Challenges of manufacturing of AAV for gene therapy products

focusing on upstream and downstream processes

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Agenda

1 Introduction of Astellas

2 USP of AAV manufacturing

3 DSP of AAV manufacturing

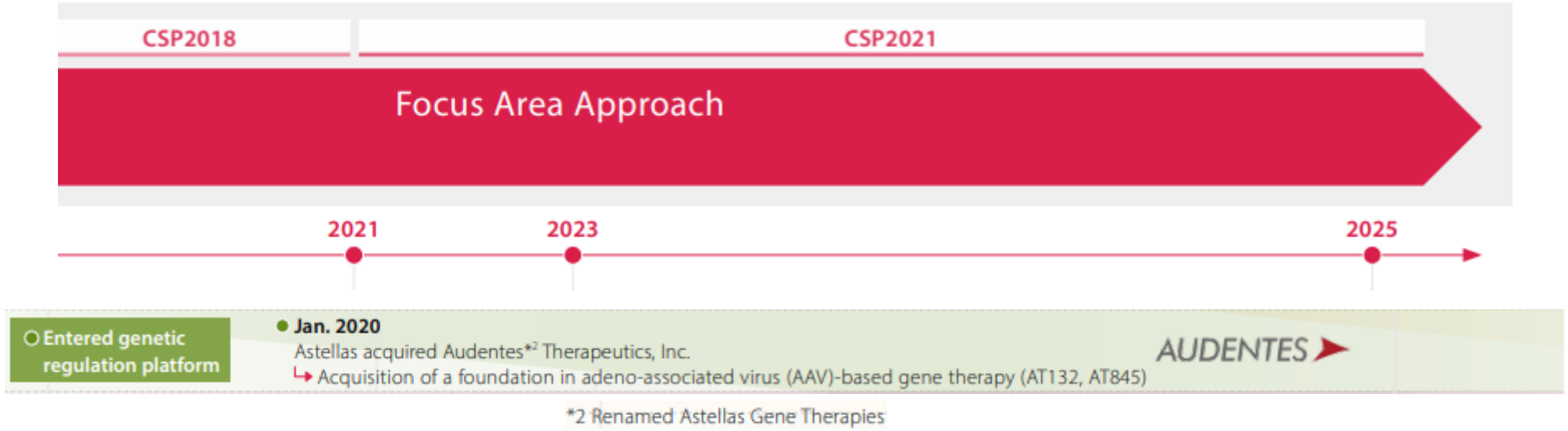
4 Summary

Introduction of Astellas

astellas

Our history of Value Creation

From Astellas' Integrated Report 2025



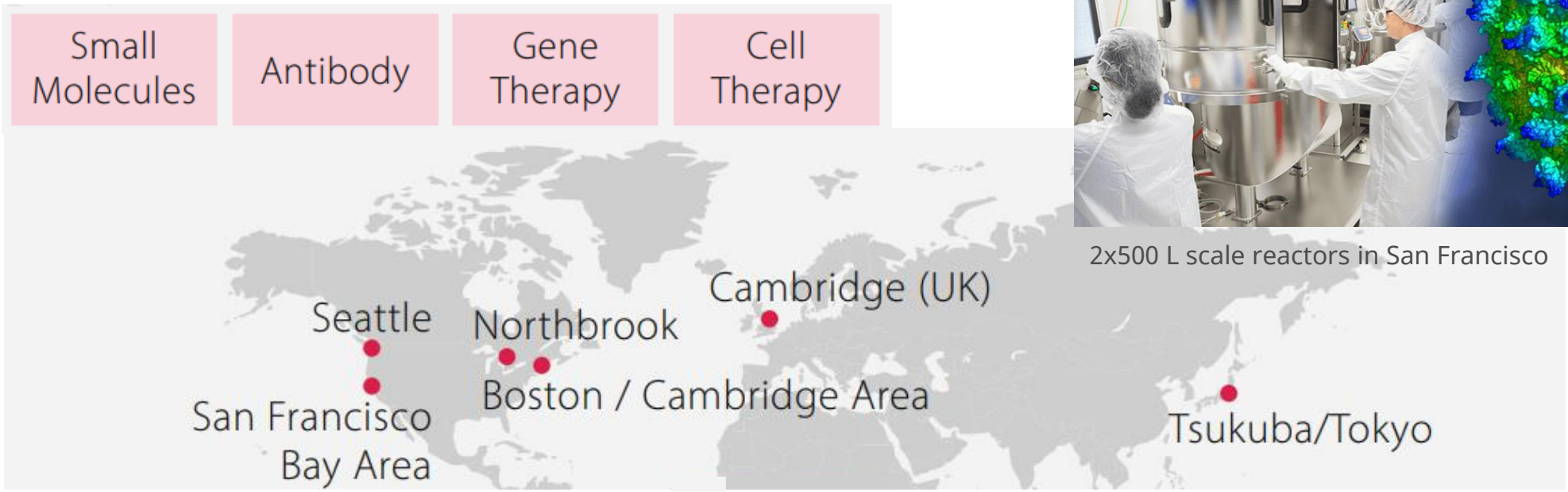
Given major changes in the healthcare industry environment, Astellas is conducting its research activities while incorporating cutting-edge science from outside the Company, and continuously creates new valuable drugs. Astellas will continue to work on Focus Areas, where we have a competitive advantage, and turn innovative science into VALUE for patients.

Astellas pharma Inc. was established in 2005, merged by two Japanese pharma companies Yamanouchi Pharma, established in 1923, and Fujisawa Pharma, founded in 1894, and was well-positioned in the areas of urological diseases and transplant medicine, then entered field of cancer biology, cell therapy and gene therapy.

Our history of Value Creation

2x500 L scale reactors in San Francisco

Major locations (as of September 2025)



The R&D bases of Astellas' gene therapy are located at Tsukuba in Japan and at South San Francisco, California in US.

The manufacturing base is located at Sanford, North Carolina in US.

From Astellas' Integrated Report 2025 and website from Astellas Gene Therapies

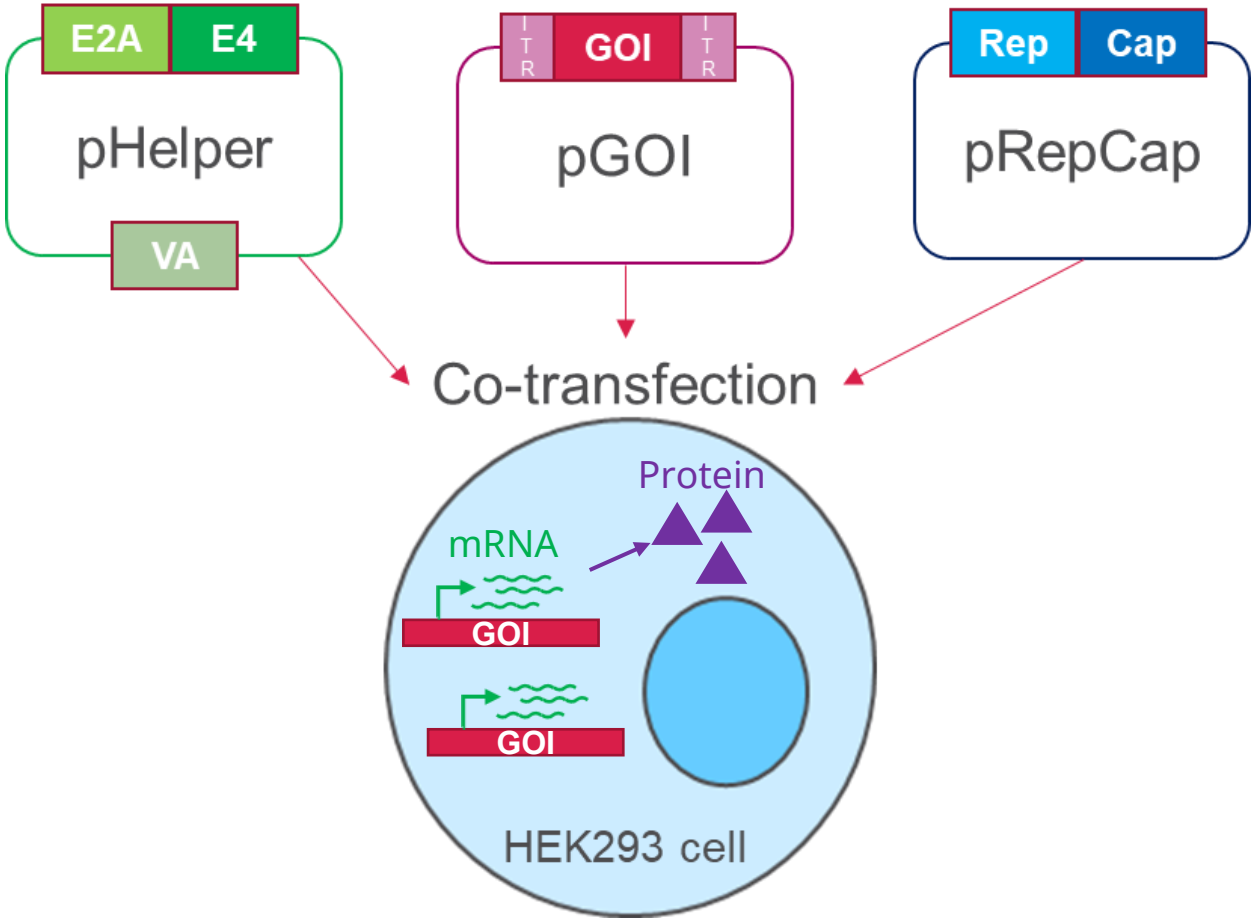
Typical manufacturing of recombinant AAV

	Process Step	Overview
USP	Cell Expansion	• Cell expansion using HEK293 cells, SF9 cells or other cell lines for AAV production
USP	Bioreactor Production	• Transient transfection (most commonly with 3 plasmids) • Optimized transfection for high % full
DSP	Clarification	• Cells lysed & clarified through depth filtration
DSP	Capsid Capture	• Bulk capture using affinity resin
DSP	Enrichment	• Optimized enrichment step to collect full capsids and separate empty capsids
DSP	Concentration & Buffer Exchange	• Concentrate to target vg/mL and exchange to formulation buffer

USP of AAV manufacturing

GOI expression may affect to AAV production

Triple-plasmid transfection method



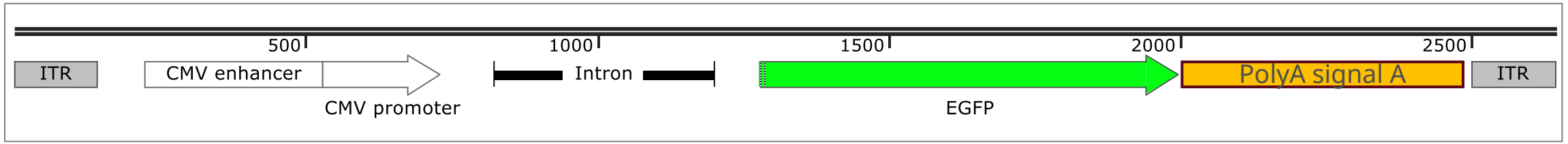
Transient protein expression from pGOI, plasmid of gene of interest, may affect AAV production.

We observed this phenomenon even when we used GFP as the GOI.

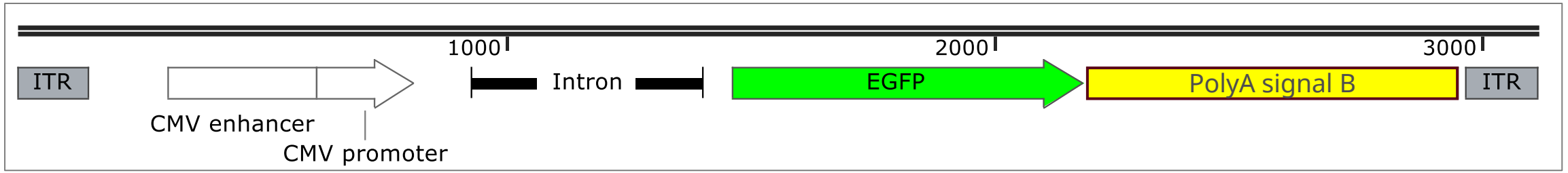
It may be because of cell stress due to over-expression of GFP.

GOI expression may affect to AAV production

Construct 1: 2.6 kb



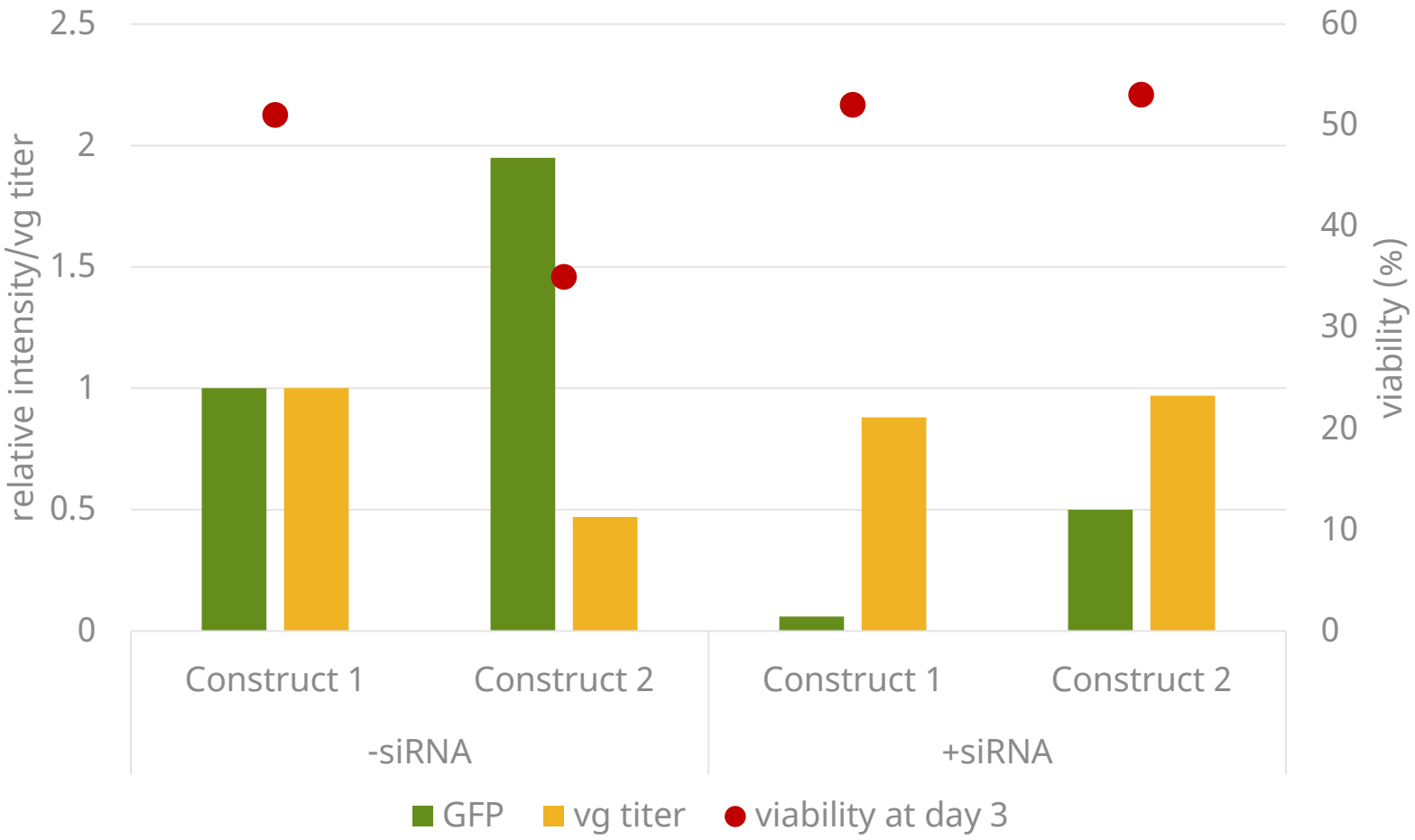
Construct 2: 3.1 kb



We compared AAV production using two different pGOI, both encoding CMV-EGFP.

The differences are origins of introns and polyA signals.

GOI expression may affect to AAV production



GFP expressed as twice as high at Construct 2 compared with Construct 1.

Due to high GFP expression, both cell viability and AAV production decreased at day 3.

GOI expression, not only GFP, may affect the amount of AAV production.

DSP of AAV manufacturing

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DNA from lysed cells may prevent down-stream purification steps.
DNase is used to digest DNA in the solution.

Different nucleases may affect AAV purification

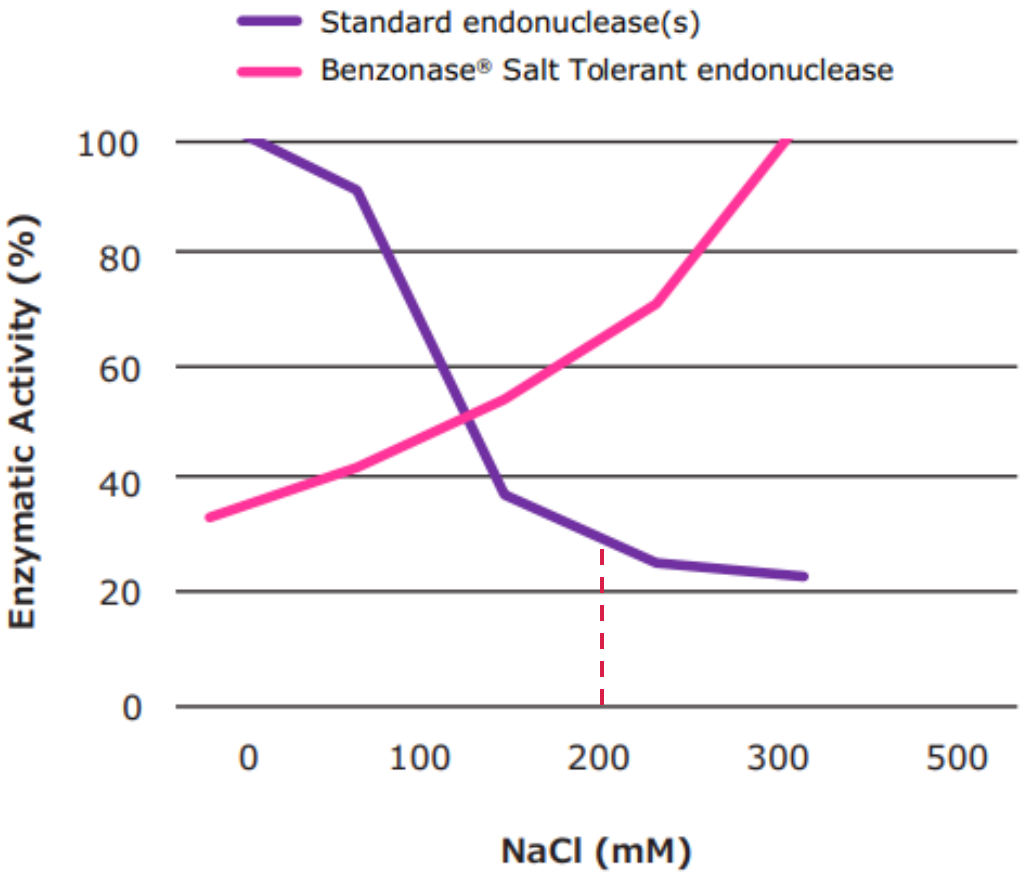


Figure 1:
Enzymatic activity (%) in the presence of NaCl (mM).

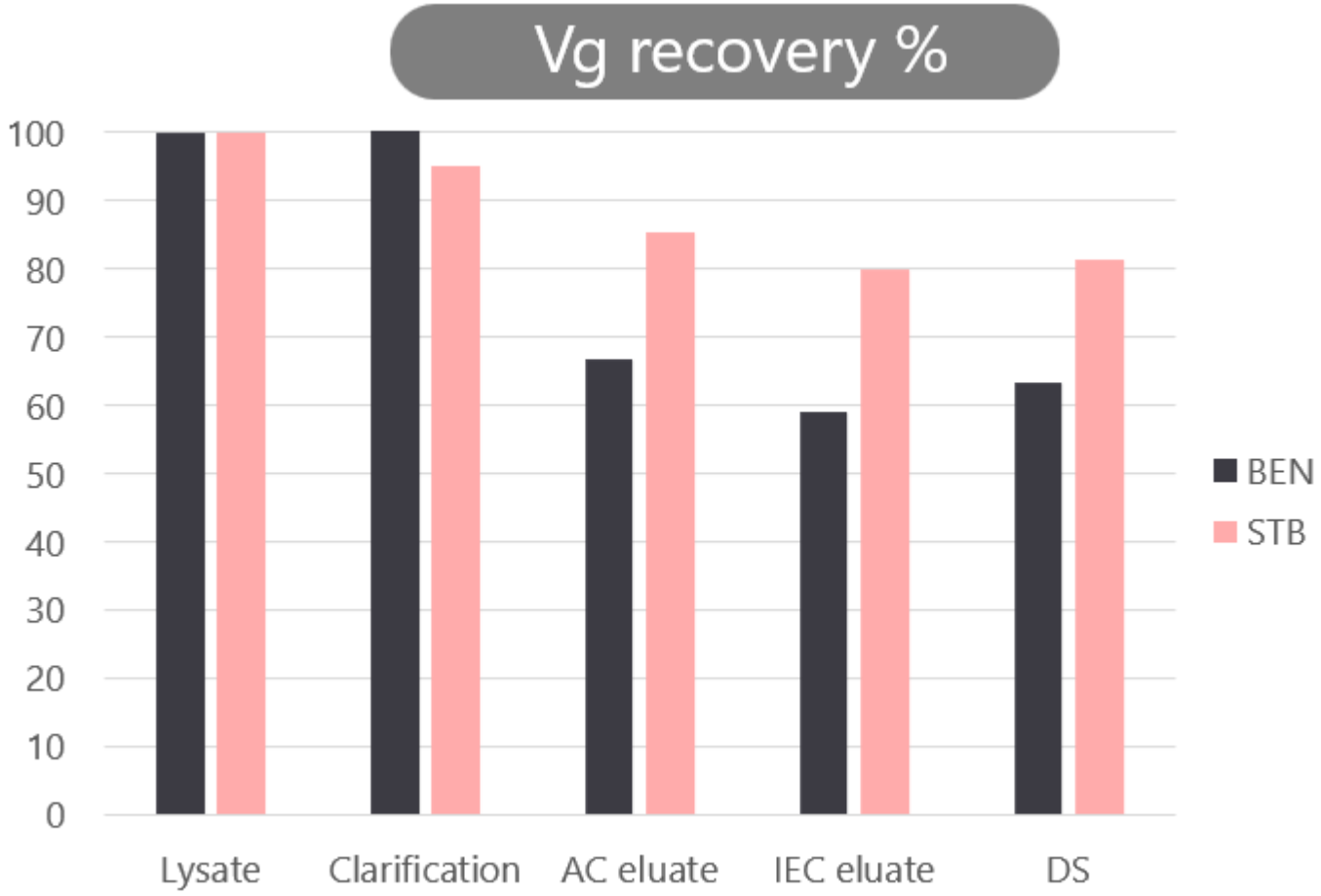
For AAV2 and AAV2 driven engineered capsid, it is known to add salt during the lysis step will increase yield of AAV. But, when salt concentration increased, the enzymatic activity of DNase, used to digest DNA decrease.

We compared Benzonase® Nuclease (BEN) and Benzonase® Salt Tolerant Endonuclease (STB) from Merck, with different salt concentrations.

Modified from STB datasheet from Merck

ds13431en-benzonase-salt-tolerant-endonuclease-emprove-expert-mk.pdf

Different nucleases may affect AAV purification

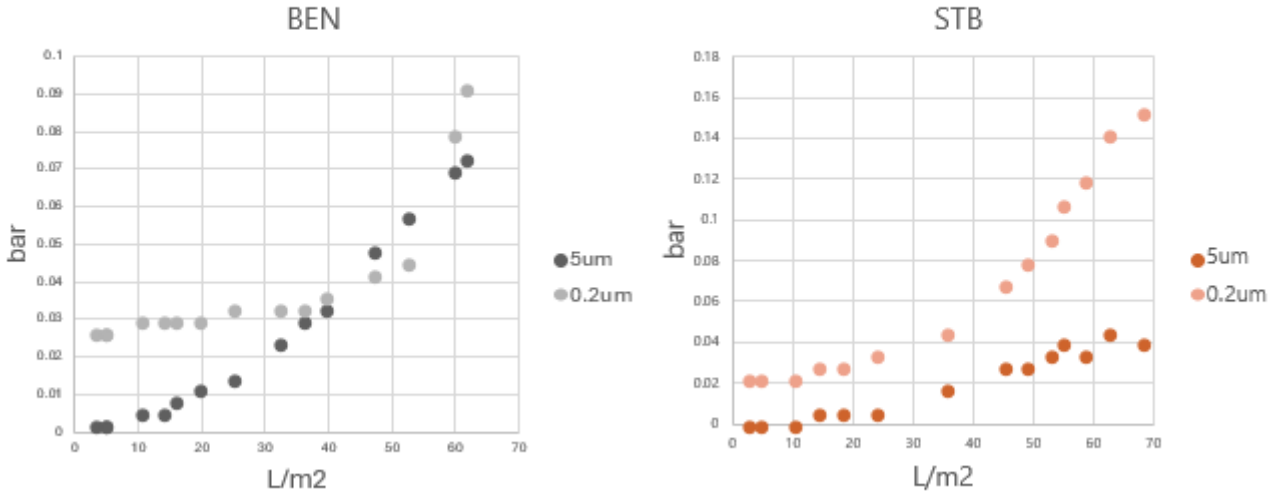


200 mM of NaCl for BEN and
500 mM of NaCl for STB
were added at the lysis step as the optimized condition for each enzyme.

To achieve higher vg yield, it was better to use STB at the lysis step.
It is better to optimize each step to achieve higher vg yield.

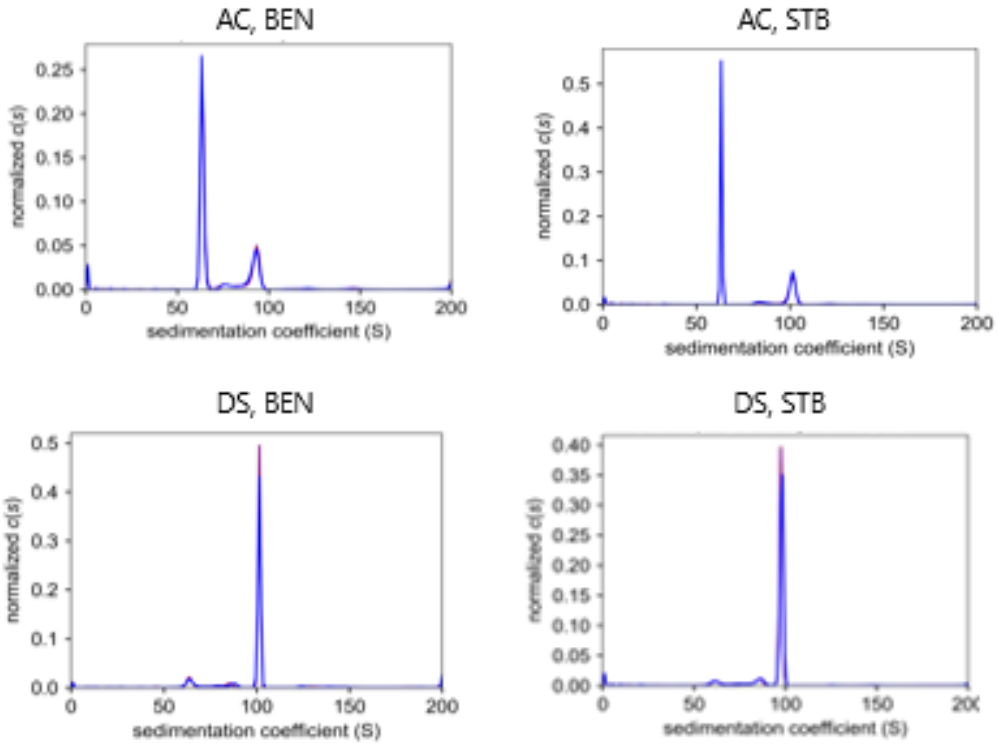
Different nucleases may affect AAV purification

Clarification trend



It seems DNase activity was not enough when we used BEN with 200 mM NaCl.

AUC



The qualities of DS were similar.

Several more additional results are in Appendix.

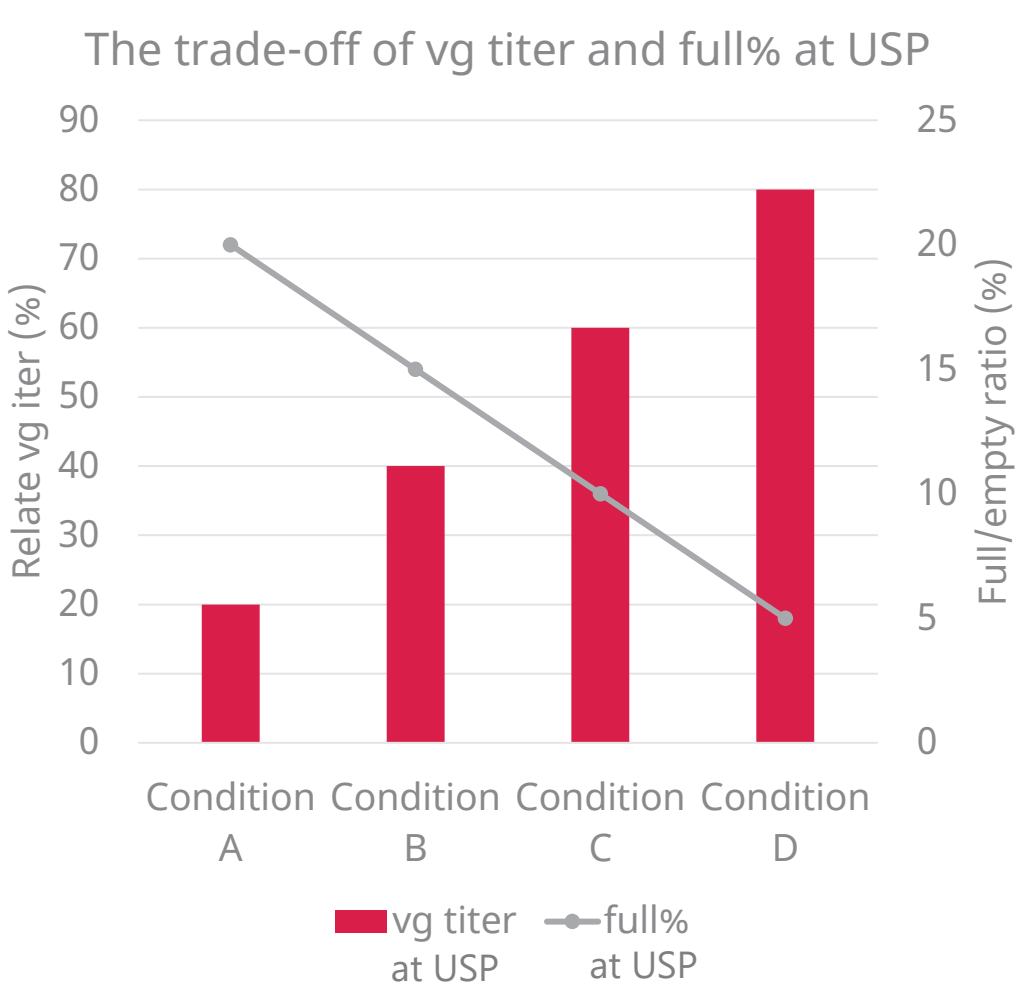
Summary

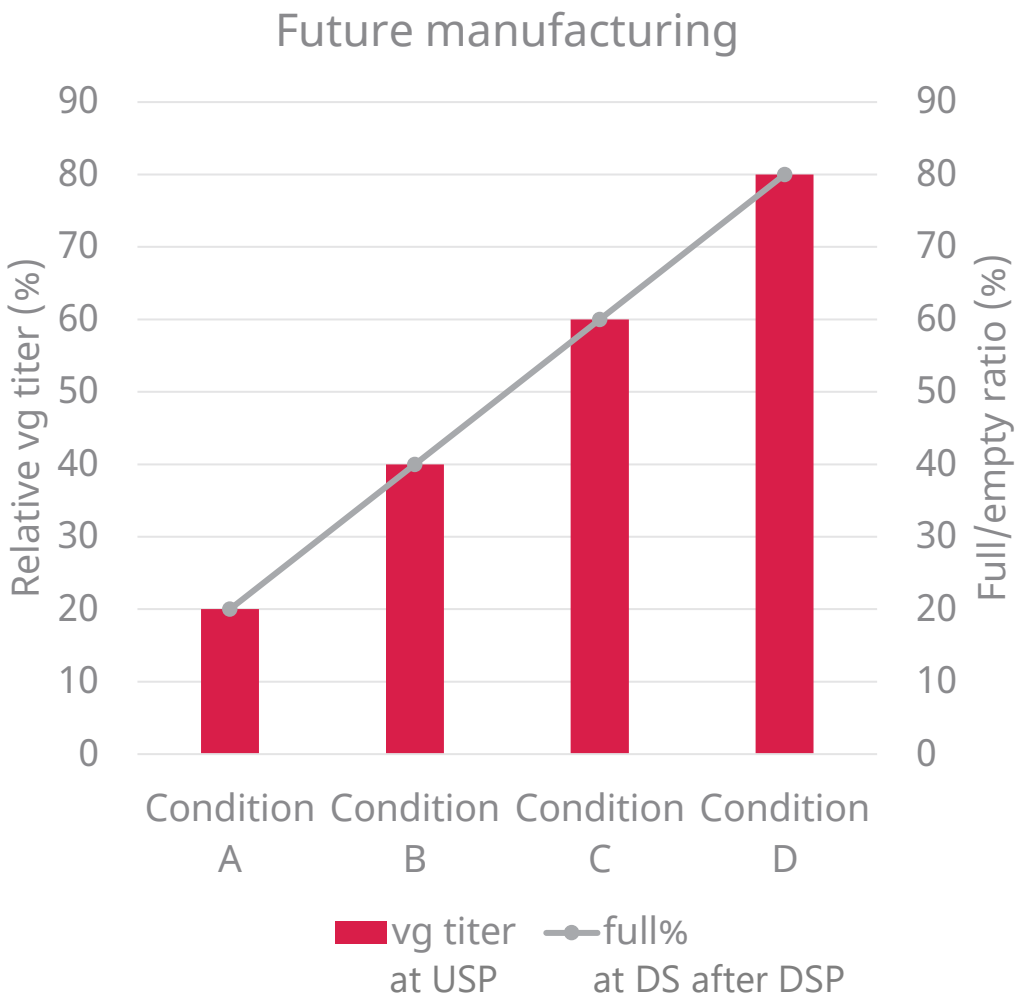
At the USP
Sometimes, the transient gene expression from pGOI during AAV production phase may affect the production of AAV. Even the same CMV-GFP with different designs resulted in different vg titers.

At the DSP
There are many steps that affect the final yield of AAV at the DSP. As shown at this presentation, different DNase with each optimized condition resulted in different yields.

It means that we need to be cautious on comparing results from different facilities using different raw materials.

An image of the ideal of manufacturing of the future





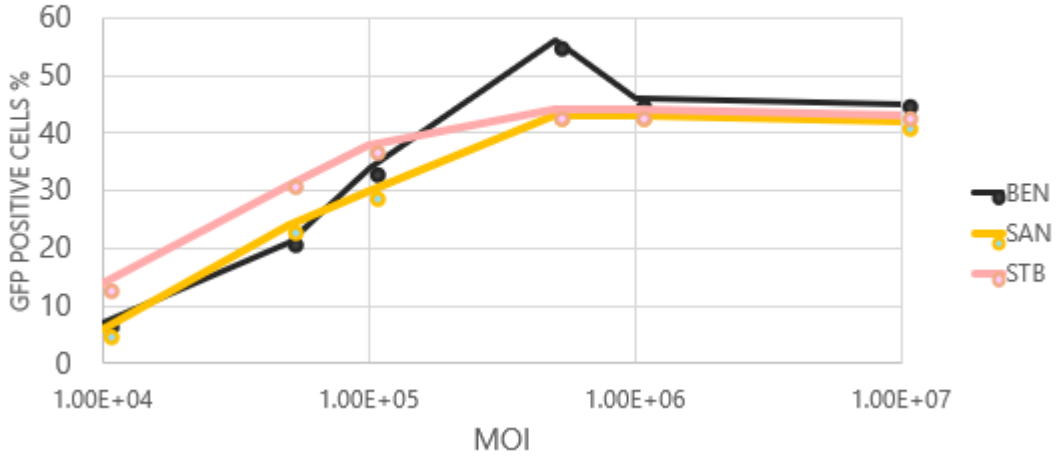
Appendix

Different nucleases may affect AAV purification

Impurities

Sample	Nuclease	HC DNA ng/vg	HCP ng/vg
DS	BEN	100%	< LOD
DS	STB	96%	< LOD

Infectivity



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