

Quantitative Multiplex Real-Time PCR analysis of Moderna (Spikevax) and Pfizer (BNT162b2) vaccines

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08.03.2025



Quantitative Multiplex Real – Time PCR

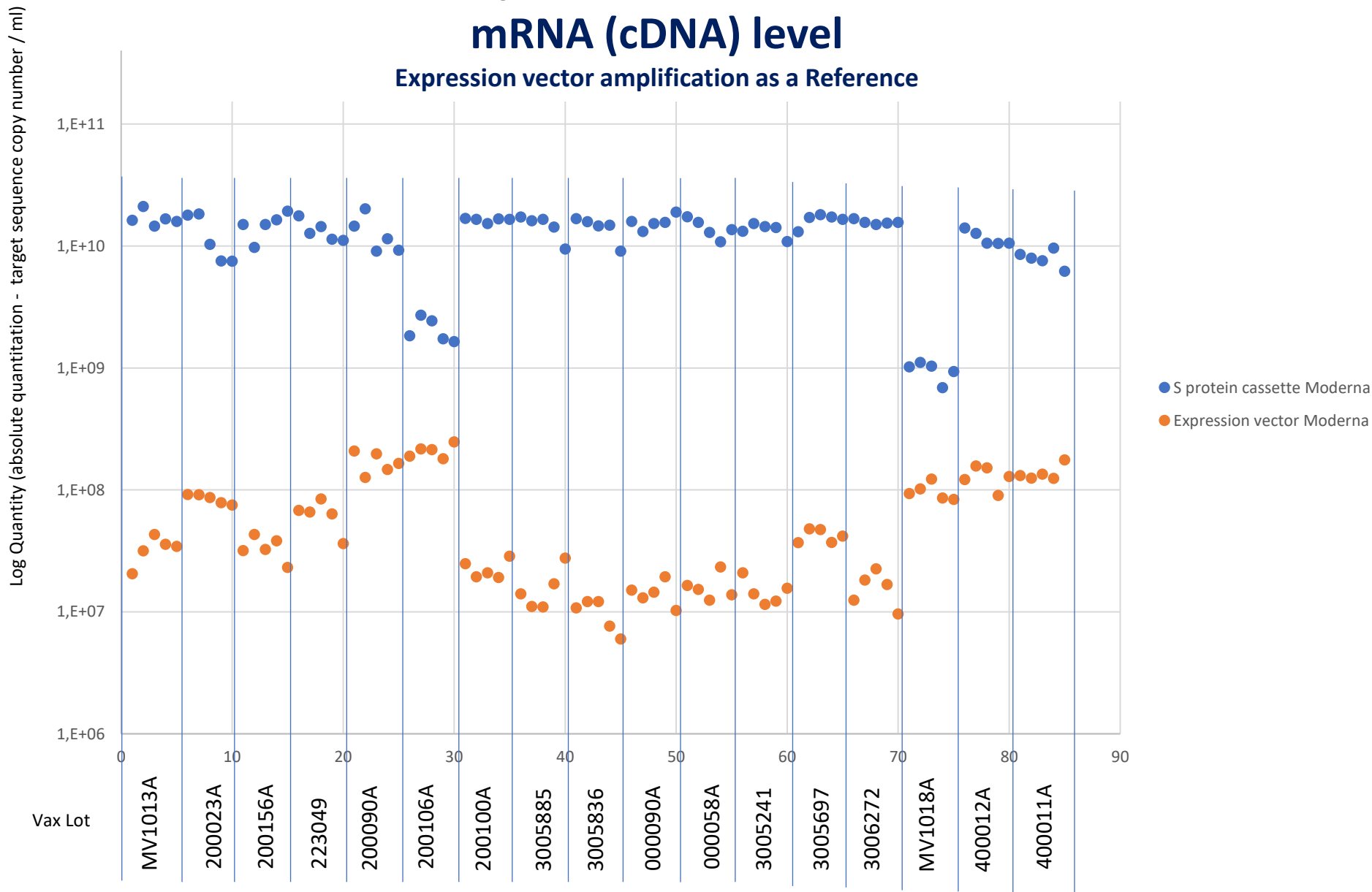
- Pfizer bivalent expression vector BNT162b2, complete sequence, circular (Ref: OR134577)
- Synthetic construct HCV1146 Moderna (mRNA-1273) SARS-CoV-2 vaccine sequence (Ref: OK120841)
- Analysis both on mRNA (cDNA) and DNA levels
- Oligonucleotides (primers and fluorescently labeled hybridization probes) obtained from Eurofins Genomics, DE
- Multiplex Quantitative Real-Time PCR (FAM – Pfizer S protein cassette, Cy5 – expression vector, ROX – Moderna S protein cassette, HEX – Escherichia coli genomic DNA)
- DNA / RNA isolation – Qiagen DNA mini kit, Qiagen, DE
- Reverse transcription - Verso cDNA kit, ThermoFisher Scientific, USA
- Rotorgene Q, 5-Plex, Qiagen, DE
- Absolute quantitation – target sequence copy number / 1 ml
- Calibration curve equation: $=10^{(-0.257 \cdot Ct + 11.897)}$
- Vaccines stored at -80°C, fresh, previously unopened lots analyzed
- Moderna Spikevax: 17 lots (5 ampules / each lot)
- Pfizer BNT162b2: 7 lots (5 ampules / each lot)

VaXX_Plex – Multiplex Real Time PCR test – primers and probes used:

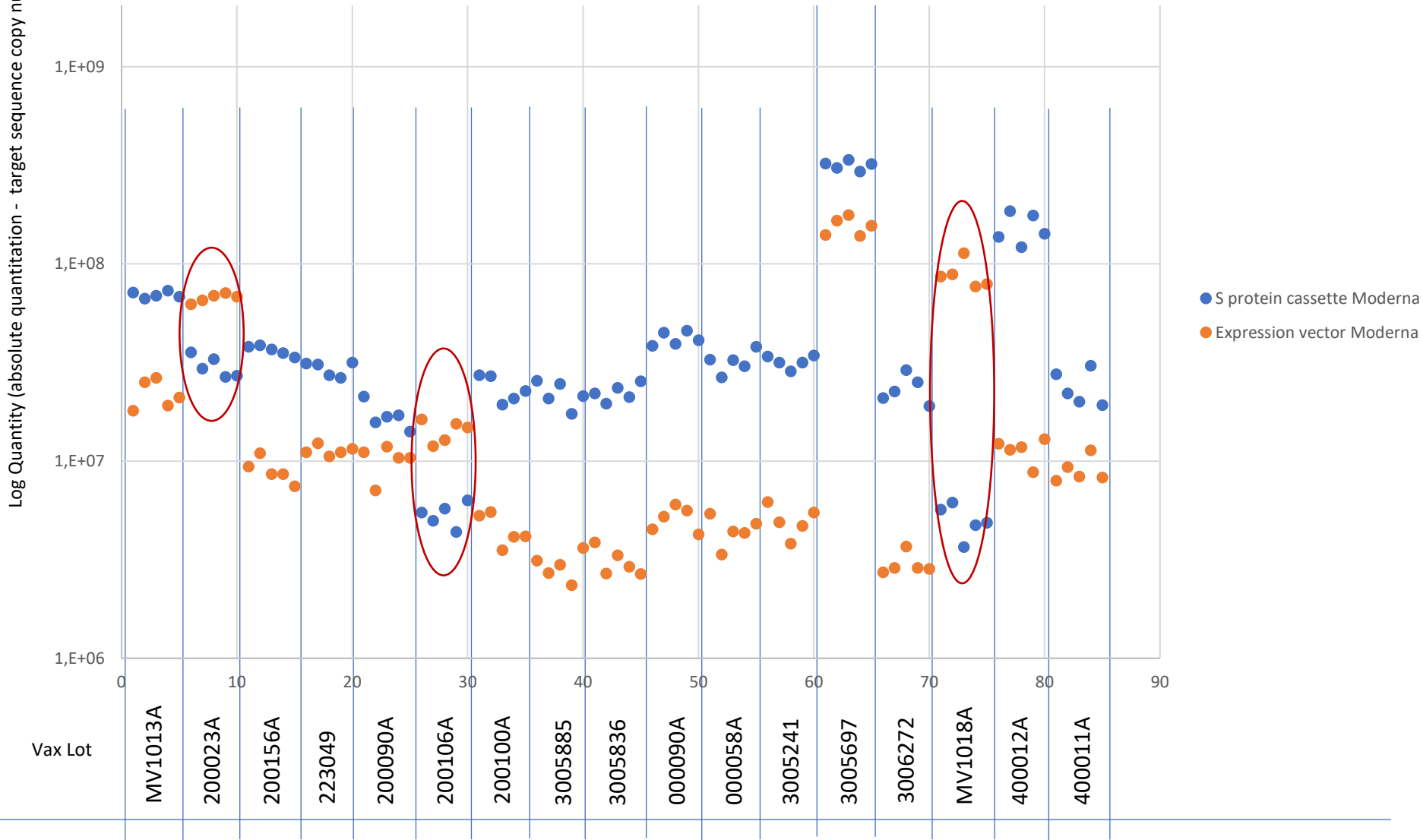
| VaXX_Plex | 100 uM (ul) | Sequence of the oligo 5'-3' |
|------------------------|-------------|--|
| PfiMo common F | 0,04 | tgcggcaagggtaccacctgatgag |
| PfiMo common R | 0,05 | ggtgttgcggtggtgatgatctgg |
| VV_Ori_F | 0,05 | ctacatacctcgtctgctaactc |
| VV_Ori_R | 0,05 | gcgcttatccggtactatc |
| EcolilTS-F03 | 0,05 | CACTCAGGCCTACCAAATTTGCA |
| EcolilTS-R02 | 0,05 | TCGCAGTGAACCTTTGCAGGTAC |
| EcolilTSProb_15 | 0,04 | HEX - CGCATAGCTCCACCATCTCTGTAGTG - BHQ1 |
| Mo Probe | 0,04 | ROX - tccccagagcgcacccccaggagtgtgtt - BHQ2 |
| VV_Ori_Probe | 0,04 | Cy5 - tgctgccagtggcgataagtctgttct - BHQ2 |
| Pfi Probe | 0,04 | FAM - ccctcagtctgccctcaggcgtgtgtt - BHQ1 |
| H2O | 0,15 | |
| celkem na jednu reakci | 0,6 | |

Moderna Spikevax - S Protein Cassette mRNA (cDNA) level

Expression vector amplification as a Reference

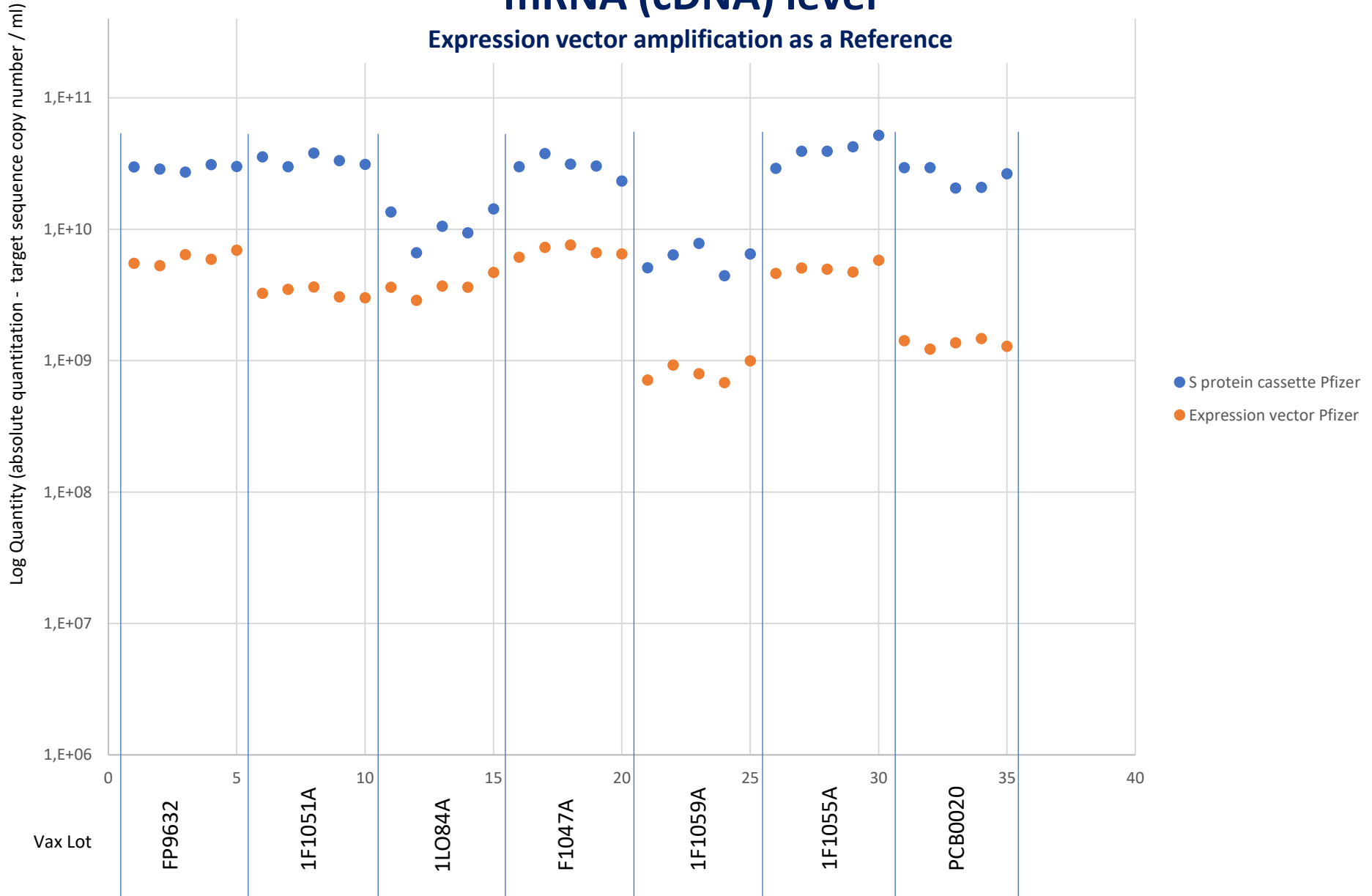


Moderna Spikevax - S Protein Cassette vs Expression Vector DNA level

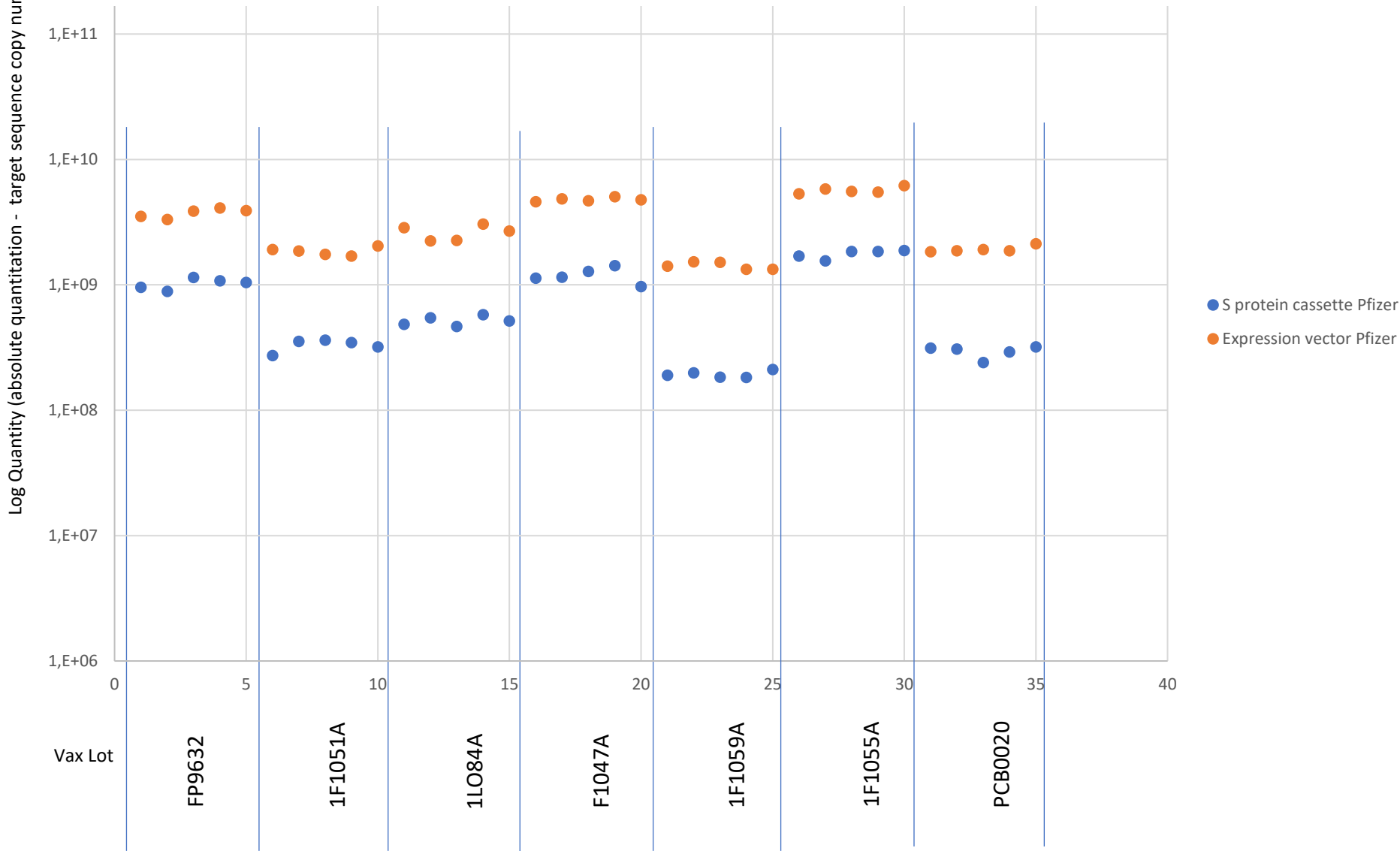


Pfizer BNT162b2 - S Protein Cassette mRNA (cDNA) level

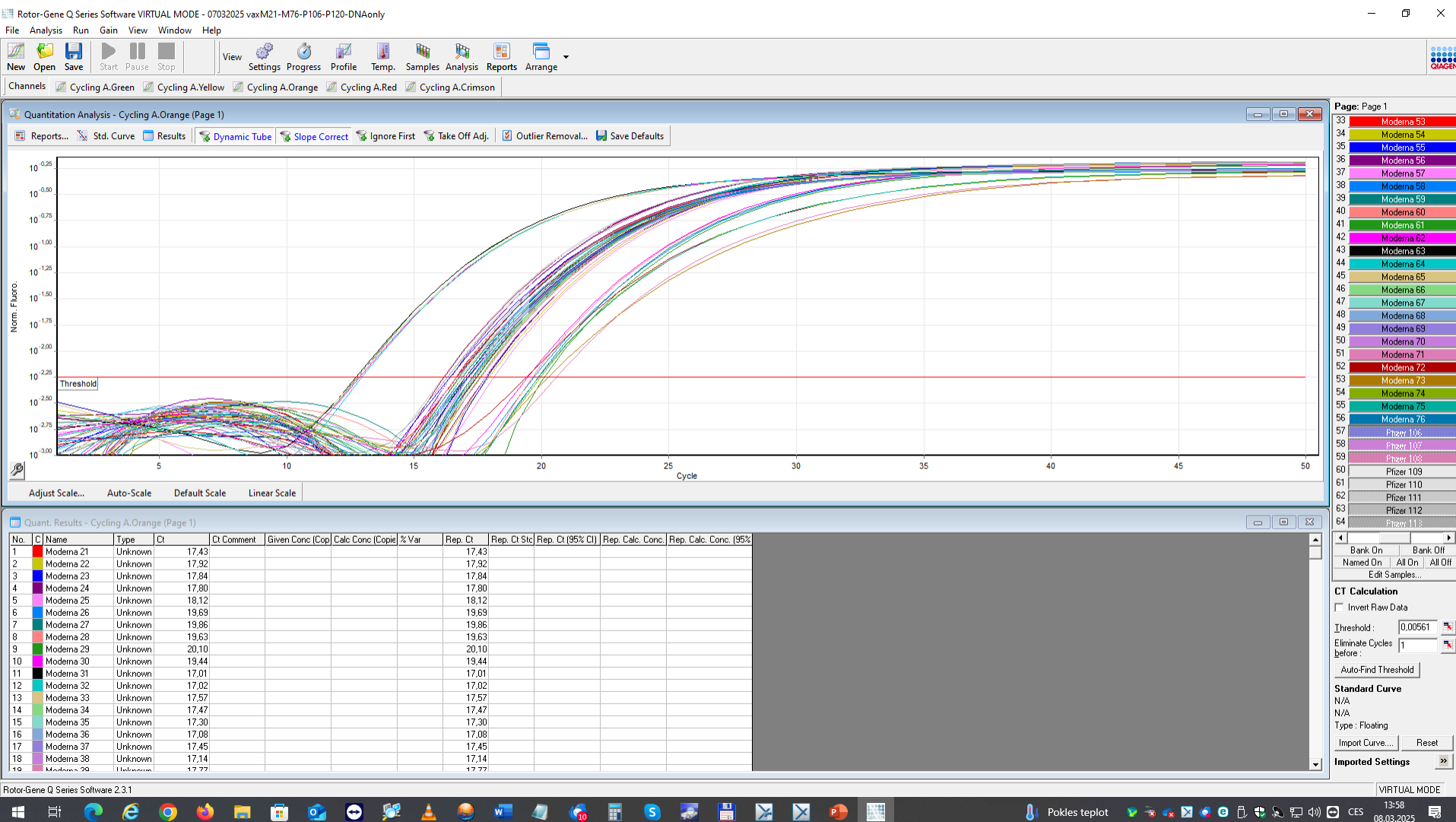
Expression vector amplification as a Reference



Pfizer BNT162b2 - S Protein Cassette vs Expression Vector DNA level



Moderna Spikevax - S Protein cassette - ROX DNA level



Moderna Spikevax - Expression Vector – Cy5 DNA level

Rotor-Gene Q Series Software VIRTUAL MODE - 07032025 vaxM21-M76-P106-P120-DNAonly

File Analysis Run Gain View Window Help

New Open Save Start Pause Stop View Settings Progress Profile Temp. Samples Analysis Reports Arrange

Channels Cycling A.Green Cycling A.Yellow Cycling A.Orange Cycling A.Red Cycling A.Crimson

Quantitation Analysis - Cycling A.Red (Page 1)

Reports... Std. Curve Results Dynamic Tube Slope Correct Ignore First Take Off Adj. Outlier Removal... Save Defaults

Adjust Scale... Auto-Scale Default Scale Linear Scale

Quant. Results - Cycling A.Red (Page 1)

| No. | C | Name | Type | Ct | Ct Comment | Given Conc. [Cop] | Calc Conc [Cop] | % Var | Rep. Ct | Rep. Ct Stc | Rep. Ct (95% CI) | Rep. Calc. Conc. | Rep. Calc. Conc. [95%] |
|-----|---|------------|---------|-------|------------|-------------------|-----------------|-------|---------|-------------|------------------|------------------|------------------------|
| 1 | | Moderna 21 | Unknown | 19.59 | | | | | 19.59 | | | | |
| 2 | | Moderna 22 | Unknown | 20.38 | | | | | 20.38 | | | | |
| 3 | | Moderna 23 | Unknown | 19.56 | | | | | 19.56 | | | | |
| 4 | | Moderna 24 | Unknown | 19.76 | | | | | 19.76 | | | | |
| 5 | | Moderna 25 | Unknown | 19.78 | | | | | 19.78 | | | | |
| 6 | | Moderna 26 | Unknown | 19.00 | | | | | 19.00 | | | | |
| 7 | | Moderna 27 | Unknown | 19.49 | | | | | 19.49 | | | | |
| 8 | | Moderna 28 | Unknown | 19.37 | | | | | 19.37 | | | | |
| 9 | | Moderna 29 | Unknown | 19.02 | | | | | 19.02 | | | | |
| 10 | | Moderna 30 | Unknown | 19.09 | | | | | 19.09 | | | | |
| 11 | | Moderna 31 | Unknown | 20.84 | | | | | 20.84 | | | | |
| 12 | | Moderna 32 | Unknown | 20.77 | | | | | 20.77 | | | | |
| 13 | | Moderna 33 | Unknown | 21.47 | | | | | 21.47 | | | | |
| 14 | | Moderna 34 | Unknown | 21.24 | | | | | 21.24 | | | | |
| 15 | | Moderna 35 | Unknown | 21.18 | | | | | 21.18 | | | | |
| 16 | | Moderna 36 | Unknown | 21.71 | | | | | 21.71 | | | | |
| 17 | | Moderna 37 | Unknown | 21.98 | | | | | 21.98 | | | | |
| 18 | | Moderna 38 | Unknown | 21.88 | | | | | 21.88 | | | | |
| 19 | | Moderna 39 | Unknown | 22.14 | | | | | 22.14 | | | | |

Page: Page 1

- 33 Moderna 53
- 34 Moderna 54
- 35 Moderna 55
- 36 Moderna 56
- 37 Moderna 57
- 38 Moderna 58
- 39 Moderna 59
- 40 Moderna 60
- 41 Moderna 61
- 42 Moderna 62
- 43 Moderna 63
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- 50 Moderna 70
- 51 Moderna 71
- 52 Moderna 72
- 53 Moderna 73
- 54 Moderna 74
- 55 Moderna 75
- 56 Moderna 76
- 57 Pfizer 106
- 58 Pfizer 107
- 59 Pfizer 108
- 60 Pfizer 109
- 61 Pfizer 110
- 62 Pfizer 111
- 63 Pfizer 112
- 64 Pfizer 113

Bank On Bank Off
Named On All On All Off
Edit Samples...

CT Calculation
 Invert Raw Data
Threshold: 0.00574
Eliminate Cycles before: 0
Auto-Find Threshold

Standard Curve
N/A
N/A
Type: Floating
Import Curve... Reset

Imported Settings

Rotor-Gene Q Series Software 2.3.1

VIRTUAL MODE
14:01
08.03.2025

Pfizer BNT162b2 - S Protein cassette - FAM DNA level

Rotor-Gene Q Series Software VIRTUAL MODE - 07032025 vaxM21-M76-P106-P120-DNAonly

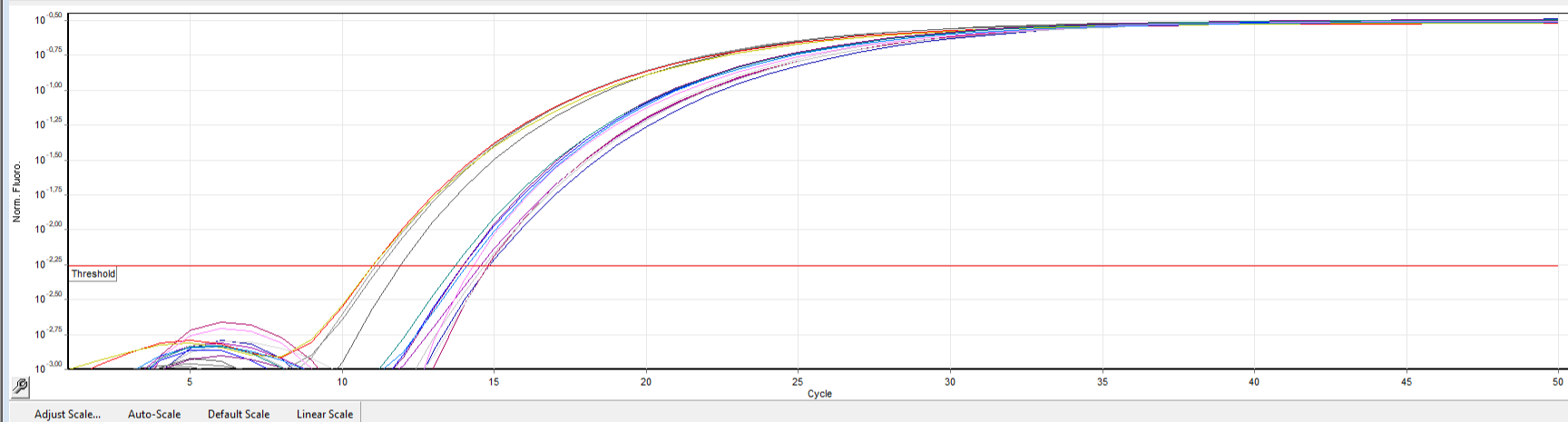
File Analysis Run Gain View Window Help

New Open Save Start Pause Stop View Settings Progress Profile Temp. Samples Analysis Reports Arrange

Channels Cycling A.Green Cycling A.Yellow Cycling A.Orange Cycling A.Red Cycling A.Crimson

Quantitation Analysis - Cycling A.Green (Page 1)

Reports... Std. Curve Results Dynamic Tube Slope Correct Ignore First Take Off Adj. Outlier Removal... Save Defaults



Quant. Results - Cycling A.Green (Page 1)

| No. | C | Name | Type | Ct | Ct Comment | Given Conc. (Cop) | Calc Conc. (Copie) | % Var | Rep. Ct | Rep. Ct Stc | Rep. Ct (95% CI) | Rep. Calc. Conc. | Rep. Calc. Conc. (95%) |
|-----|---|------------|---------|-------|------------|-------------------|--------------------|-------|---------|-------------|------------------|------------------|------------------------|
| 57 | | Pfizer 106 | Unknown | 14,83 | | | | | 14,83 | | | | |
| 58 | | Pfizer 107 | Unknown | 14,54 | | | | | 14,54 | | | | |
| 59 | | Pfizer 108 | Unknown | 14,78 | | | | | 14,78 | | | | |
| 60 | | Pfizer 109 | Unknown | 14,81 | | | | | 14,81 | | | | |
| 61 | | Pfizer 110 | Unknown | 14,66 | | | | | 14,66 | | | | |
| 62 | | Pfizer 111 | Unknown | 11,10 | | | | | 11,10 | | | | |
| 63 | | Pfizer 112 | Unknown | 11,26 | | | | | 11,26 | | | | |
| 64 | | Pfizer 113 | Unknown | 11,89 | | | | | 11,89 | | | | |
| 65 | | Pfizer 114 | Unknown | 10,99 | | | | | 10,99 | | | | |
| 66 | | Pfizer 115 | Unknown | 11,00 | | | | | 11,00 | | | | |
| 67 | | Pfizer 116 | Unknown | 13,95 | | | | | 13,95 | | | | |
| 68 | | Pfizer 117 | Unknown | 13,95 | | | | | 13,95 | | | | |
| 69 | | Pfizer 118 | Unknown | 14,28 | | | | | 14,28 | | | | |
| 70 | | Pfizer 119 | Unknown | 14,09 | | | | | 14,09 | | | | |
| 71 | | Pfizer 120 | Unknown | 13,69 | | | | | 13,69 | | | | |

Page: Page 1

- 41 Moderna 61
- 42 Moderna 62
- 43 Moderna 63
- 44 Moderna 64
- 45 Moderna 65
- 46 Moderna 66
- 47 Moderna 67
- 48 Moderna 68
- 49 Moderna 69
- 50 Moderna 70
- 51 Moderna 71
- 52 Moderna 72
- 53 Moderna 73
- 54 Moderna 74
- 55 Moderna 75
- 56 Moderna 76
- 57 Pfizer 106
- 58 Pfizer 107
- 59 Pfizer 108
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- 61 Pfizer 110
- 62 Pfizer 111
- 63 Pfizer 112
- 64 Pfizer 113
- 65 Pfizer 114
- 66 Pfizer 115
- 67 Pfizer 116
- 68 Pfizer 117
- 69 Pfizer 118
- 70 Pfizer 119
- 71 Pfizer 120
- 72 neg. VaxxFlex

Bank On Bank Off
Named On All On All Off
Edit Samples...

Rotor-Gene Q Series Software 2.3.1

VIRTUAL MODE
18:12
08.03.2025



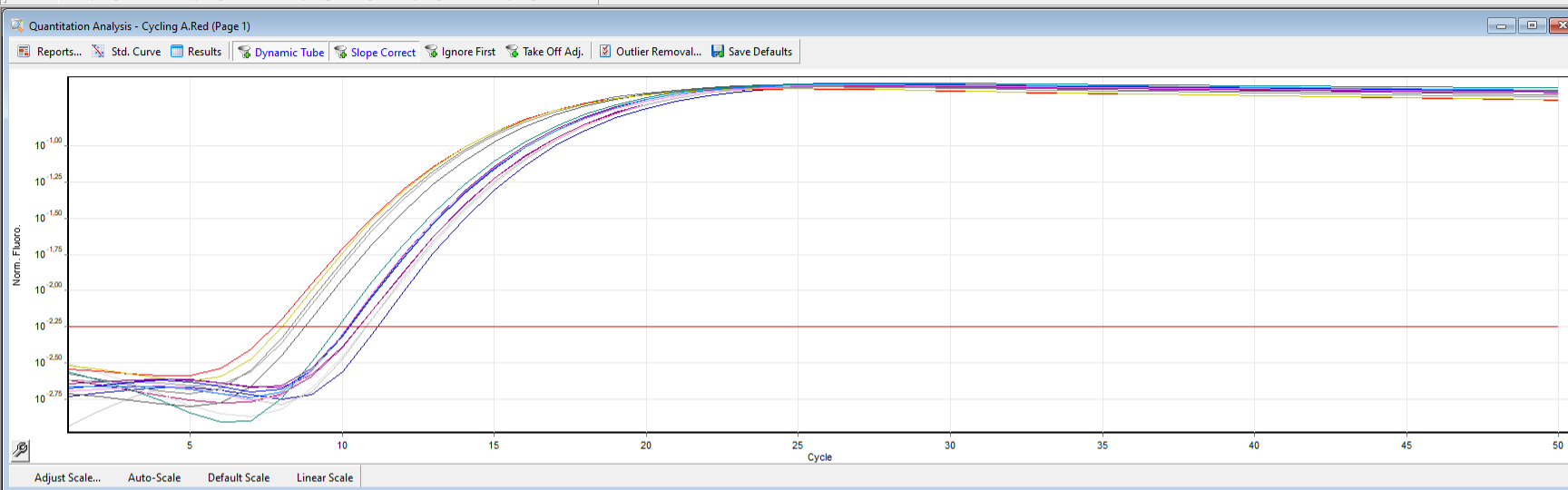
Pfizer BNT162b2 – Expression vector – Cy5 DNA level

Rotor-Gene Q Series Software VIRTUAL MODE - 07032025 vaxM21-M76-P106-P120-DNAonly

File Analysis Run Gain View Window Help

New Open Save Start Pause Stop View Settings Progress Profile Temp. Samples Analysis Reports Arrange

Channels Cycling A.Green Cycling A.Yellow Cycling A.Orange Cycling A.Red Cycling A.Crimson



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 - 52 Moderna 72
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 - 54 Moderna 74
 - 55 Moderna 75
 - 56 Moderna 76
 - 57 Pfizer 106
 - 58 Pfizer 107
 - 59 Pfizer 108
 - 60 Pfizer 109
 - 61 Pfizer 110
 - 62 Pfizer 111
 - 63 Pfizer 112
 - 64 Pfizer 113

Quant. Results - Cycling A.Red (Page 1)

| No. | C Name | Type | Ct | Ct Comment | Given Conc. [Cop] | Calc Conc. [Copie] | % Var | Rep. Ct | Rep. Ct Stc | Rep. Ct (95% CI) | Rep. Calc. Con |
|-----|------------|---------|-------|------------|-------------------|--------------------|-------|---------|-------------|------------------|----------------|
| 57 | Pfizer 106 | Unknown | 11.18 | | | | | 11.18 | | | |
| 58 | Pfizer 107 | Unknown | 10.56 | | | | | 10.56 | | | |
| 59 | Pfizer 108 | Unknown | 10.57 | | | | | 10.57 | | | |
| 60 | Pfizer 109 | Unknown | 10.79 | | | | | 10.79 | | | |
| 61 | Pfizer 110 | Unknown | 10.79 | | | | | 10.79 | | | |
| 62 | Pfizer 111 | Unknown | 8.45 | | | | | 8.45 | | | |
| 63 | Pfizer 112 | Unknown | 8.30 | | | | | 8.30 | | | |
| 64 | Pfizer 113 | Unknown | 8.78 | | | | | 8.78 | | | |
| 65 | Pfizer 114 | Unknown | 7.77 | | | | | 7.77 | | | |
| 66 | Pfizer 115 | Unknown | 8.03 | | | | | 8.03 | | | |
| 67 | Pfizer 116 | Unknown | 10.25 | | | | | 10.25 | | | |
| 68 | Pfizer 117 | Unknown | 10.22 | | | | | 10.22 | | | |
| 69 | Pfizer 118 | Unknown | 10.18 | | | | | 10.18 | | | |
| 70 | Pfizer 119 | Unknown | 10.22 | | | | | 10.22 | | | |
| 71 | Pfizer 120 | Unknown | 9.86 | | | | | 9.86 | | | |

CT Calculation

Invert Raw Data

Threshold: 0.00564

Eliminate Cycles before: []

Auto-Find Threshold

Standard Curve

N/A

Type: Floating

Import Curve... [] Reset []

Imported Settings []

Rotor-Gene Q Series Software 2.3.1

VIRTUAL MODE

Quantities of S protein cassettes on mRNA (cDNA) and DNA levels in Moderna Spikevax and Pfizer BNT162b2 vaccines

| | S protein cassette mRNA (cDNA) (copy number / ml) | S protein cassette DNA (copy number / ml) |
|------------------|--|--|
| Moderna Spikevax | 10e9 – 10e10 | 10e7 – 10e9 |
| Pfizer BNT162b2 | 10e10 – 10e11 | 10e8 – 10e9 |

No DNA content has been declared by either manufacturer to be a regular constituent of the vaccine formulations. The high quantities of DNA present in the samples tested exclude a possibility of a mere contamination during the process of manufacturing.

Summary

- On the mRNA (cDNA) level, using S protein cassettes as detection targets, remarkable difference in the mRNA quantity was observed in both Moderna Spikevax and Pfizer BNT162b2 vaccines (1-2 orders of magnitude; blue dots in the respective graphs).
- In Moderna lots 200106A, MV1018A, and 20023A, given the inverted ratio of the expression vector and S protein cassette quantities, other DNA targets cloned in the expression vector might be suspected to be present in the lots tested. The respective Moderna lots are highlighted by red ovals. More detailed analysis would be needed to clarify the issue.
- Both Moderna Spikevax and Pfizer BNT162b2 vaccines contain high quantities of the S protein DNA expression cassettes, with quantities comparable to the quantity of mRNA, which is the only declared active molecular ingredient (as per the package inserts provided by the manufacturers).
 - Moderna Spikevax vaccines contain **10e7 - 10e9** copies of **DNA** S protein expression cassette / ml
 - Pfizer BNT162b2 vaccines contain **10e8 - 10e9** copies of **DNA** S protein expression cassette / ml

These amounts of DNA, quantitatively comparable to the amounts of the active mRNA, cannot be considered a „contamination“. Contamination, typically, would be expected at quantities several orders of magnitude lower.