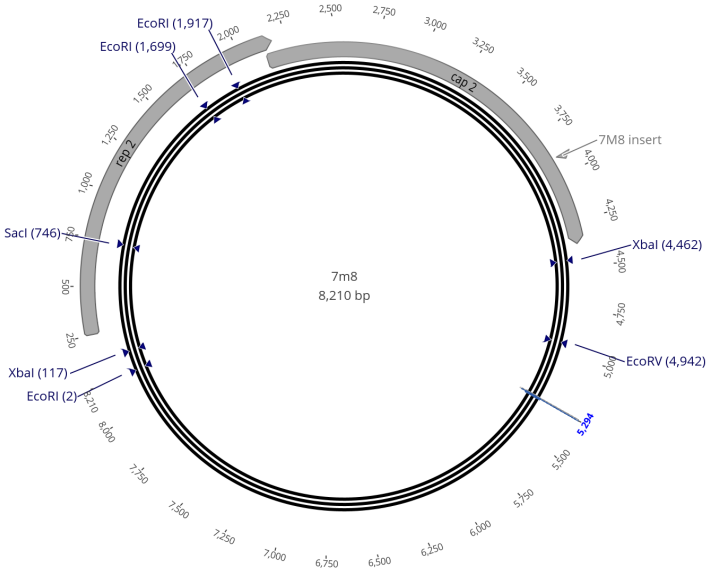
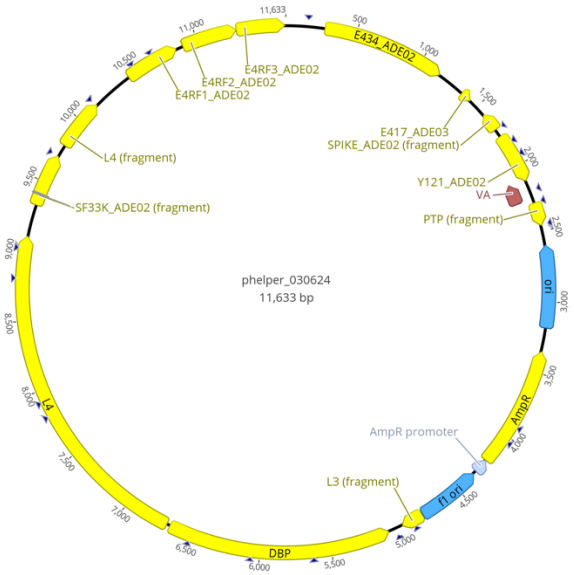
AAV Batch #: 7m8-20241115-scCAG-GFP
Serotype: 7m8
AAV construct: pAAV-scCAG-GFP

Plasmid map:

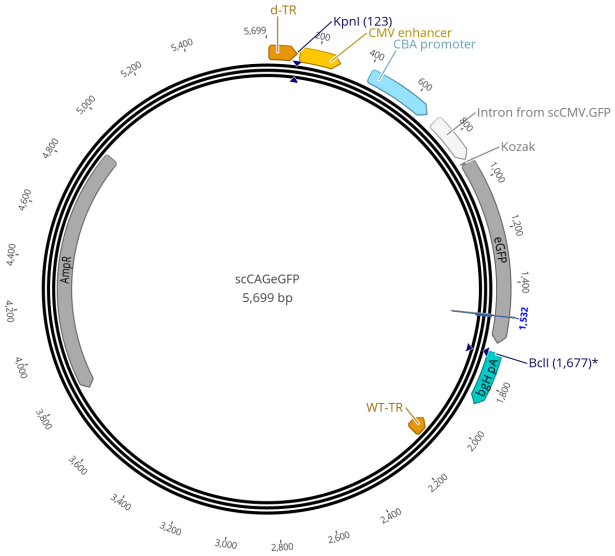
7m8(rep/cap):



pHelper:



scCAG-GFP-BC43:
scCAG-GFP-BC138:
scCAG-GFP-BC208:
scCAG-GFP-BC224:
scCAG-GFP-BC128:
scCAG-GFP-BC225:
scCAG-GFP-BC209:



Packaging protocol and Titering:
Barcoded AAVs were purchased commercially from Packgene and diluted in-house. The titers were measured using qPCR AAV titer kit (ABM, catalog# G931), following the manufacturer's protocol.

AAV construct	Titer (Vg/mL)
7**m8**-CAG-BC4**3 GFP**	1.00e+13
7**m8**-CAG-BC138 **GFP**	1.53e+12
7**m8**-CAG-BC208 **GFP**	2.44e+11
7**m8**-CAG-BC224 **GFP**	2.06e+10
7**m8**-CAG-BC128 **GFP**	1.21e+9
7**m8**-CAG-BC225 **GFP**	1.71e+8
7**m8**-CAG-BC209 **GFP**	4.22e+7
F**inal 7m8 library pool**	2.99E+12

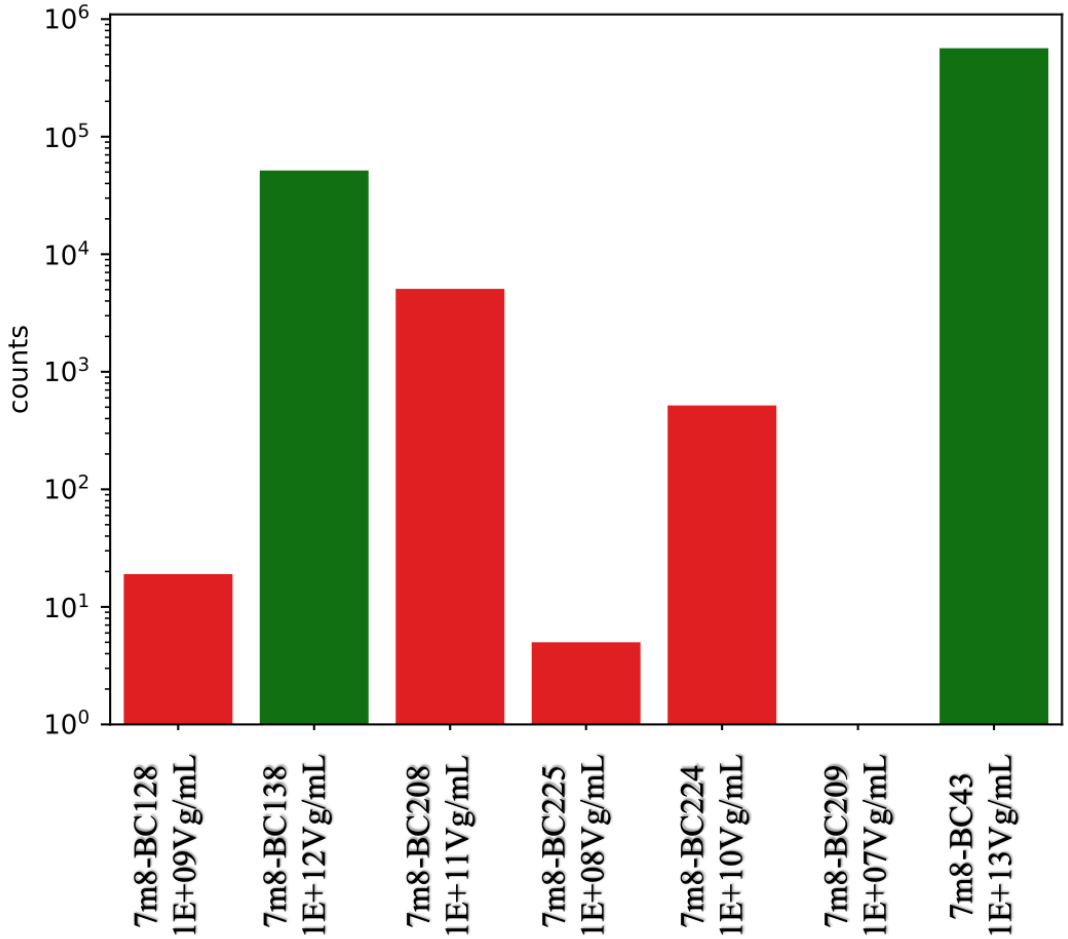
Endotoxin levels:
Bacterial endotoxin was detected by gel-clot method. The sample is diluted with water for BET to the maximum dilution factor. Take the sample dilution and mix it with an equal volume of LAL reagent (e.g., 0.1 ml aliquot). Continue incubating the reaction mixture (at 37 ± 1 °C for 60 ± 2 min) without vibration. To test the integrity of the gel, remove each tube directly from the incubator and invert it through approximately 180° in one smooth motion. If a firm gel is formed that remains in place upon

inversion, record the result as positive. If no intact gel is formed, the result is negative.

AAV construct	Value (EU/mL)
7**m8**-CAG-BC4**3 GFP**	<0.2
7**m8**-CAG-BC138 **GFP**	<0.2
7**m8**-CAG-BC208 **GFP**	<0.2
7**m8**-CAG-BC224 **GFP**	<0.2
7**m8**-CAG-BC128 **GFP**	<0.2
7**m8**-CAG-BC225 **GFP**	<0.2
7**m8**-CAG-BC209 **GFP**	<0.2
Final 7m8 library pool	<0.5

Dilution curve analysis:

Final titer of the pooled dilution curve was 2.99E+12 vg/mL. Barcodes from the pooled library were amplified by PCR and then subjected to sequencing on an Illumina iSeq100. Quantification of barcodes revealed the following library balance:



QC provided by Packgene is copied below.

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 128]	**Lot#**	19341-2E1
Order#	USJW241008942-2	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：4.38E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19341-2E1	3.18E+11	0.69	20	4.38E+12	4.38E+12

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

Figure 2 Amplification curves

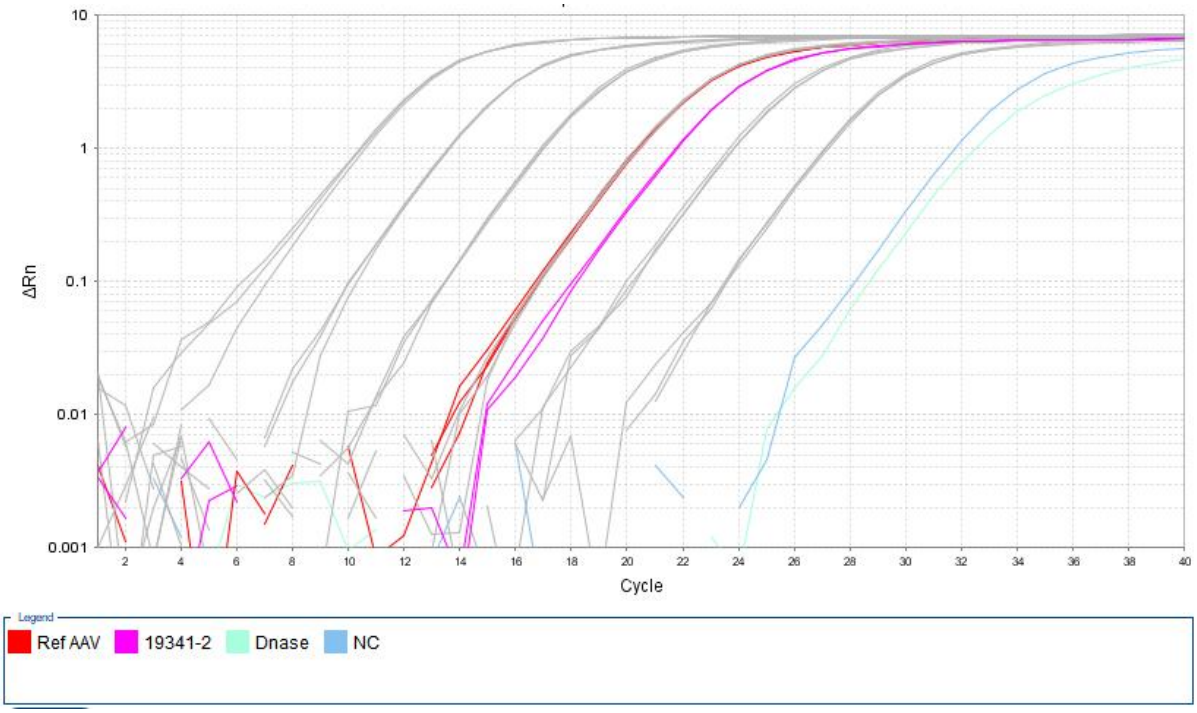
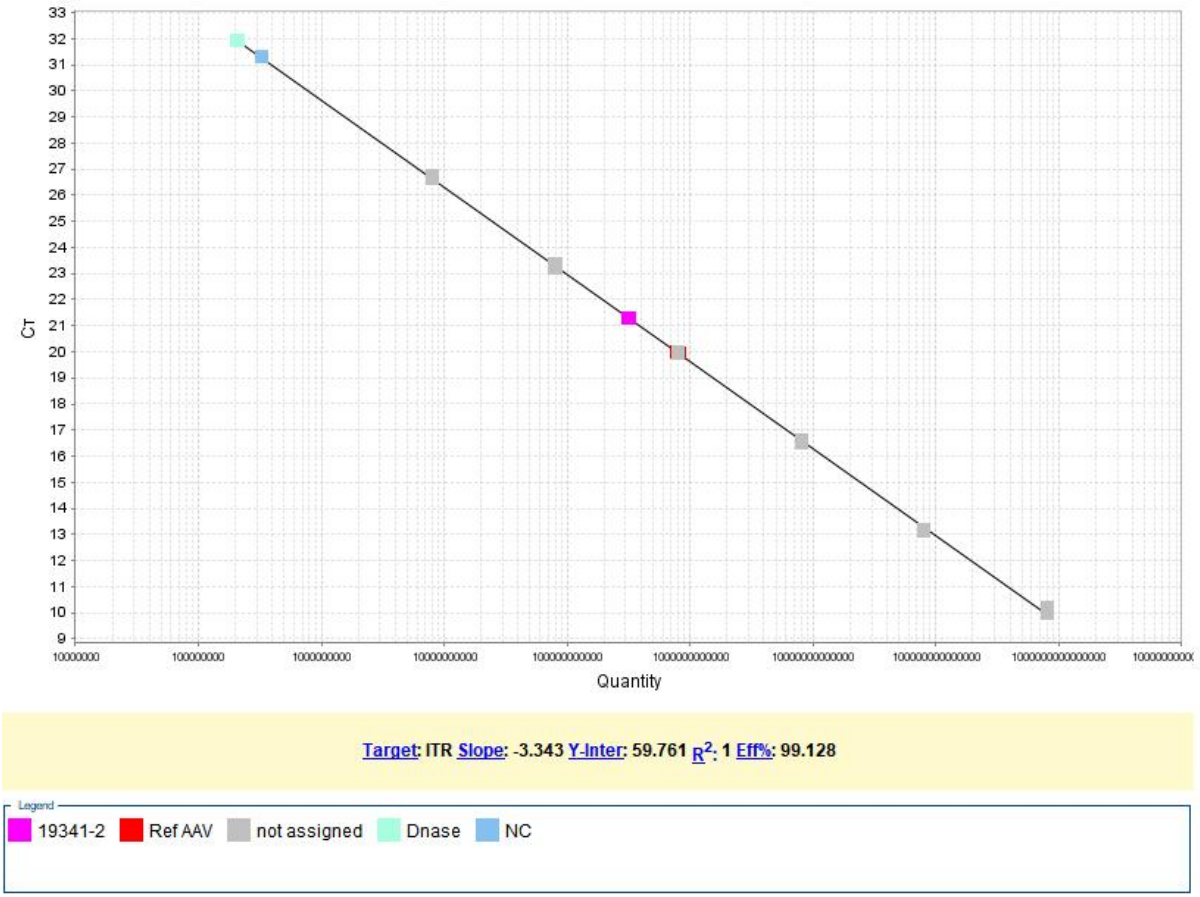


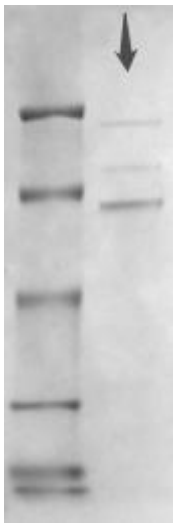
Figure 3 Standard Curve



Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 208]	**Lot#**	19343-2E1
Order#	USJW241008942-6	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：5.67E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19343-2E1	4.12E+11	0.69	20	5.67E+12	5.67E+12

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

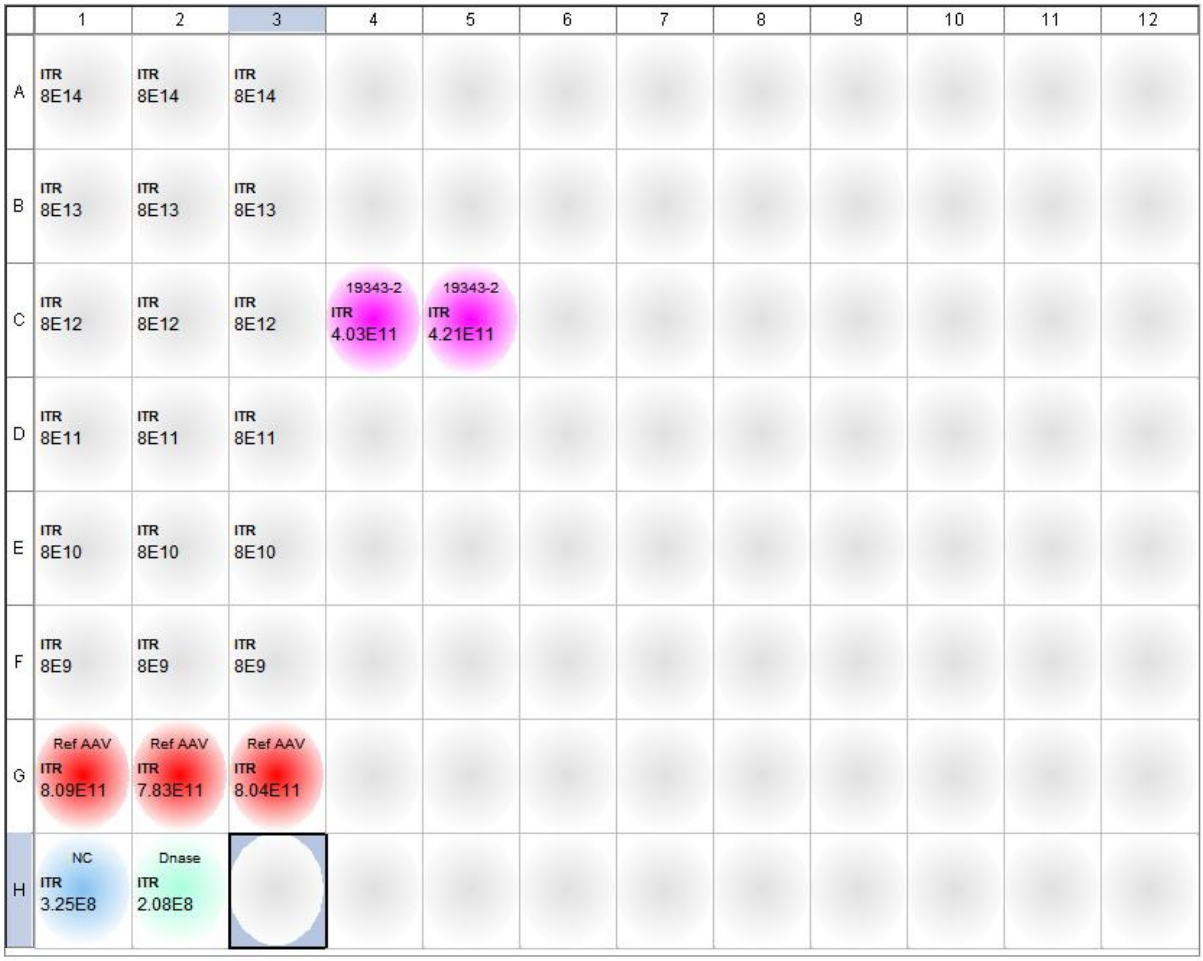


Figure 2 Amplification curves

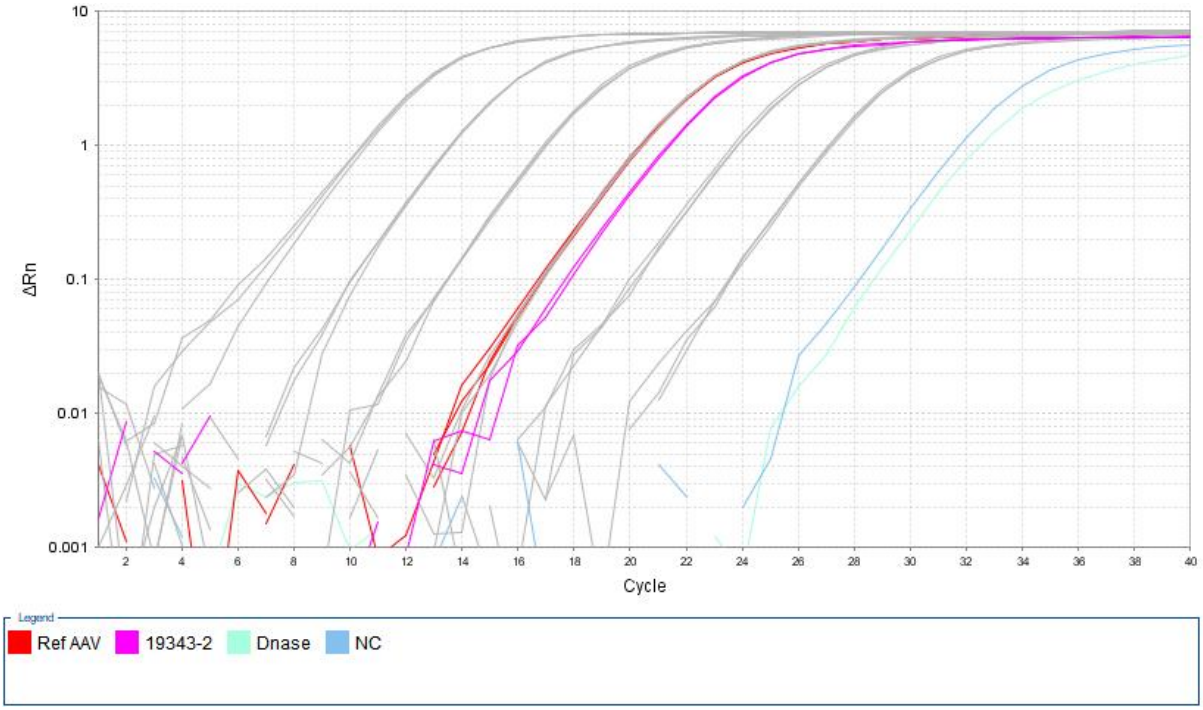
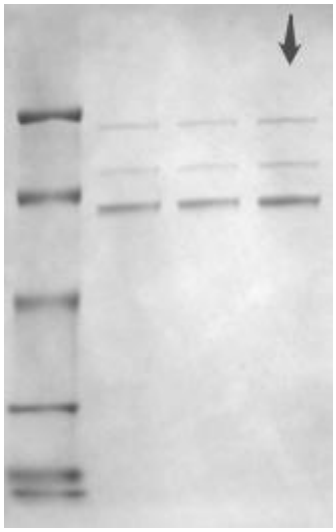


Figure 3 Standard Curve

Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 209]	**Lot#**	19344-2E1
Order#	USJW241008942-8	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：2.65E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19344-2E1	1.92E+11	0.69	20	2.65E+12	2.65E+12

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

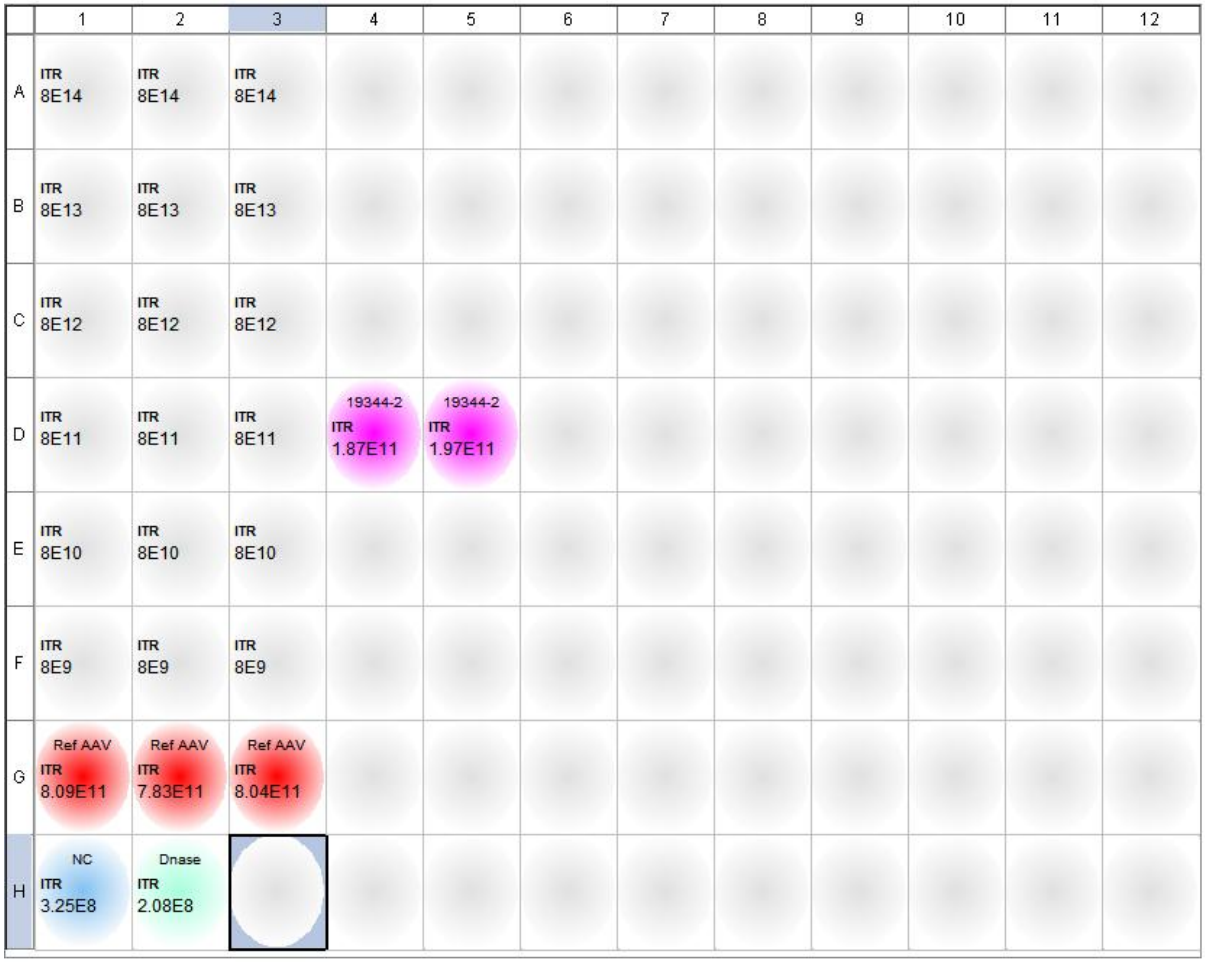


Figure 2 Amplification curves

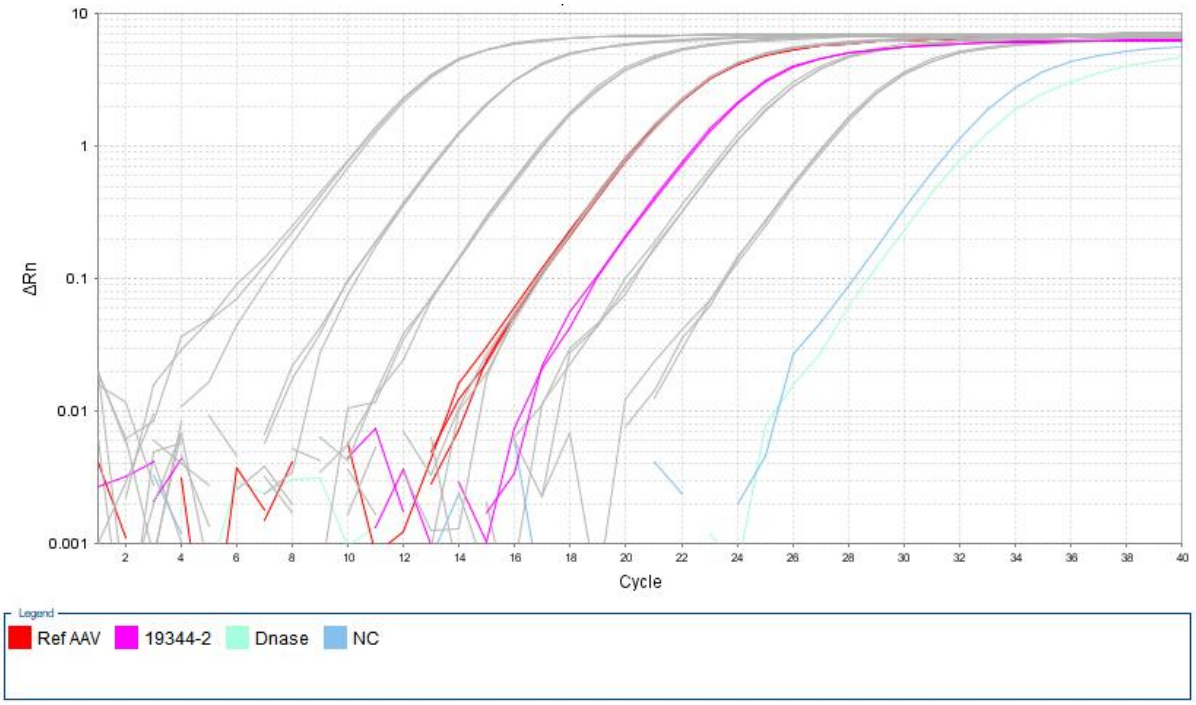
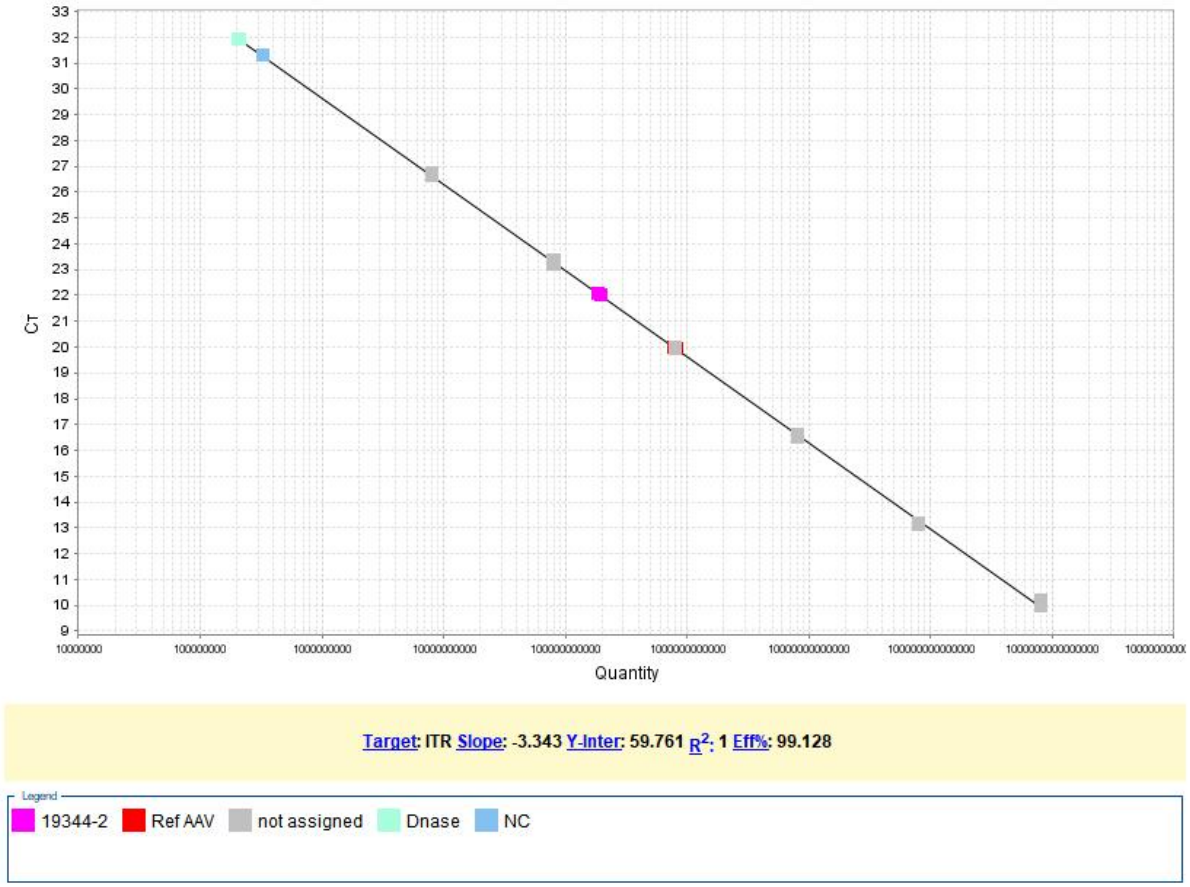


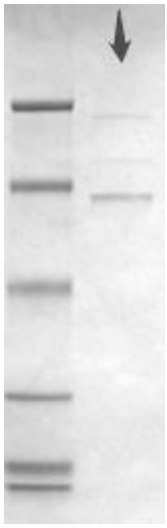
Figure 3 Standard Curve



Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 224]	**Lot#**	19345-2E1
Order#	USJW241008942-10	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：5.61E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19345-2E1	4.07E+11	0.69	20	5.61E+12	5.61E+12

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

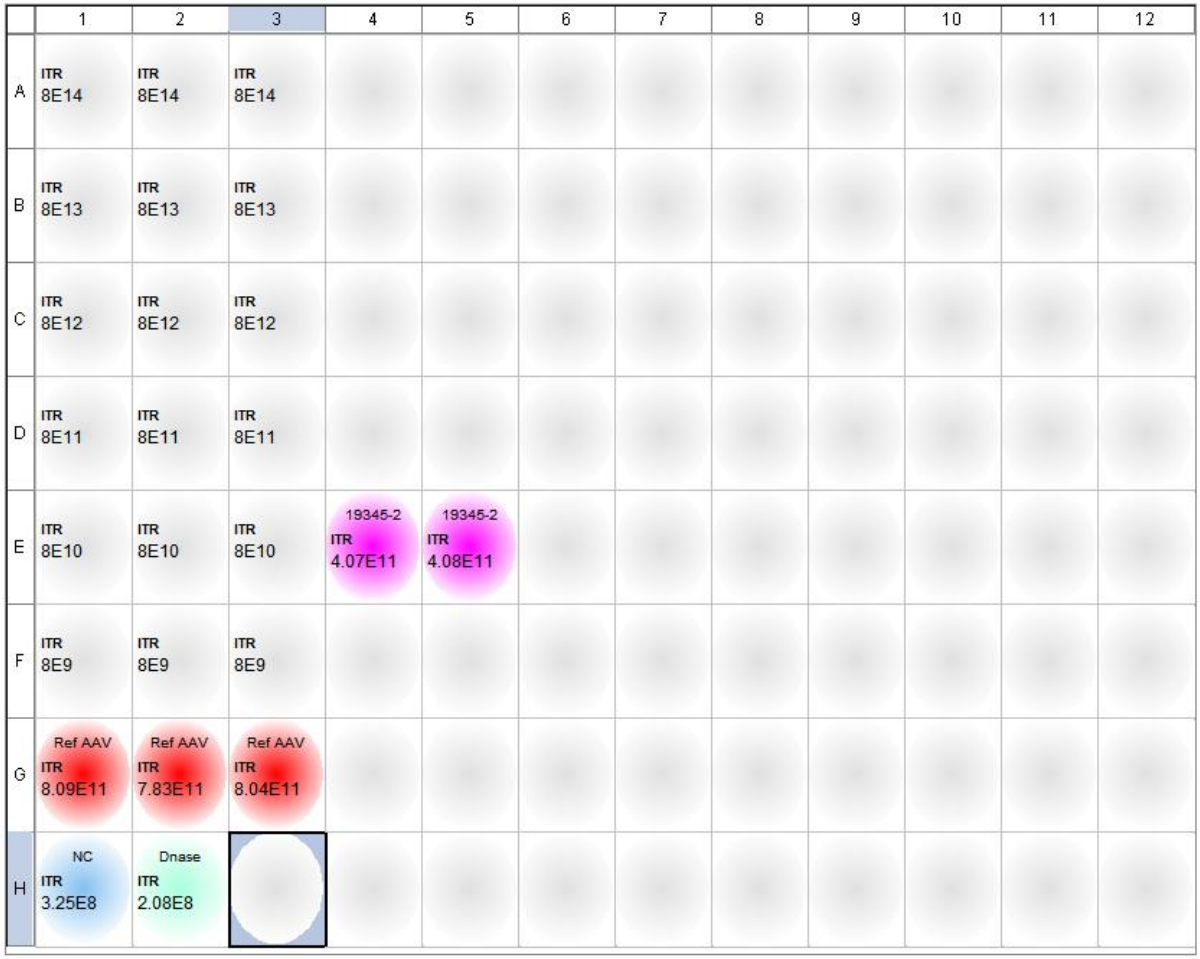


Figure 2 Amplification curves

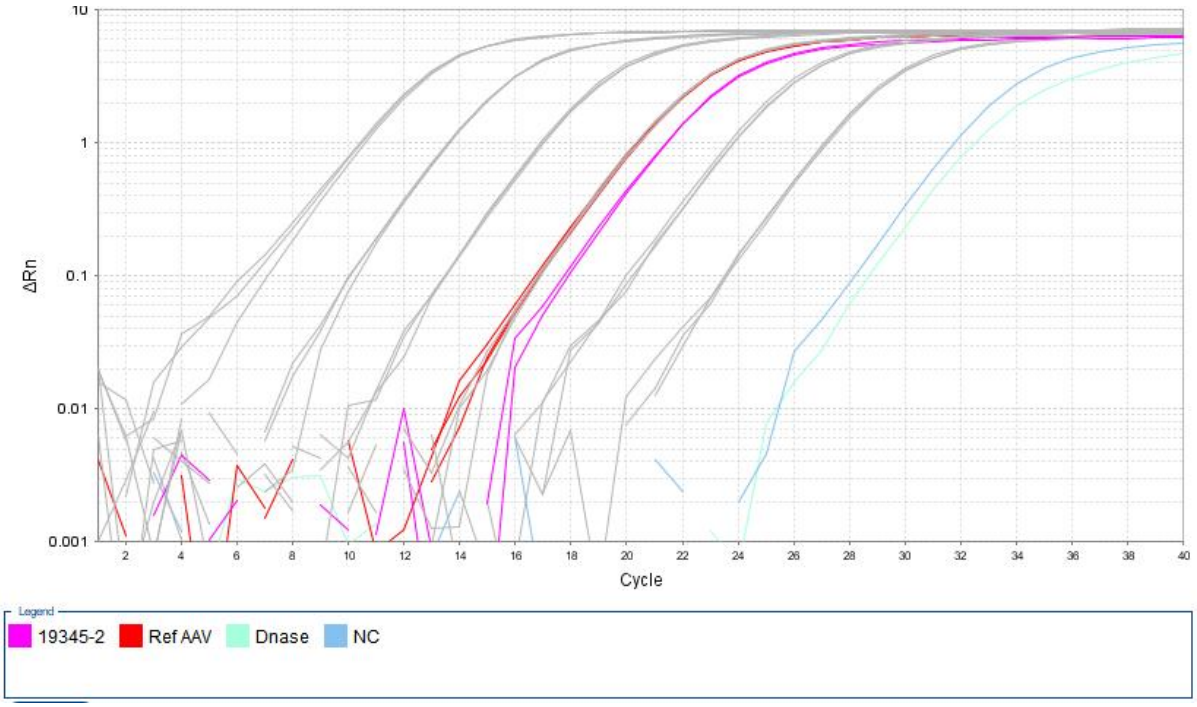
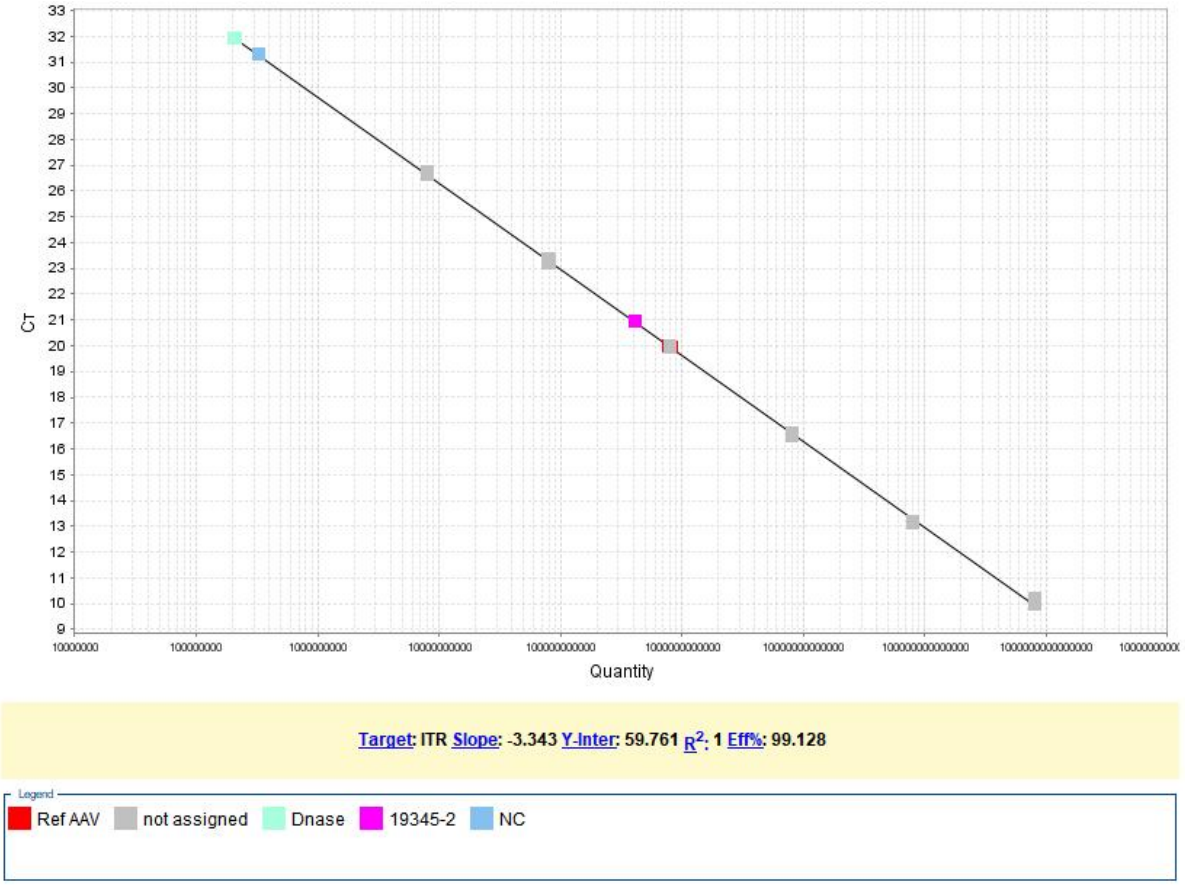


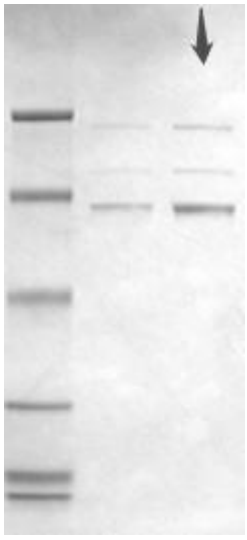
Figure 3 Standard Curve



Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 225]	**Lot#**	19346-2E1
Order#	USJW241008942-12	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：4.36E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19346-2E1	3.17E+11	0.69	20	4.36E+12	4.36E+12

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

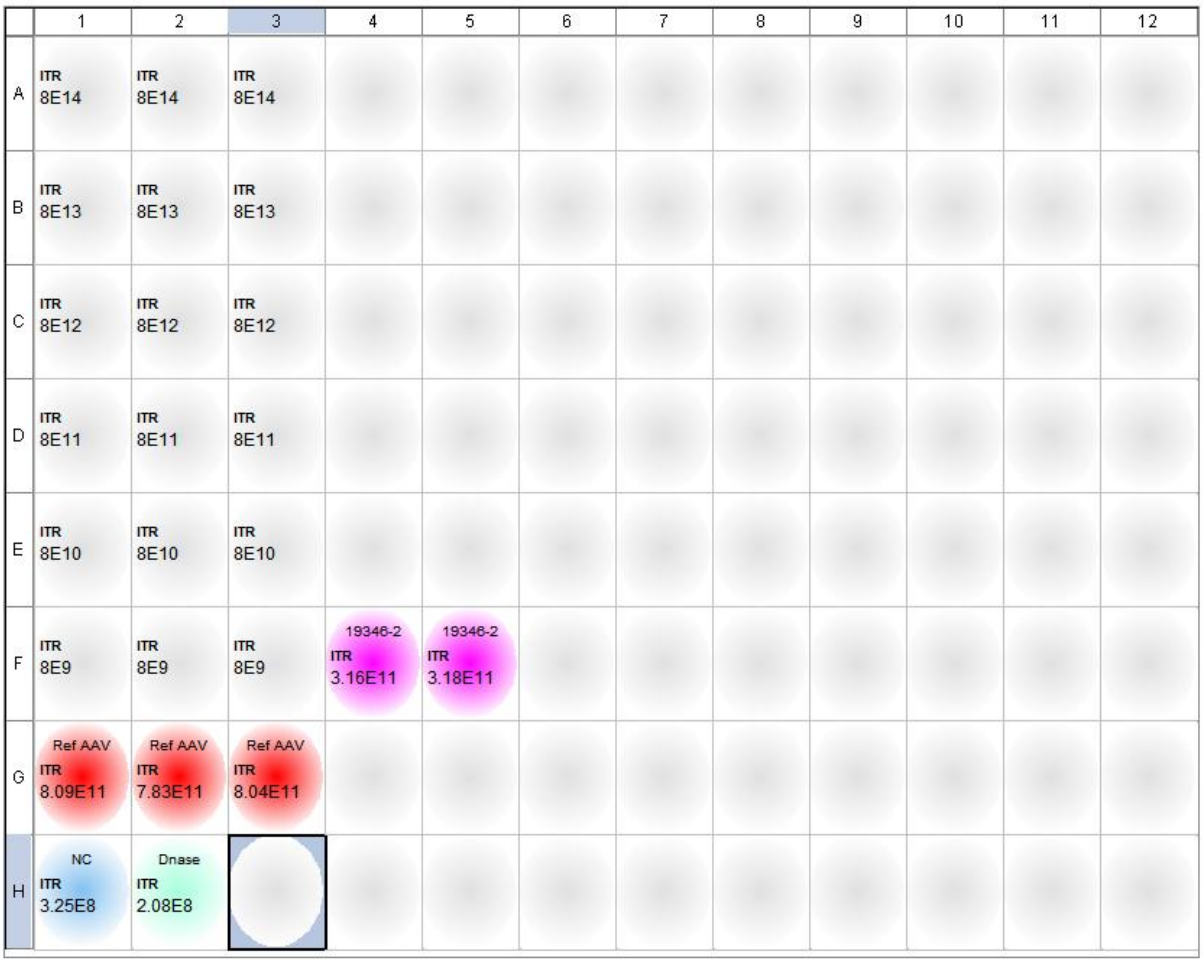


Figure 2 Amplification curves

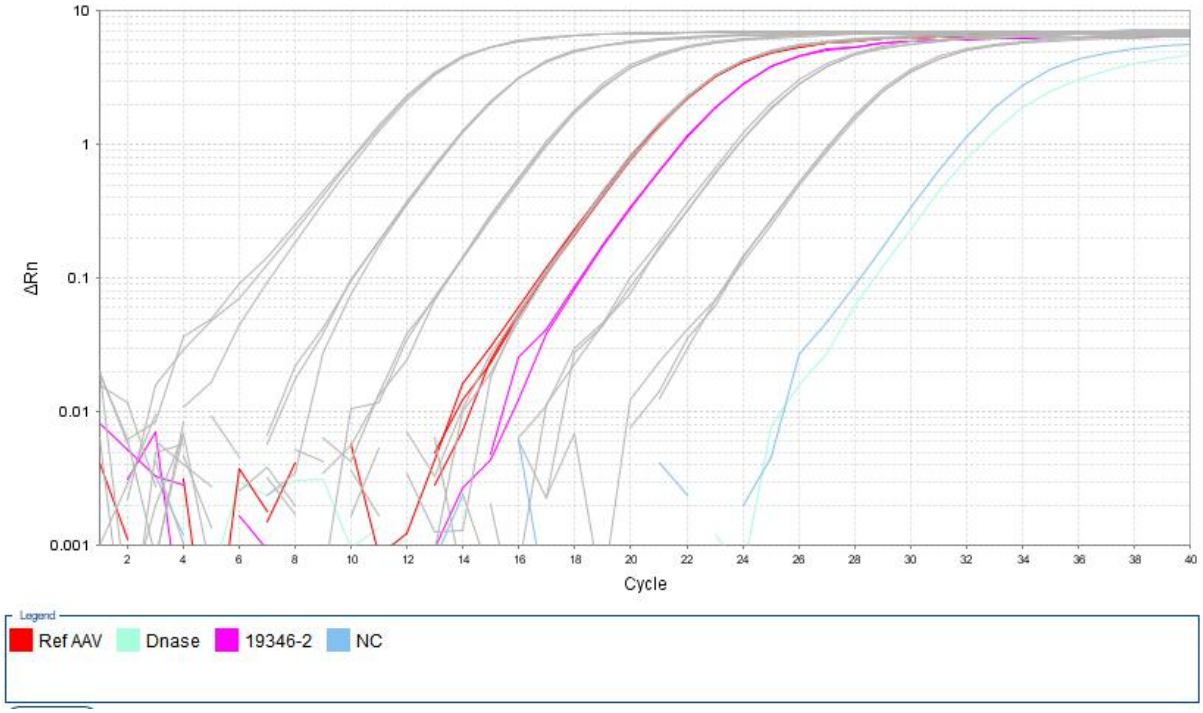
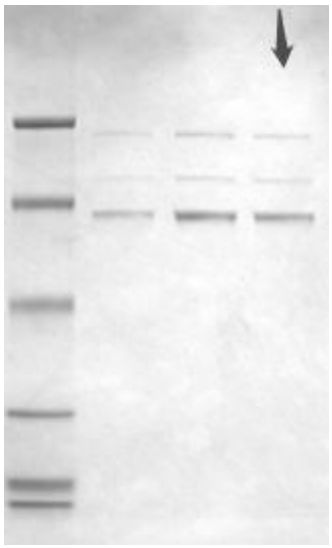


Figure 3 Standard Curve

Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band

Certificate of Analysis （COA）

Name	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 43]	**Lot#**	19368-2T
Order#	USJW241008942-84	**Date**	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer：1E+13GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	＜0.2EU/mL

Conclusion:Qualified

* Adjusted Titer：Titers adjusted to order requirements.

Item 1：Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19368-2T	1.40E+12	0.52	20	1.44E+13	1E+13

* **Reference AAV**:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer：5.5E+11(GC/mL)

Figure 1：Sample Location

Figure 2 Amplification curves

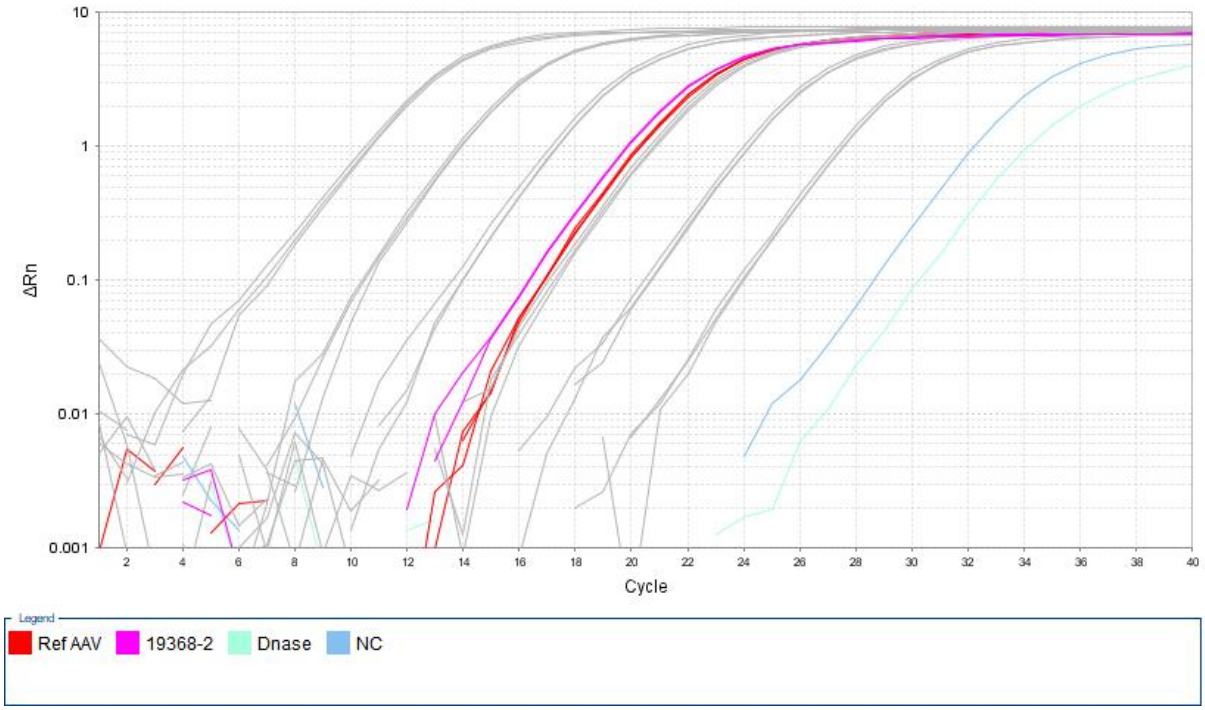
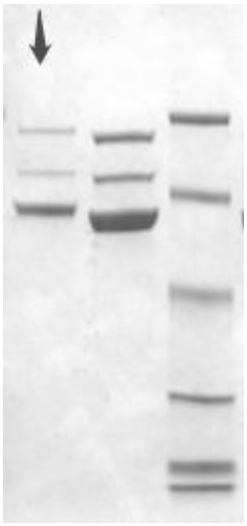


Figure 3 Standard Curve

Item 2：Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

Item 3：Purity（SDS-PAGE）



Maker:97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

Conclusion：No other significant band