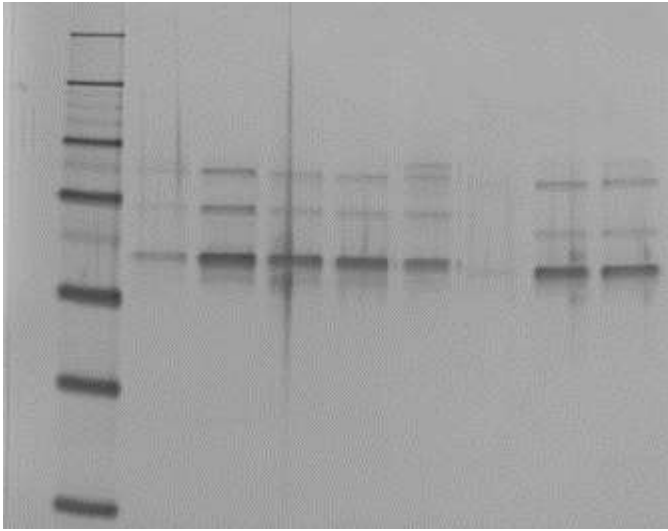


Supplemental information

**Characterization of residual microRNAs in AAV
vector batches produced in HEK293 mammalian
cells and Sf9 insect cells**

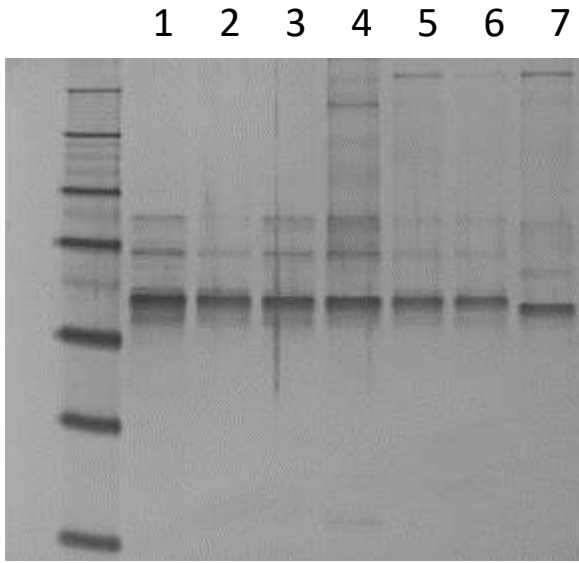
Magalie Penaud-Budloo, Emilie Lecomte, Quentin Lecomte, Simon Pacouret, Frédéric Broucque, Aurélien Guy-Duché, Jean-Baptiste Dupont, Laurence Jeanson-Leh, Cécile Robin, Véronique Blouin, Eduard Ayuso, and Oumeya Adjali

1 2 3 4 5 6 7 8



Lane	1	2	3	4	5	6	7	8
Batch	9	7	6	1	2	5	3	4
Sample	AAV8	AAV8	AAV8	AAV8	AAV8 empty	AAV2	AAV2	AAV2 empty
Purification	IA	IA+CsCl	CsCl	CsCl	CsCl	CsCl	CsCl	CsCl
Purity (%)	93.2	85.6	73.7	85.2	76.0	nd	100	95.5

Figure S1: Quality controls of rAAV batches produced in HEK293 cells. Purity was determined by SDS-PAGE realized from 2×10^{10} total particles per rAAV sample, and expressed in percentage of AAV VP1, VP2 and VP3 proteins to total proteins. nd: not determined.



Lane	1	2	3	4	5	6	7
Batch	15	13	14	10	11	12	16
Serotype	AAV8	AAV8	AAV8 empty	AAV8	AAV8	AAV8	AAV2
Purification	IA	IA+CsCl	IA+CsCl	CsCl	CsCl	CsCl	CsCl
Purity (%)	79.5	84.7	79.5	56.1	84.5	85.7	78.9

Figure S2: Quality controls of rAAV batches produced in Sf9 cells. Purity was determined by SDS-PAGE realized from 2×10^{10} total particles per rAAV sample, and expressed in percentage of AAV VP1, VP2 and VP3 proteins to total proteins.

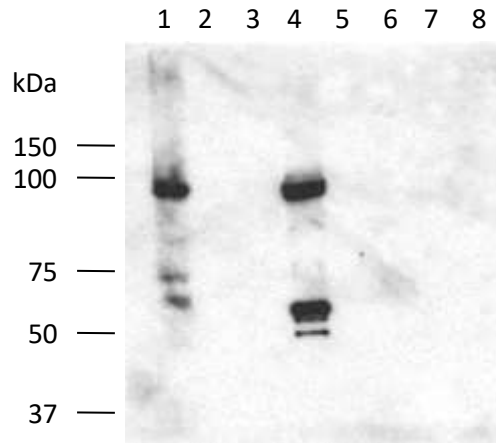


Figure S3: Detection of Ago-2 in AAV samples by Western Blot.

5 μ g of HEK293 total protein extract (1) and 25 μ L of purified empty or full rAAV were loaded on a 4-12% acrylamide tris gel. AAV2 (5) and AAV8 (2, 3, 4, 6, 7, 8) vectors were produced in HEK293 cells and purified by CsCl gradient ultracentrifugation (2 to 5), IA column (6) or CsCl followed by IA (7, 8). After SDS-PAGE, western blot was realized using the anti-Argonaute-2 antibody (Abcam, ab186733) diluted at 1:1000 and the donkey anti-rabbit IgG-HRP (Jackson ImmunoResearch, 711-035-152) at 1:20000 as secondary antibody. Predicted Ago-2 molecular weight is 97 kDa. The anti-Ago-2 antibody does not recognize Argonaute proteins of Sf9 insect cells. For rAAV batch correspondence, refer to Table S3: batch 6 (2), batch 1 (3), batch 2 (4), batch 3 (5), batch 9 (6), batch 7 (7) and batch 8 (8).

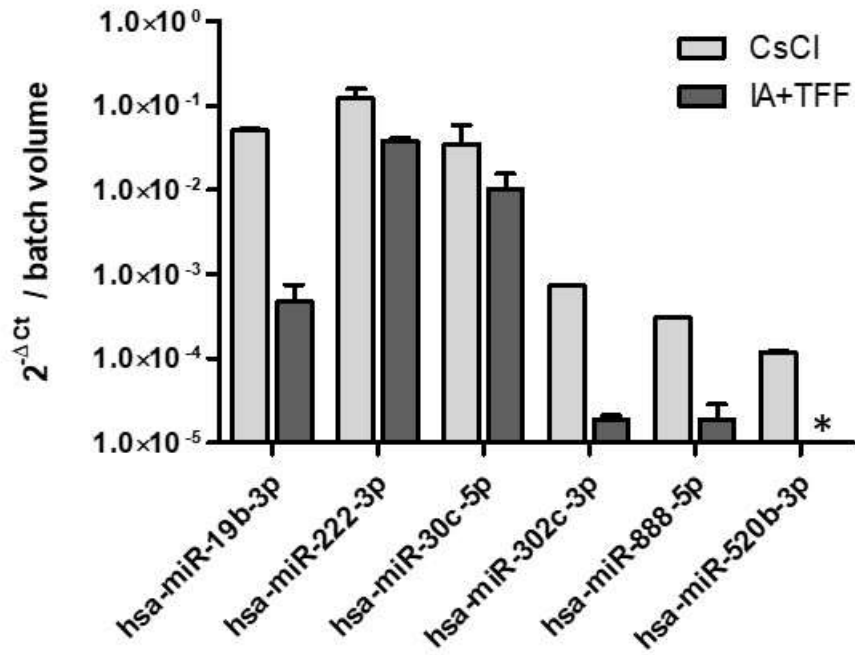


Figure S4: Impact of the purification process on the residual miRNA content. A 5-L bulk of AAV8 produced in Sf9 insect cells by dual-baculovirus infection was divided into two equal parts before purification by CsCl or IA-TFF. The asterisk indicates that Ct is below the qPCR threshold. The mean and standard deviation of two biological replicates are presented.

Table S1: Quantification of miRNA in rAAV batches using the Qubit fluorometer.

Platform	Platform	Serotype	Fraction	Purification	miRNA concentration (ng/μL)	Quantity of miRNA (ng) per 1E10 vg
Batch 17	HEK293	AAV8	full	CsCl	1.86	3.2
Batch 18	HEK293	AAV8	full+empty	IA	0.22	0.8
Batch 19	HEK293	AAV8	full	CsCl	0.69	0.5
Batch 20	HEK293	AAV8	full+empty	IA	2.67	2.9
Batch 21	Sf9	AAV8	full	CsCl	5.95	5.4
Batch 22	Sf9	AAV8	full	CsCl	2.26	3.7
Batch 23	Sf9	AAV8	full+empty	IA	2.49	9.6

Table S2: MicroRNAs selected for RT-qPCR quantification.

Cells (organism)	miRNA name	miRBase accession number	miR Sequence 5' to 3'
HEK293 (<i>Homo sapiens</i>)	hsa-miR-19b-3p	MIMAT0000074	UGUGCAAUCCAUGCAAACUGA
	hsa-miR-222-3p	MIMAT0000279	AGCUACAUCUGGCUACUGGGU
	hsa-miR-30c-5p	MIMAT0000244	UGUAAACAUCCUACACUCUCAGC
	hsa-miR-302c-3p	MIMAT0000717	UAAGUGCUUCCAUGUUUCAGUGG
	hsa-miR-888-5p	MIMAT0004916	UACUCAAAAAGCUGUCAGUCA
	hsa-miR-520b-3p	MIMAT0002843	AAAGUGCUUCCUUUUAGAGGG
Sf9 (<i>Spodoptera frugiperda</i>)	hsa-miR-184	MIMAT0000454	UGGACGGAGAACUGAUAAAGGGU
	sfr-miR-bantam-3p	na	UGAGAUCAUUGUGAAAGCUGAU
	hsa-miR-100-5p	MIMAT0000098	AACCCGUAGAUCCGAACUUGUG
	hsa-miR-10a-5p	MIMAT0000253	UACCCUGUAGAUCCGAAUUUGUG
	hsa-let-7a-5p	MIMAT0000062	UGAGGUAGUAGGUUGUAUAGUU
	hsa-miR-33a-5p	MIMAT0000091	GUGCAUUGUAGUUGCAUUGCA

Sf9 miRNAs were selected from Mehrabadi *et al.* Gen Virol 2013. na: not applicable.

Table S3: rAAV batches analyzed in the study.

Platform	Batch number	Bulk number	Serotype	Fraction	Purification	vg/mL	p/mL	p/vg
HEK293	1	6788	AAV8	full	CsCl	2.1E+13	2.1E+13	1.0
	2	6788	AAV8	empty	CsCl	7.1E+10	5.0E+13	704.2
	3	6789	AAV2	full	CsCl	2.7E+12	5.1E+12	1.9
	4	6789	AAV2	empty	CsCl	3.0E+10	3.3E+12	110.0
	5	BACTRANS008	AAV2	full	CsCl	3.8E+11	5.8E+11	1.6
	6	BACTRANS027	AAV8	full	CsCl	2.6E+13	3.5E+13	1.3
	7	BACTRANS035	AAV8	full	IA+CsCl	7.1E+12	6.1E+12	0.9
	8	BACTRANS035	AAV8	empty	IA+CsCl	2.0E+10	nd	nd
	9	BACTRANS035	AAV8	full+empty	IA	7.7E+12	2.0E+13	2.6
	17	6855	AAV8	full	CsCl	5.9E+12	nd	nd
	18	6855	AAV8	full+empty	IA	2.6E+12	nd	nd
	19	6856	AAV8	full	CsCl	1.5E+13	nd	nd
	20	6856	AAV8	full+empty	IA	9.2E+12	nd	nd
Sf9	10	AAVBAC243	AAV8	full	CsCl	1.8E+12	4.7E+12	2.6
	11	AAVBAC312	AAV8	full	CsCl	7.9E+12	1.6E+13	2.0
	12	AAVBAC314	AAV8	full	CsCl	8.9E+12	1.9E+13	2.1
	13	AAVBAC307	AAV8	full	IA+CsCl	1.1E+13	2.6E+13	2.4
	14	AAVBAC307	AAV8	empty	IA+CsCl	2.5E+11	5.5E+13	220.0
	15	AAVBAC307	AAV8	full+empty	IA	3.2E+13	1.7E+14	5.4
	16	AAVBAC313	AAV2	full	CsCl	2.2E+12	6.9E+12	3.1
	21	AAVBAC390	AAV8	full	CsCl	1.1E+13	nd	nd
	22	AAVBAC391	AAV8	full	CsCl	6.1E+12	nd	nd
	23	AAVBAC386	AAV8	full+empty	IA	2.6E+12	nd	nd

AAV vectors were generated with the CMV-eGFP-HBBpA cassette. The concentration in vector genome per mL (vg/mL) was determined by qPCR targeting ITRs. Capsid titer in particle per mL (p/mL) was determined by ELISA. nd: not determined.

Table S4: Correlation analysis of the miRNA profiles for HEK293-derived AAV vectors.

$r^{(1)}$	HEK293	batch 1	batch 2	batch 3	batch 4	batch 5	batch 6	batch 7	batch 8	batch 9
HEK293	na	na	na	na	na	na	na	na	na	na
batch 1	0.95	na	na	na	na	na	na	na	na	na
batch 2	0.96	0.94	na	na	na	na	na	na	na	na
batch 3	0.20	0.38	0.04	na	na	na	na	na	na	na
batch 4	0.69	0.78	0.54	0.84	na	na	na	na	na	na
batch 5	0.23	0.43	0.10	0.99	0.85	na	na	na	na	na
batch 6	0.23	0.51	0.18	0.99	0.90	0.99	na	na	na	na
batch 7	0.78	0.86	0.65	0.76	0.99	0.77	0.83	na	na	na
batch 8	0.99	0.98	0.95	0.29	0.76	0.33	0.42	0.84	na	na
batch 9	0.41	0.55	0.24	0.97	0.94	0.96	0.98	0.89	0.50	na

⁽¹⁾ The Pearson correlation coefficient r was calculated from RT-qPCR data for the 6 selected miRNAs.

Table S5: Correlation analysis of the miRNA profiles for Sf9-derived AAV vectors.

$r^{(1)}$	Sf9	batch 10	batch 11	batch 12	batch 13	batch 14	batch 15	batch 16
Sf9	na	na	na	na	na	na	na	na
batch 10	0.63	na	na	na	na	na	na	na
batch 11	0.75	0.98	na	na	na	na	na	na
batch 12	0.62	0.94	0.96	na	na	na	na	na
batch 13	0.67	1.00	0.99	0.95	na	na	na	na
batch 14	0.67	1.00	0.99	0.95	1.00	na	na	na
batch 15	0.64	1.00	0.99	0.95	1.00	1.00	na	na
batch 16	0.84	0.94	0.99	0.93	0.96	0.96	0.95	na

⁽¹⁾ The Pearson correlation coefficient r was calculated from RT-qPCR data for the 6 selected miRNAs.