Exhibit 33

The Vax Gene Files: An Accidental Discovery https://www.printfriendly.com/p/g/9nukNi

Pfizer and Moderna bivalent vaccines contain 20-35% expression vector and are transformation competent in E. coli

Kevin McKernan

Cannabis Genome Project,2011. Developed the SOLiD sequencer. R&D lead Human Genome Project at MIT/WIBR. Founder- Medicinal Genomics

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The Vax-Gene Files: An Accidental Discovery

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By Julie Sladden, Julian Gillespie May 27, 2023 Vaccines 6 minute read

In 1928 scientist Alexander Fleming returned to his laboratory after a 2-week holiday. A petri dish of bacteria accidentally left on the lab bench, somehow became cross-contaminated with *Penicillium notatum* mould. Fleming noticed the mould inhibited the growth of the bacteria. This accidental discovery marked the dawn of the antibiotic era and a turning point in medical, and perhaps human history.

Recently, another accidental discovery has scientists wondering whether we have turned another corner in history.

The story begins with Kevin McKernan, a scientist with 25 years experience in the genomic field and a leading expert in sequencing methods for DNA and RNA. He has worked on the Human Genome Project and more recently in medicinal genomics involving DNA sequencing.

In the process of trying to sort out a sequencing problem, McKernan used anonymously sent, Pfizer and Moderna Covid-19 bivalent vaccines to act as mRNA controls.

'Somebody sent me these thinking, this is the perfect control... It should be pure. So, if you get this to work, you'll sort out your mRNA sequencing problems,' McKernan explains in a recent <u>interview</u>. 'They were right about that. It did sort out our problems. But what we discovered in the process is that they weren't pure mRNA. They actually had a lot of DNA in the background.'

McKernan was shocked, 'It's not what we were looking for... I had this hunch that the new modified nucleotides they have in the mRNA may have a higher error rate, and therefore we would see more mistakes in the mRNA. So, I knew we would have to sequence like a millionfold deep... over and over again to find these mistakes. When we did that DNA popped up and I thought "Oh, that's a bigger problem. We have to focus on that." ... I kind of went into panic mode, realizing that I didn't budget any time to look into this, and the world has to know about it.'

Let's pause here and look at what we're told about the Covid-19 mRNA injections. We're assured:

- The injections are safe. Meanwhile, <u>adverse event reporting systems</u> around the world record previously unseen rates of adverse events and injuries;
- *The injections are effective*. We would ask: Effective for what? Not stopping transmission. We're not sure about preventing serious illness either evidenced by recent <u>data</u> and New South Wales Health <u>reports</u> which show a disproportionate number of hospital and ICU admissions amongst the vaccinated.
- *The injection materials stay at the injection site*. Recently released <u>documents</u> obtained under FOI show the lipid nanoparticles become widely distributed notably to the liver, spleen, adrenal glands, ovaries, and testes;
- The injections won't change your DNA.

Let's look at that last one a little bit closer.

The Australian TGA states you can find reputable information about Covid-19 vaccines on their '<u>Is it true</u>' section of the website. It is worth a look. In answer to the question 'Can COVID-19 vaccines alter my DNA?' the TGA is clear: 'No, COVID-19 vaccines do not alter your DNA.'

They <u>explain</u>, 'mRNA vaccines use a synthetic genetic code called RNA to give our cells instructions about how to make the coronavirus' unique spike protein. When our body has made the protein encoded by the mRNA vaccine, it then recognises the spike protein as being foreign and launches an immune response against it. The RNA from the vaccine does not change or interact with our DNA in any way.'

Phew. Well, that's ok then, right?

Possible routes for mRNA to convert to DNA (including a process known as reverse transcription) were discounted. Until the publication of an annoying little paper in 2022 by <u>Alden et al</u>, an in vitro study involving human liver cells which showed Pfizer's mRNA was expressed as DNA within six hours.

At the time, this was assumed due to reverse transcription of the mRNA. However, in light of McKernan's discovery, there's a whole new possibility to consider. What if the vaccines *already* contained DNA? Then arguments about whether the mRNA could reverse transcribe into DNA become irrelevant.

Let's return to McKernan and take a closer look at what he found. In addition to the *expected* mRNA, he also found mRNA fragments, other pieces of RNA, and two forms of DNA: linearised and circular. The significance of the circular – or plasmid – DNA is important. The plasmid DNA is the 'complete recipe' used to program bacterial cells to mass produce the mRNA. This DNA should not be there. Further investigation by McKernan showed the plasmid DNA contained in the vaccines was indeed viable and <u>capable of transformation</u> in bacterial cells.

So, the Pfizer and Moderna vials of bivalent vaccine that McKernan tested were contaminated with DNA. DNA encoding the spike gene and *potentially* capable of inserting into the genome of an organism.

The question is, does this DNA have the potential to become part of the genome of a *human* organism and if so what might be the consequences? This would have required looking at 'genotoxicity,' something Australia's <u>TGA</u> says the (Pfizer) injections were not tested for, and the TGA did not ask for.

In case you are wondering, there *are* strict guidelines about DNA contamination levels in mRNA products. The European Medicines Agency (EMA) and FDA stated limits are 330 nanograms of DNA per milligram of RNA. In Australia, the <u>TGA</u> says it should be no more than 10 nanograms *per dose*.

(It's unclear how these limits were decided. Personally, we'd be hoping for zero DNA in our mRNA injections.)

This means that DNA should not be more than 0.033 per cent of the total nucleic acids in the dose. But McKernan's analysis demonstrated DNA contamination of up to 35 percent in the bivalent injection samples. This is up to 1,000 times higher than deemed to be 'acceptable' by the regulating authorities.

Next, McKernan analysed the monovalent (earlier) injections. The Pfizer monovalent injections were also found to be contaminated with DNA, though not as much. The levels of DNA in the Pfizer monovalent injections were 18-70 times higher than the EMA limit.

So, what happens now?

These results are in the process of being further verified by the scientific community. In the essence of speed, McKernan published his findings and methods publicly on <u>Substack</u> and <u>online</u>. He explains, 'The publication system, during the pandemic, is politicised. So, that's probably not going to get the word out very quickly. I had to do my best to document this all and make the data public.'

If McKernan's findings are verified, the implications are serious. Widespread DNA contamination would bring into question the quality of the entire mRNA injection manufacturing process, safety systems, and regulatory oversight. In addition, DNA might not be the only contaminant.

This contamination discovery begs a question. What does Australia's Office of the Gene Technology Regulator (OGTR) know about the <u>safety of these mRNA</u> injections? And what discussions have occurred between the TGA and the OGTR regarding the safety of these injections?

Some of these questions are being <u>asked</u> and will hopefully get answers. Soon, we hope.

Another question weighs heavily. What does this 'accidental discovery' mean for those who've had the mRNA injections, in terms of their health, their offspring, and future of the human genome?

Scientists and genomics experts are shocked by the discovery. McKernan too, 'I didn't expect to find Pfizer's entire blueprint for how they manufacture this thing sitting in the vial.'

Neither did we.

Authors



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Dr Julie Sladden is a medical doctor and freelance writer with a passion for transparency in healthcare. Her op-eds have been published in both The Spectator Australia and The Daily Declaration. In 2022, she was elected as a Local Government Councillor for West Tamar in Tasmania.

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Julian Gillespie

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Julian Gillespie is a lawyer and former barrister in Australia, known for his Covid-19 research and advocacy. His work includes seeking to have the provisional approval of Covid-19 vaccines declared legally invalid due to failures to meet regulatory standards. Julian is also a director of Children's Health Defense, Australia.

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Preamble- Follow on estimations of DNA/RNA contamination with alternative methods and more lots has reproduced contamination above the EMA limit but are less extreme than 20-35%. Please be certain to read the <u>follow on substacks</u>. These substacks look at Qubit readings and qPCR and found 43:1 - 161:1 Ratio of RNA:DNA in the Pfizer monovalent vaccines. Limitations- All lots evaluated to date are expired and this could lead to faster RNA decay than DNA decay. It is also possible, the Agilent Tape station DNA gels are staining some RNA and inflating the DNA estimates in the below study. <u>Previous work</u> demonstrated that the Pfizer and Moderna bivalent vaccines are contaminated with the expression vectors used to manufacture the mRNAs. While EMA documents suggest these double stranded DNAs (dsDNAs) are linearized, no data is provided to quantitate this important step. Linear plasmids are less replication competent than circular plasmids. With early estimates of billions of potentially contaminating molecules of DNA, even 99% efficient linearization reactions could still leave millions of replication competent plasmids behind.

The initial sequencing of the mRNA vaccines did not sequence deeply enough to ascertain the completeness of the linearization reaction. The initial sequencing survey of these vaccines also utilized a directional RNA-Seq library construction method that contained <u>actinomycin D</u>. This additive is used to suppress DNA amplification in the RNA-Seq method as it intercalates with DNA and suppresses DNA dependent RNA polymerase <u>activity of many polymerases</u>. Thus the original 1:350 -1:3000 DNA:RNA read count levels likely **under estimate** the level of plasmid contamination present in the Moderna and Pfizer bivalent vaccines.

We explored several methods to properly assess the ratio of circular to linear DNA contaminating the vaccines. One method utilized RNase A treatment of the vaccine to remove the modified mRNA. These RNase A treated samples were then DNA purified. Once we had DNA from the vaccine, we could evaluate it with multiple orthogonal assays to triangulate a quantitative measurement of the nucleic acids.



Figure 1

Method 1) Transform vaccine derived DNA into Kanamycin sensitive *E.coli* cells and plate on Kanamycin selective plates. Circular plasmid will provide antibiotic resistance to E.coli and thus present colonies on Kan plates. Linear DNA is less likely to provide antibiotic selection.

Method 2) Design a multiplexed qPCR assay that targets the vector and the spike mRNA insert. These can be used to estimate the plasmid to mRNA ratio more quantitatively than actinomycin D based RNA-sequencing kits that bias the sequencing reads towards RNA.

Method 3) Electrophoresis of purified DNA and RNA from the vaccines to estimate the relative amounts and size of each nucleic acid.

Method 4) Whole genome shotgun sequence the DNA to afford higher coverage over the vector DNA than was obtainable with mRNA present in the library. The 2 million fold sequencing coverage over the vaccine only provided 500-4000 fold coverage over the vector. If we remove the RNA and focus the sequencers on the just the DNA, we can obtain 2 million fold coverage over the plasmid to look for rare circles.

Introduction

The manufacturing of vaccine based mRNAs is best described by Nance et al.



Figure 3. Production of m1 Ψ mRNAs by *in vitro* transcription. Left: Components of *in vitro* transcription reaction. Right: Incorporation of m1 Ψ -triphosphate into RNA is guided by m1 Ψ 's ability to form a canonical base pair with adenine of the DNA template in the T7 RNA polymerase active site.

Figure2 -Reproduction of figure 3 from Nance et al. modified to identify the plasmid DNA in the T7 RNA polymerase.

This process can lead to residual plasmid DNA being left in the vaccine mRNAs. The method can also lead to truncated mRNA synthesis as seen in many RNA integrity (RIN plots) data listed as a concern by the EMA and TGA. The presence of *E.coli* based plasmids is a canary for Lipopolysaccharide (LPS or endotoxin) contamination. Whenever you see high levels of plasmid contamination derived from gram negative bacteria like *E.coli*, you should expect high levels of endotoxin contamination. Injecting endotoxin can lead to anaphylaxis and toxic shock syndrome.

The vector sequences discovered in <u>previous sequencing</u> work do not contain the long Poly A tracts described by Nance *et al.* This implies the poly-adenylation is performed after mRNA synthesis. This could be achieved with a polyA polymerase or a RNA ligase. The later method is more compatible with the unique poly-A tail described in Nance *et al.*

However, RNA ligases would also poly-adenylate truncated mRNAs from the original T7 polymerase manufacturing step. The polyadenlyation of truncated mRNAs would ensure the translation of truncated proteins. These questions should be further explored as poly A tracts are also notorious regions for assembly error. (See March 10th update below).

Methods

RNase A treatment of the Vaccines

RNase A cleaves both uracils and cytosines. N1-methylpseudouridine is known to be <u>RNAse-L resistant</u> but RNase A will cleave cytosines which still exist in the mRNAs. This leaves predominantly DNA for sequencing. Vaccine mRNA that was previously sequenced and <u>discussed here</u>, was treated at 37C for 20 minutes with 3ul of 20 Units/ul Monarch RNase A from NEB. The RNase reaction was purified using 1.5X of SenSATIVAx (Medicinal Genomics #420001). Sample were eluted in 20ul ddH20 after DNA purification. 15ul was used for DNA sequencing and 5ul used for transformation of E.coli.

Transformation of E.coli

50ul of NEBExpress competent *E.coli* cells (NEB#C2523I) were heat shocked at 42C for 20 seconds with 5ul of DNA (RNase treated vaccine) according to the manufacturers instructions. Cells were recovered in SOC for 1 hour with shaking at 37C. 200-800ul of SOC was plated on Kanamycin 25 plates (Teknova) and grown at 37C for 20 hours. Images were taken at both 20 hours and 44 hours.



Figure 3. Transformation of NEBExpress Kan sensitive E.coli cells. Plates were grown at 37C for 48 hours. 200ul and 800ul of SOC were plated in order to capture the entire transformation on 1 plate.

Colonies were picked into 250ul of ddH20 and lysed with 2% LiDS at 37C for 5 minutes. DNA was purified with 1.5X Ampure and 2x 70% EtOH washes. DNA was run on a Agilent Tape station to assess gDNA and presence of plasmids. *E.coli* qPCR (Medicinal Genomics) was utilized to confirm the colonies were in fact *E.coli*.



Figure 4. Top - E.coli qPCR was performed using Medicinal Genomics PathoSEEK E.coli detection qPCR kit (#420102). Bottom- Agilent Tape Station Electropherogram of gDNA isolations of vaccine-transformed E.coli colonies.

Watchmaker Genomics fragment libraries were constructed from these E.coli colonies for further sequence confirmation of the transformation.



Figure 5. Watchmaker Genomics Illumina Whole Genome Shotgun libraries from vaccine transformed E.coli colonies. Electropherogram generated from an Agilent Tape Station D5000 high sensitivity tape.

Whole genome shotgun of Pre-transformation RNase'd Vaccines.

15ul of the DNA (prior to E.coli transformation) was converted into sequence ready libraries using Watchmakers Genomics <u>WGS library construction kit</u>.



Figure 6. Pre-Transformation Fragment libraries generated with Watchmaker genomics Library construction kits. Electropherogram generated from an Agilent Tape Station D5000 high sensitivity tape.

RT-qPCR of plasmid and insert of mRNA vaccines prior to transformation

Due to the use of actinomycin D in the RNA-seq first strand synthesis, RT-qPCR and qPCR was used to assess the insert to plasmid ratio. qPCR primers were designed using IDTs Primer Quest software targeting the Kanamycin gene in the plasmid (HEX) and the Spike protein in the plasmid and the mRNA (FAM). The primer coordinates are depicted in vector map produced by SnapGene.



Figure 7. Vector map the Pfizer bivalent vial 1 depicted with SnapGene. The Kanmycin qPCR primers are depicted in Green (HEX) and the Spike primers are depicted in blue (FAM). Of note is the 3' SNP in the T7 Promoter.



Figure 8. T7 Promoter in the Pfizer bivalent vaccine has a 3 prime single nucleotide polymorphism (in red) compared to Moderna vector.



Figure 9. RT-qPCR of the Pfizer spike sequence in blue (FAM) and the Pfizer Kanamycin gene in the plasmid in Green (HEX). Near equivalent CT scores for the vector and the spike sequence imply lots of contaminating vector.

CTAB/Chloroform/SPRI purification of Vaccines

Some variability in qPCR performance was noted with our LiDs/SPRI purification method of the vaccines. This left some samples opaque and is believed to be residual LNPs in the purification. A CTAB/Chloroform/SPRI isolation was optimized to address this and used for further qPCR and Agilent electrophoresis in Figures 10, 11 and 12. Briefly, 300ul of Vaccine was added to 500ul of CTAB (MGC solution A in SenSATIVAx MIP purification kit. #420004). The sample was then vortexed and heated for 5 minutes at 37C. 800ul of chloroform was added, vortexed and spun at 19,000 rpms for 3 minutes. The top 250ul of aqueous phase was collected and added to 250ul of solution B and 1ml of magnetic binding buffer. Samples were vortexed and incubated for 5 minutes and magnetically separated. The supernatant was removed and the beads washed with 70% Ethanol two times. Samples were finally eluted in 300ul of MGC elution buffer.



Figure 10. qPCR (No Reverse Transcription so DNA only) of the Pfizer bivalent vaccine with a multiplex qPCR assay that targets Kanamycin gene in the vector (red) and the Spike protein also in the vector (Blue). 10 Fold serial dilutions are performed in triplicate for each dilution. This assay only amplifies DNA in the vaccines and it CTs under 20 demonstrate high levels of DNA contamination.



Figure 11. qPCR (No Reverse Transcriptase so DNA only) of Pfizer and Moderna bivalent vaccines with a spike targeted assay in blue (FAM). Tenfold serial dilutions were performed to understand linearity of qPCR and relative quantities of DNA contamination for both manufacturers. Pfizer has slightly lower concentrations of DNA consistent with lower dosages of mRNA in their vaccine compared to Moderna.



Figure 12. Agilent High Sensitivity RNA Screen Tape of 1:10 diluted DNA purified from the vaccines. 300ul of vaccines were CTAB purified and eluted in 300ul MGC elution buffer (Tris based) to maintain original vaccine volumes. 23-56ng/ul are reported.



Figure 13. Agilent Genomic DNA Screen Tape of a non diluted vaccine prep. 300ul of vaccine were CTAB purified and eluted in 300ul of MGC elution buffer (Tris based) to maintain original vaccine volumes. 7.5-11.3 ng/ul are reported.

Conclusions

Previous RNA-Seq based estimates of the double stranded DNA contamination in the vaccines significantly under reported the magnitude of the contamination. Using qPCR and electrophoresis, we demonstrate the dsDNA contamination levels are 100 fold higher and imply trillions of DNA molecules per dose. The Pfizer DNA contamination ranges from 8.19-11.3 ng/ul with 23-28ng/ul of mRNA. The Moderna DNA contamination ranges from 7.5 - 9.5ng/ul with 25.7ng/ul - 55.9ng/ul of mRNA. This

averages to 9.1ng/ul mean DNA concentration versus 33.4ng/ul mean RNA concentration.

This equates to 27.3% (9.1/33.4) of the nucleic acid in each vaccine being expression vector. This is several orders of magnitude over the the EMAs limit of 330ng/mg.

An unknown portion of these dsDNA contaminants are replication competent plasmids that can transform *E.coli* with a simple 20 second 42C heat shock treatment. These plasmids provide antibiotic resistance on LB-Kan plates and can be isolated from *E.coli* cultures. It is unlikely these plasmids will express spike protein in non-laboratory modified *E.coli* as the ribosomal signals in the vaccine mRNA are designed for mammalian translation. The T7 promoter is known to leak in mammalian cell lines and some laboratory *E.coli* genotypes but is not expected to leak in wild type *E.coli*. This may enable mRNA to be expressed from these plasmids in mammalian cells but unless the plasmids are integrated into the human genome, they are unlikely to be replicated to high copy number.

While bacteria are unlikely to express this spike protein, bacteria can replicate this plasmid and serve as a <u>bactofection</u> source for introduction of these <u>mammalian</u> <u>expression plasmids</u> to human cells.

Given the near equimolar contamination, studies evaluating the reverse transcriptase capacity of LINE-1 should be reconsidered. If each injection provides trillions of dsDNA contaminants, LINE-1 RT activity is not a necessary step for genome integration. The critiques of <u>Alden *et al*</u> focused primarily on the fact that LINE-1 is predominantly expressed in cancer cells lines and that the LINE-1 observation shouldn't be extrapolated to patients. The vaccines are providing trillions of dsDNAs containing a potentially leaky T7 promoter encoding a spike protein with a Kozak consensus sequence. With these levels of contamination, RT activity from LINE-1 is not a prerequisite for genome integration. These data cannot inform on genome integration and further IRB reviewed deep sequencing work is required to address rare mosaic integration exceeds the EMA specifications by several orders of magnitude and further scrutiny should be applied to the endotoxin levels and dsRNA levels in these vaccines.

These data are preliminary and further sequencing confirmation will be made publicly available once complete. Quantitative PCR assays are now available for detection of both Moderna and Pfizer monovalent and bivalent mRNA vaccines and their respective contaminating vector DNAs. This assay also detects the Janssen adenovirus spike sequence. These may be helpful for screening blood, semen, serum and other tissues for blood blanks and fertility clinics concerned with vaccine contamination.

Comments regarding Peer Review- This article will be critiqued for not submitting to the church of academic gatekeeping (Peer Review). The pandemic has revealed many of the warts of this process. You can read about how we can transform peer review with Bitcoin on <u>this substack.</u> While these platforms are being built, the best one can do publishing early and preliminary work is to propose and enable rapid review by the marketplace. Presenting scientific work that contains an easy to verify or falsify hypothesis is the key to migrating to a more decentralized science. This work was careful to present a hypothesis that can be confirmed or falsified in a high school biology lab. All that is needed is an electrophoresis assay that measures RNA and DNA and these results can be readily reproduced. The market will validate this finding long before traditional peer review even puts its boots on. Independent wet lab reproduction trumps 3 anonymous readers every time.

Update March 10th.

While it is notable that the vector has a short poly A tract, we should wait until the 'RNase A' sequencing libraries are back before to we speculate too much about the manufacturing process ligating on poly A tracts. The RNase A libraries focus the sequencer on the DNA in the vaccine and allow us to examine those molecules without the 1000X higher signal from the mRNAs.

It is an important area of focus as it may be critical for interpreting the smeary western blots discussed by the EMA and others. Public sequence QC of the plasmids or the vaccine lots would greatly reduce public anxiety over this topic. We once enjoyed rapid data release policies in genomics with the Human genome project and the <u>Bermuda</u> <u>accord.</u> Some of this spirit persists today with over 6.7 millions SARs-CoV-2 <u>genomes</u> <u>public in NCBI</u>. Vaccine genomics, however defies this scientific sprit.

The vaccine sequences to date can be counted on 1 hand and can only be found in the depths of github or substack. A few accidental vaccine genomes exist due to sequencing patients plasma but there is no concerted effort to shine a spot lot on the variability of this novel genomic manufacturing process.

We have sequences of the viral genomes that involuntarily enter our body but none on the ones we can actually control via injection? The fidelity of the ExoN/RdRp polymerase gene in SARs-CoV-2 is higher than the error prone T7 polymerase with N1 methylpseudouridine suggesting sequence surveillance of the vaccine supply chain is more important than the sequencing the virus. This is a incoherent position given the RO of the vaccine traversed the globe via social media transmission as quickly as a biological virus.

Why caution is in order-

Poly A tracts are notorious locations for error in assembly as they have low complexity and often little signature to algorithmically organize. Sequences are assembled from pairs of 150 base pair reads. Imagine a jigsaw puzzle where all the pieces are the same color. You really only have the edge pieces to work with and in the case of sequencing a mixture of RNA and DNA you have conflicting edge pieces (at the poly A tail) that want to break the puzzle into two different puzzles.

The plasmid DNA sequences being circular, will disagree the most with the mRNA sequences right at the end of the poly A. So this may be an assembly artifact that DNA only sequencing clarifies. More to come on the next stack.

¹ <u>EMA documentation on 330ng/mg DNA/RNA limit. Page 74.</u>

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