Supplementary information

Systematic analysis of COVID-19 mRNA vaccines using four orthogonal approaches demonstrates no excessive DNA impurities

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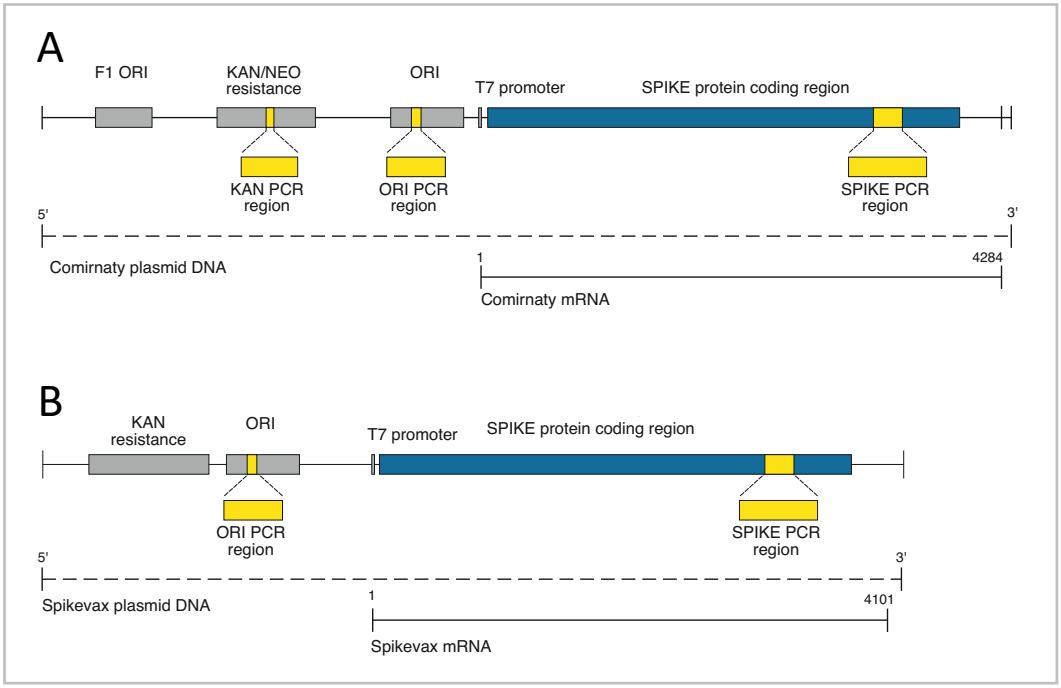


Figure S1. | Schematic illustrations of DNA templates used for production of mRNA for Comirnaty and Spikevax vaccines with indicated position of regions amplified in qPCR.
Template structures were derived from the sequences obtained in this study and were used for the design of primers and probes.

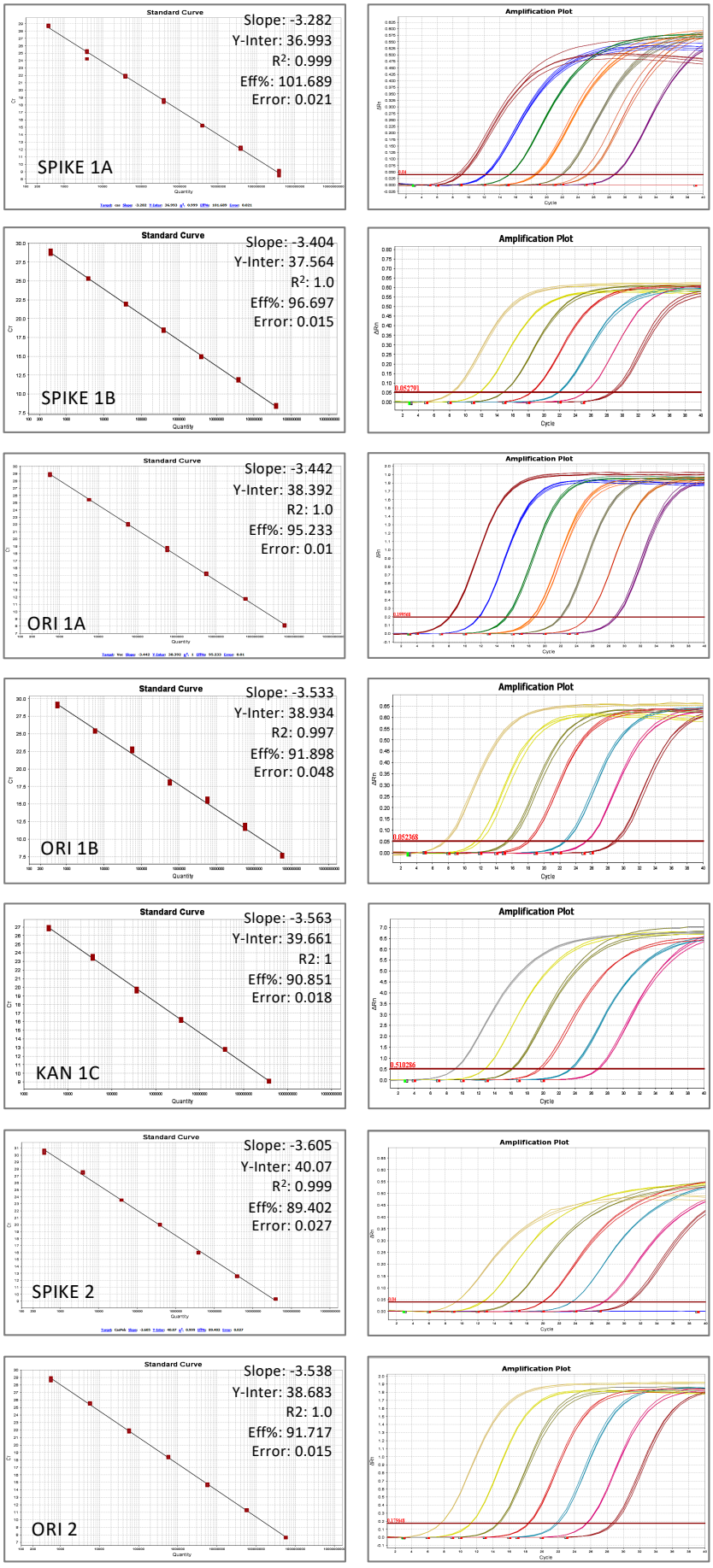


Figure S2. | Calibration graphs and amplification curves for assessing qPCR efficiency of the primers used in the vaccine analysis and ensuring reliable quantification of target regions.

Calibration graphs were generated from serial tenfold dilutions of known concentrations of synthetic DNA standards (from 100 pg to 0.1 fg) corresponding to individual PCR amplicons.

Inlets in calibration graphs indicate parameters of each designed qPCR assay. Amplification curves show the progress of qPCR reactions.

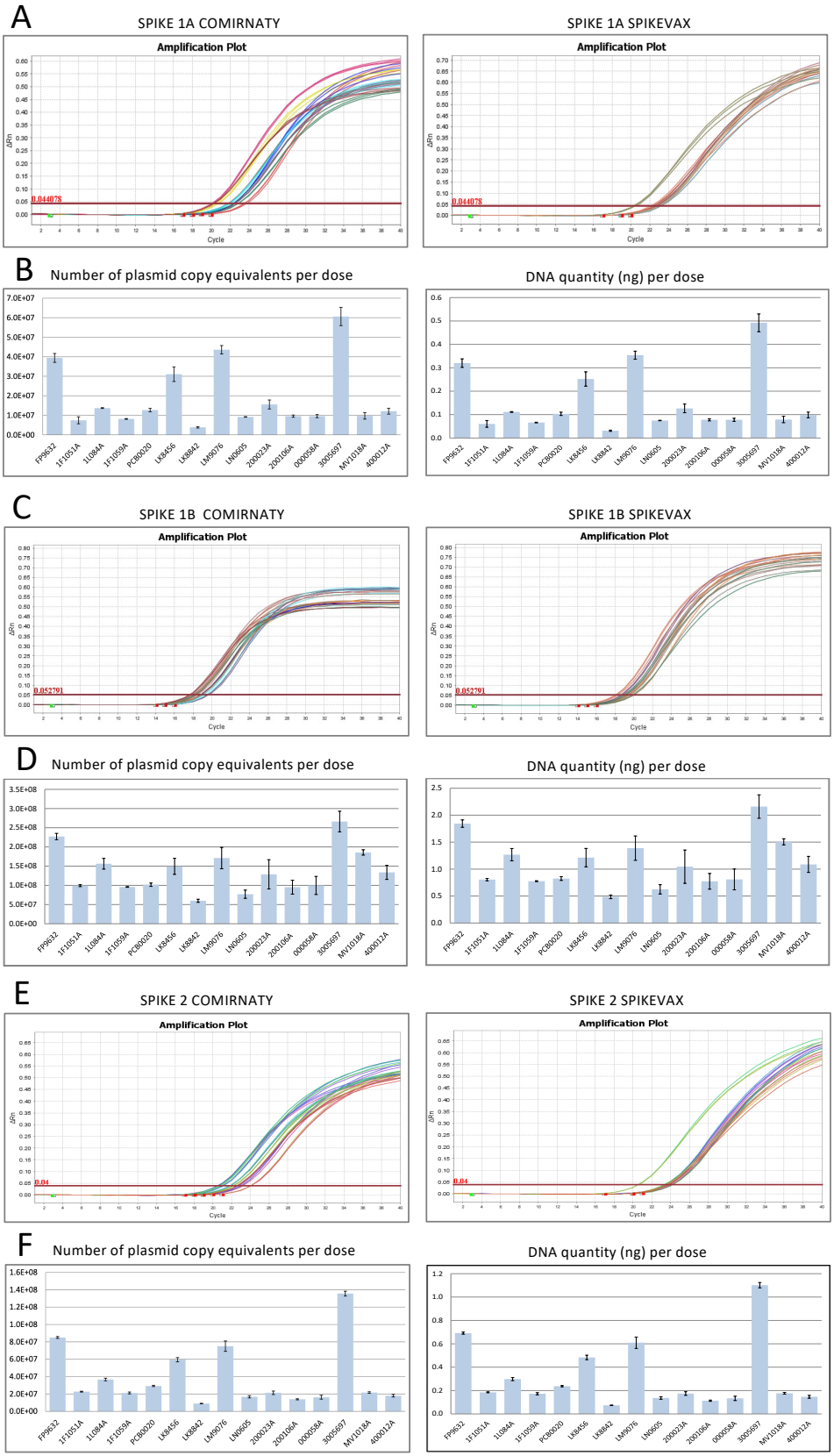


Figure S3. | Analysis of all vaccine batches by qPCR SPIKE assays.
(A, C, E) Amplification curves of the 3' end of S protein coding sequence using SPIKE 1A, SPIKE 1B and SPIKE 2 primers. (B, D, F) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose.

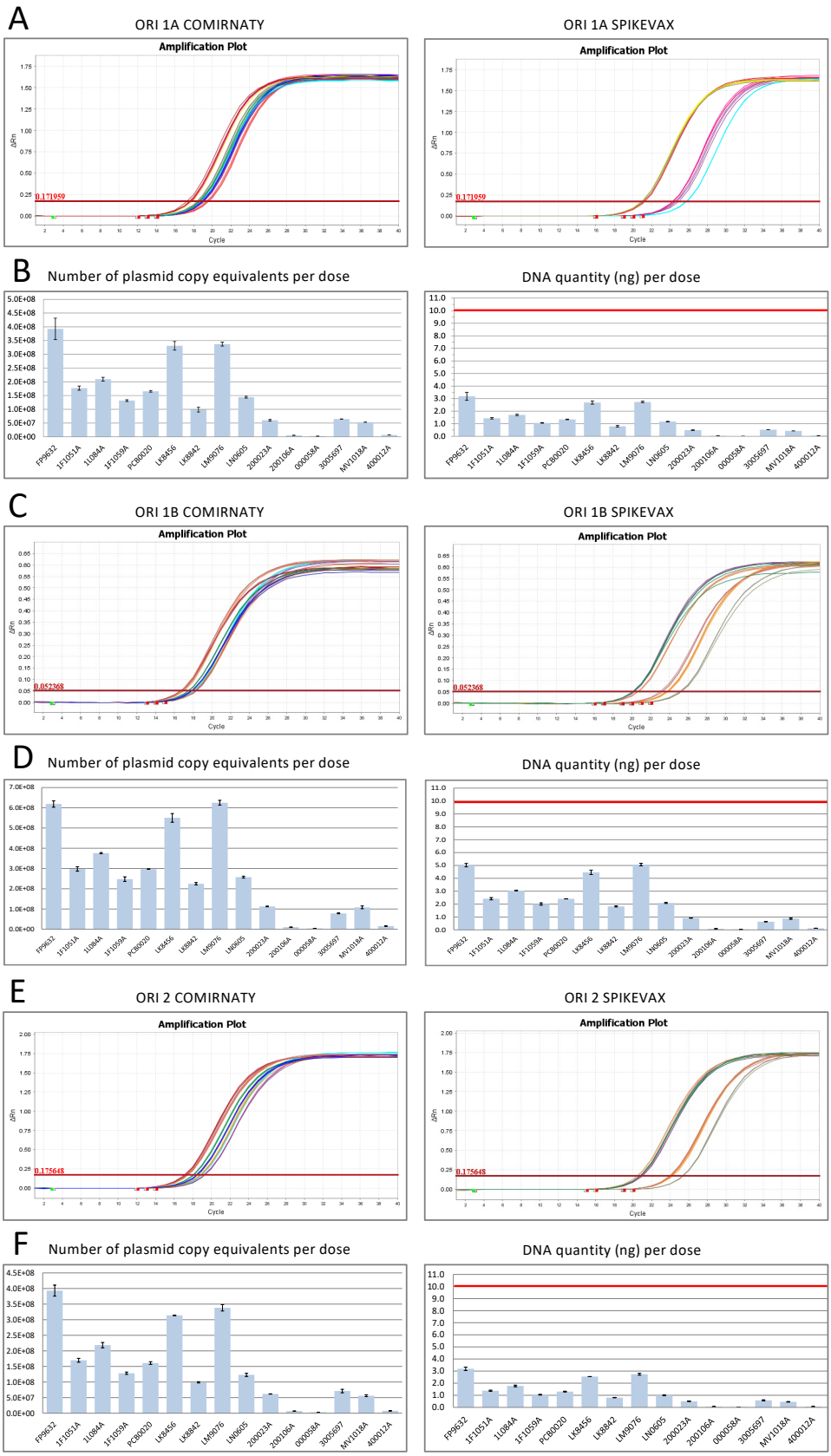


Figure S4. | Analysis of all vaccine batches by qPCR ORI assays.
(A, C, E) Amplification curves of the region containing origin of plasmid replication using ORI 1A, ORI 1B and ORI 2 primers. (B, D, F) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose. Red lines in (B, D, F) indicate a limit of 10 ng/dose.

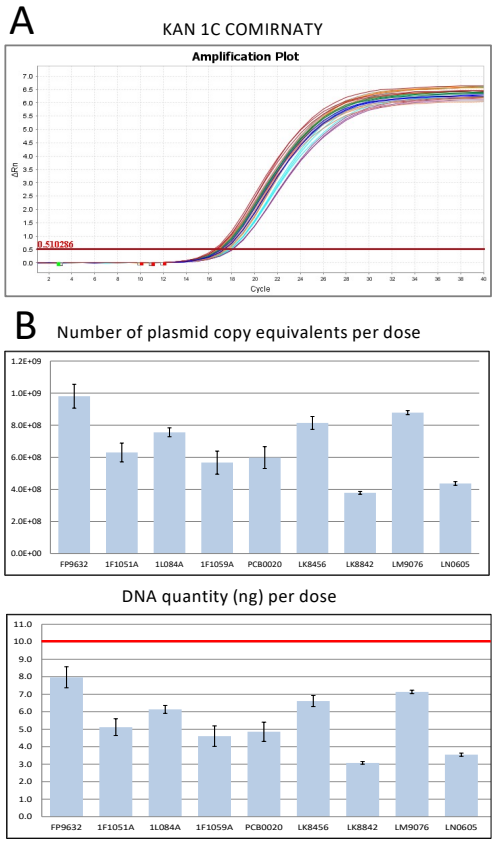
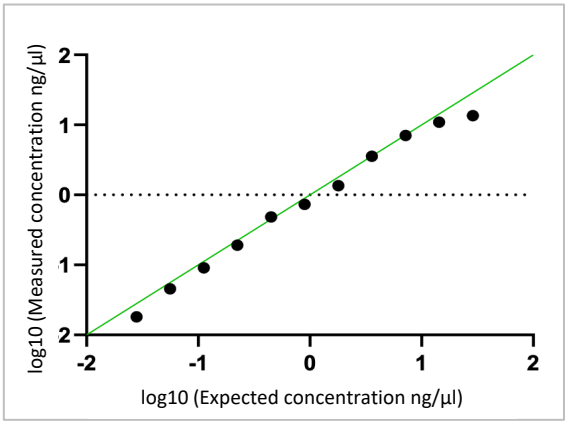


Figure S5. | Analysis of Comirnaty vaccine batches by qPCR KAN assay.
(A) Amplification curves of the region that confers resistance to kanamycin using KAN 1C primers. (B) Graphs illustrating calculated numbers of plasmid copy equivalents and their conversion to quantity of residual DNA in nanograms per vaccine dose. Red line in (B) indicates a limit of 10 ng/dose.

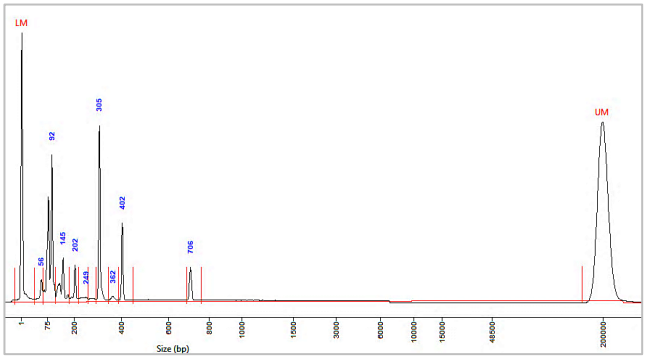
A

Dilution	Expected concentration	Measured concentration
10x	28,7	13,5181
20x	14,35	10,9040
40x	7,175	7,0478
80x	3,5875	3,5657
160x	1,79375	1,3567
320x	0,896875	0,7333
640x	0,4484375	0,4835
1280x	0,22421875	0,1919
2560x	0,112109375	0,0907
5120x	0,0560546875	0,0456
10240x	0,02802734375	0,0181

B



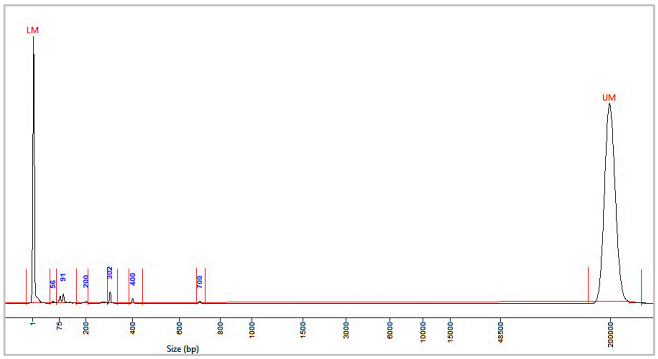
C



D

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0147		7,4072	0	38	3	234,65
2	56	0,0211	4,9	0,6406	38	61	54	10,68
3	92	0,1644	37,9	3,2513	61	110	83	12,74
4	145	0,0527	12,2	0,6233	110	177	139	10,81
5	202	0,0237	5,5	0,1947	177	218	200	4,08
6	249	0,0103	2,4	0,0709	218	260	238	4,90
7	305	0,0906	20,9	0,4857	293	344	307	2,08
8	362	0,0079	1,8	0,0355	344	390	365	3,09
9	402	0,0409	9,4	0,1664	390	449	404	2,21
10	706	0,0217	5,0	0,0504	688	758	709	1,72
11	200000 (UM	0,0230		0,0002	172225	265364	201166	3,81
	TIC:	0,4333	ng/uL					
	TIM:	5,5187	nmole/L					
	Total conce	0,4835	ng/uL					

E



F

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0147		19,1651	0	48	1	232,36
2	56	0,0008	4,7	0,0235	48	67	57	5,01
3	91	0,0092	52,6	0,1795	67	155	84	12,68
4	200	0,0006	3,4	0,0049	155	211	200	0,92
5	302	0,0049	27,9	0,0265	293	335	303	0,84
6	400	0,0016	9,4	0,0068	386	443	400	0,55
7	700	0,0004	2,0	0,0008	683	727	700	0,34
8	200000 (UM	0,0279		0,0002	170542	244278	200813	3,50
	TIC:	0,0175	ng/uL					
	TIM:	0,2420	nmole/L					
	Total conce	0,0181	ng/uL					

Figure S6. | Quantification of control ladder DNA by capillary electrophoresis on Fragment Analyser System 5200 using DNF-464 HS DNA Fragment Kit.

(A) List of control DNA dilutions, expected concentrations and actually measured concentrations. (B) Graphical comparison of expected and measured values. (C) Densitogram resulting from the fragment analysis of ladder DNA diluted 640-fold (LM – lower marker of 1 bp, UM – upper marker of 20,000 bp) and (D) table with corresponding quantitative values for each size marker of the ladder DNA. (E) Densitogram of control ladder DNA diluted 10,240-fold and (F) corresponding table with quantitative values for measurable size markers.

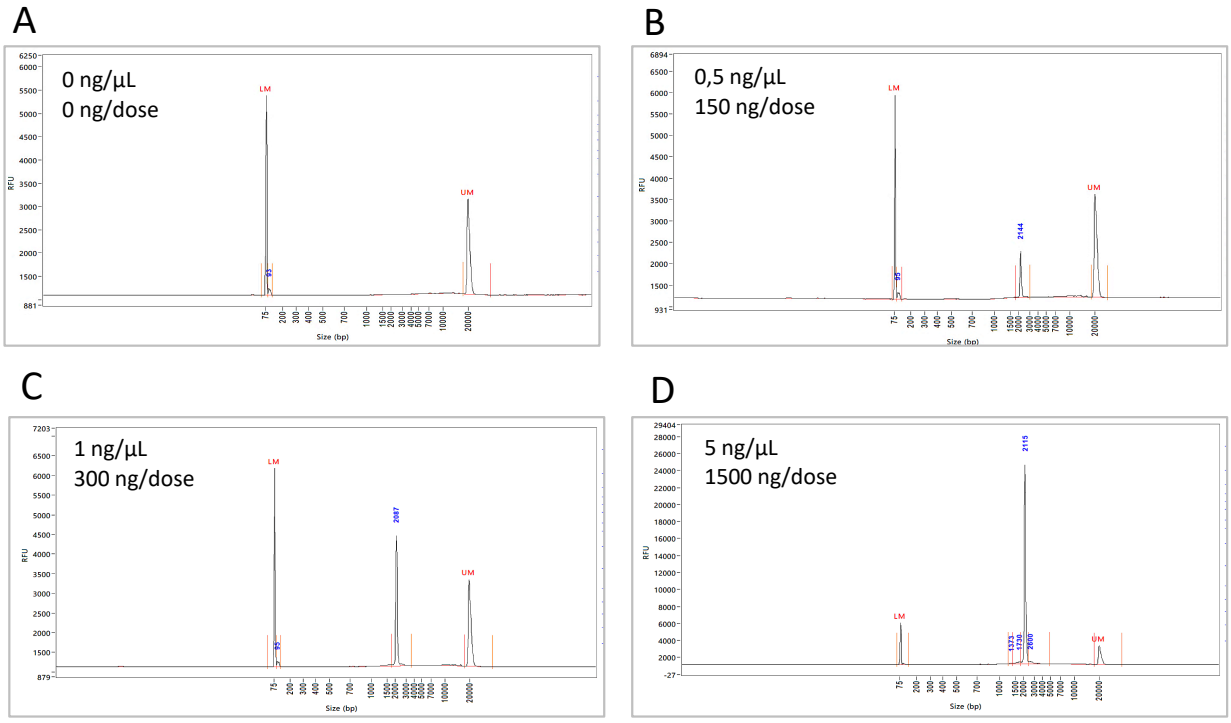


Figure S7. | Visualisation of spiked external DNA fragment added to negative control.
Capillary electrophoresis was performed on Fragment Analyzer System 5200 using DNF-930 Large Fragment DNA Kit. (A) Blank, negative control with no added DNA (LM depict 75 bp, UM depicts 20,000 bp). (B – D) Samples with increasing quantities of externally added DNA fragment. DNA signal was detected as a single peak of 2,100 bp with the height corresponding to the amount of added DNA.

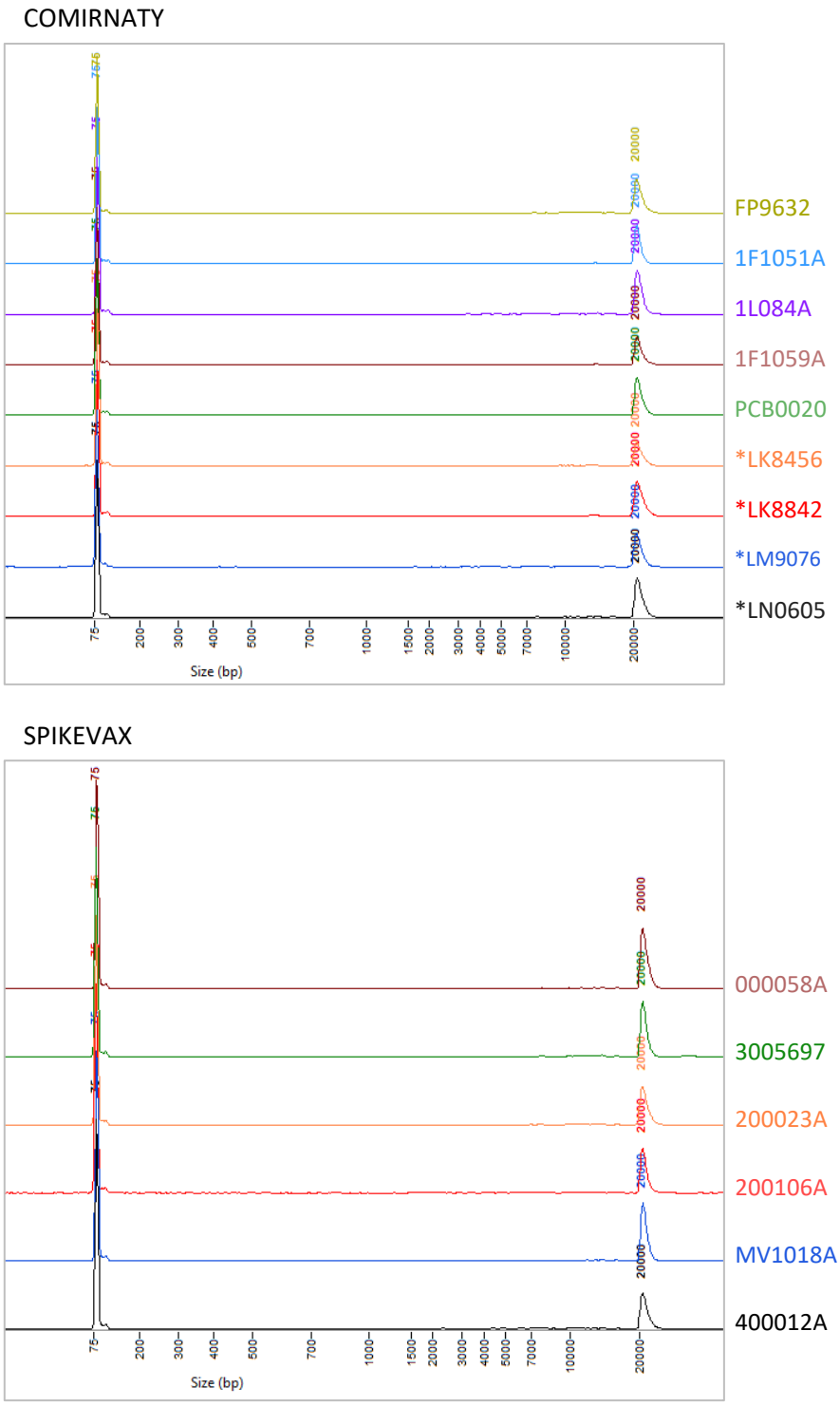
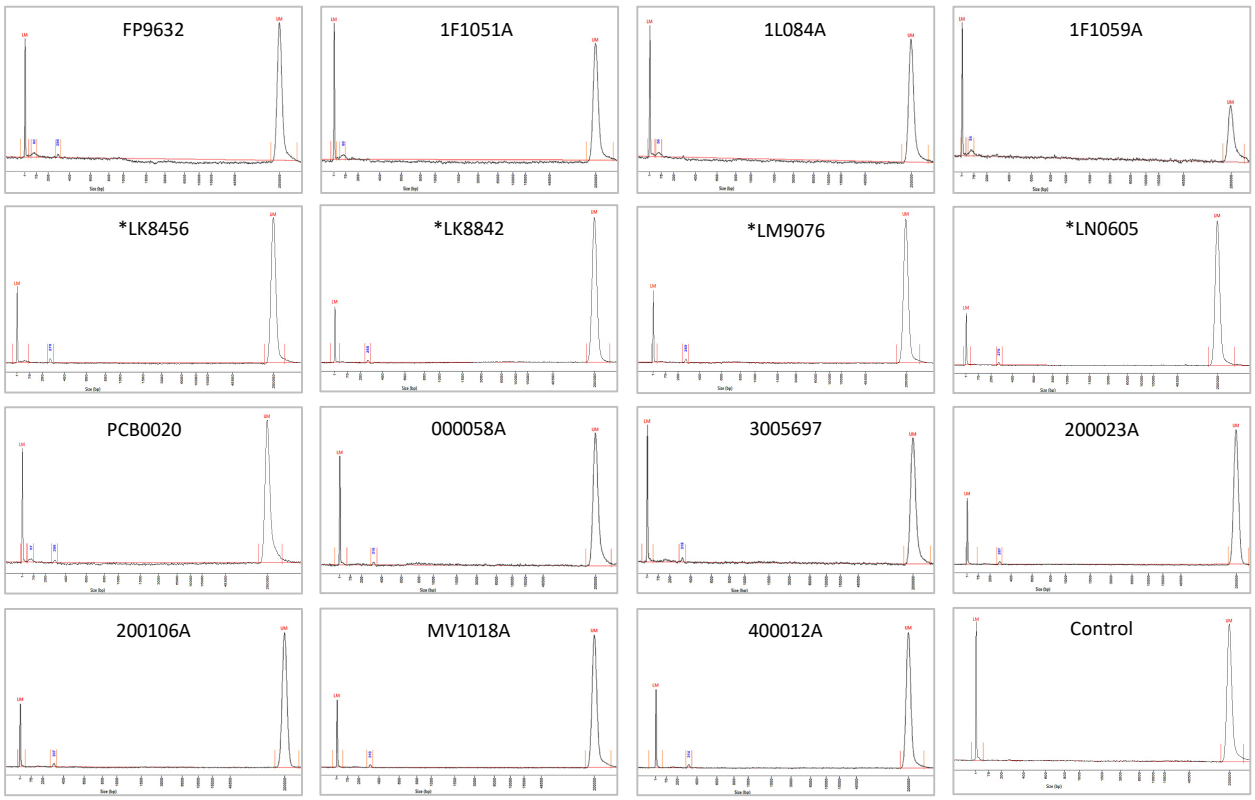


Figure S8. | Fragment analysis of Comirnaty and Spikevax vaccines using the DNF-930 dsDNA 930 Reagent Kit. Representative densitograms of all batches show no specific peaks indicating absence of DNA quantities within the detection range of the kit.

A



B

FP9632

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0155		20,2359	0	24	1	307,27
2	60	0,0190	85,6	0,5359	41	76	58	14,72
3	286	0,0032	14,4	0,0181	264	315	290	1,53
4	200000 (UM	0,0471		0,0004	171652	259997	202049	4,57
	TIC:	0,0222	ng/uL					
	TIM:	0,5541	nmole/L					
	Total conce	0,0243	ng/uL					

1F1051A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0155		20,2359	0	17	1	249,32
2	60	0,0165	100,0	0,4757	36	71	57	14,52
3	200000 (UM	0,0363		0,0003	170258	258136	202245	4,62
	TIC:	0,0165	ng/uL					
	TIM:	0,4757	nmole/L					
	Total conce	0,0184	ng/uL					

1L084A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0155		11,2818	0	35	2	185,97
2	200000 (UM	0,0386		0,0003	169793	256741	202575	5,03
	TIC:	0,0000	ng/uL					
	TIM:	0,0000	nmole/L					
	Total conce	0,0144	ng/uL					

1F1059A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0155		20,2359	0	26	1	212,35
2	54	0,0214	100,0	0,6153	38	73	57	13,96
3	200000 (UM	0,0194		0,0002	174440	244183	204254	5,69
	TIC:	0,0214	ng/uL					
	TIM:	0,6153	nmole/L					
	Total conce	0,0236	ng/uL					

*LK8456

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0165		5,1782	0	69	5	275,42
2	270	0,0138	100,0	0,0835	248	300	271	2,10
3	200000 (UM	0,0742		0,0006	167574	242504	201393	4,81
	TIC:	0,0138	ng/uL					
	TIM:	0,0835	nmole/L					
	Total conce	0,0146	ng/uL					

*LK8842

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0165		21,6176	0	28	1	186,75
2	265	0,0074	100,0	0,0461	240	293	266	1,54
3	200000 (UM	0,1179		0,0010	172358	257382	201347	4,60
	TIC:	0,0074	ng/uL					
	TIM:	0,0461	nmole/L					
	Total conce	0,0088	ng/uL					

*LM9076

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0165		21,6176	0	25	1	180,45
2	269	0,0113	100,0	0,0688	242	294	269	1,90
3	200000 (UM	0,0900		0,0007	164916	252600	201387	5,13
	TIC:	0,0113	ng/uL					
	TIM:	0,0688	nmole/L					
	Total conce	0,0160	ng/uL					

*LN0605

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0165		12,0521	0	27	2	201,8
2	270	0,0128	100,0	0,0777	249	303	270	1,99
3	200000 (UM	0,1204		0,0010	166511	263226	201643	5,36
	TIC:	0,0128	ng/uL					
	TIM:	0,0777	nmole/L					
	Total conce	0,0200	ng/uL					

PCB0020

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0159		20,8147	0	30	1	172,32
2	57	0,0100	95,7	0,3095	36	71	53	13,31
3	286	0,0005	4,3	0,0026	262	316	287	0,82
4	200000 (UM	0,0593		0,0005	167894	254148	204189	5,88
	TIC:	0,0105	ng/uL					
	TIM:	0,3121	nmole/L					
	Total conce	0,0105	ng/uL					

000058A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		20,0093	0	51	1	367,94
2	316	0,0021	100,0	0,0107	293	354	319	1,23
3	200000 (UM	0,0516		0,0004	172282	246062	202479	4,45
	TIC:	0,0021	ng/uL					
	TIM:	0,0107	nmole/L					
	Total conce	0,0046	ng/uL					

3005697

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		11,1555	0	39	2	149,37
2	318	0,0039	100,0	0,0200	289	350	319	1,30
3	200000 (UM	0,0365		0,0003	174560	249107	201734	4,04
	TIC:	0,0039	ng/uL					
	TIM:	0,0200	nmole/L					
	Total conce	0,0073	ng/uL					

200023A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		20,0093	0	69	1	204,73
2	287	0,0116	100,0	0,0659	259	312	289	2,15
3	200000 (UM	0,0782		0,0006	178357	234260	200145	3,05
	TIC:	0,0116	ng/uL					
	TIM:	0,0659	nmole/L					
	Total conce	0,0130	ng/uL					

200106A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		20,0093	0	36	1	342,86
2	307	0,0142	100,0	0,0763	273	332	305	2,07
3	200000 (UM	0,0841		0,0007	173421	239971	200505	3,22
	TIC:	0,0142	ng/uL					
	TIM:	0,0763	nmole/L					
	Total conce	0,0154	ng/uL					

MV1018A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		11,1555	0	43	2	155,57
2	310	0,0095	100,0	0,0501	278	339	311	1,95
3	200000 (UM	0,0821		0,0007	173801	248346	202089	4,60
	TIC:	0,0095	ng/uL					
	TIM:	0,0501	nmole/L					
	Total conce	0,0095	ng/uL					

400012A

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0153		11,1555	0	48	2	146,89
2	314	0,0126	100,0	0,0661	285	346	313	2,39
3	200000 (UM	0,0690		0,0006	176459	246062	201745	4,20
	TIC:	0,0126	ng/uL					
	TIM:	0,0661	nmole/L					
	Total conce	0,0173	ng/uL					

Control

	Size (bp)	ng/uL	% (Conc.) (ng/uL)	nmole/L	From (bp)	To (bp)	Avg. Size	CV%
1	1 (LM)	0,0168		21,8901	0	42	1	266,13
2	200000 (UM	0,0418		0,0003	168252	252290	202637	5,71
	TIC:	0,0000	ng/uL					
	TIM:	0,0000	nmole/L					
	Total conce	0,0026	ng/uL					

Figure S9. | Densitograms and corresponding quantitative data from the fragment analysis of the batches of Comirnaty and Spikevax vaccines on Fragment Analyzer 5200 using DNF-464 HS DNA Fragment Kit. Measurements were accomplished with 3 biological replicated (3 vials for each batch) and 2-3 technical replicates (measurements of each biological replicate). (A) Representative densitograms from each analysed vaccine batch. (B) Corresponding quantitative assessment of the total DNA concentrations per 1 µl of vaccine sample).

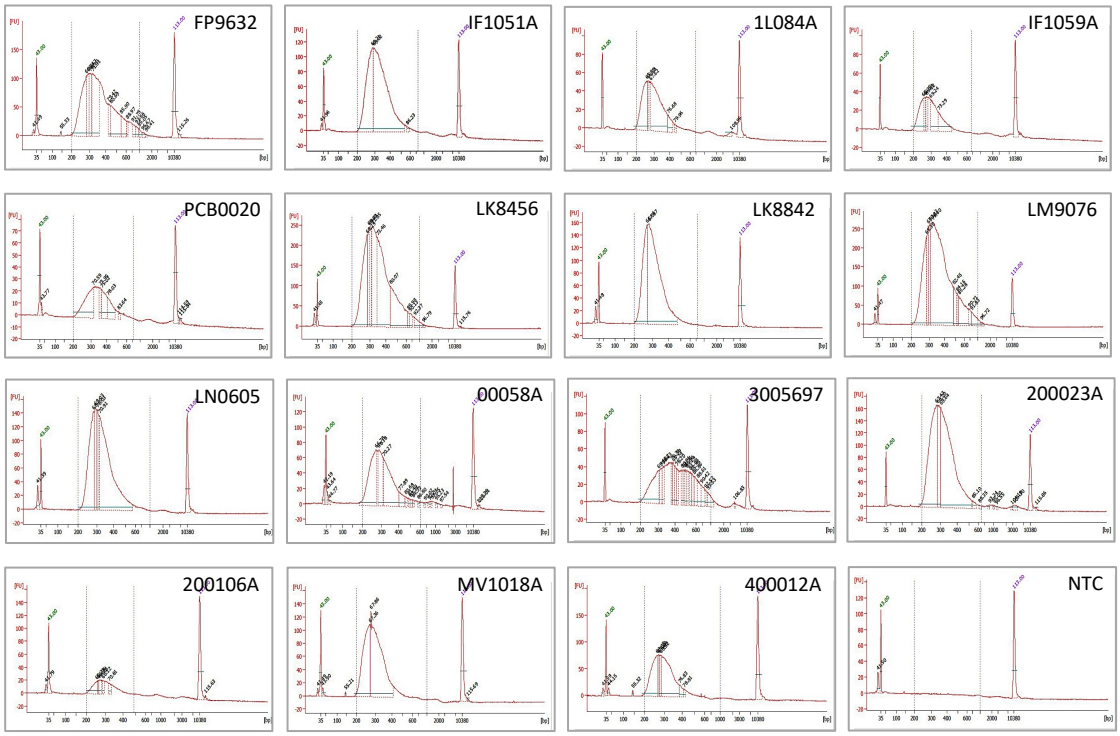


Figure S10. | Fragment lengths profiles of sequencing libraries prepared using TruSeq DNA Nano kit according to modified protocol from the analysed vaccine batches.
Fragment analysis was performed on Agilent Bioanalyzer 2100 using High Sensitivity DNA kit with detection range of 100 pg/µl for the fragmented DNA.