Deep sequencing of the Moderna and Pfizer bivalent vaccines identifies contamination of expression vectors designed for plasmid amplification in bacteria

ANANDAMIDE

FEB 15, 2023

Deep sequencing of the Moderna and Pfizer bivalent vaccines identifies contamination of expression vectors designed for plasmid amplification in bacteria.

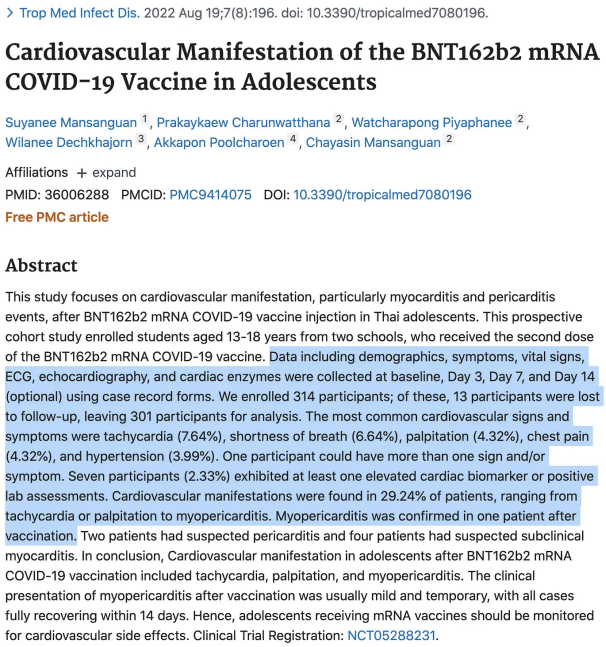
February 16th 2023

Introduction

As universities in the United States continue to mandate liability-free injections (COVID vaccines) for students at limited risk of contracting COVID, it becomes imperative that more public information be made available for the ingredients of these experimental vaccines. Both the EMA and the TGA have made note of fragmented RNA and smeary western blots suggesting the vaccine manufacturing process lacks fidelity and transparency. Shortly after the TGA data was released, Patel *et al.* (Pfizer) published a paper attempting to defuse these concerns. Jessica Rose has covered this topic here.

Informed consent cannot be obtained with poorly characterized therapeutics.

We are now entering the third year of COVID and its has become increasingly clear which demographics are at risk. The student age group (under 25) has repeatedly been shown to have very low risk of COVID yet the vaccine induced adverse events for students in this age bracket is higher than any vaccine ever administered. Krug *et al.* observed a risk of 1:6250 risk for myo/pericarditis in 16-17 year olds (Krug et al).



> Trop Med Infect Dis. 2022 Aug 19;7(8):196. doi: 10.3390/tropicalmed7080196.

Cardiovascular Manifestation of the BNT162b2 mRNA COVID-19 Vaccine in Adolescents

Suyanee Mansanguan [1], Prakaykaew Charunwatthana [2], Watcharapong Piyaphanee [2], Wilanee Dechkhajorn [3], Akkapon Poolcharoen [4], Chayasin Mansanguan [2]

Affiliations + expand

PMID: 36006288 PMCID: PMC9414075 DOI: 10.3390/tropicalmed7080196

Free PMC article

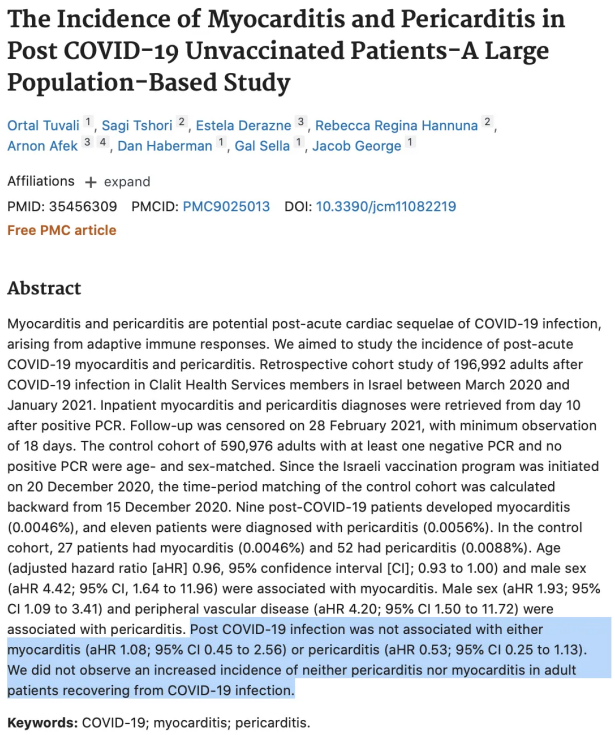
Abstract

This study focuses on cardiovascular manifestation, particularly myocarditis and pericarditis events, after BNT162b2 mRNA COVID-19 vaccine injection in Thai adolescents. This prospective cohort study enrolled students aged 13-18 years from two schools, who received the second dose of the BNT162b2 mRNA COVID-19 vaccine. Data including demographics, symptoms, vital signs, ECG, echocardiography, and cardiac enzymes were collected at baseline, Day 3, Day 7, and Day 14 (optional) using case record forms. We enrolled 314 participants; of these, 13 participants were lost to follow-up, leaving 301 participants for analysis. The most common cardiovascular signs and symptoms were tachycardia (7.64%), shortness of breath (6.64%), palpitation (4.32%), chest pain (4.32%), and hypertension (3.99%). One participant could have more than one sign and/or symptom. Seven participants (2.33%) exhibited at least one elevated cardiac biomarker or positive lab assessments. Cardiovascular manifestations were found in 29.24% of patients, ranging from tachycardia or palpitation to myopericarditis. Myopericarditis was confirmed in one patient after vaccination. Two patients had suspected pericarditis and four patients had suspected subclinical myocarditis. In conclusion, Cardiovascular manifestation in adolescents after BNT162b2 mRNA COVID-19 vaccination included tachycardia, palpitation, and myopericarditis. The clinical presentation of myopericarditis after vaccination was usually mild and temporary, with all cases fully recovering within 14 days. Hence, adolescents receiving mRNA vaccines should be monitored for cardiovascular side effects. Clinical Trial Registration: NCT05288231.

Mansanguan et al.

The "Thailand study" (Mansanguan *et al*) implies even higher rates of cardiac risk for students, where 29.24% of students (n=301) experienced cardiovascular manifestations. Studies including 23 Million Nordic patients observed a significant rate of myocarditis in this age group as well. This study, while larger, was not as controlled as the Thailand study in that Mansanguan *et al.* took baseline measurements of the patients and explored more than just myo/pericarditis.

These risks are not seen with C19 itself.



The Incidence of Myocarditis and Pericarditis in Post COVID-19 Unvaccinated Patients-A Large Population-Based Study

Ortal Tuvali [1], Sagi Tshori [2], Estela Derazne [3], Rebecca Regina Hannuna [2], Arnon Afek [3 4], Dan Haberman [1], Gal Sella [1], Jacob George [1]

Affiliations + expand

PMID: 35456309 PMCID: PMC9025013 DOI: 10.3390/jcm11082219

Free PMC article

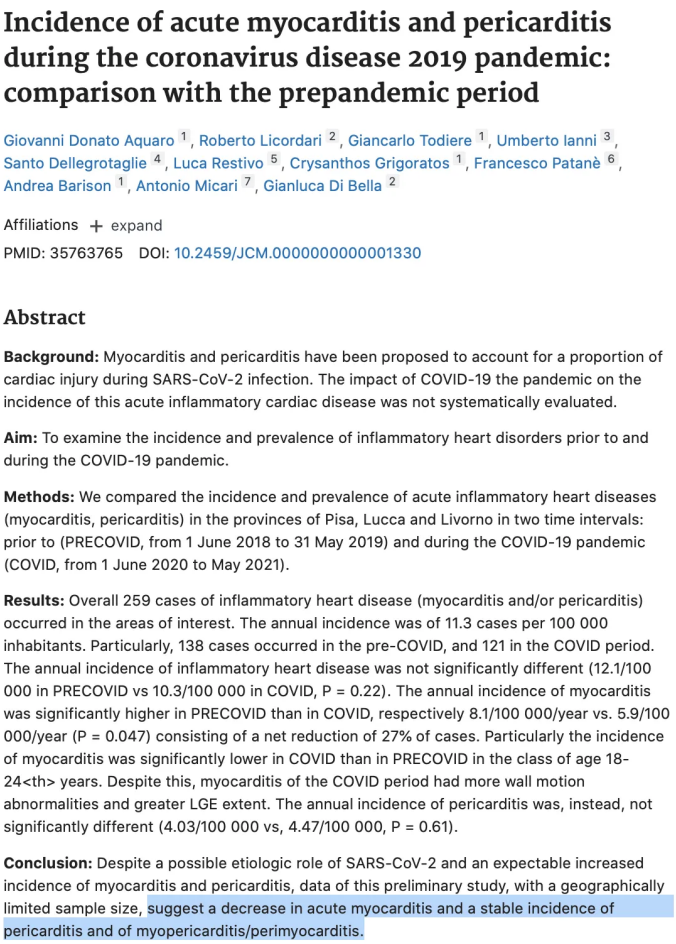
Abstract

Myocarditis and pericarditis are potential post-acute cardiac sequelae of COVID-19 infection, arising from adaptive immune responses. We aimed to study the incidence of post-acute COVID-19 myocarditis and pericarditis. Retrospective cohort study of 196,992 adults after COVID-19 infection in Clalit Health Services members in Israel between March 2020 and January 2021. Inpatient myocarditis and pericarditis diagnoses were retrieved from day 10 after positive PCR. Follow-up was censored on 28 February 2021, with minimum observation of 18 days. The control cohort of 590,976 adults with at least one negative PCR and no positive PCR were age- and sex-matched. Since the Israeli vaccination program was initiated on 20 December 2020, the time-period matching of the control cohort was calculated backward from 15 December 2020. Nine post-COVID-19 patients developed myocarditis (0.0046%), and eleven patients were diagnosed with pericarditis (0.0056%). In the control cohort, 27 patients had myocarditis (0.0046%) and 52 had pericarditis (0.0088%). Age (adjusted hazard ratio [aHR] 0.96, 95% confidence interval [CI]; 0.93 to 1.00) and male sex (aHR 4.42; 95% CI, 1.64 to 11.96) were associated with myocarditis. Male sex (aHR 1.93; 95% CI 1.09 to 3.41) and peripheral vascular disease (aHR 4.20; 95% CI 1.50 to 11.72) were associated with pericarditis. Post COVID-19 infection was not associated with either myocarditis (aHR 1.08; 95% CI 0.45 to 2.56) or pericarditis (aHR 0.53; 95% CI 0.25 to 1.13). We did not observe an increased incidence of neither pericarditis nor myocarditis in adult patients recovering from COVID-19 infection.

Keywords: COVID-19; myocarditis; pericarditis.

Tuvali et al.

Similar results are seen in Aquaro *et al.* and Sechi *et al.* where C19 derived Myocarditis is no different than background rates.



Incidence of acute myocarditis and pericarditis during the coronavirus disease 2019 pandemic: comparison with the prepandemic period

Giovanni Donato Aquaro [1], Roberto Licordari [2], Giancarlo Todiere [1], Umberto Ianni [3], Santo Dellegrotaglie [4], Luca Restivo [5], Crysanthos Grigoratos [1], Francesco Patanè [6], Andrea Barison [1], Antonio Micari [7], Gianluca Di Bella [2]

Affiliations + expand

PMID: 35763765 DOI: 10.2459/JCM.0000000000001330

Abstract

Background: Myocarditis and pericarditis have been proposed to account for a proportion of cardiac injury during SARS-CoV-2 infection. The impact of COVID-19 the pandemic on the incidence of this acute inflammatory cardiac disease was not systematically evaluated.

Aim: To examine the incidence and prevalence of inflammatory heart disorders prior to and during the COVID-19 pandemic.

Methods: We compared the incidence and prevalence of acute inflammatory heart diseases (myocarditis, pericarditis) in the provinces of Pisa, Lucca and Livorno in two time intervals: prior to (PRECOVID, from 1 June 2018 to 31 May 2019) and during the COVID-19 pandemic (COVID, from 1 June 2020 to May 2021).

Results: Overall 259 cases of inflammatory heart disease (myocarditis and/or pericarditis) occurred in the areas of interest. The annual incidence was of 11.3 cases per 100 000 inhabitants. Particularly, 138 cases occurred in the pre-COVID, and 121 in the COVID period. The annual incidence of inflammatory heart disease was not significantly different (12.1/100 000 in PRECOVID vs 10.3/100 000 in COVID, P = 0.22). The annual incidence of myocarditis was significantly higher in PRECOVID than in COVID, respectively 8.1/100 000/year vs. 5.9/100 000/year (P = 0.047) consisting of a net reduction of 27% of cases. Particularly the incidence of myocarditis was significantly lower in COVID than in PRECOVID in the class of age 18-24<th> years. Despite this, myocarditis of the COVID period had more wall motion abnormalities and greater LGE extent. The annual incidence of pericarditis was, instead, not significantly different (4.03/100 000 vs, 4.47/100 000, P = 0.61).

Conclusion: Despite a possible etiologic role of SARS-CoV-2 and an expectable increased incidence of myocarditis and pericarditis, data of this preliminary study, with a geographically limited sample size, suggest a decrease in acute myocarditis and a stable incidence of pericarditis and of myopericarditis/perimyocarditis.

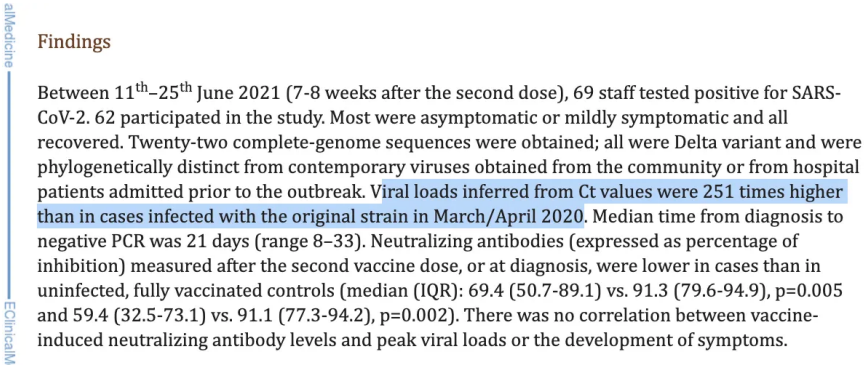
Aquaro et al.

A meta analysis confirms this.

This difference in cardiac risk between the vaccine and the virus should come as no surprise. Intramuscular (IM) administration comes with immediate potential access to the vasculature system. Studies evaluating skilled nurses using aspiration techniques have a 1.9% rate of hitting a vein or an artery. Accidental IV injection in Dentistry is even higher at 4% with aspiration. SARs-CoV-2 vaccines do not even require aspiration and likely have a higher accidental IV injection rate. Marc Girodot has covered this in detail.

On the flip side of this risk equation we find infection from C19 has been shown to provide more durable immunity than the narrow spike protein focused vaccines. Natural immunity provides mucosal antibodies and T-Cell recognition of the proteome derived from the entire 30kb viral genome where the vaccines are focused on a small ~4kb (1273 amino acids) region of the virus.

This narrow-epitope vaccine strategy, now has documented escape mutants where the majority of the mutations from Delta to Omicron are amino acid changing variants in the spike domain targeted by the vaccine program. This enrichment in amino acid changing variants over synonymous variants is the hall mark of selection. Vaccines that don't stop transmission and fail to limit the viral load of the patient, leave the evolutionary clock of the virus (RdRp polymerase) intact but merely steer evolution around the paddle you placed in the river. Chau *et al*. demonstrated higher viral loads in the vaccinated. The study traversed multiple variants but other studies suggest equal to slightly lower viral loads in the vaccinated. Even in these cases the variance is a few CTs and in the 10^8 range. Dahdouh *et al.* showed up to 10 CT variances in swabbing alone suggesting many confounders to these studies. The small CT change is in the 100 million molecule range and is so high in both the vaccinated and unvaccinated that a 2 CT difference is irrelevant given the orders of magnitude lower requirements for a minimum infective dose (10^6).



Findings

Between 11th–25th June 2021 (7-8 weeks after the second dose), 69 staff tested positive for SARS-CoV-2. 62 participated in the study. Most were asymptomatic or mildly symptomatic and all recovered. Twenty-two complete-genome sequences were obtained; all were Delta variant and were phylogenetically distinct from contemporary viruses obtained from the community or from hospital patients admitted prior to the outbreak. Viral loads inferred from Ct values were 251 times higher than in cases infected with the original strain in March/April 2020. Median time from diagnosis to negative PCR was 21 days (range 8–33). Neutralizing antibodies (expressed as percentage of inhibition) measured after the second vaccine dose, or at diagnosis, were lower in cases than in uninfected, fully vaccinated controls (median (IQR): 69.4 (50.7-89.1) vs. 91.3 (79.6-94.9), p=0.005 and 59.4 (32.5-73.1) vs. 91.1 (77.3-94.2), p=0.002). There was no correlation between vaccine-induced neutralizing antibody levels and peak viral loads or the development of symptoms.

Chau et al. 'Vietnam study'

It is is well established that these vaccines do not stop transmission and recent studies from the Cleveland clinic (preprint) even demonstrate negative vaccine efficacy with each additional vaccine. They also demonstrate a dose dependent effect or a 'Biological gradient' which is one of the tenets of the Bradford Hill conditions for causality. This implies the vaccines are weakening patients immune systems and making them more susceptible to C19 and other infections.

Thus the vaccination policies at universities appear to violate fundamental medical ethics as they are asking students to absorb a negative risk/benefit medical intervention to shield older faculty. This is using their student body as human shields while failing to

inform that the shield has a 'Russian Roulette' price for its user. This is mis-informed coercion not informed consent.

This is particularly true for vaccines that do not stop transmission and in several studies show signs of negative vaccine efficacy (Barnstable Mass). The Barnstable Mass study run by the CDC showed higher infection rates amongst the vaccinated. Australia is now 96% vaccinated (16+ 2 Doses) and the hospitals are enriched above 96% for vaccinated patients. Excess mortality in Australia is higher post vaccination than during the pre-vaccination pandemic.

The bivalent boosters were never adequately studied in this student age group. Paul Offit was quoted as saying "The fix was in" regarding the approval of these vaccines. Instead of large scale RCTs, mouse data was predominantly used as for the bivalent booster approval. Even the RCTs that were performed on BNT162b2 and mRNA1273 have been reanalyzed by independent researchers (Fraiman et al) and found to have no benefit (Bardosh et al).

Byram Bridle explains how the selective marketing of Pfizers Relative Risk reduction score was a violation of FDA policy. Yet it was met with thunderous applause.

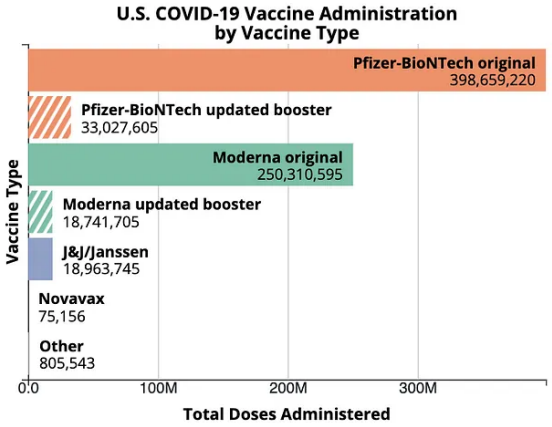
The initial vaccines that targeted Wuhan-1 spike protein have never provided lot to lot DNA sequencing quality control. They have never provided any evidence of transcriptional or translational fidelity of these pro-drugs. This is of utmost importance as the vaccines incorporated an error prone nucleotide known as N1-methyl-pseudouridine (m1Ψ) known to increase the transcriptional error rate to 250-300/Million or 1 error every 4,000 nucleotides (Chen *et al*). This translates into an error in every vaccine molecule synthesized and 14-34 trillion are injected with Pfizer and Moderna respectively. If the single molecule (Pacific Biosciences sequencing) assays used to estimate this error rate manifest in real human studies, this is an extraordinary degree of complexity.

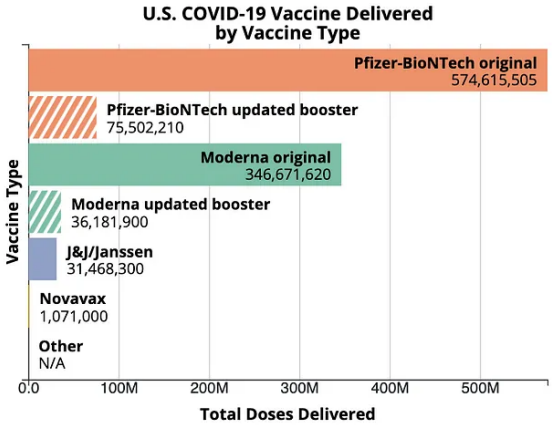
To make matters even worse, the impact of this base on ribosome fidelity is unknown but the published attempts to model pseudouridines (not N1-methyl-pseudoU or m1Ψ) impact on fidelity have shown substantial increases in ribosomal frame-shifting, stop

codon ablation and translational error (Fernandez et al). A study that attempted to unsuccessfully challenge this is discussed here.

Andrew Fire's lab sequenced the earlier vaccines but failed to ever disclose the raw sequencing reads. These data are needed to address concerns over transcriptional error rates and heteroplasmies.

No public sequence data exists for the novel bivalent vaccines being administered to children. Over 50M of the bivalent vaccines have been administered according to the CDC as of this writing.

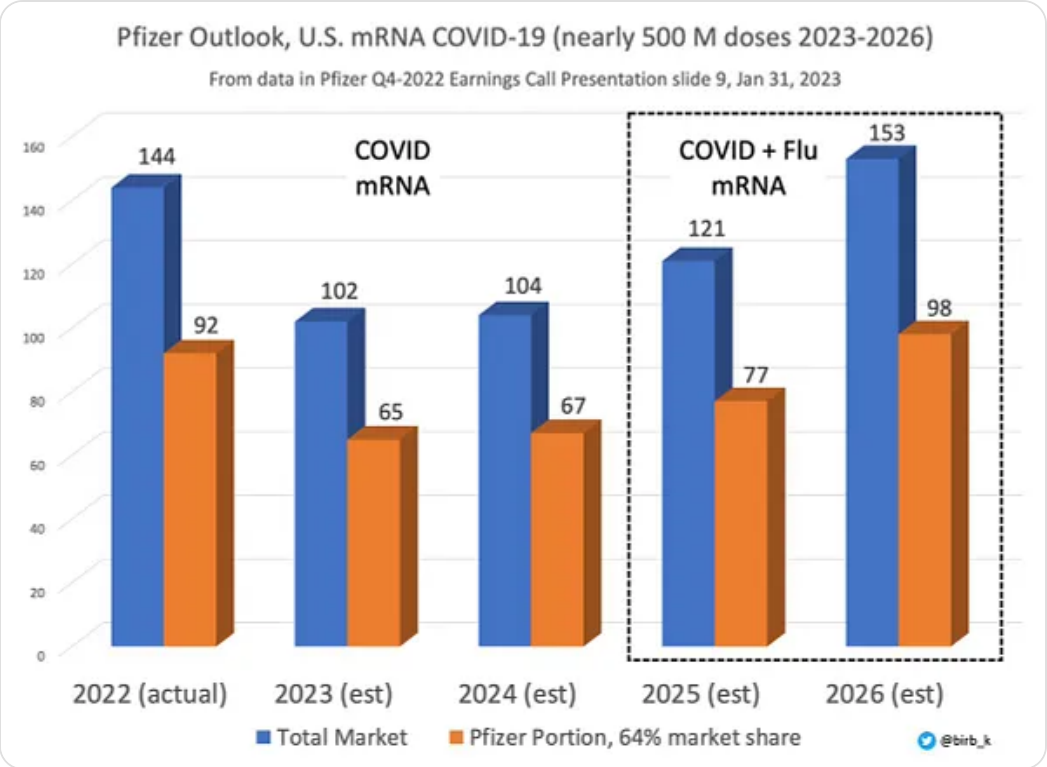




Pfizer is forecasting strong sales for 3 years.

KBirb
@birb_k

Pfizer forecasting nearly 500 million mRNA COVID doses in 2023-2026, with years 2025 & 2026 being a combo COVID/Flu mRNA. Pfizer's share, at recent rumored price of $120/dose is $37 billion just for the U.S. They foresee a huge CASH COW. Source: investors.pfizer.com/Investors/Even...



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Since there is limited public QA/QC data regarding these bivalent vaccines, we sought to independently monitor the RNA integrity of Pfizer and Moderna bivalent vaccines. Sample vials were purified and evaluated via electrophoresis. Directional RNA-seq libraries were constructed and sequenced on Illumina sequencers.

EMA documents from August 2022 exist for the Pfizer Bivalent vaccine. These documents state that NGS (Next Generation Sequencing) data exists but were not supplied to the EMA. The EMA also made note of there being only Western Blots available for characterization of the translated product and that the bands did not match the anticipated sizes.

characterisation data on the omicron variant, which is considered mandatory to guarantee safety of the product.42

In response, the Applicant has provided characterisation data for the Omicron (BA.1) variant. The package includes confirmation of primary structure, 5'-Cap structure, higher order structure and biological activity. Essentially, the same methods as those used for characterisation of the original variant have been applied. It is noted that primary structure analysis by NGS has been excluded. However, the HPLC-UV and LC-MS/MS studies are found sufficient to confirm the primary structure.

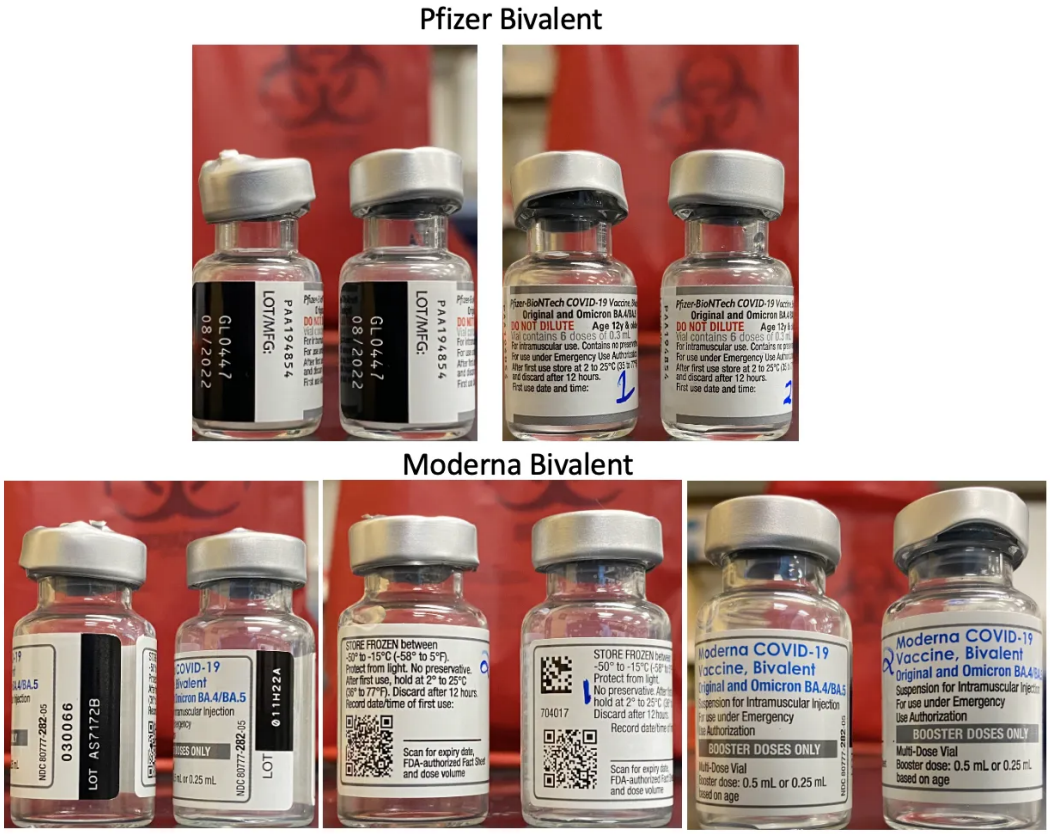
Biological activity is confirmed by western blot analysis and cell-free in vitro translation. This is found acceptable. However, some details for the western blot analysis are lacking and the identity of the observed bands are not clear. It is recommended that the applicant provide this information post-approval.

- The expressed protein size for BNT162b2 Omicron (B.1.1.529) DS is evaluated by western blot. The Applicant claims that the protein size is consistent with the expected size of the translated protein. However, the theoretical protein sizes of the mature protein and variants thereof are not presented in the dossier. This information should be provided, and the bands observed by WB should be assigned. In addition, the antibody used for western blot should be further described, i.e., it should be stated if it targets the S1 or S2 domain of the protein. The

Caption from the EMA.

Methods

Images and lot numbers of vaccine vials. Pfizer vials are from the same lot. Moderna lots are different.



Vaccine Lot numbers and QR codes.

Methods

Purifying the mRNA from the LNPs.

100ul of each vial was sampled (1/3rd to 1/5th of a dose)

- 5ul of 2% LiDs was added to 100ul of Vaccine to dissolve LNPs
- 100ul of 100% Isopropanol
- 233ul of Ampure (Beckman Genomics)
- 25ul of 25mM MgCl2 (New England Biolabs)

Samples were tip mixed 10X and incubated for 5 minutes for magnetic bead binding.

Magnetic Beads were separated on a 96-well magnet plate for 10 minutes and washed twice with 200ul of 80% EtOH.

The beads were left to air dry for 3 minutes and eluted in 100ul of ddH20. 2ul of eluted sample was run on an Agilent Tape Station.

Library Construction

50ul of each 100ul sample was converted into RNA-Seq libraries for Illumina sequencing using the NEB NEBNext UltraII Directional RNA library Kit for Illumina (NEB#E7760S).

To enrich for longer insert libraries the fragmentation time was reduced from 15 minutes to 10 minutes and the First strand synthesis time was extended at 42C to 50 minutes per the long insert recommendations in the protocol.

No Ribo depletion or PolyA enrichment was performed as to provide the most unbiased assessment of all fragments in the library. The library was amplified for 16 cycles according to the manufacturers protocol. A directional library construction method was used to evaluate the single stranded nature of the mRNA. This is an important quality metric in the EMA and TGA disclosure documents as dsRNA (>0.5%) can induce an innate immune response. dsRNA content is often estimated using an ELISA. Directional

DNA sequencing offers a more comprehensive method for its estimation and was previously measured and 99.99% in Jeong et al. It is unclear how this may vary lot to lot or within the new manufacturing process for the newer bivalent vaccines.

Results

Fragment analysis of each vial is depicted in Figure 1. RNA fragmentation is evident in both brands and all lots but is particularly notable in Pfizer vials. Surprisingly, mRNA products longer than the anticipated length of the mRNA were also observed in the bivalent vaccines.

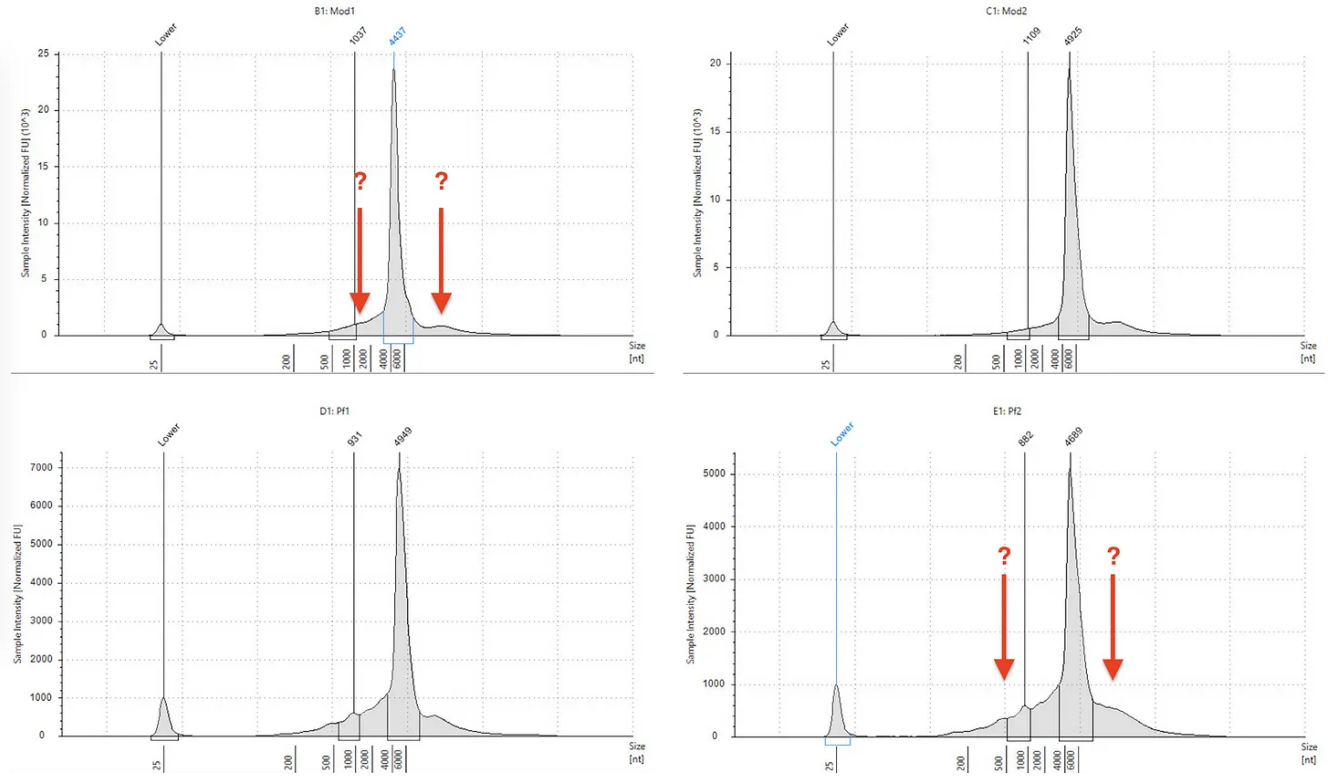
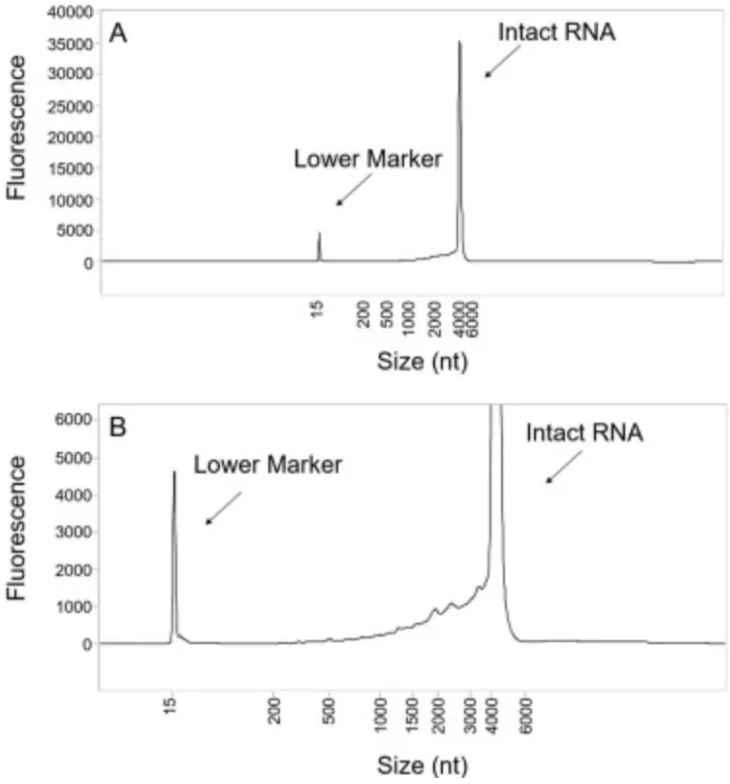


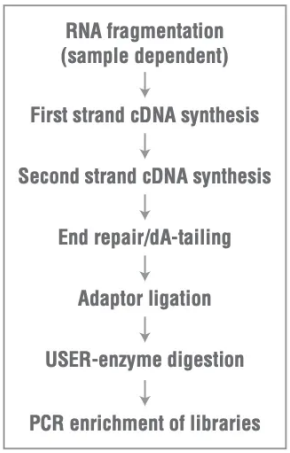
Figure 1. Agilent Tape Station Electrophoresis of the Bivalent vaccines. Moderna mRNA-1273.214 (Top) and the Pfizer bivalent vaccine (Bottom).

These longer fragments are note seen in Patel et al. with the monovalent vaccines.



Patel et al electrophoresis of the BNT162b2 mRNA vaccine.

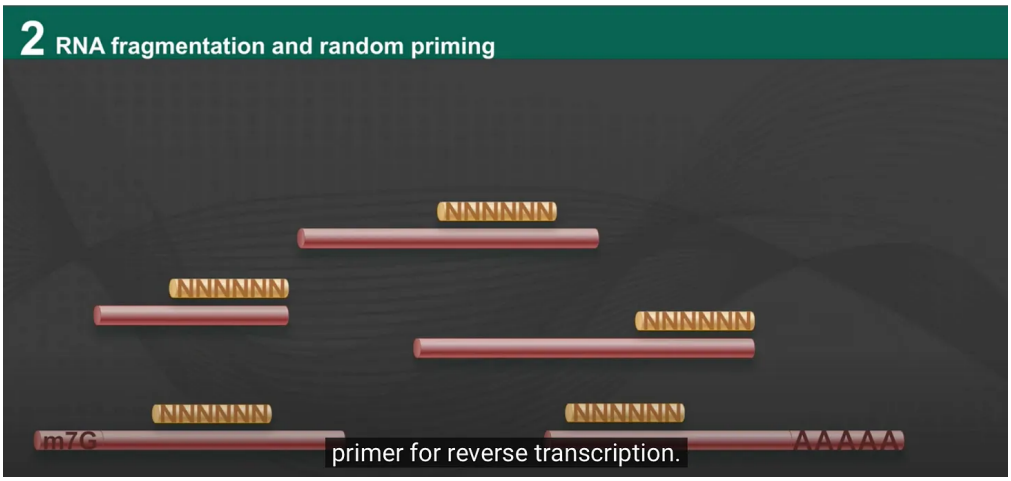
The first step in making RNA-Seq libraries is to fragment these mRNAs and convert them into DNA.



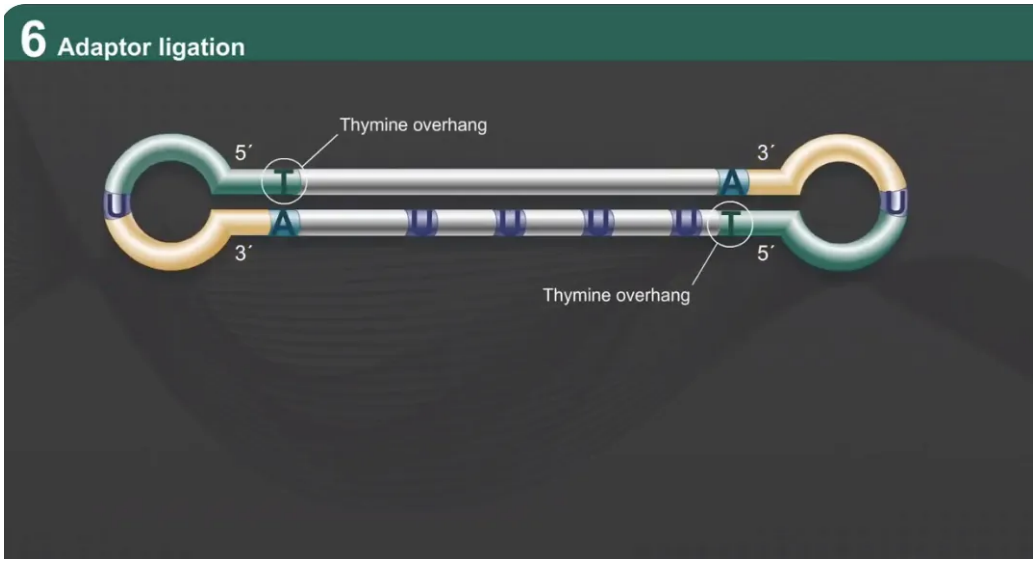
The 'directional' library construction method is described here. Directional libraries ensure that knowledge of the strand (watson vs crick strand) is captured and as a result

one can better estimate double-stranded DNA or RNA contamination in the mRNA synthesis of the vaccine.

The 1st strand synthesis step may introduce additional error based on Reverse Transcriptase sensitivity to m1Ψ. The synthesis begins with a Reverse Transcriptase (RT polymerase) and random primers (5'NNNNNN-3'OH). Once these random primers hybridize to RNA, the RT enzyme incorporates nucleotide that best match the template. These templates have a base known to disrupt base pairing fidelity (m1Ψ).



Following first strand synthesis, there is an ephemeral second DNA strand synthesis that incorporates an enzymatically 'erase-able' base (DNA based Uracil). This facilitates the ligation of double stranded sequencing adaptors to the dsDNA molecules. After adaptor ligation, this second strand is erased using UDG/UNG or a USER enzyme from New England BioLabs.



Of note, this USER enzyme digests DNA based uracils, not RNA based uracils. As a result, only the first strand synthesis is used as a template for library PCR. Since PCR based polymerase errors are an order of magnitude less frequent (1:100,000) than Reverse Transcriptase errors (1:10,000), we should see errors predominantly from the 1st strand synthesis attempting to replicate a m1Ψ RNA template. The final result of this approach will have primarily 2 modes of error.

1)T7 RNA polymerase is used to synthesize mRNA vaccines from a DNA based expression vector. T7 RNA polymerase will have increased incorporation error with m1Ψ.

2)Reverse Transcription error from turning the vaccine m1Ψ modified mRNA into DNA for Illumina sequencing.

Figure 2 depicts the final library sizes produced from these methods. These do not reflect on the manufacturing process of either vaccine provider but are the expected results from an RNA-Seq based kit.

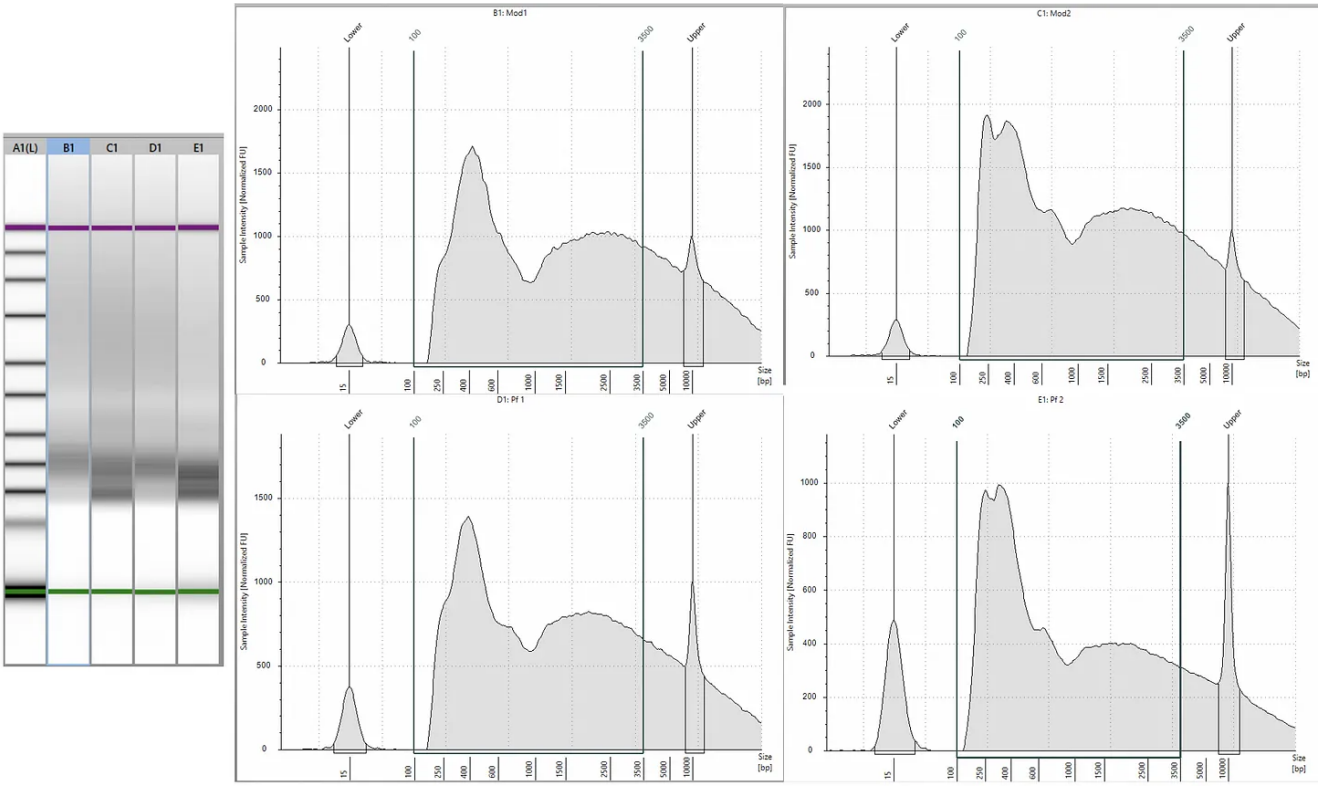
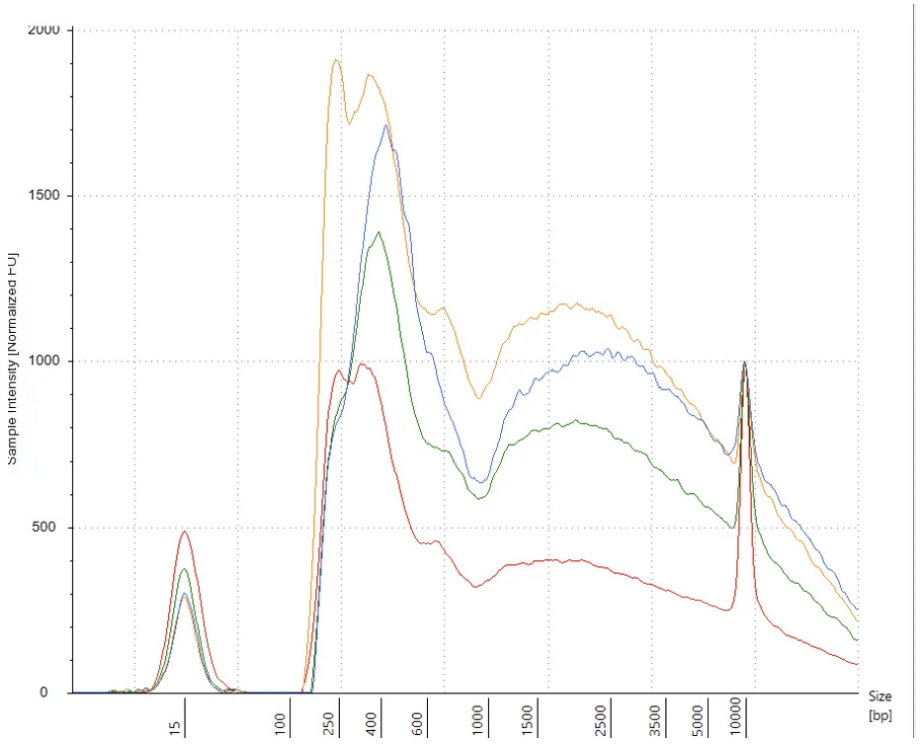


Figure 2. Illumina Libraries after 16 cycles of PCR. The libraries can likely be obtained with 12 cycles of PCR as the bimodal distribution of amplicons is a sign of too much input DNA into the Library PCR. The Illumina clustering will favor shorter amplicons but a higher number of improperly paired reads may result with bimodal amplification.



4 Libraries overlaid for comparison

Analysis pipeline

Reads were demultiplexed and processed with

- Trimgalore - Removes Illumina Sequencing adaptors.
- Megahit- assembles reads into contigs.
- Megahit for SARs-CoV-2
- Samtools- generates BAM files for viewing in IGV.
- BWA-mem- Short read mapper used to align reads back to the assembled references.
- SnapGene software- (www.snapgene.com)- Used to visualize and annotate expression vectors
- IGV- Integrated Genome Viewer used to visualize Illumina sequencing reads.

Sequencing results

Since these mRNA targets are so small (4Kb), they can be DNA barcoded and easily fit into any sequencing run. Illumina HiSeq 4000 lanes usually produce over 300M 150bp reads per lane. 0.5% of these reads (1.5 million reads) produces ~225Mb of sequence across a 4Kb fragment. 50,000X coverage across a 4Kb fragment is an error bar on current sequencer output, underscoring the affordability of sequencing every lot. The list price for 300M reads from many sequencing service centers is ~$1300. The sequencing costs for these samples was $7 per vial and used a mere 1/3rd of a dose (100ul). The library construction costs may have added an additional $50 in expense.

Two different expression vectors are found in the Moderna bivalent vaccines. Two different lots were sequenced and there may different background expression plasmids in each lot. Note, since these vaccines have bivalent inserts, the assembler is often splitting the inserts into separate contigs (omicron vs wuhan-1). I've included the entire output file from megahit to afford further polishing of these references by the public. These draft assemblies have been left in raw form delivered directly from megahit to afford reproduction of the work.

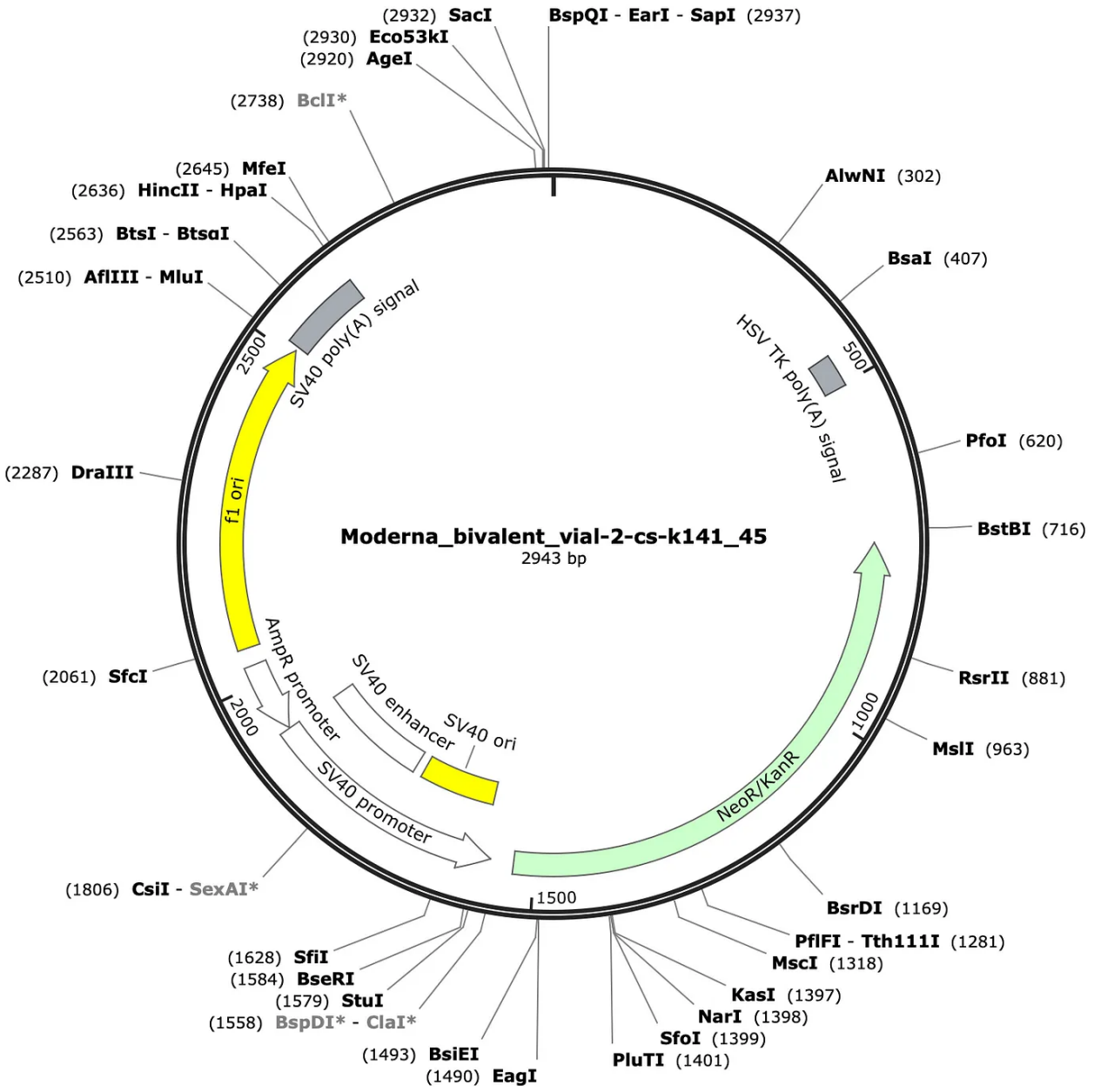


Figure 3. Moderna bivalent vial #2. This may be an empty vector or an assembly artifact that can occur when the insert is 1000X higher in coverage than the vector. Notice the Coverage is only 60X and other vectors are 13,000 X. Likely an index hopping artifact.

>k141_45 flag=1 multi=60.0000 len=2943
CTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTT
GCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACT
CACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAG
TATATATGAGTAACCTGAGGCTATGGCAGGGCCTGCCGCCCCGACGTTGGCTGCGAGCCCTGGGCCTTCACCCGAACTTGGGGGGTGGGG
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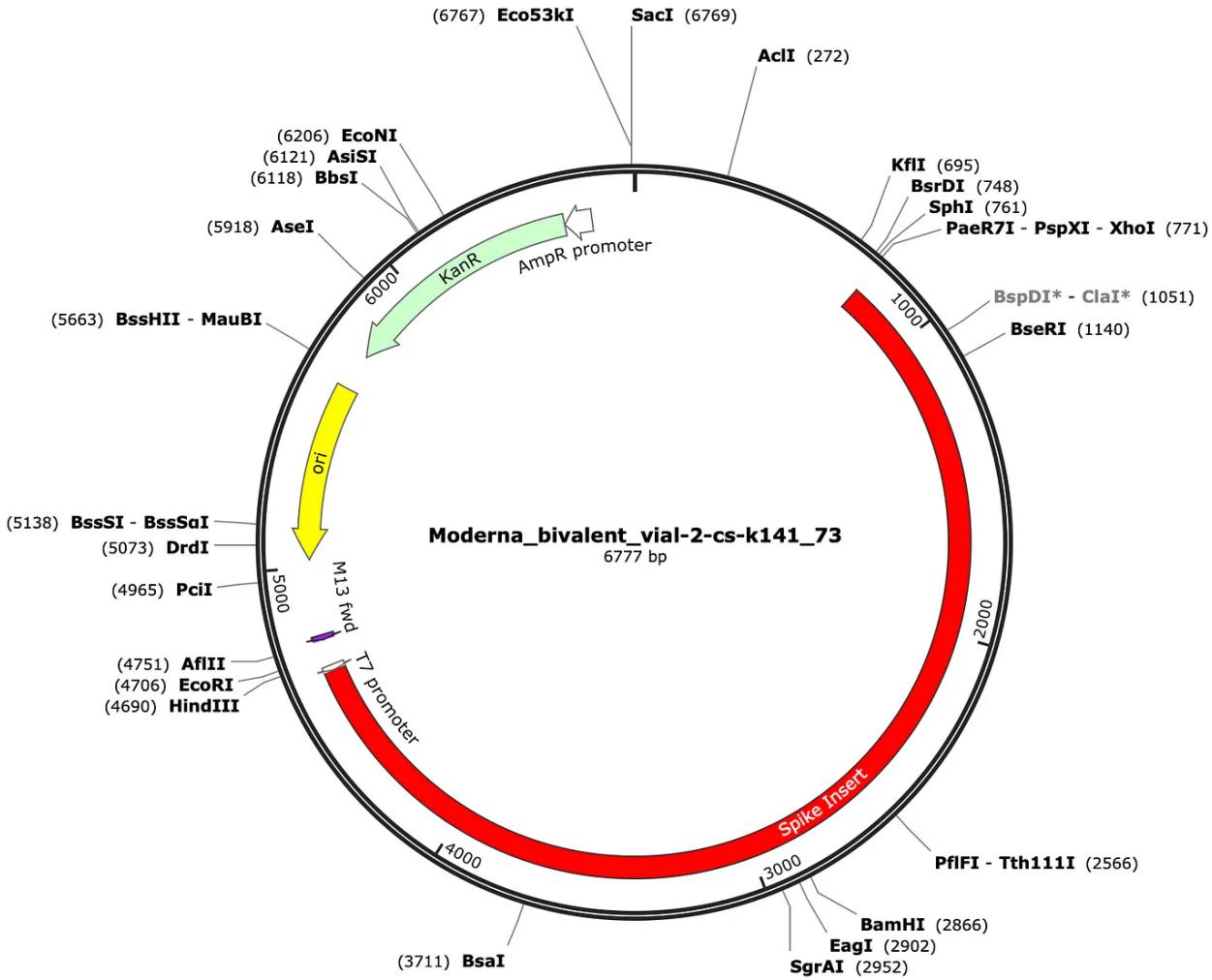

Figure 4. A Moderna vector with a spike insert from the same Vial #2 as in figure 3.

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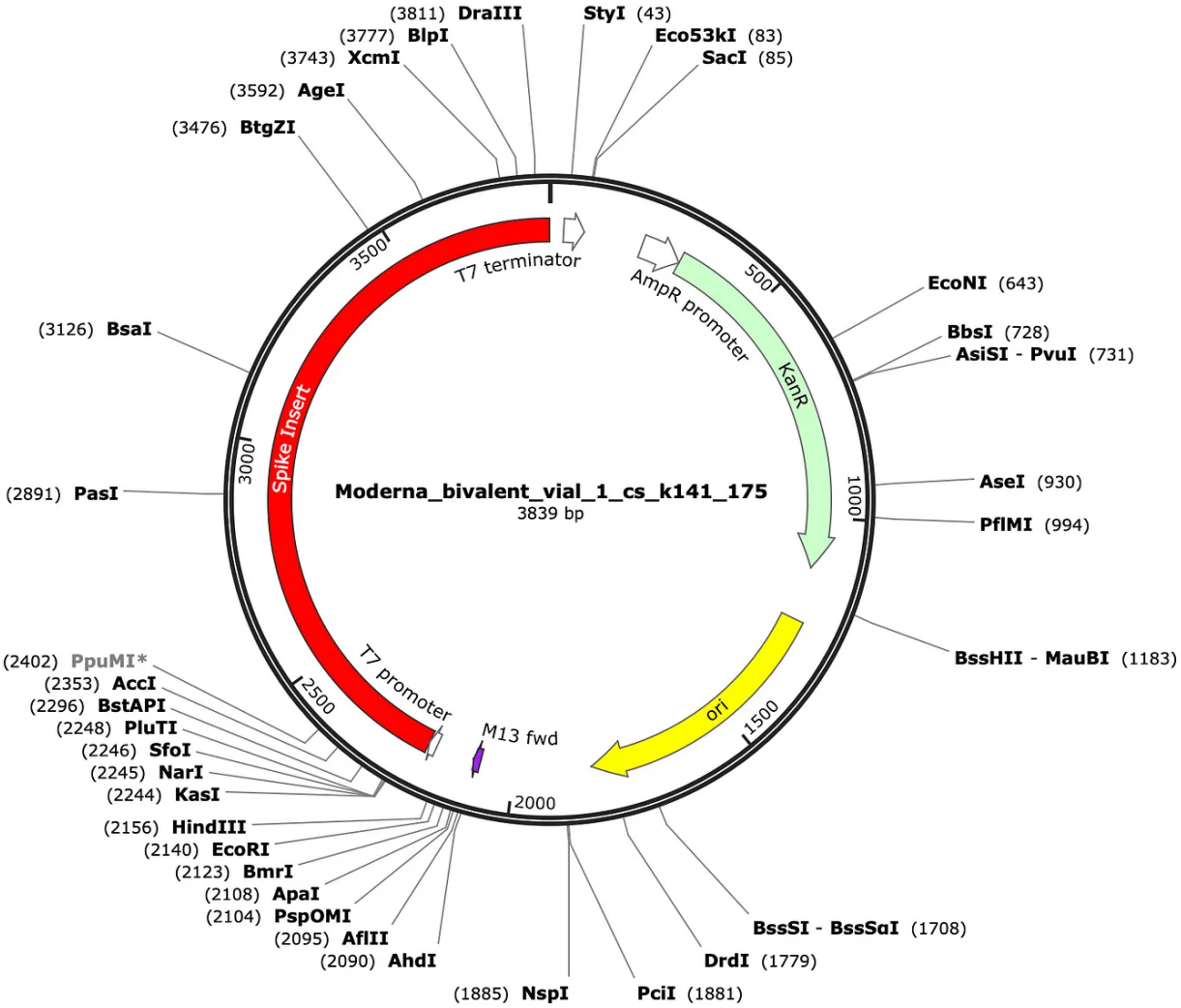

Figure 5. Moderna bivalent vaccine vector from vial 1. This has a partial Spike sequence. Likely broken due to Omicron and Wuhan-1 divergence.

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Expression vector found in the Pfizer bivalent vaccine

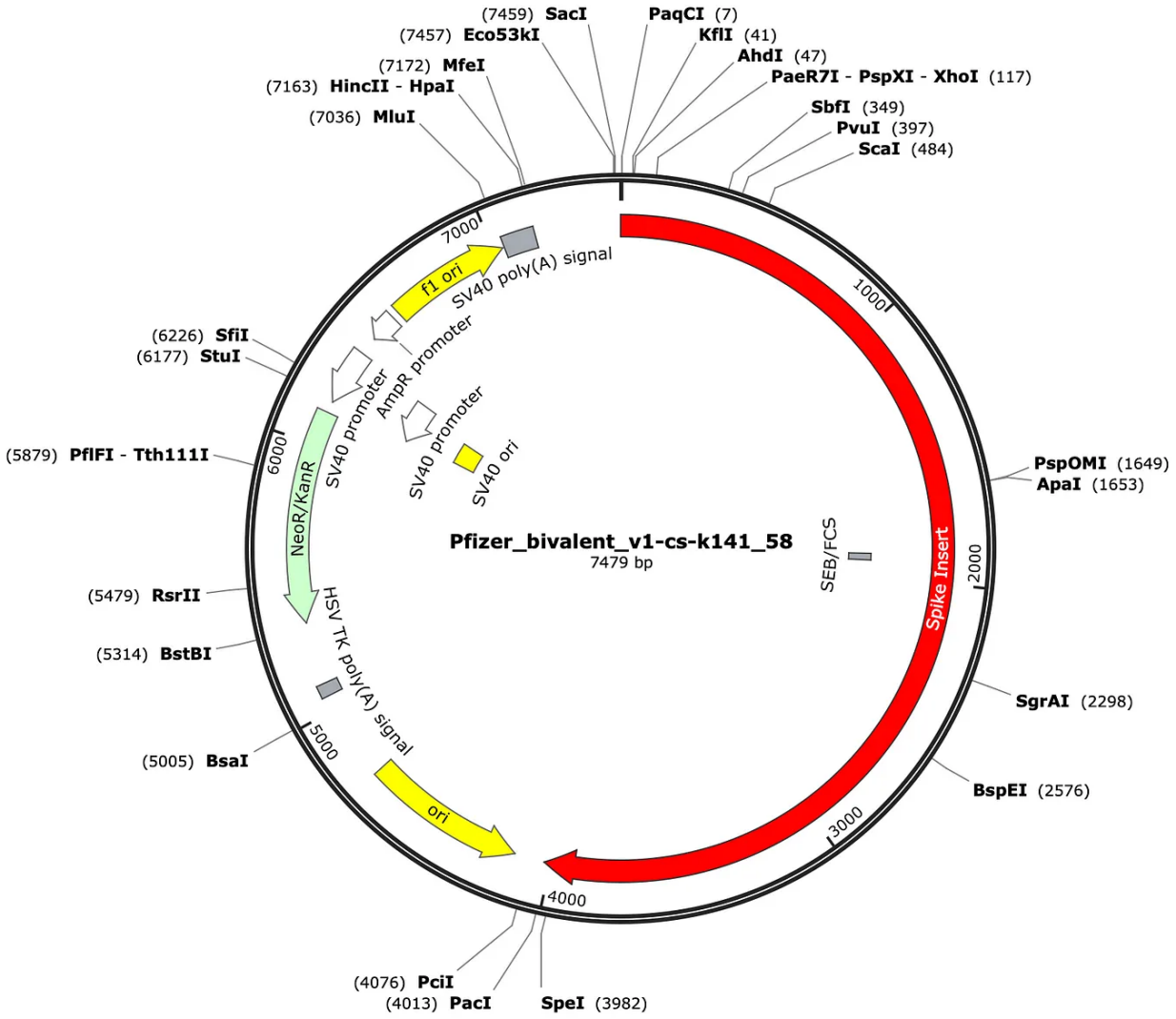

Figure 6. Pfizer bivalent vaccine vector with SEB/FCS annotated.

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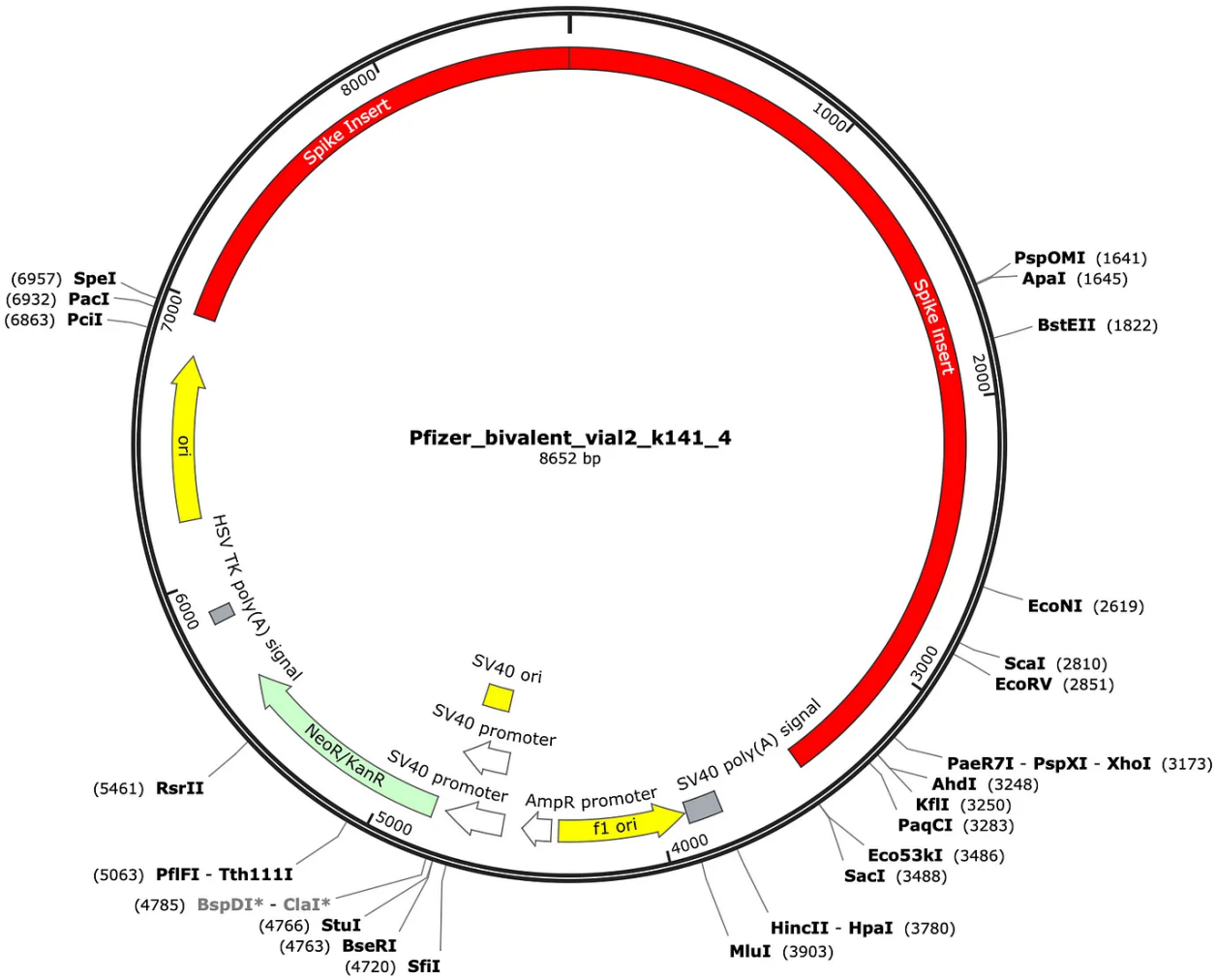

Figure 7. This assembly split the spike protein likely due to omicron and Wuhan-1s divergence. As a result there is 702bp of spike protein duplicated on the ends of the molecule at the noon hash mark.

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Of interest, this Moderna vaccine vector sequence has 99.8% identical sequence to the plasmids discovered in *Pseudomonas aeruginosa* samples which were famously edited from NCBI after spike protein sequence was identified in them. At the time, there was a healthy debate regarding if these plasmids would remain at high copy number in their host without any antibiotic selective pressure. The depth of coverage for the spike containing plasmids was 10X lower than the pseudomonas genome reads while other native pseudomonas plasmids were 50X higher in coverage than the pseudomonas genome. This led me to believe this spike plasmid was more likely a contamination event during the sequencing process as high copy plasmids should have higher copy number than their host genomes as evidenced by the native pseudomonas plasmids present in the assembly. This remains an unsettled debate.

I had not considered if the patients in this pseudomonas study had been vaccinated with a vaccine containing high amounts of contaminating expression plasmids. How long do these plasmids replicate in mammalian systems without Neo or Kan selection? Were these patients ever treated with such antibiotics due to their pseudomonas infection? Would this selection have worked given many pseudomonas are natively resistant to Neo and Kan already? The lack of public transparency on these liability free products leaves many questions.

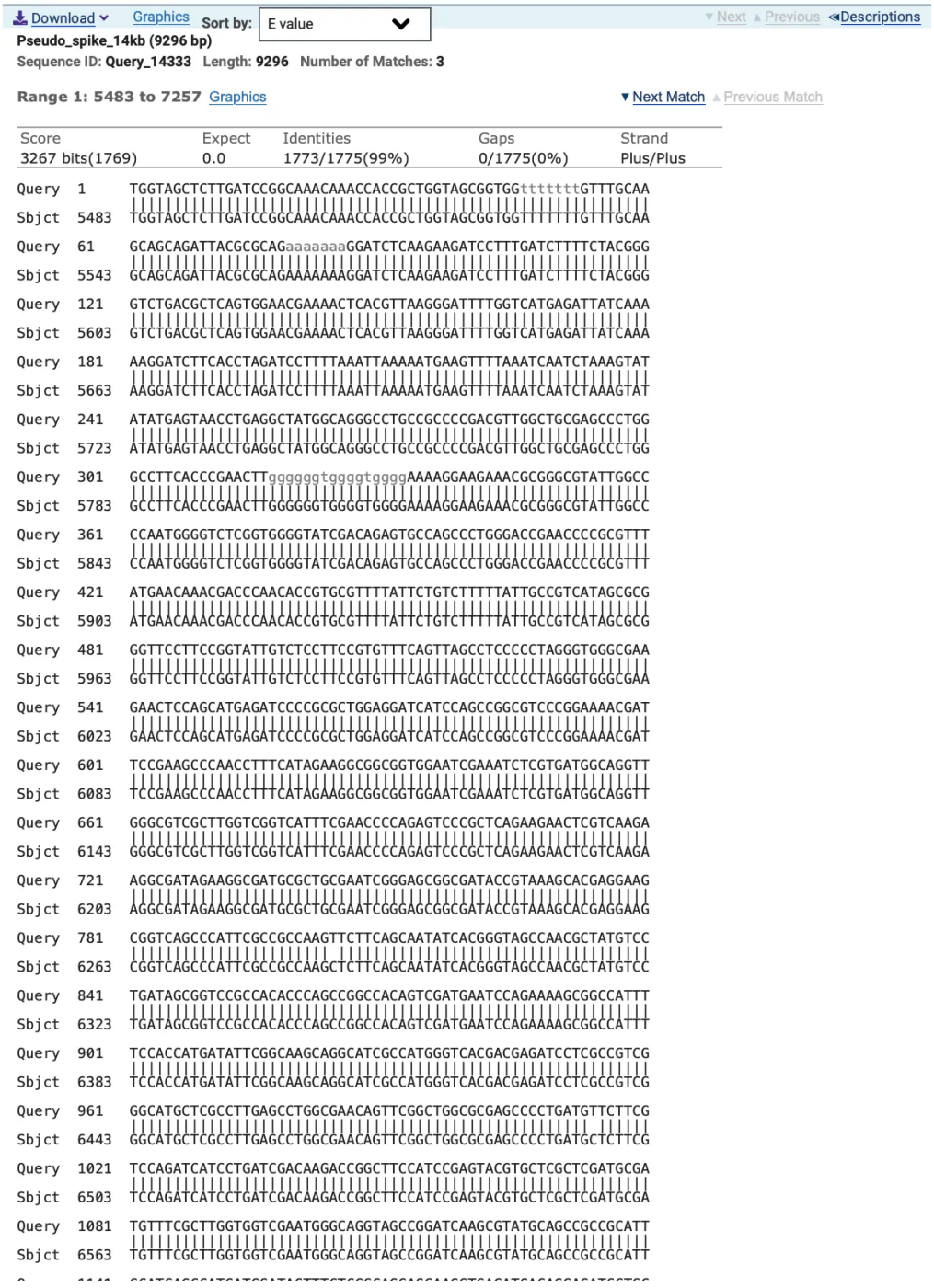
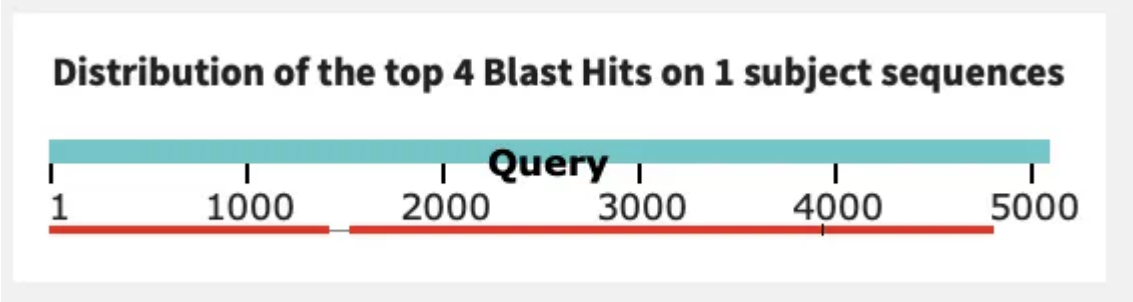


Figure 8. BLAST alignment of the Moderna vector to the Pseudomonas vector.

Pfizer expression vector blasted to *Pseudomonas aeruginosa* spike plasmid.



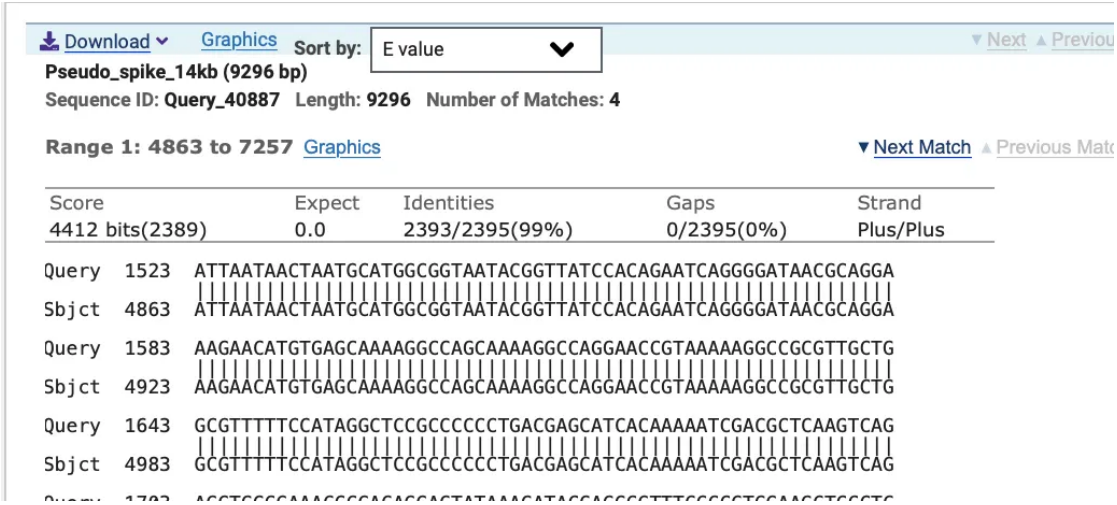


Figure 9. BLAST analysis of the Pfizer vector to the Pseudomonas vector.

The EMA set limits for dsDNA contamination at less than 330ng/mg RNA. This is roughly 1 part per 3,030 mRNA molecules. It is not clear how they set these standards. For instance, a shot containing 34 trillion mRNAs with a 1 part per 3,000 plasmid contamination rate would equate to over 10 billion antibiotic resistant plasmids being transfected per patient. The sequencing evidence we now have on hand confirms that most of this DNA is in-fact the expression plasmid DNA, complete with spike protein, SV40 mammalian expression promoters, aminoglycoside antibiotic resistance and high copy origins of replication that are compatible with both mammalian expression and bacterial amplification.

One can make a quick estimate of the relative ratio of the vector to insert nucleic acids by looking at the maximum depth of coverage of the insert versus the vector. Moderna vial 2 has 739X maximum coverage over the vector and 2.2 million X coverage over the insert. This equates to 1 vector for every 3,000 mRNAs.

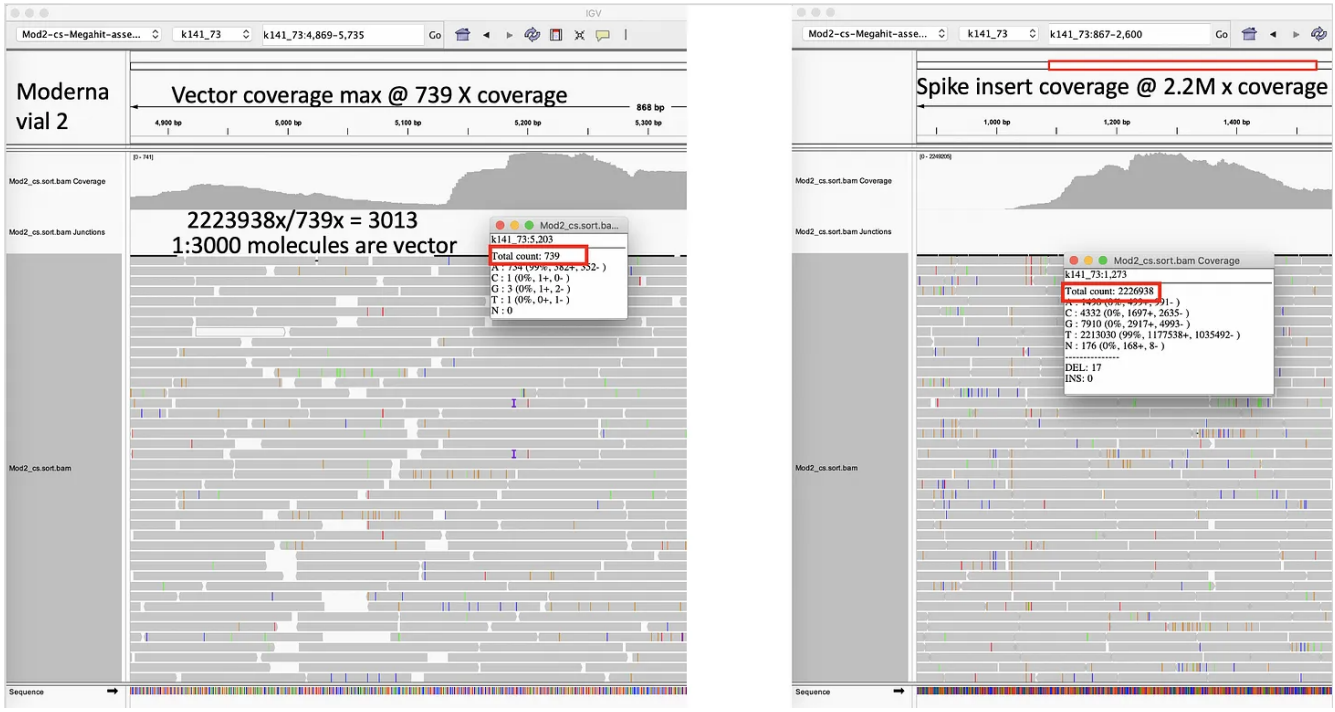


Figure 10. Read depth analysis of Moderna vial 2 for the vector and insert sequences.

The Pfizer vials have an order of magnitude higher rates of contamination. This is consistent with the fragment analysis having more off-target peaks.

Figure 11. Read Depth analysis of the Pfizer bivalent vial 1.

Since both DNA and RNA are being measured with this method, these estimates should be confirmed with qPCR using DNase and RNases to refine the ratio. Now that the sequence of the contaminating nucleic acid is published above, these assays can be built and used to monitor lot to lot contamination.

One can take the strandedness information in the sequencing data and discern which parts of the assembly are comprised of watson and crick strands (DNA) and which parts contain mostly crick strands (sense strands or mRNA). You can see this analysis pinpoints the T7 Promoter at base 2200 in the Moderna vial 1 vector. This is unequivocal evidence that the contaminating vector sequence is double stranded DNA and not RNA.

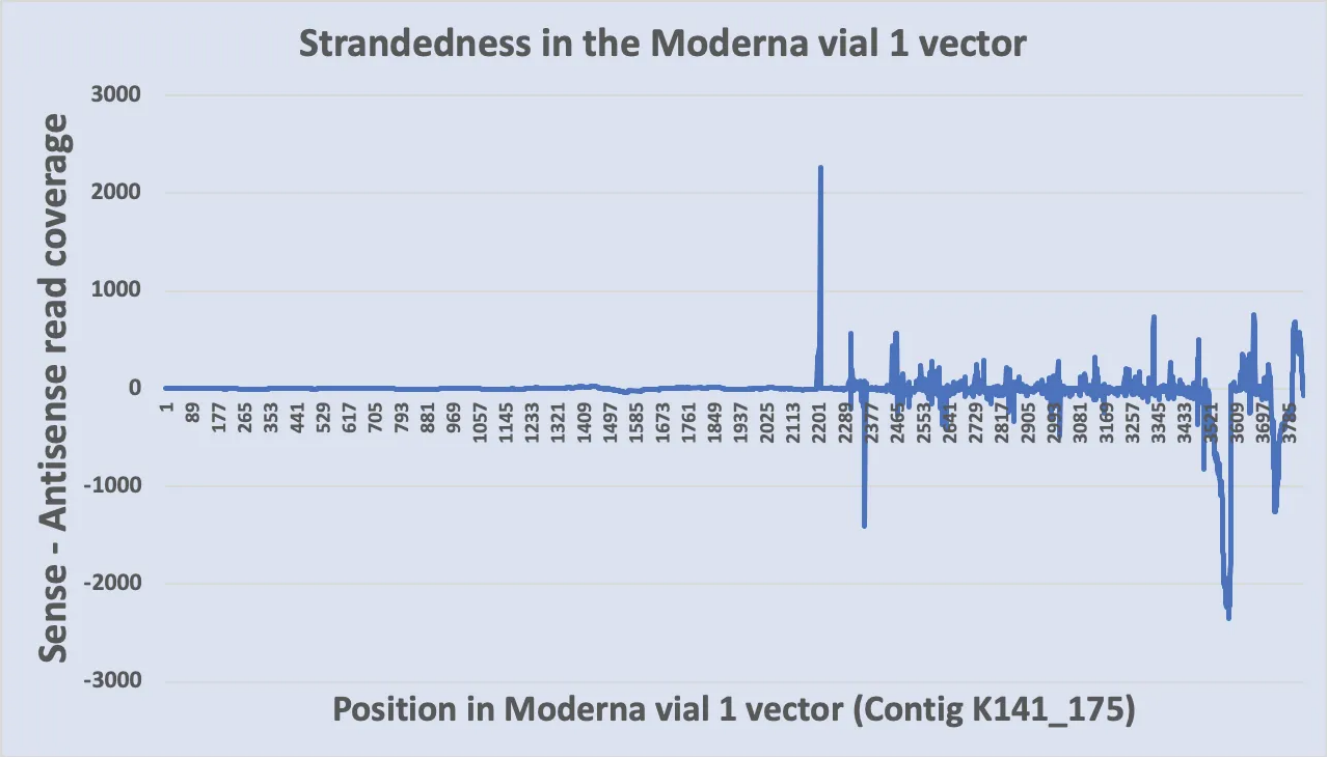
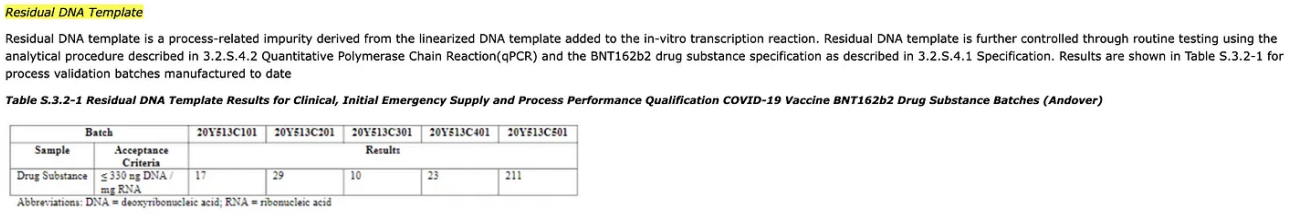


Figure 12. Watson vs Crick Read depth analysis. When Crick strand coverage minus Watson strand coverage = 0, you have DNA. When it's imbalanced, you have mostly sense strand mRNA.

What is not shown in the EMA documents is the efficiency of linearizing this plasmid which may help to limit its capacity to amplify in the host. Many linear amplifiable plasmids exist and this in no way guarantees faster clearance of the plasmid but methods should be described to ascertain the efficiency and completion of this step of the vaccine manufacturing process.

The paired-end reads in this study may help to [answer this question] as the assembler we utilized was not programmed to assess circularity of the contigs.



Residual DNA Template

Residual DNA template is a process-related impurity derived from the linearized DNA template added to the in-vitro transcription reaction. Residual DNA template is further controlled through routine testing using the analytical procedure described in 3.2.S.4.2 Quantitative Polymerase Chain Reaction(qPCR) and the BNT162b2 drug substance specification as described in 3.2.S.4.1 Specification. Results are shown in Table S.3.2-1 for process validation batches manufactured to date

Table S.3.2-1 Residual DNA Template Results for Clinical, Initial Emergency Supply and Process Performance Qualification COVID-19 Vaccine BNT162b2 Drug Substance Batches (Andover)

Batch		20Y513C101	20Y513C201	20Y513C301	20Y513C401	20Y513CF01
Sample	Acceptance Criteria	Results				
Drug Substance	≤ 330 ng DNA / mg RNA	17	29	10	23	211

Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid

Figure 13. EMA residual DNA template guidelines are not met with the contamination seen in Pfizer vials sequenced.

Transcriptional error

The sequencing data exceeds 1 million X coverage over the mRNAs. The vertical colored hash marks in the grey horizontal reads (below IGV screenshot) are errors. These errors can be a result of multiple factors.

1)T7 polymerase mis-incorporations while synthesizing vaccine mRNA from the expression plasmids found contaminating these vaccines. Since this step uses the error prone nucleotide m1Ψ, transcriptional errors are expected to increase 200-300% (Chen *et al*).

2)Reverse Transcriptase used to turn this modified mRNA into DNA for Illumina sequencing. These RT enzymes are known to be error prone (10^-4). Their error rate likely increases with the use of m1Ψ as a template.

3)PCR and sequencing error. These errors can be detected and addressed by eliminating duplicate reads, optical duplicates and quality filtering the sequencing date.

It will be interesting to monitor the sequencing errors in the DNA plasmid backbone compared to the sequencing errors seen in the spike protein as this would help to resolve the source of the error. The plasmid backbone is more faithfully replicated as DNA and has never been derived from m1Ψ templates. The mRNA, will have gone through 2 polymerase steps with a low fidelity nucleotide (m1Ψ). A comparison of the error rates in the backbone of the plasmid vs the error rates in the transcribed region of

the plasmid would help itemize the error induced with the use of an error prone nucleotide.

Given the high depth of coverage, future work will focus on Q30 filtering (1 error per 10^3) all of the reads to eliminate errors that could introduced by the sequencer and reassess if transcriptional error can be detected without UMIs.

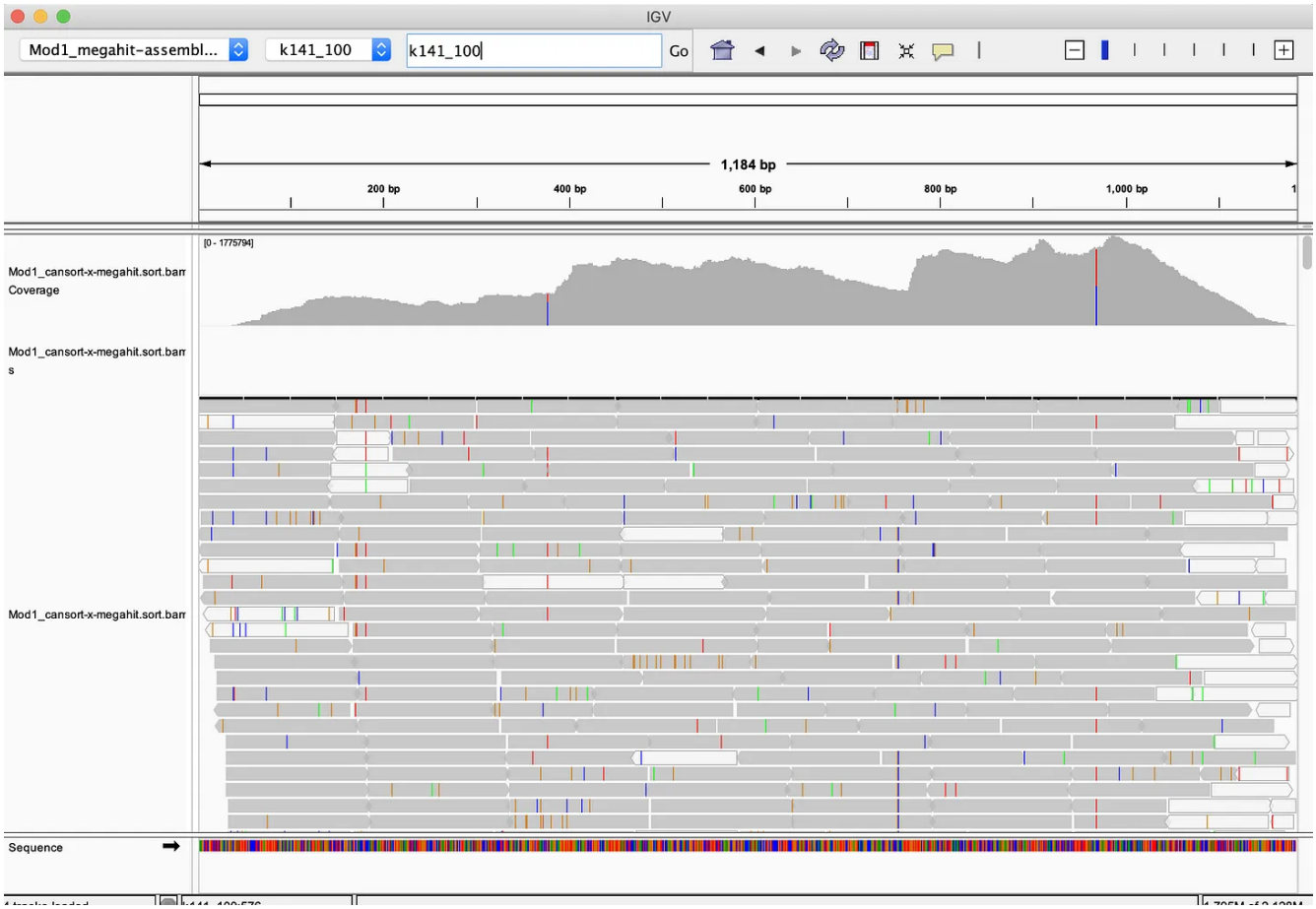


Figure 14. Reads mapped back to the Megahit assembly of the Moderna Bivalent vaccine vial 1.

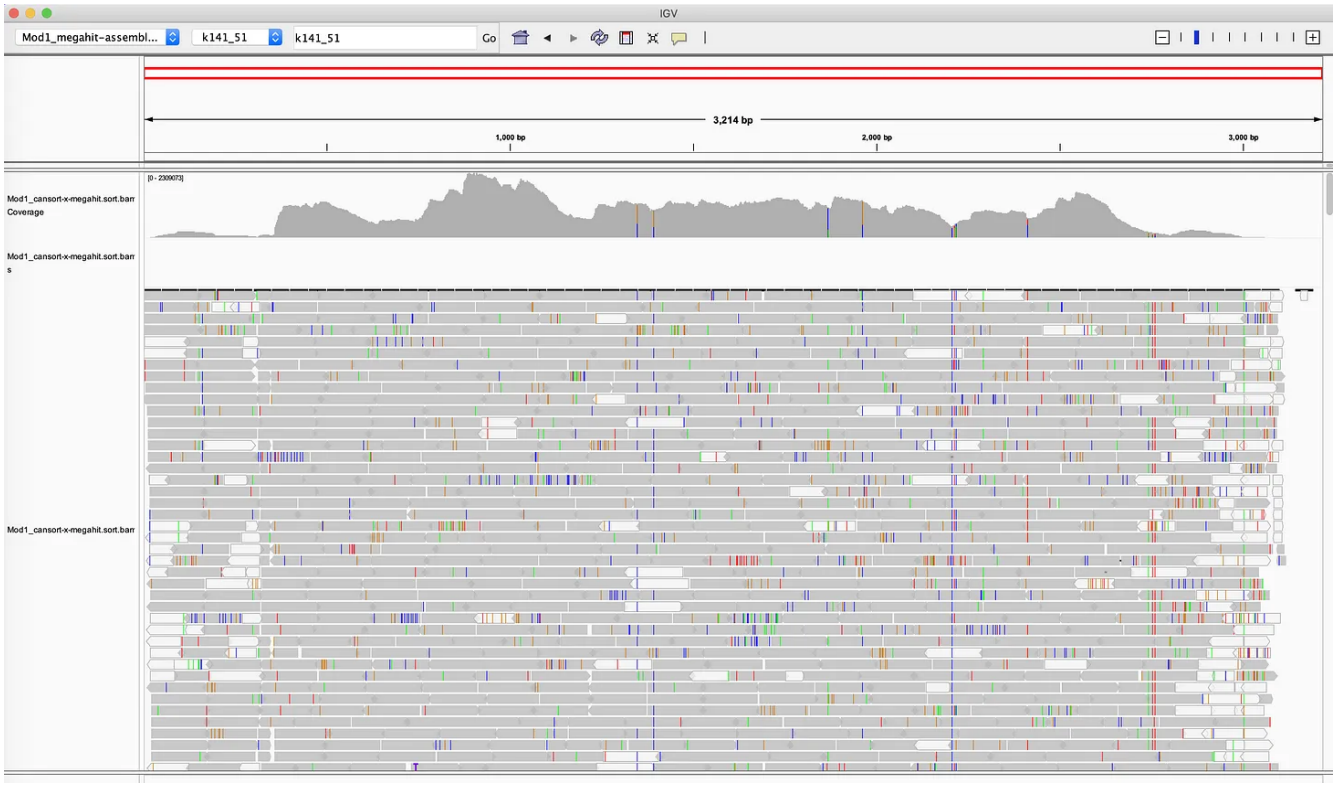


Figure 15. Reads mapped back to the megahit assemblies delivers two haplotypes for the spike protein. Wuhan-1 and Omicron are diverged enough that the assembler splits these Spike protein sequence into two contigs.

To survey transcriptional error rates estimated to be 1:3,000-4,000 in frequency described by Chen *et al.*, Unique Molecular Identifiers are often (UMIs) required.

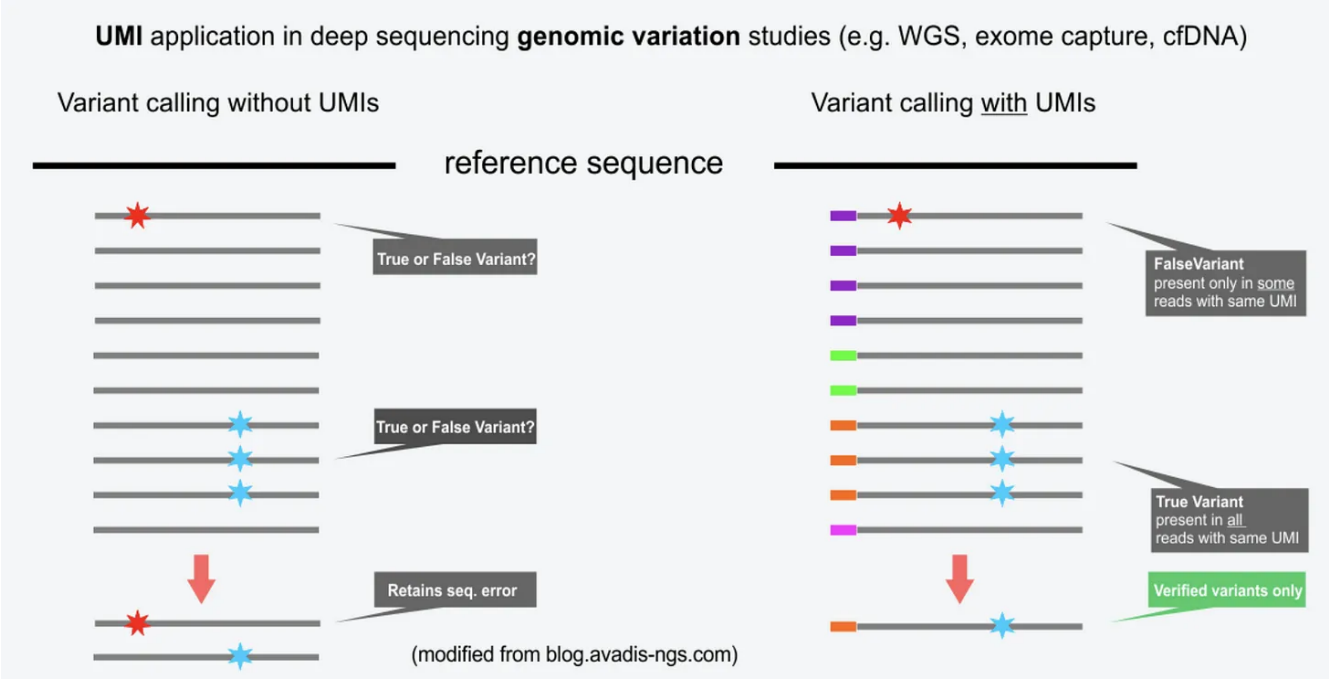


Figure 16. depiction of UMIs for error correcting reads.

This work was performed to establish a baseline sequence for the bivalent vaccines and was not designed with UMIs. Future work may explore this.

Discussion

Contamination of double stranded DNA (dsDNA) in these vaccines is a significant concern. The EMA specified dsDNA limits in this vaccines below 0.33% (330pg/mg). This is roughly 1 DNA molecule for 3,000 mRNA molecules. While the Moderna vaccines are meeting this specification, the Pfizer vaccines are 10 fold higher in contamination with 1 DNA molecule per 350 mRNAs. This is 1 replication competent plasmid per 350 mRNA molecules and equates to billions of antibiotic resistant plasmids injected per person per shot.

There are library construction artifacts that might skew the assessment of transcriptional error. m1Ψ is known to stall polymerases and induce higher rates of polymerase error. This may enable more efficient first strand synthesis from the plasmid DNA compared to the modified RNA. However, such high levels of polymerase inhibition would raise questions regarding the fidelity of the transcription using m1Ψ

The EMA had good reason to monitor the dsDNA levels in the vaccines. DsDNA injections can induce type I interferon responses via STING in mammals. If these dsDNAs are packaged into LNPs, they can transfect and transform both mammalian and bacterial cells in the patients microbiome. Its not clear how the EMA settled on their acceptable dsDNA contamination and if they had considered contaminating DNA that was capable of amplifying inside the host.

The vectors contain mammalian promoters, bacterial origins of replication in addition to the neomycin and kanamycin resistance genes. Circular plasmids like this are used for stable transfection and continued expression of genes in mammalian cells with the strong SV40 promoter. This could lead to prolonged spike expression in patients injected with these constructs. Bacteria transformed with these plasmids would replicate 50-300 copies of the plasmid per cell. It is not know if the bacteria would also

express the spike protein in these plasmids but the presence of T7 promoters in some of the vectors implies this is likely.

Patient use of neomycin or kanamycin after vaccination with these plasmids could enable the the selection of neomycin and kanamycin resistant bacteria in the gut microbiome. It is unclear if the spike protein in these expression vectors is expressed in bacteria. Nevertheless, inoculating billions of people with dual antibiotic resistance, high-copy number plasmids could be a step backwards in our fight against antibiotic resistance. Do these expression vectors transform the gut microbiome? How many copies of the mammalian plasmid expression vectors are replicated post vaccine transfection?

Arkmedic covers a biodistribution study that demonstrates LNP localization to the large intestine in 48hrs. Even if 1% of the LNPs localize to the intestines, bacteria could amplify these plasmids to far higher levels given the high copy origin of replication in the vectors. While LNPs are more effective at transfection of mammalian cell membranes than bacterial cell walls, most *E.coli* is readily transformed at 37C with naked DNA. Patients with fevers will improve the transformation efficiency.

Jessica Rose noted (personal communication) there are over 40 patient submission to VAERs with Neomycin or Kanamycin in their description. This number is hard to place into context as not all VAERs submissions include the antibiotic use of the victims.

In summary, there is a paucity of public information on the sequence fidelity and nucleic acid purity used in these vaccines. To our knowledge, this is the first deep sequencing of these products and the first time expression plasmids have been discovered in the vaccines. These are potent contaminants in the vaccines being administered to children. Billions of these contaminants per injection is likely an under estimate of their the entire burden as these plasmids can self replicate in bacterial hosts. Multiple studies have demonstrated prolonged vaccine mRNA clearance. This could be the result of the m1Ψ in the mRNA or the transfection or transformation of DNA based expression vectors. The introduction of billions of antibiotic resistance genes in high copy replication competent plasmids should evoke concerns over accelerating global antibiotic resistance.

Acknowledgements.

I'd like to thank Jessica Rose, Sabine Hazan, Jikkyleaks, @pathogenetics, Steve Massey, Valentine Bruttel , Lynn Fynn, Sasha Latypova and Pharmacoconuts for helpful comments on this topic. These data should be considered draft assemblies. Further work is required to split the Omicron and Wuhan-1 haplotypes and refine the estimates of linear to circular DNA that is present in the vaccines. Error rate analysis and background sequencing artifacts may be further refined through a community effort. Feel free to leave comments below.

Sequencing Data

Raw Illumina Reads

- Pfizer Bivalent Vial 1 Forward reads
- Pfizer Bivalent Vial 1 Reverse reads
- Pfizer Bivalent Vial 2 Forward reads
- Pfizer Bivalent Vial 2 Reverse reads
- Moderna Vial 1 Forward reads
- Moderna Vial 1 Reverse reads
- Moderna Vial 2 Forward reads
- Moderna Vial 2 Reverse reads

Read files are run through sha256 (Hash and stash) and etched onto the DASH blockchain. The SHA256 hash of the read file is spent into the OP_RETURN of an immutable ledger. If the hash of the file doesnt match the hash in these transactions, the file has been tampered with.

- Pfizer Vial 1 Forward hash
- Pfizer Vial 1 Reverse hash
- Pfizer Vial 2 Forward hash

- Pfizer Vial 2 Reverse hash
- Moderna Vial 1 Forward hash
- Moderna Vial 1 Reverse hash
- Moderna Vial 2 Forward hash
- Moderna Vial 2 Reverse hash

Megahit Assemblies

- Pfizer Vial 1
- Pfizer Vial 2
- Moderna Vial 1
- Moderna Vial 2

Illumina Reads mapped back to Megahit Assemblies

- Pfizer Vial 1 BAM File. Index File
- Pfizer Vial 2 BAM File. Index File
- Moderna Vial 1 BAM File. Index File
- Moderna Vial 2 BAM File. Index File

Q30 Filtered Illumina Reads (use these for transcriptional error rate estimates)

FastQ-Filter download: usage> fastq-filter -e 0.001 -o output.fastq input.fastq

- Pfizer bivalent Vial 1 Forward Reads
- Pfizer bivalent Vial 1 Reverse Reads
- Pfizer bivalent Vial 2 Forward Reads
- Pfizer bivalent Vial 2 Reverse Reads

- Moderna bivalent Vial 1 Forward Reads
- Moderna bivalent Vial 1 Reverse Reads
- Moderna bivalent Vial 2 Forward Reads
- Moderna bivalent Vial 2 Reverse Reads

Q30 BAM files. Q30 Reads mapped against Megahit assemblies

- Pfizer Vial 1 q30-BAM file. Index File
- Pfizer Vial 2 q30-BAM file. Index File
- Moderna Vial 1 q30-BAM file. Index File
- Moderna Vial 2 q30-BAM file. Index File

IGVtools error by base on q30 reads

Fields = Position in contig, Positive stand (+)A, +C, +G, +T, +N, +Deletion, +Insertion, Negative strand -A, -C, -G, -T, -N, -Deletion, -Insertion

Moderna Vial 1

Moderna Vial 2

Pfizer Vial 1

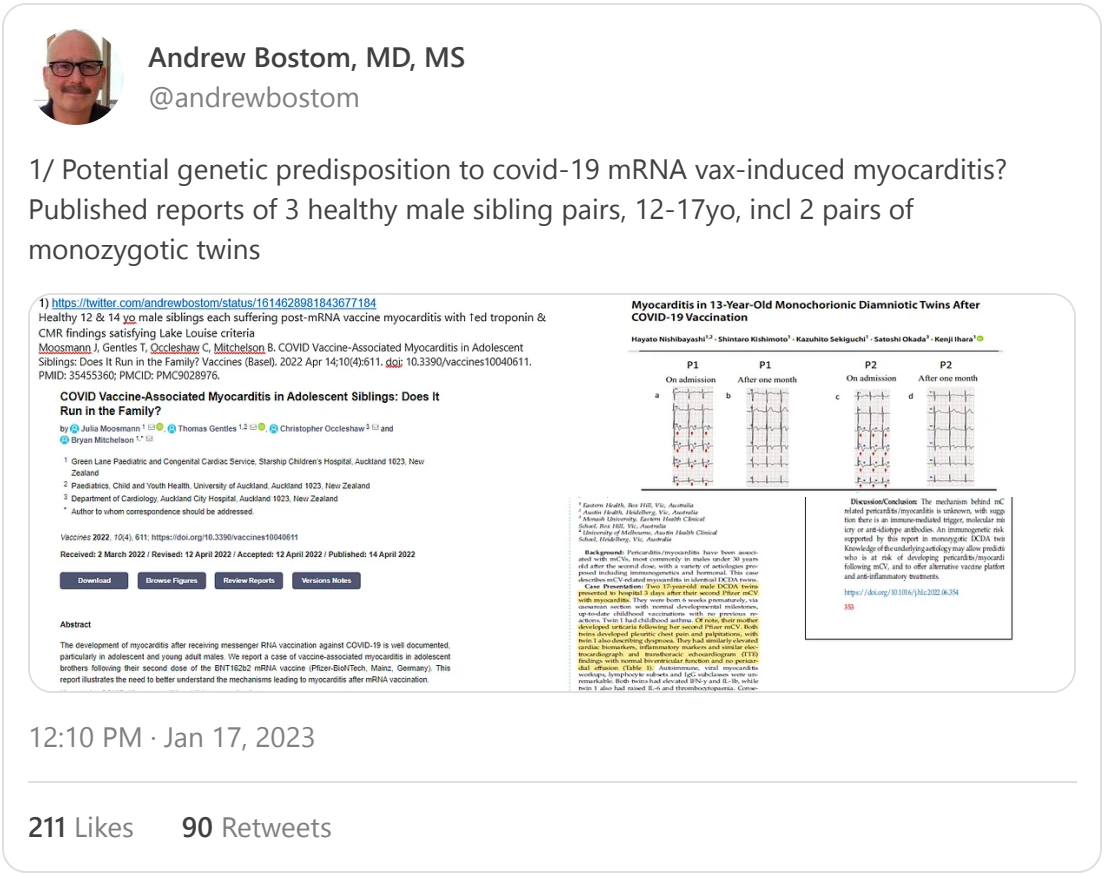
Pfizer Vial 2

Other references-

1. BNT-CMC-Peer-Reviewer
2. BNT162b2_EMA-Peer-Review
3. BNT162b2-Analytical_Methods_Transfer

4. BMJ_review
5. Rapporteur-Rolling-Review-Report 1
6. Rapporteur-Rolling-Review-Report 2
7. Rapporteur-Rolling-Review-Report 3
8. Other links from COVID_TRUTHS
9. Peer Review Comments

Andrew Bostom is a great follow on the C19 vs mRNA myocarditis debate-



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164 Comments

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Alan Richards Feb 16 Liked by Anandamide

You took quite a risk doing this work, tampering with government property. On behalf of humanity, thank you.

LIKE (72) REPLY SHARE

6 replies by Anandamide and others

Jessica Rose Writes Unconditional Jessica Feb 15 Liked by Anandamide

Gonna Substack this Substack. Man oh man.

LIKE (62) REPLY SHARE

162 more comments...