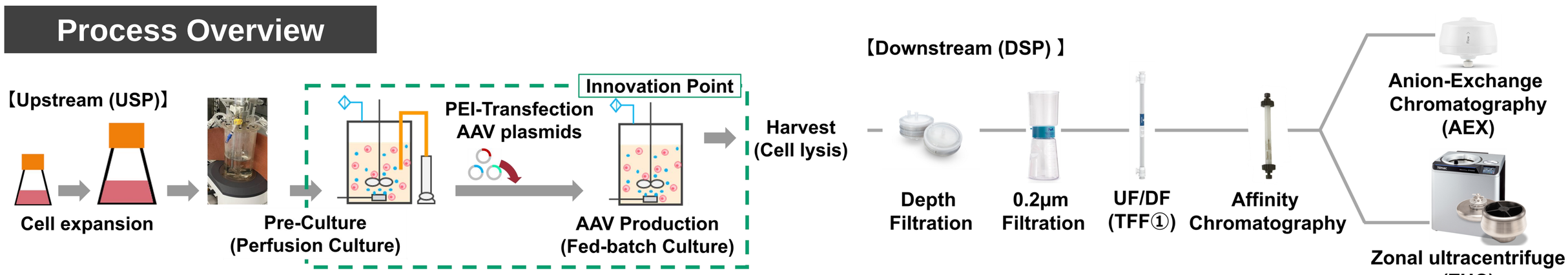
Partners for *Life*

Cost-effective rAAV mass manufacturing by high-density cell with small-scale equipment

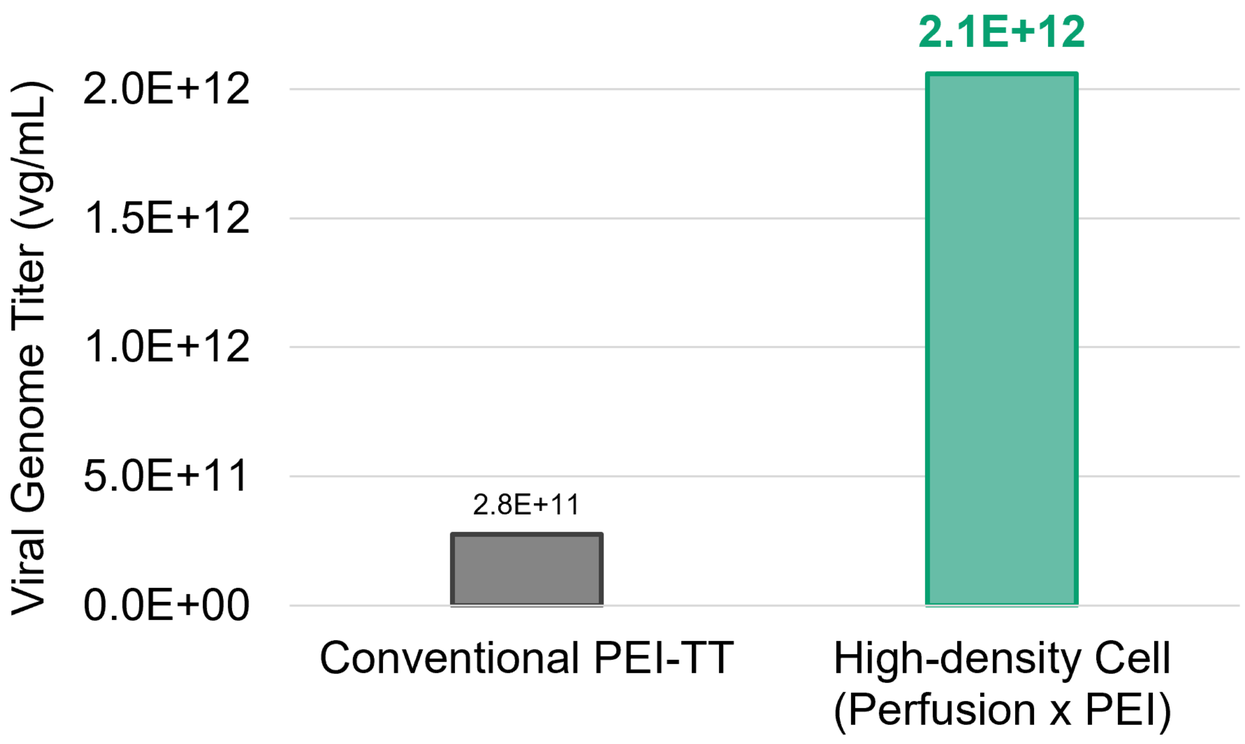
Background Conventional PEI-TT methods (PEI-Transfection to 2E6 cell/mL) have been considered low in rAAV productivity due to the limitation of cell density. As a result, scale-up production in a large-scale equipment is required for AAV mass production.

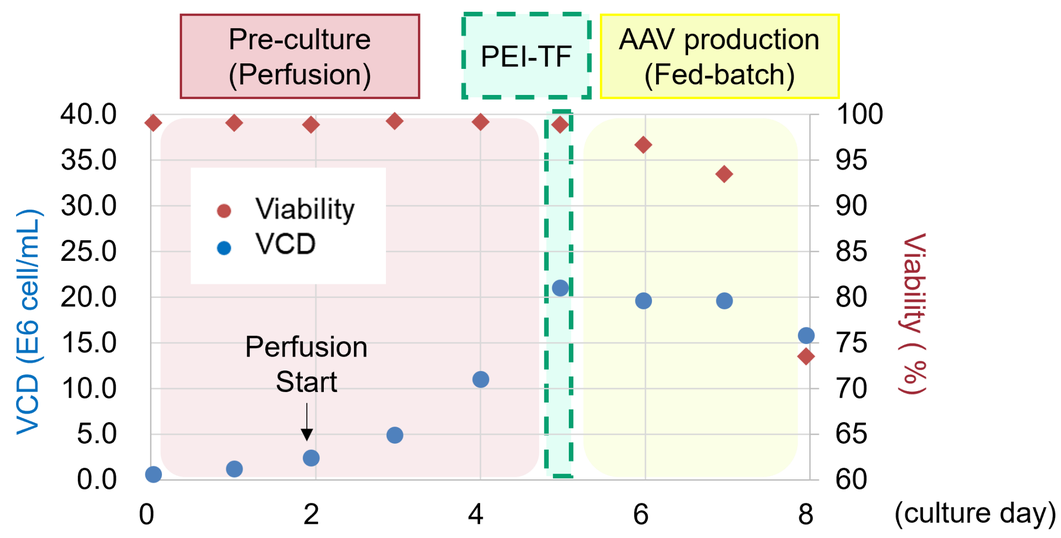
Highlights To increase productivity, our intensified AAV production method enables PEI-mediated transfection into high-density cells by increasing cell density through perfusion culture. It offers approx.10-fold higher rAAV5 productivity in a single transfection batch. This enables us to provide clients with high-yield AAV titers using small-scale equipment, which are equivalent to results obtained with large-scale equipment.
We also demonstrated several downstream purification processes using samples derived from high-density cells. Even with high-density cells, hcDNA and HCP were reduced to the low-density level as those obtained by conventional PEI-TT methods. By replacing the Full/Empty separation with Zonal Ultracentrifugation (ZUC) method, the full ration is significantly improved.

Process Overview



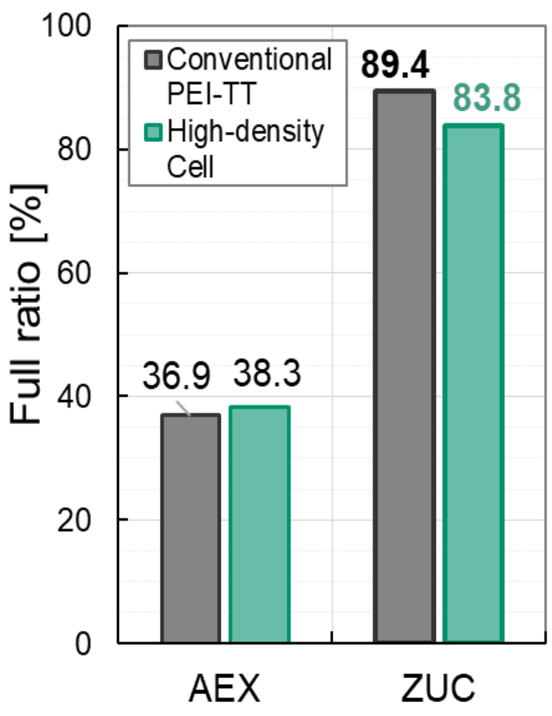
Intensified rAAV5 production from high-density cell





Method	VCD @TF (cell/mL)	AAV5 genome titer (Vg/mL)	Productivity fold change: Vg/mL base	Productivity fold change: Vg base
Conventional PEI-TT	2.0E+06	2.8E+11	1	1
High-density Cell (Perfusion x PEI)	2.1E+07	**2.1E+12**	**7.5**	**9.9**

Impurities clearance and full particle ratio at ZUC



	HCP [ng/mL]		hcDNA [ng/2.1E+12Vg]	
	AEX	ZUC	AEX	ZUC
Conventional PEI-TT	<200	<200	130	82
High-density Cell	<200	<200	148	182

- HCP : Even with High-density Cells, HCP removed to below the detection limit (<200ng/mL, over 99.9% clearance across DSP).
- hcDNA : Even with High-density cells, it was confirmed that hcDNA could be reduced to the same as Conventional PEI-TT.
- Full ratio : With ZUC method, a full ratio of nearly 90% is achieved, regardless of USP conditions

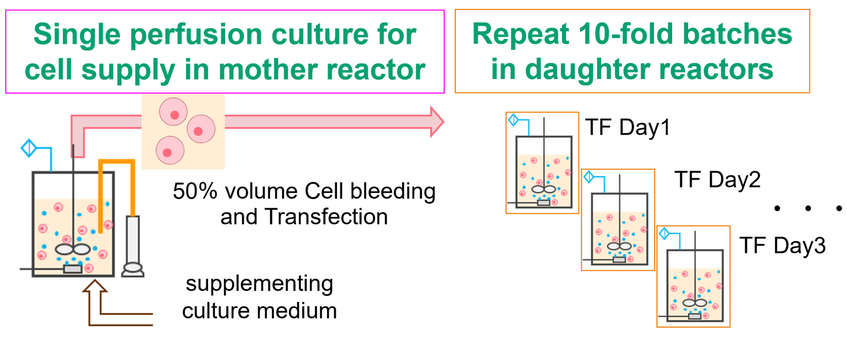
* Above is our lab scale data. Full/Empty separation by AEX can be further improved depending on the yield.

Potential reduction amount of plasmid

	Scale (L)	Titer (Vg/L)	Total VG @USP (Vg)	Plasmid amount (mg per 2.8E16vg)
Conventional-TT	100	2.8E+14	2.8E+16	100
HCD-PEI	10	**2.1E+15**	2.1E+16	**49** ▲51%

This method is estimated to reduce the plasmid by 51% to obtain the same amount of rAAV produced in a 100L batch at a 10L scale without any equipment and steps other than perfusion culture.

Further improvement



This method enabling 10-fold rAAV production with high-density cells can be repeatedly implemented by preparing another reactors. It could potentially provide productivity equivalent to equipment over 100 -fold larger, even with a small-scale reactor.

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