

A Direct Comparison of rAAV5 Variants Derived from the Baculovirus Expression System Using LC-MS Workflows Demonstrates Key Differences in Overall Production Yield, Product Quality and Vector Efficiency

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Supplementary Material

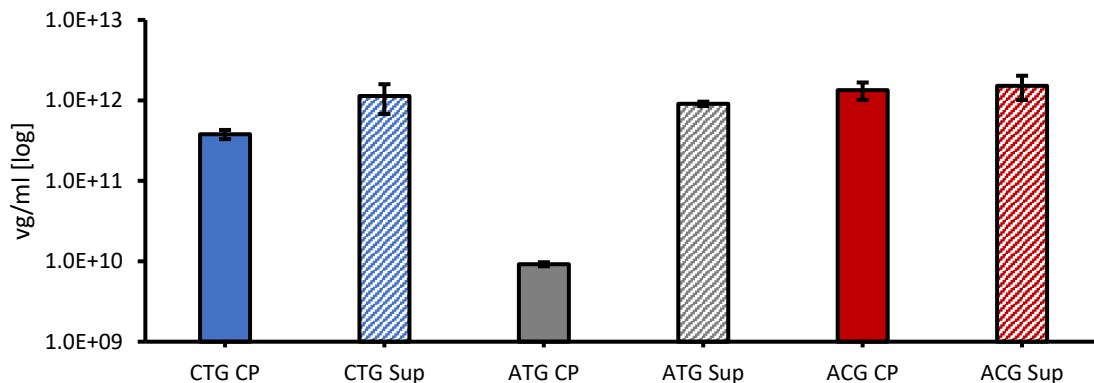


Figure S1: Total yield measured by qPCR representing material generated from recombinant and wild type AAV5 with harvest taking place after 72hr.

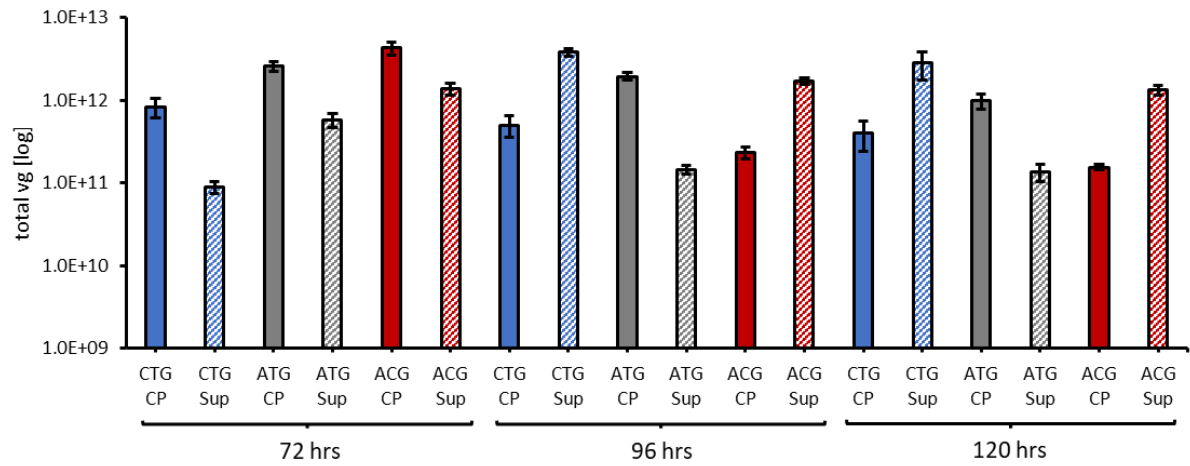


Figure S2: Total yield measured by qPCR representing material generated from recombinant and wild type AAV5 repeats, with harvest taking place after 72, 96 and 120 hr.

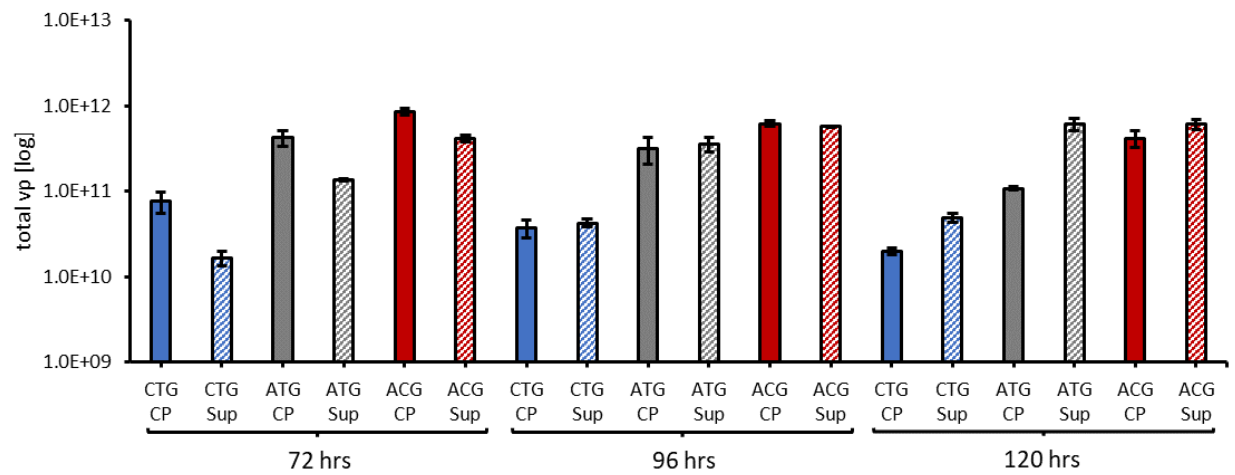


Figure S3: Total titre/yield measured by capsid ELISA representing material generated from recombinant and wild type AAV5, with harvest taking place after 72, 96 and 120 hr.

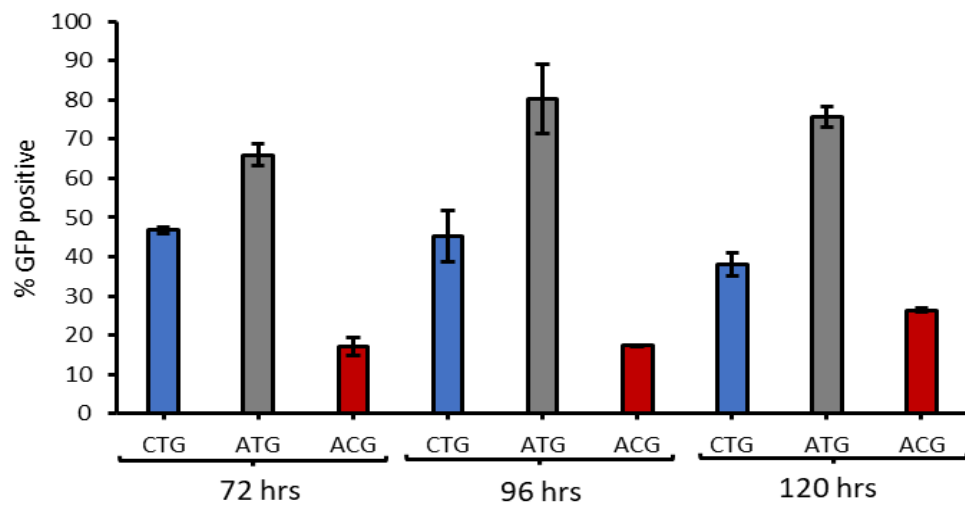


Figure S4: *In vitro* potency assay of wild type and variant capsids in CHO-K1 cells. Measurements taken using the cell pellet samples only.

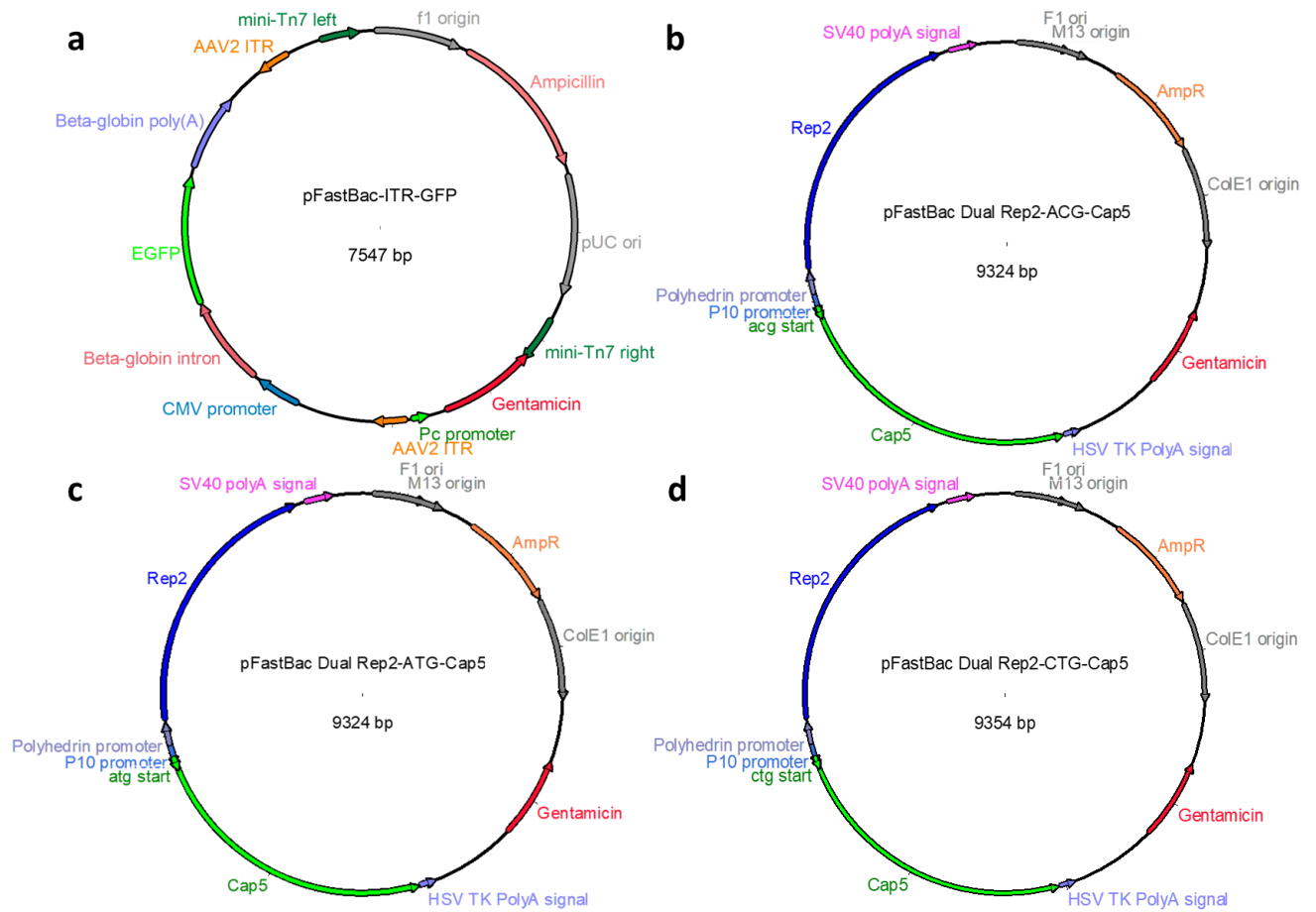


Figure S5: Plasmid maps of constructs used to generate Baculovirus, which were subsequently used to produce AAV. **a**, gene-of-interest carrying construct containing the GFP gene flanked by AAV2 ITR sequences. **b**, ACG start codon variant of the Rep/Cap carrying construct. **c**, ATG start codon variant of the Rep/Cap carrying construct. **d**, CTG start codon variant of the Rep/Cap carrying construct. GenBank accession numbers are as follows: BankIt2794929pFastBac-ITR-GFP: PP328554, BankIt2794929pFastBacDual-Rep2-ACG-Cap5: PP328555, BankIt2794929pFastBacDual-Rep2-ATG-Cap5: PP328556, BankIt2794929pFastBacDual-Rep2-CTG-Cap5: PP328557.

Table S1: VP ratio analysis per replicate of rAAV based on a total ratio theoretical value of 12 (1:1:10, VP1:2:3) calculated using peak area quantitation based on the FLR signal during HILIC separation and used to plot bar charts available in the main text.

	VP1					VP2					VP3				
	Rep 1	Rep 2	Rep 3	Average	SD	Rep 1	Rep 2	Rep 3	Average	SD	Rep 1	Rep 2	Rep 3	Average	SD
CTG CP	0.710	0.742	0.736	0.729	0.014	0.786	0.749	0.767	0.767	0.015	10.504	10.509	10.497	10.504	0.005
CTG SUP	0.370	0.413	0.543	0.442	0.074	0.486	0.364	0.686	0.512	0.133	11.144	11.223	10.771	11.046	0.197
ATG CP	0.396	0.339	0.291	0.342	0.043	0.403	0.365	0.328	0.366	0.030	11.201	11.295	11.380	11.292	0.073
ATG SUP	0.547	0.484	0.540	0.524	0.028	0.445	0.388	0.500	0.444	0.046	11.008	11.128	10.960	11.032	0.071
ACG CP	0.338	0.338	0.336	0.337	0.001	0.785	0.805	0.832	0.808	0.019	10.877	10.857	10.831	10.855	0.018
ACG SUP	0.365	0.362	0.361	0.363	0.002	0.790	0.784	0.744	0.772	0.020	10.845	10.855	10.895	10.865	0.022

Table S2: Percentage of empty and full capsids per replicate of rAAV quantified by native mass spectrometry and AEX-LC and used to plot bar charts available in the main text.

Native MS										
	<i>Rep 1</i>	<i>Rep 2</i>	<i>Rep 3</i>	<i>Rep 1</i>	<i>Rep 2</i>	<i>Rep 3</i>	<i>Average</i>		<i>SD</i>	
		%E			%F		%E	%F	%E	%F
CTG CP	41.30	41.20	40.40	58.70	58.80	59.60	40.97	59.03	0.40	0.40
CTG SUP	31.90	32.70	32.20	68.10	67.30	67.80	32.27	67.73	0.33	0.33
ATG CP	30.30	31.30	30.00	69.70	68.70	70.00	30.53	69.47	0.56	0.56
ATG SUP	30.90	30.60	30.60	69.10	69.40	69.40	30.70	69.30	0.14	0.14
ACG CP	52.60	56.00	56.80	47.40	44.00	43.20	55.13	44.87	1.82	1.82
ACG SUP	52.40	71.70	71.90	47.60	28.30	28.10	65.33	34.67	9.15	9.15

AEX-LC-FLR										
CTG CP	23.25	22.16	21.92	76.74	77.84	78.08	22.44	77.55	0.58	0.58
CTG SUP	21.12	22.53	20.65	78.88	77.47	79.35	21.43	78.57	0.80	0.80
ATG CP	26.34	26.31	26.63	73.66	73.69	73.37	26.43	73.57	0.14	0.14
ATG SUP	26.13	24.76	26	73.87	75.24	74	25.63	74.37	0.62	0.62
ACG CP	46.22	46.66	46.01	53.78	53.34	53.99	46.30	53.70	0.27	0.27
ACG SUP	52.8	53.53	52.52	47.2	46.47	47.48	52.95	47.05	0.43	0.43

Table S3: MS acquisition parameters used for charge detection mass spectrometry based on the Direct Mass Technology™ (DMT) mode provided by Thermo Scientific on a Q Exactive Ultra High Mass Range (UHMR) mass spectrometer (Thermo Scientific, Bremen, Germany).

Parameter	Setting
Spray voltage	0.9 kV
Capillary temperature	275°C
S-lens RF level	200
Resolution setting at m/z 400	50,000
Trapping gas	Nitrogen
UHV pressure (mbar)	4.6e-10 mab
IST desolvation voltage	-150 V
Averaging	Off
Detector m/z optimization	High
Ion transfer target	High
Ion Injection	IT = 200