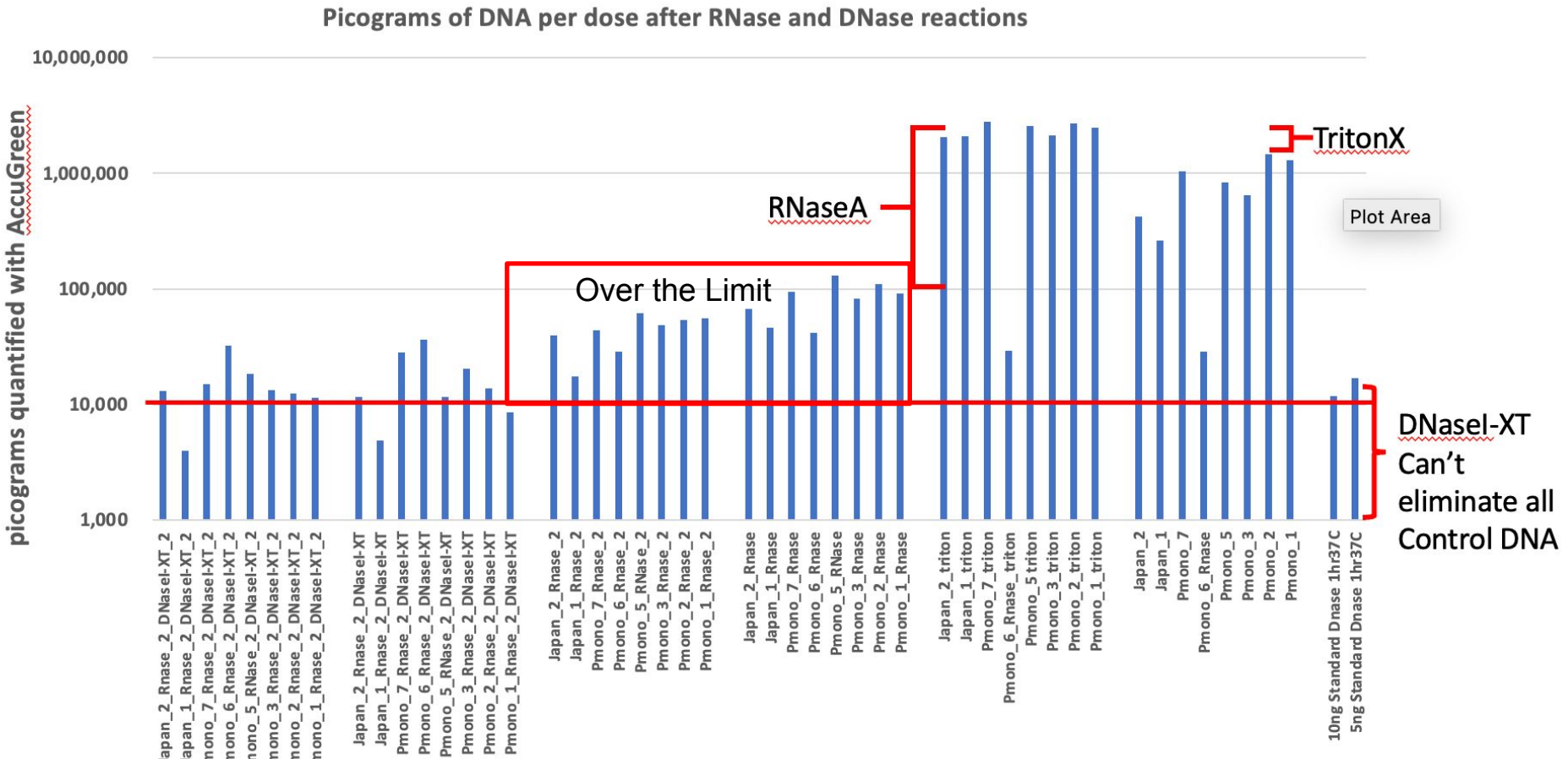
Pfizers primers target RNA and DNA?



Why did Pfizer not use qPCR for measuring RNA and DNA?
Because they can't pass the spec and regulators are too inept to understand they are getting played

Fluorometry with RNase A

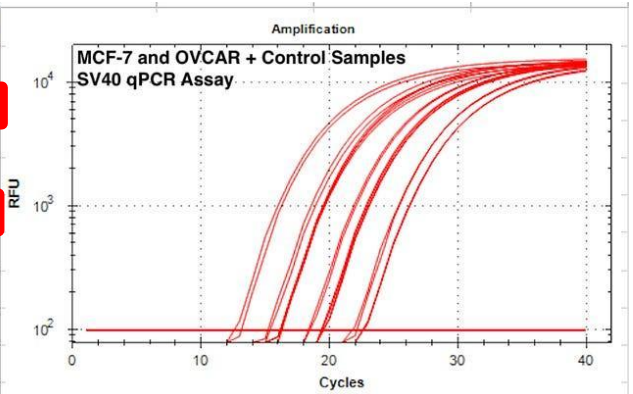


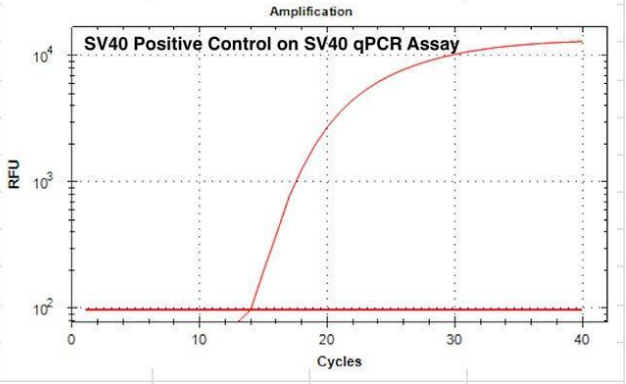

Picograms of DNA per dose after RNase and DNase reactions
picograms quantified with AccuGreen
10,000,000
1,000,000
100,000
10,000
1,000
RNaseA
TritonX
Over the Limit
Plot Area
DNaseI-XT
Can't
eliminate all
Control DNA
Japan_2_Rnase_2_DNaseI-XT_2
Japan_1_Rnase_2_DNaseI-XT_2
Pmono_7_Rnase_2_DNaseI-XT_2
Pmono_6_Rnase_2_DNaseI-XT_2
Pmono_5_RNase_2_DNaseI-XT_2
Pmono_3_Rnase_2_DNaseI-XT_2
Pmono_2_Rnase_2_DNaseI-XT_2
Pmono_1_Rnase_2_DNaseI-XT_2
Japan_2_Rnase_2_DNaseI-XT
Japan_1_Rnase_2_DNaseI-XT
Pmono_7_Rnase_2_DNaseI-XT
Pmono_6_Rnase_2_DNaseI-XT
Pmono_5_RNase_2_DNaseI-XT
Pmono_3_Rnase_2_DNaseI-XT
Pmono_2_Rnase_2_DNaseI-XT
Pmono_1_Rnase_2_DNaseI-XT
Japan_2_Rnase_2
Japan_1_Rnase_2
Pmono_7_Rnase_2
Pmono_6_Rnase_2
Pmono_5_RNase_2
Pmono_3_Rnase_2
Pmono_2_Rnase_2
Pmono_1_Rnase_2
Japan_2_Rnase
Japan_1_Rnase
Pmono_7_Rnase
Pmono_6_Rnase
Pmono_5_RNase
Pmono_3_Rnase
Pmono_2_Rnase
Pmono_1_Rnase
Japan_2_triton
Japan_1_triton
Pmono_7_triton
Pmono_6_Rnase_triton
Pmono_5 triton
Pmono_3_triton
Pmono_2_triton
Pmono_1_triton
Japan_2
Japan_1
Pmono_7
Pmono_6_Rnase
Pmono_5
Pmono_3
Pmono_2
Pmono_1
10ng Standard Dnase 1hr37C
5ng Standard Dnase 1hr37C

~20 fold higher than previous vaccines
Lot 1F1042A BioNtech

Ulrike Kaemmerer

Sample	Dilution of Lysate	Target	Cq (TexRed)
MCF-7 9715	1uL direct	SV40	16.11
MCF-7 1042A	1uL direct	SV40	13.09
MCF-7 8967	1uL direct	SV40	15.15
OVCAR 9715	1uL direct	SV40	16.23
OVCAR 1042A	1uL direct	SV40	12.72
OVCAR 8967	1uL direct	SV40	15.39
MCF-7 9715	1ul 1:10	SV40	19.36
MCF-7 1042A	1ul 1:10	SV40	16.12
MCF-7 8967	1ul 1:10	SV40	18.34
OVCAR 9715	1ul 1:10	SV40	19.64
OVCAR 1042A	1ul 1:10	SV40	16.11
OVCAR 8967	1ul 1:10	SV40	18.54
MCF-7 9715	1ul 1:100	SV40	22.68
MCF-7 1042A	1ul 1:100	SV40	19.62
MCF-7 8967	1ul 1:100	SV40	21.85
OVCAR 9715	1ul 1:100	SV40	22.73
OVCAR 1042A	1ul 1:100	SV40	19.43
OVCAR 8967	1ul 1:100	SV40	22.09
Positive Control	N/A	SV40	14.00
NTC	N/A	SV40	ND





FDA guidelines were derived from Cell Substrate gDNA

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 6×10^5 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

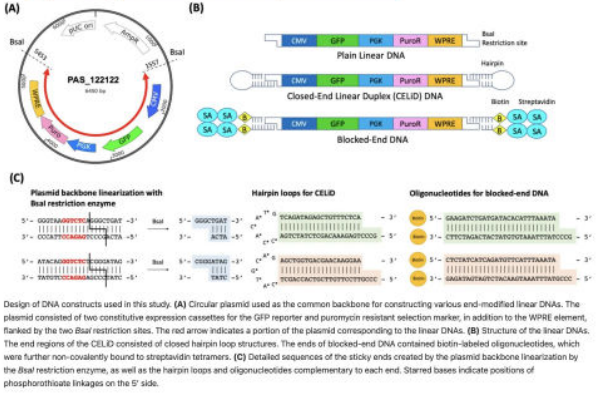
5153 Accesses | 199 Altmetric | Metrics

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.

modRNA is found in Heart Tissue 30 days after vax

npj | vaccines

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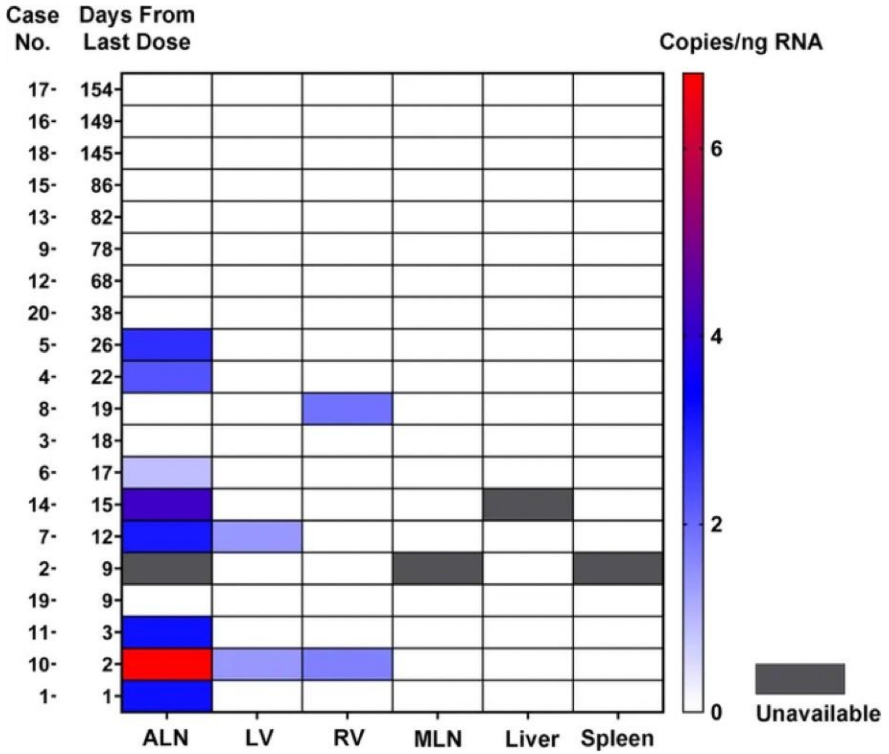
Published: 27 September 2023

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



Spike nucleic acid persistance

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

Journal Article

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

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

Found in Plasma 28 days later

Cancer?

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

The 3 hit hypothesis

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)

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

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... AA

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.

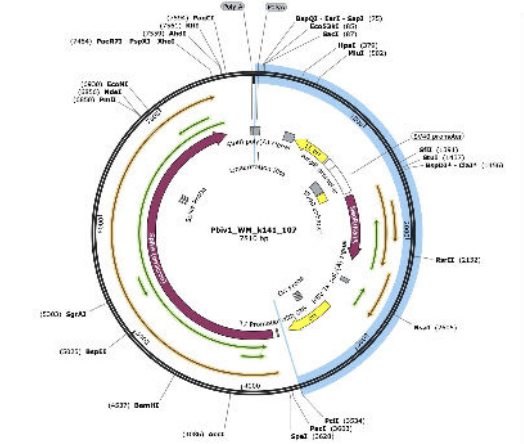
The identity of the plasmid after transfection into the bacterial cell to be used for production should be confirmed in addition to the phenotype of the cell.

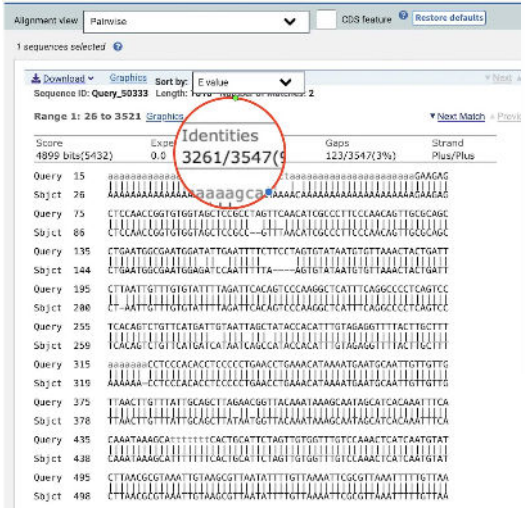
A. Product Manufacture

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.

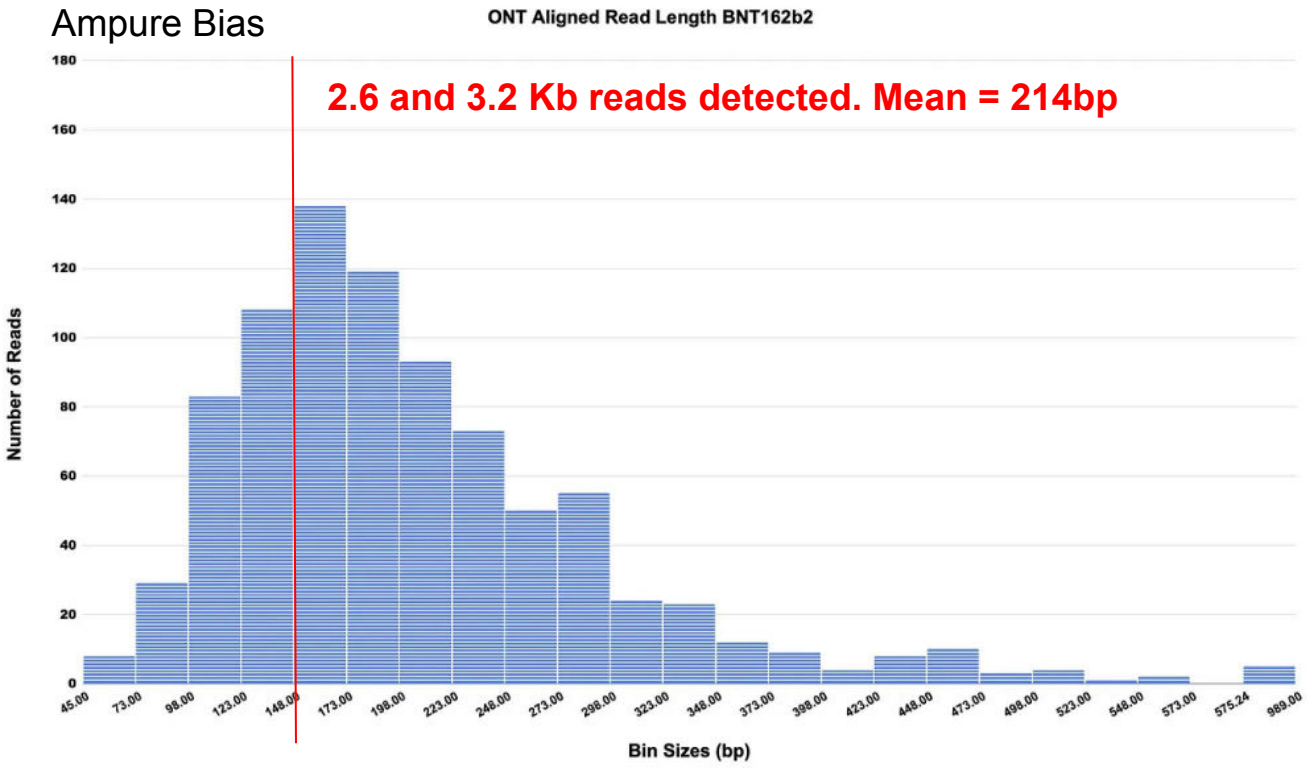
The description of the manufacturing process should be sufficiently detailed to enable an assessment of the safety of the product. If lots produced for preclinical safety studies are

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





Ampure Bias



Oxford Nanopore Sequencing

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

(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.

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(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

DNA is non Functional

Prothrombotic

SCIENTIFIC REPORTS
nature research

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PMCID: PMC5430798
PMID: 28442771

Double-stranded DNA induces a prothrombotic phenotype in the vascular endothelium

Erik Gaitzsch,[#1,2] Thomas Czermak,[#3] Andrea Ribeiro,[1] Yvonn Heun,[4] Monica Bohmer,[4] Monika Merkle,[1] Hanna Mannell,[4] Christian Schulz,[3,5] Markus Wörnle,[1] and Joachim Pircher[3,5]

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Abstract

Go to:

Double-stranded DNA (dsDNA) constitutes a potent activator of innate immunity, given its ability to bind intracellular pattern recognition receptors during viral infections or sterile tissue damage. While effects of dsDNA in immune cells have been extensively studied, dsDNA signalling and its pathophysiological implications in non-immune cells, such as the vascular endothelium, remain poorly understood. The aim of this study was to characterize prothrombotic effects of dsDNA in vascular endothelial cells. Transfection of cultured human endothelial cells with the synthetic dsDNA poly(dA:dT) induced upregulation of the prothrombotic molecules tissue factor and PAI-1, resulting in accelerated blood clotting *in vitro*, which was partly dependent on RIG-I signalling. Prothrombotic effects were also observed upon transfection of endothelial cells with hepatitis B virus DNA-containing immunoprecipitates as well human genomic DNA. In addition, dsDNA led to surface expression of von Willebrand factor resulting in increased platelet-endothelium-interactions under flow. Eventually, intrascrotal injection of dsDNA resulted in accelerated thrombus formation upon light/dye-induced endothelial injury in mouse cremaster arterioles and venules *in vivo*. In conclusion, we show that viral or endogenous dsDNA induces a prothrombotic phenotype in the vascular endothelium. These findings represent a novel link between pathogen- and danger-associated patterns within innate immunity and thrombosis.

DNA integration risk- Keith Peden/FDA

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Biologicals 37 (2009) 190–195

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Issues associated with residual cell-substrate DNA in viral vaccines

Li Sheng-Fowler, Andrew M. Lewis Jr., Keith Peden*

Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, Food and Drugs Administration, Bethesda, MD 20892, USA

Received 2 February 2009; accepted 2 February 2009

Abstract

The presence of some residual cellular DNA derived from the production-cell substrate in viral vaccines is inevitable. Whether this DNA represents a safety concern, particularly if the cell substrate is derived from a tumor or is tumorigenic, is unknown. DNA has two biological activities that need to be considered. First, DNA can be oncogenic; second, DNA can be infectious. As part of our studies to assess the risk of residual cell-substrate DNA in viral vaccines, we have established assays that can quantify the biological activities of DNA. From data obtained using these assays, we have estimated the risk of an oncogenic or an infectious event from DNA. Because these estimates were derived from the most sensitive assays identified so far, they likely represent worst-case estimates. In addition, methods that inactivate the biological activities of DNA can be assessed and estimations of risk reduction by these treatments can be made. In this paper, we discuss our approaches to address potential safety issues associated with residual cellular DNA from neoplastic cell substrates in viral vaccines, summarize the development of assays to quantify the oncogenic and infectivity activities of DNA, and discuss methods to reduce the biological activities of DNA.
Published by Elsevier Ltd on behalf of The International Association for Biologicals.

Keywords: Oncogenic DNA; Infectious DNA; Risk evaluation

1. Introduction: potential concerns associated with DNA

The variety of cell substrates that have been used for the manufacture of viral vaccines licensed in the United States is limited to primary cells of avian or monkey origin, to the diploid cell lines (formerly termed diploid cell strains [1]) WI-38, MRC-5, and FRhL-2, and to one continuous cell line, the VERO line (derived from African green monkey kidney cells) [2]. While these cell substrates have produced vaccines of proven safety and efficacy, it is increasingly apparent that this repertoire is insufficient for the production of the next generation of viral vaccines, such as those against HIV/AIDS, against emerging infectious diseases (*e.g.*, SARS), and against agents of bio-terrorism. In addition, the potential of a pandemic influenza outbreak caused by influenza viruses that either cannot be propagated to high titers in eggs or that are pathogenic for chickens, such as H5N1 avian influenza viruses, has prompted additional cell substrates to be evaluated for influenza vaccines, such as the Madin-Darby canine kidney cell line [3–5]. So far, many of the new mammalian cell substrates that are being evaluated for viral vaccine manufacture are considered to be neoplastic, since they have been immortalized by various mechanisms, and some are tumorigenic. The fear that components derived from the production-cell substrate could induce cancer in vaccine recipients was one of the reasons that the Armed Forces Epidemiological Board recommended in 1954 against the use of tumorigenic cells or cells derived from human tumors for the manufacture of vaccines for human use [6,7]. That this recommendation remained for over 40 years was due at least in part to the inability to evaluate this concern scientifically, which was due first to the inability to identify the risk factors and second, once these risk factors were identified, to a lack of assays capable of quantifying the risk posed by these factors.

It should be pointed out that this manuscript discusses potential risks associated with use of novel highly tumorigenic neoplastic cells for the manufacture of viral vaccines, and any discussion about other types of cell substrates or products is beyond its scope.

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E-mail address: keith.peden@fda.hhs.gov (K. Peden).

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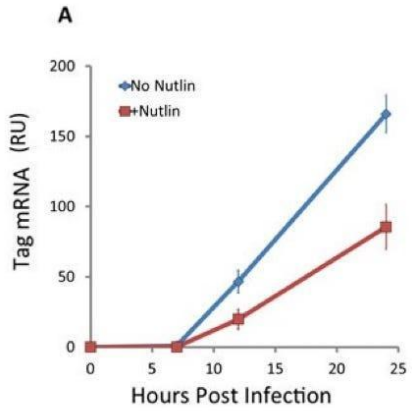
doi:10.1016/j.biologicals.2009.02.015

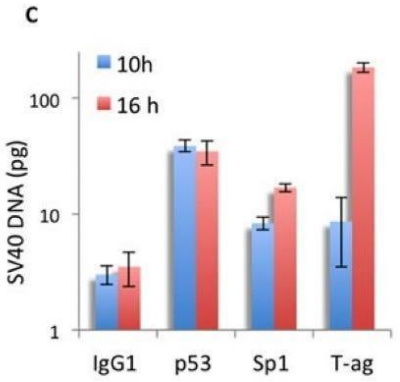
SV40 Promoter Binds to p53 Tumor Suppressor gene

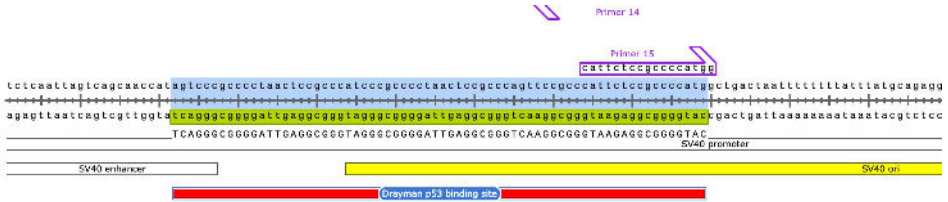
Figure 7

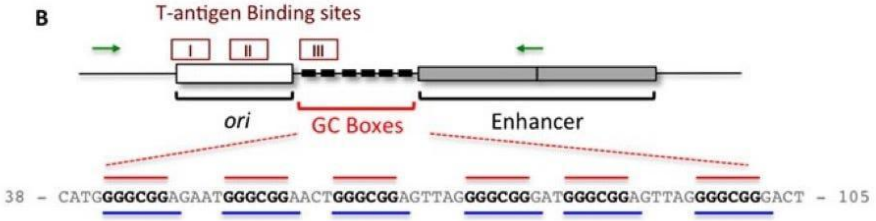
p53 binds to the SV40 early promoter, correlating with a decrease in T-ag mRNA

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.







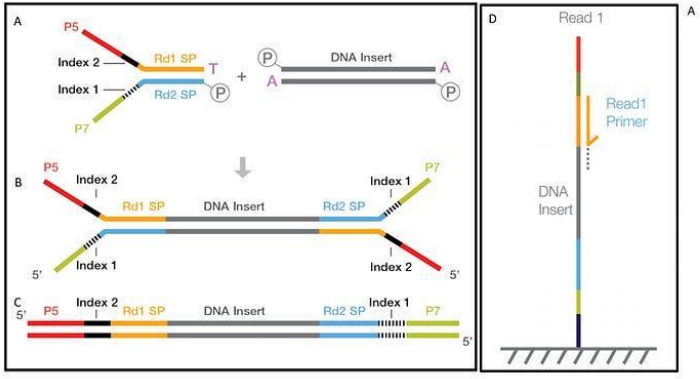


Preliminary evidence of integration in Cancer cell lines

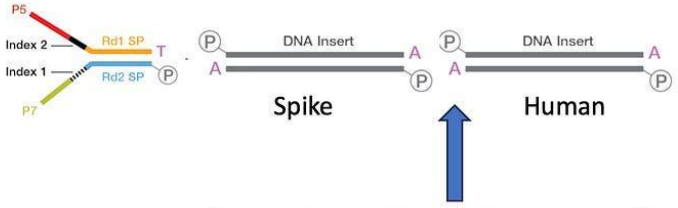
Methods

- qPCR screen OVCAR3 and MCF7 cells that have been +/- vaccine treated.
- Prioritize qPCR positive cell passages (& neg controls) for Illumina Whole Genome Sequencing.
- Sequence 250 million reads per sample and look for reads that map to both human and vaccine.
- PolyA and Human Mitochondrial sequences in the vaccine are filtered out as they create false positive integration events

Illumina Library Prep



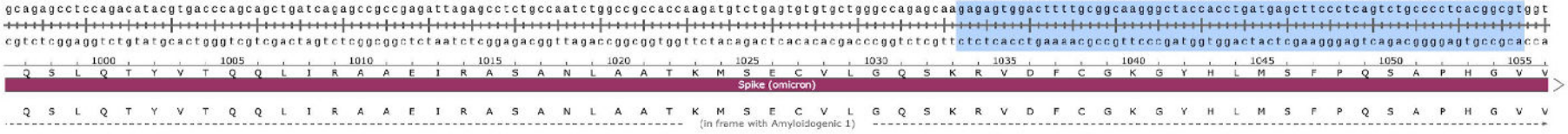
Rare Side reactions –
Chimeric ligation during Sequencing Adaptor ligation

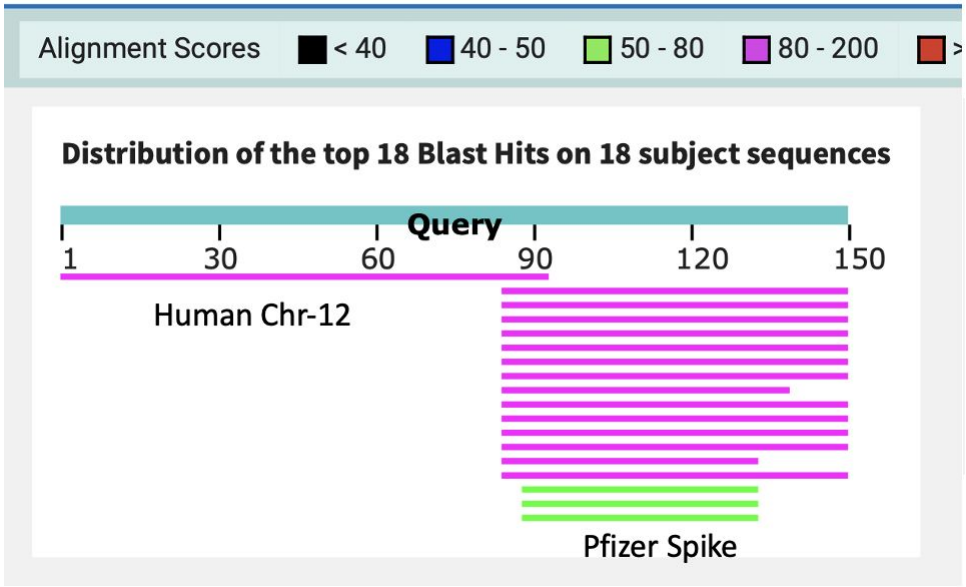


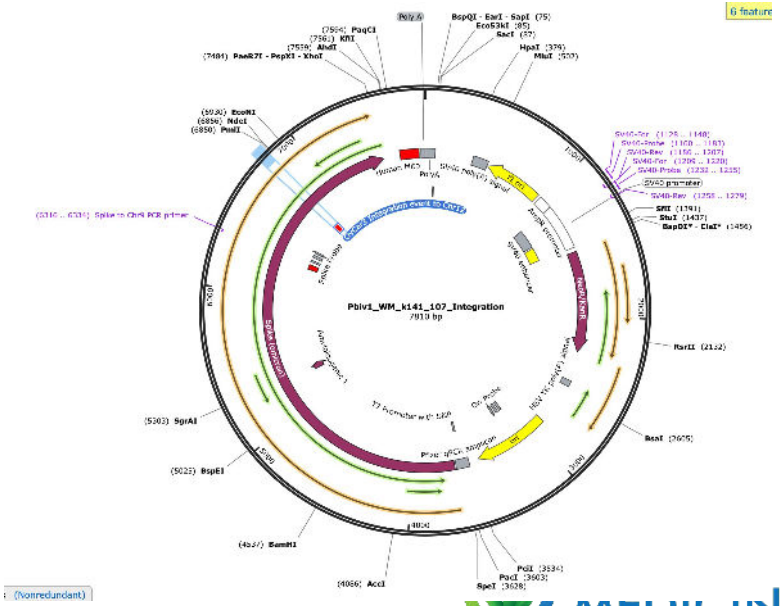
If Plus A fails, gDNA could ligate to itself

MEDICINAL GENOMICS

BNT162b2 Spike Integration Chr.12

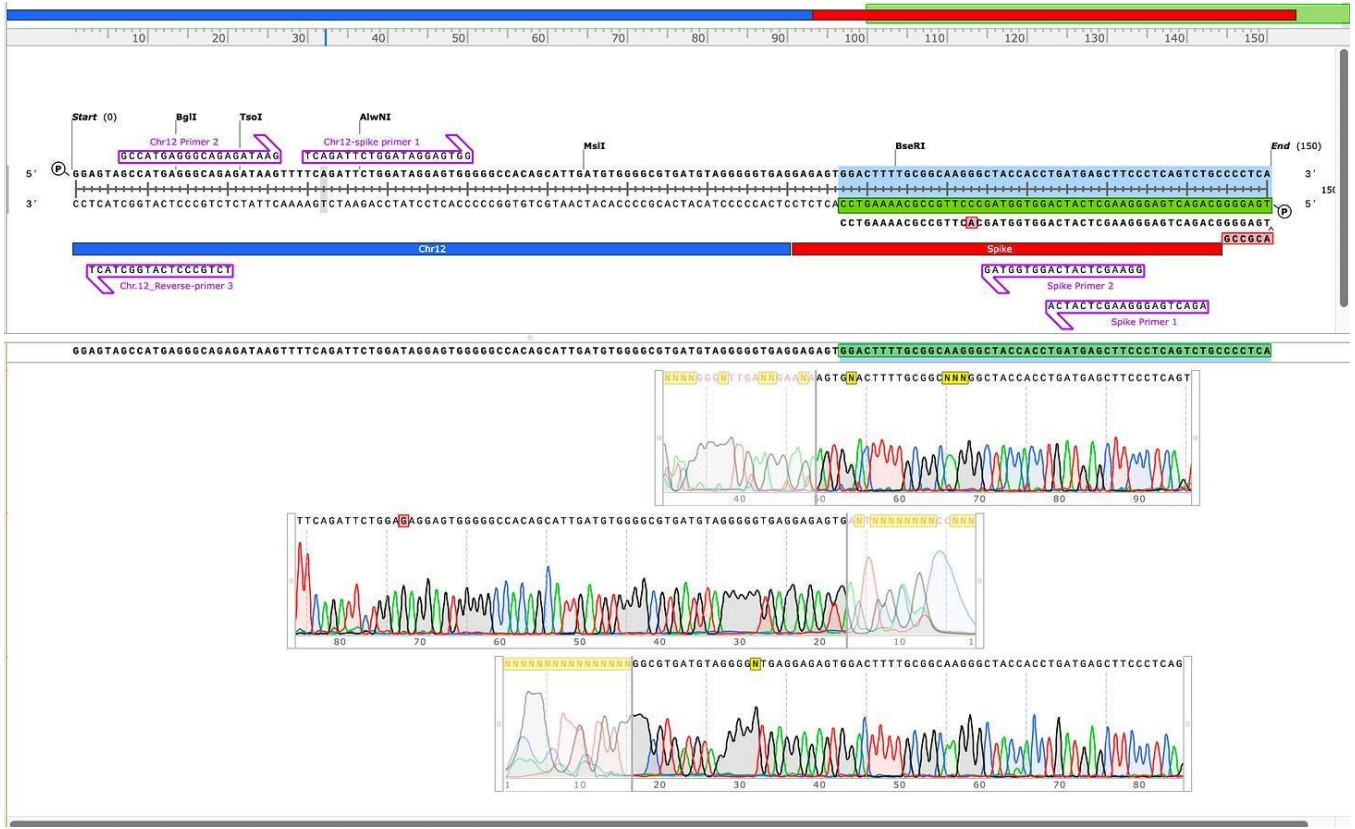






MEDICINAL GENOMICS

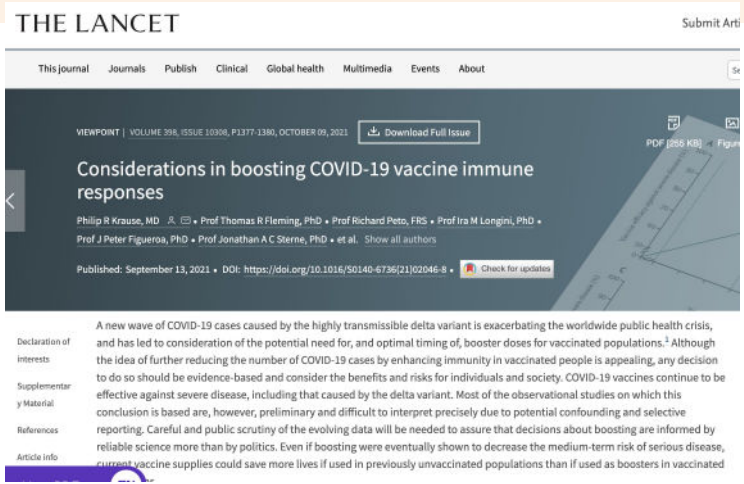
Sanger validation of Integration on Chr12 and Chr9



Revolving Door Culture at the FDA

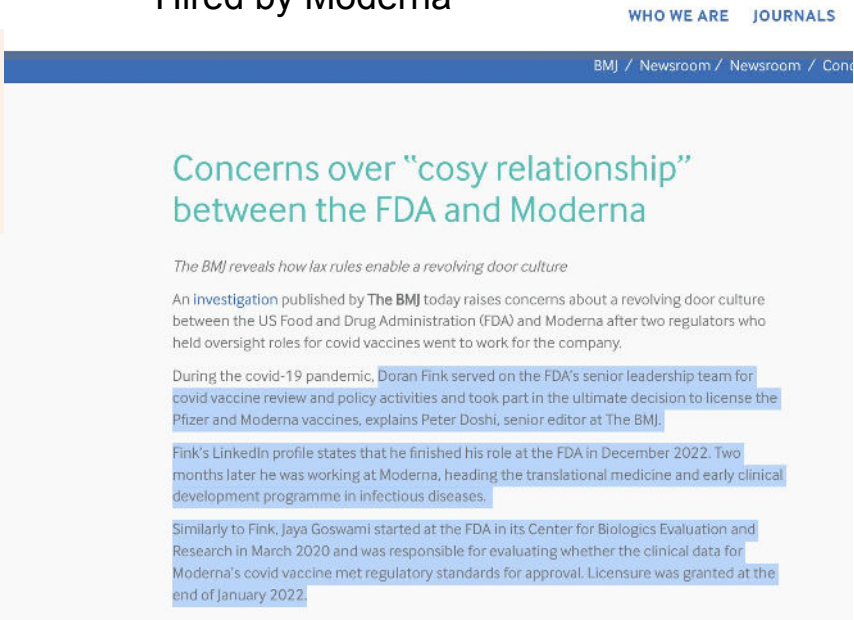
Resigned in Protest

Philip Krause and Marion Gruber, who resigned from the Food and Drug Administration two weeks ago, are among the authors of a scathing critique of widespread booster shots, which was published in The Lancet on Monday.



BMJ

Hired by Moderna



Does Massachusetts have the same conflicts with the large Pfizer and Moderna tax base?

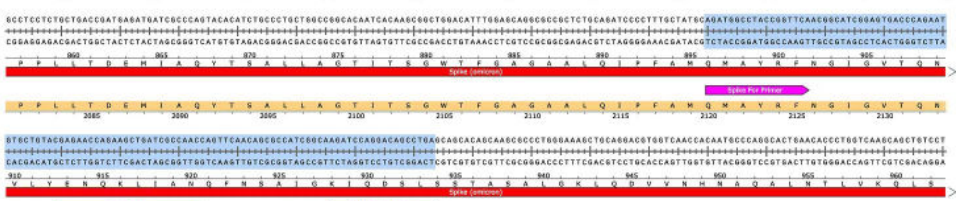
qPCR for tissues of concern

- Blood Banks
- Sperm Banks/Fertility clinics
- Breast Milk
- Transplant organs
- Biopsies

qPCR tests are now kitted and can screen patients tissues to prioritize sequencing.

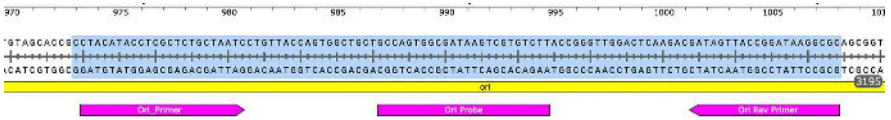
Its time to start sequencing vaccine injured patients.
We need CLIA labs to help with this.
We have the qPCR reagents to get this moving

Call to Action: Manufacture Kits



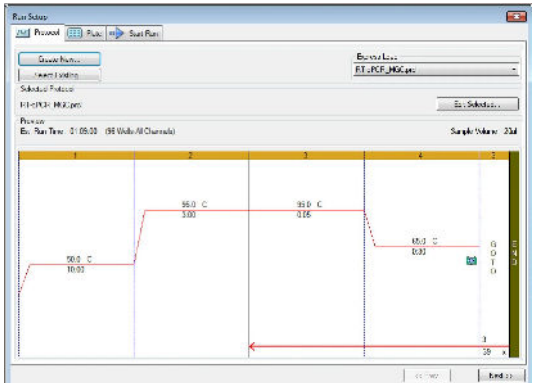
Spike Assay

- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Forward
- >AGATGGCCTACCGGTTCA
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Reverse
- >TCAGGCTGTCCTGGATCTT
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Probe
- >/56-FAM/CGAGAACCA/ZEN/GAAGCTGATCGCCAA/3IABkFQ/



Vector Origin Assay

- MedGen_Vax-vector_Ori_Forward
- >CTACATACCTCGCTCTGCTAATC
- MedGen_Vax-vector_Ori_Reverse
- GCGCCTTATCCGGTAACTATC
- MedGen_Vax-vector_Ori_Probe
- /5HEX/AAGACACGA/ZEN/CTTATCGCCACTGGC/3IABkFQ/



In 60 minutes, dsDNA contamination can be measured with direct qPCR of the vaccine vials for less than $100/vial
Using 1/300th of a dose.

Thank you for your time and consideration
Please end this deception

Kevin.McKernan@medicinalgenomics.com
https://anandamide.substack.com/