SANOFI

Engineering cell lines for AAV cell-based potency assays

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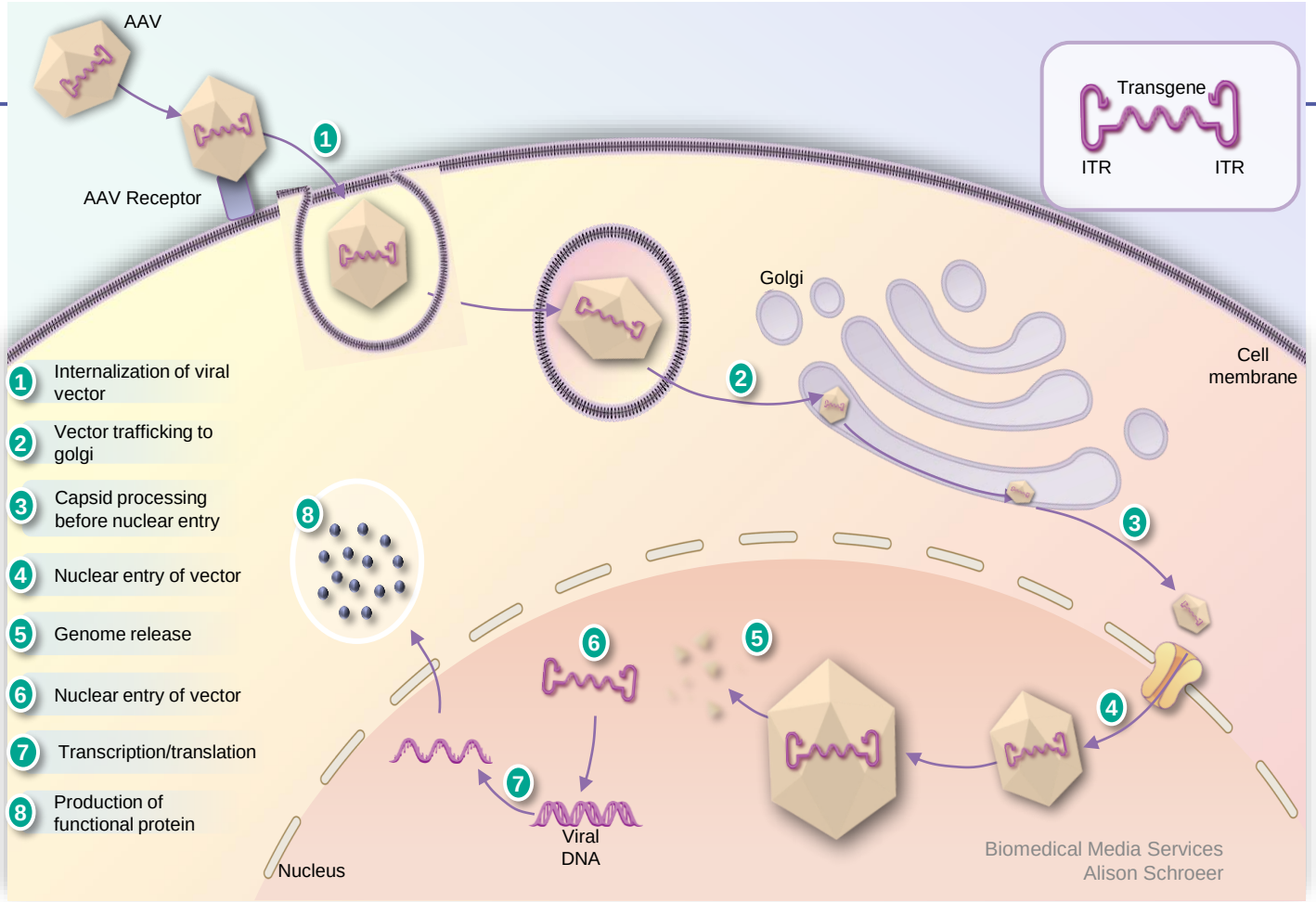
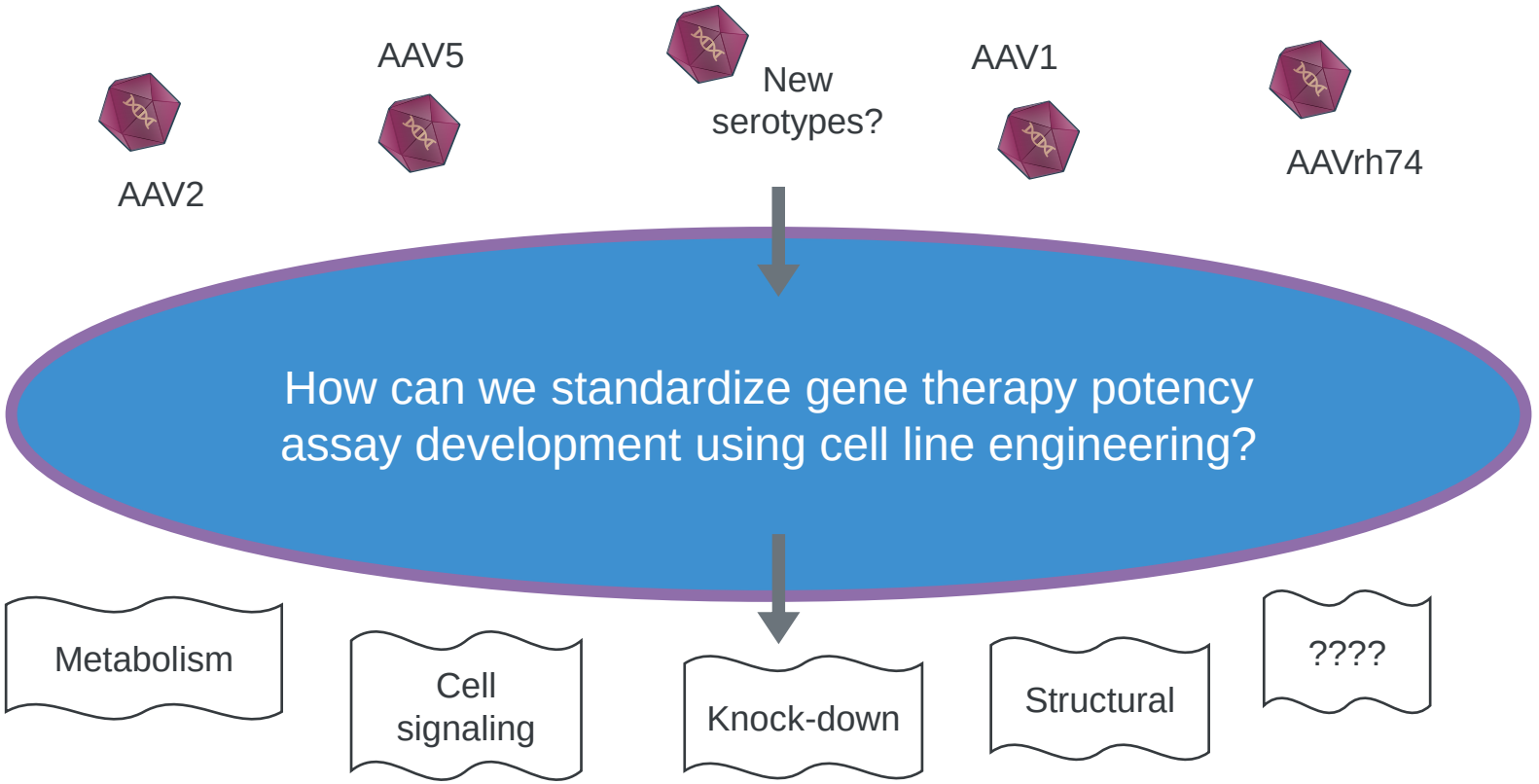
20Apr2020


AAV
AAV Receptor
Transgene
ITR
ITR
Golgi
Cell membrane
1 Internalization of viral vector
2 Vector trafficking to golgi
3 Capsid processing before nuclear entry
4 Nuclear entry of vector
5 Genome release
6 Nuclear entry of vector
7 Transcription/translation
8 Production of functional protein
Nucleus
Viral DNA
Biomedical Media Services
Alison Schroeer

Project goal

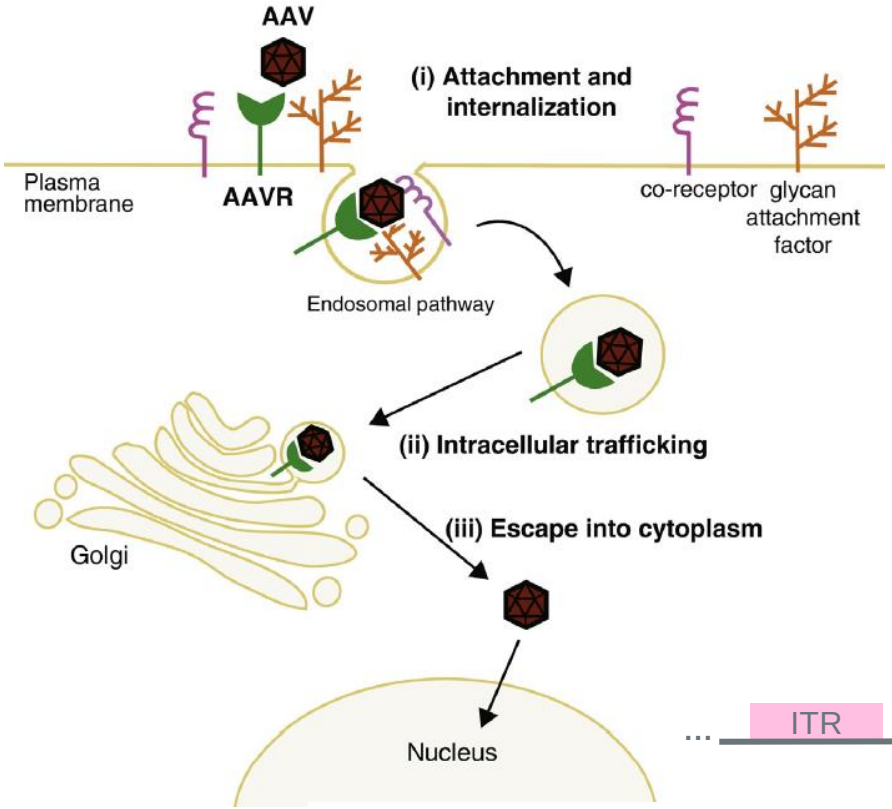


AAV receptors

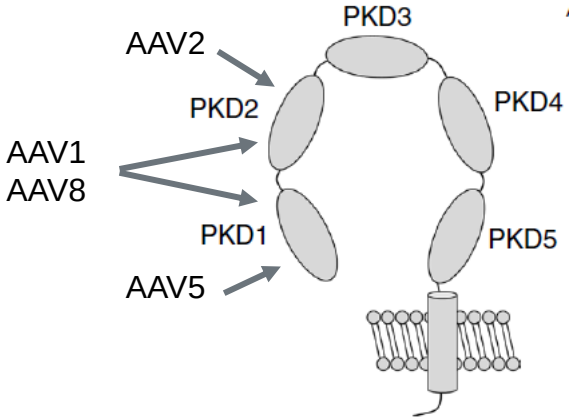
AAV	Lamin Receptor	Heparan Sulfate proteoglycan	Sialic acid	AAV Receptor (AAVR)	G protein-coupled receptor 108	HeLa
1			X	X	X	X
2	X	X		X	X	X
3	X	X		X	X	X
4			X		X	X
5			X	X		X
6		X	X	X		X
7						X
8	X			X	X	X
9	X			X	X	X
Rh.10				X	X	
po1						
12						
13		X				

Adapted from Herrmann 2018

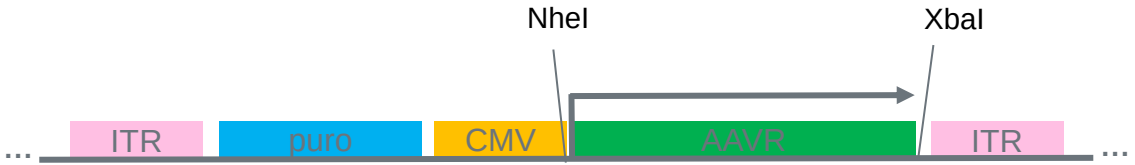
AAVR – a tool for gene therapy potency assays



Pillay and Carette, 2017



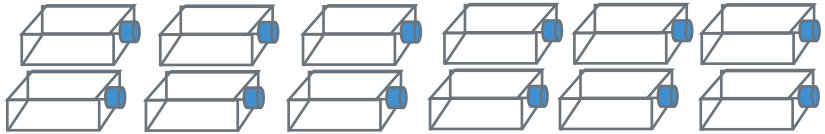
Adapted from Zhang et al, 2019



Piggybac vector

AAVR cells: Cell line development

1. 10-15 clones frozen down as pre-MCBs



2. Test for gene **expression**: choose top 4 expressers

3. Test for **cell growth**: choose best 2

4. Test in assay for **signal** and dynamic range: choose best 1

5. After 2 months in culture, repeat **expression and growth** for **stability**

✓

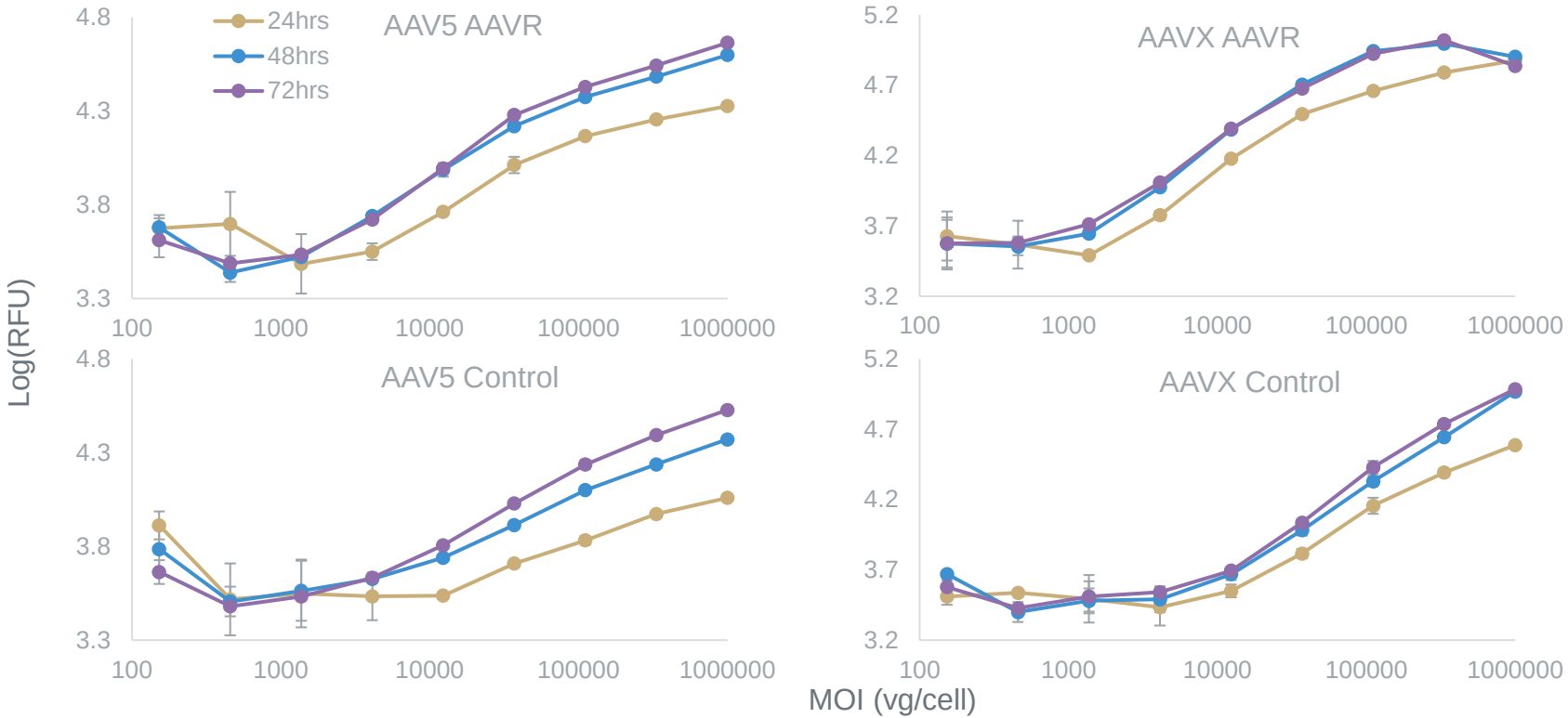
6. Test pre-MCB and MCB to alongside continuous culture in **assay** for **stability**

✓

AAVR cells: Assay development

Time points

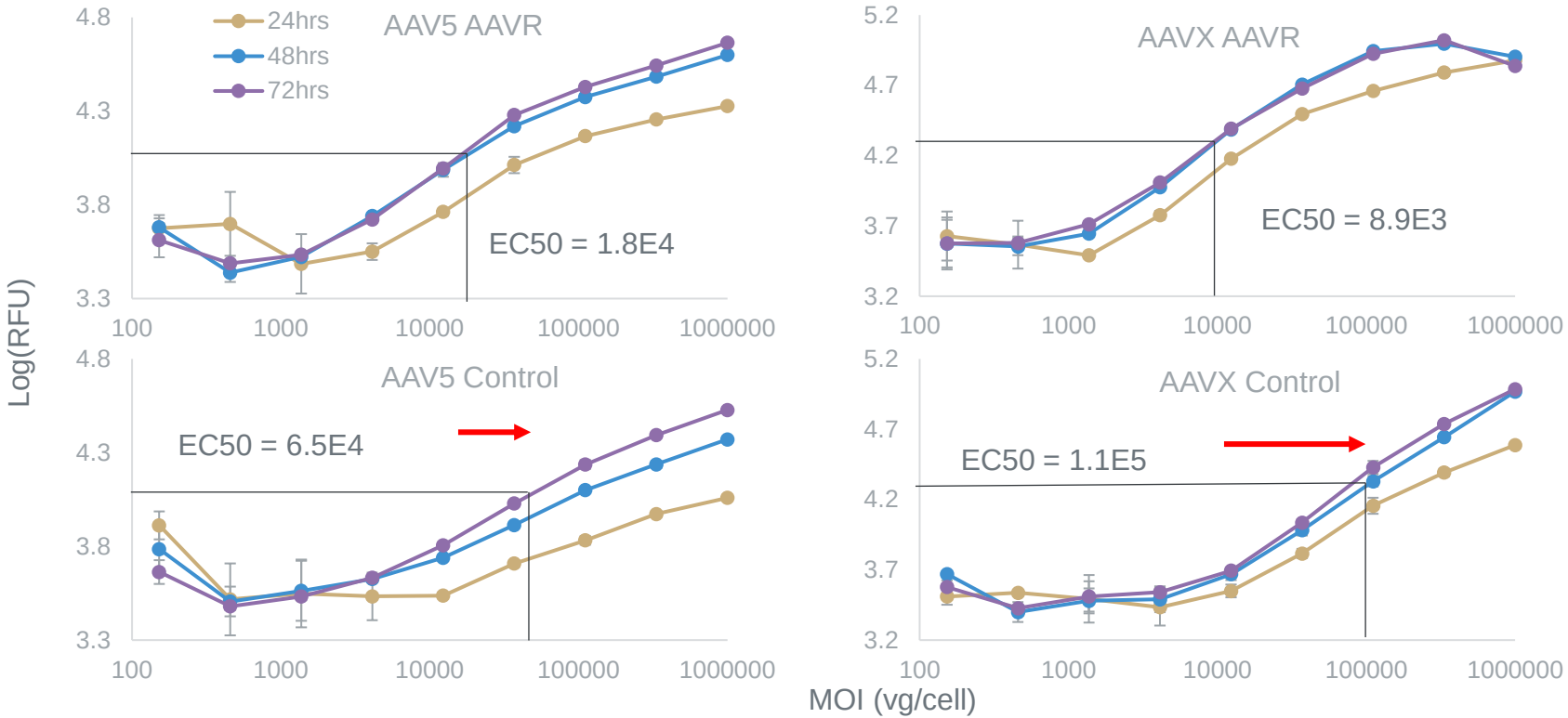
Intra-assay CV ≤5%



AAVR cells: Assay development

Time points

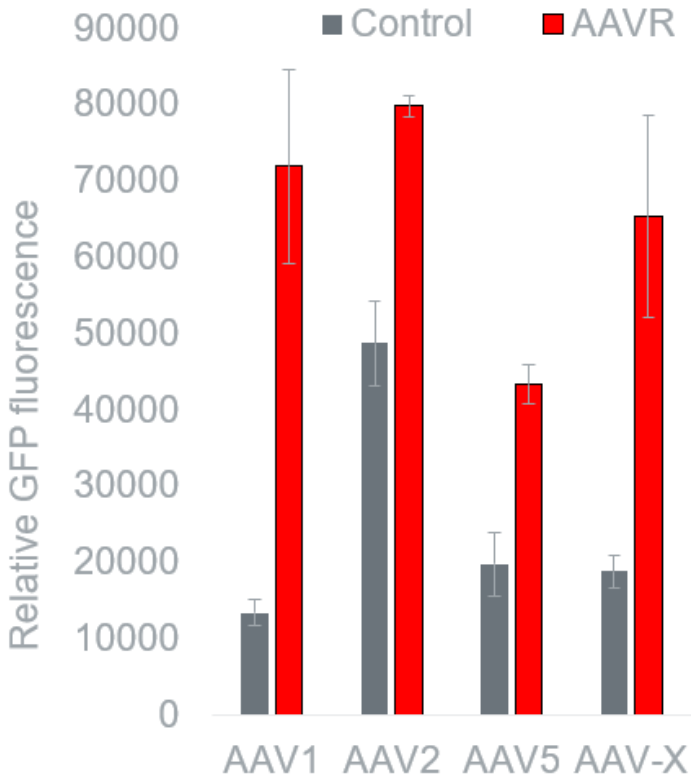
Intra-assay CV ≤5%



AAVR cells: Assay development

AAVR vs Control
- Lower EC50
- Lower %CV on EC50, asymptotes and slope
- Improved F test and R^2
- Potency mimics

✓ Cell seeding density
✓ Plate uniformity
✓ Linearity
✓ Platformable

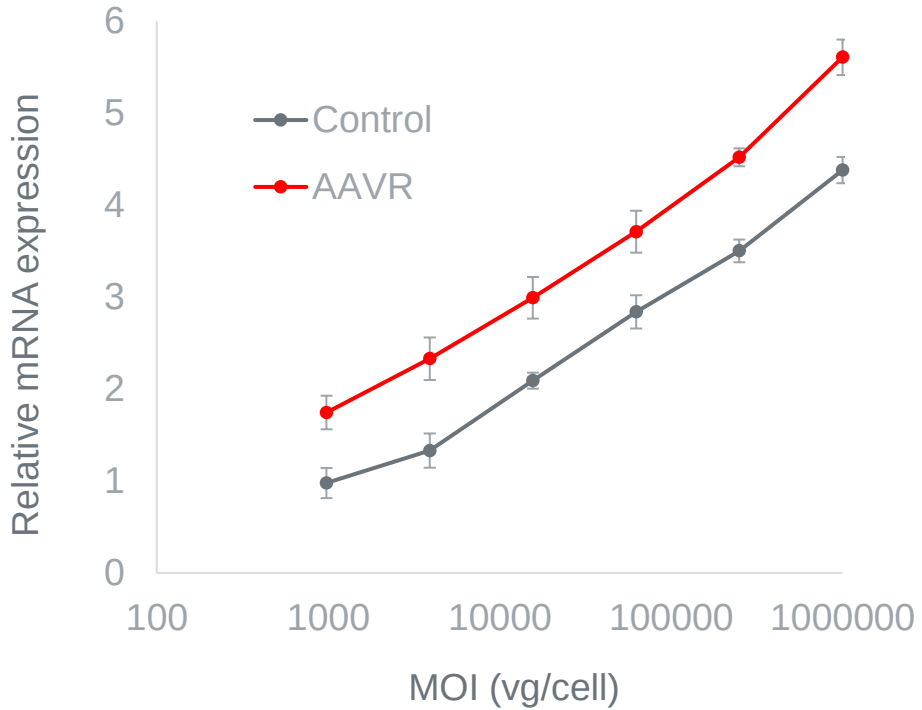


AAVR cells: mRNA expression assay

→ Infection with AAV-X

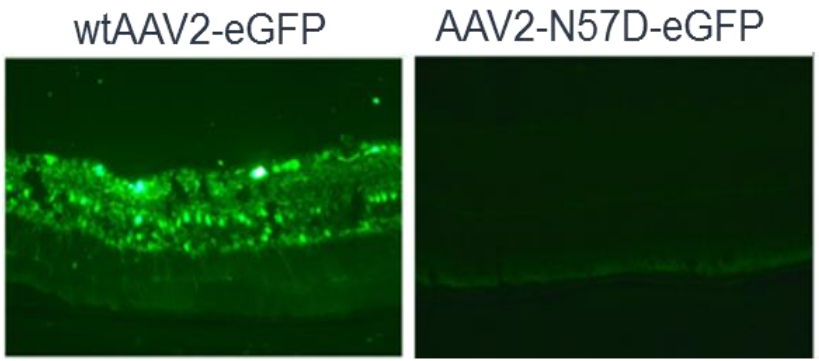
→ mRNA semi-automated King Fisher Flex extraction

→ RT and qPCR



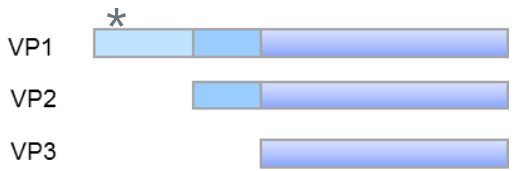
AAVR cells: Stability indicating

Deamidation of N57D – a CQA?



Adapted from Frederick, 2020

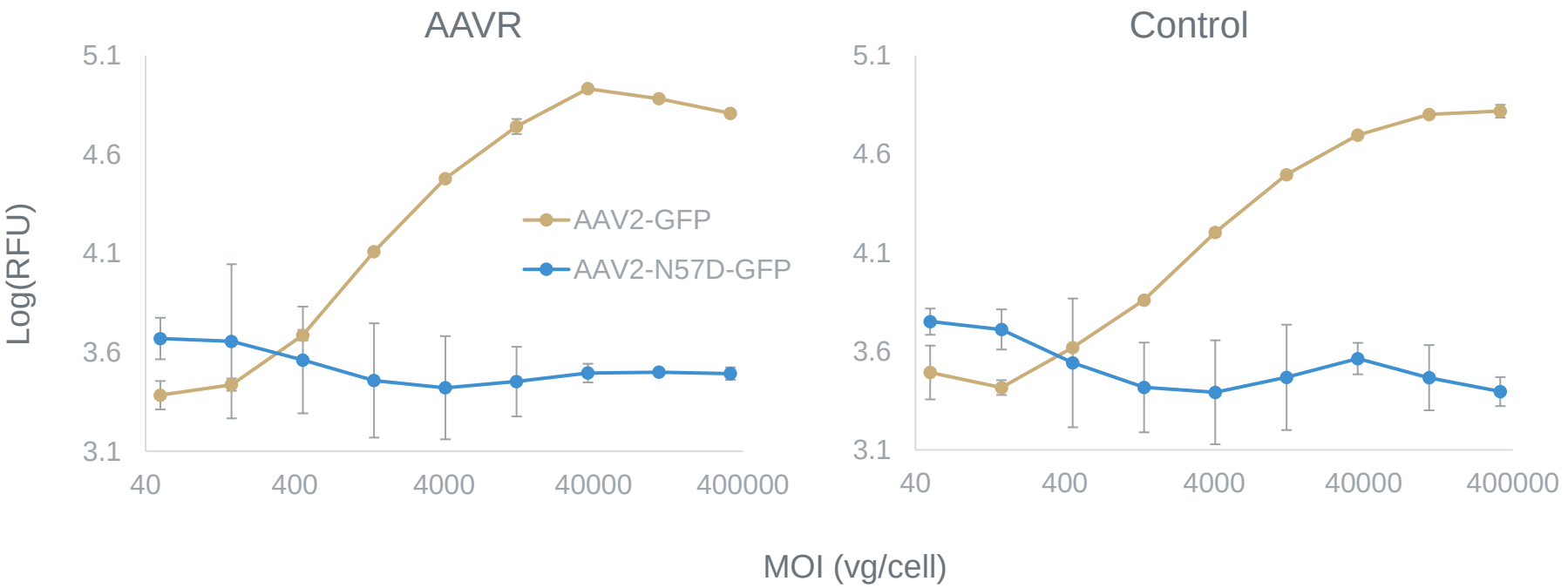
AAV capsid proteins



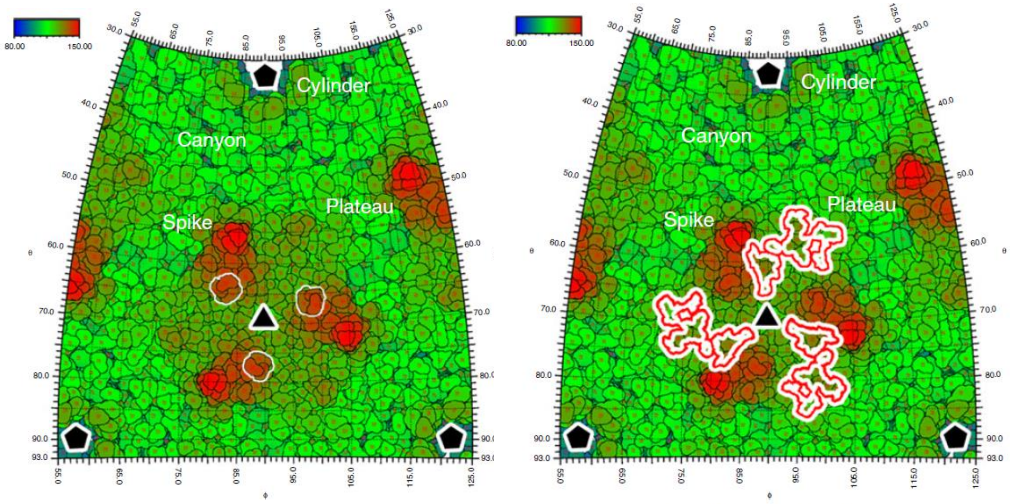
*PLA2 domain
Endosomal escape

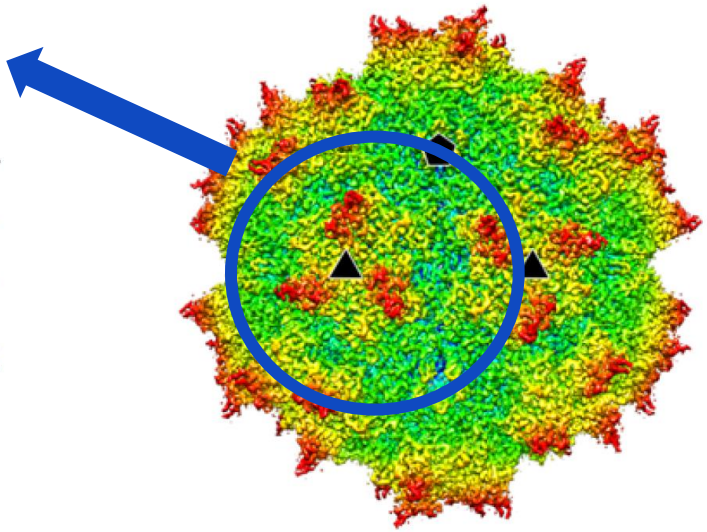
AAVR cells: Stability indicating

Sensitive to deamidation modification



AAVR cells: AAVR and heparan sulfate receptor



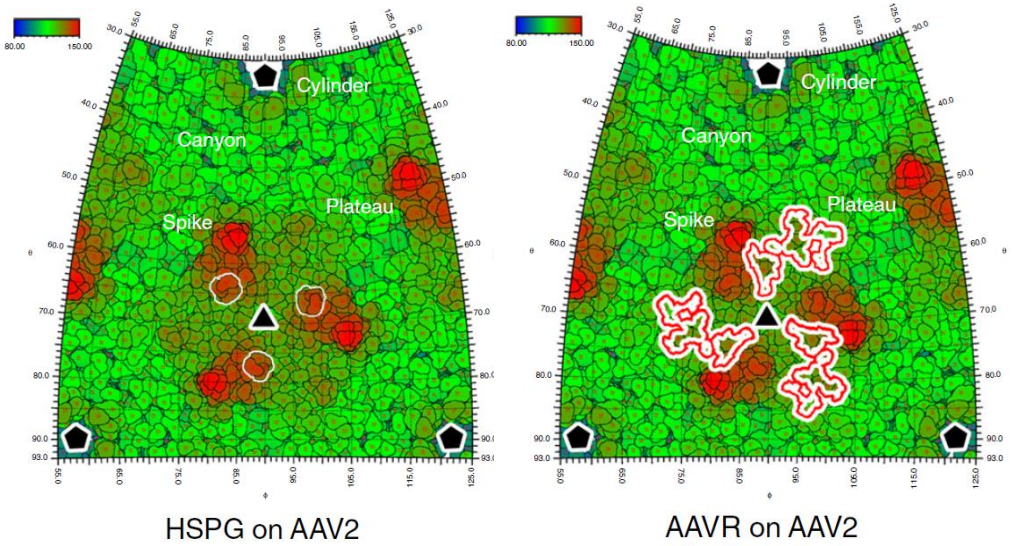


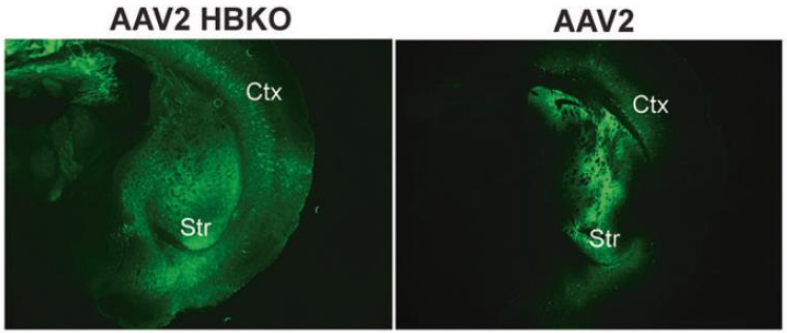
AAV2

HSPG binds R484, R487, K532, R585, R588
AAVR binds via four loops on PKD1, four loops on PKD2 incl R588

Adapted from Zhang et al, 2019

AAVR cells: AAVR and heparan sulfate receptor





Mouse brain, adapted from Sullivan et al, 2018

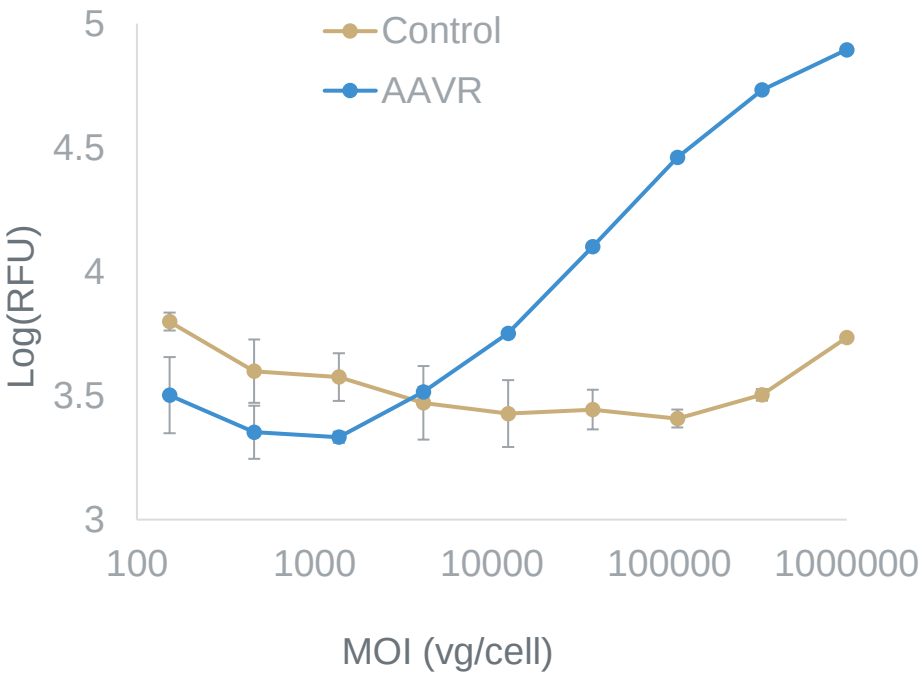
HSPG binds R484, R487, K532, R585, R588
AAVR binds via four loops on PKD1, four loops on PKD2 incl R588

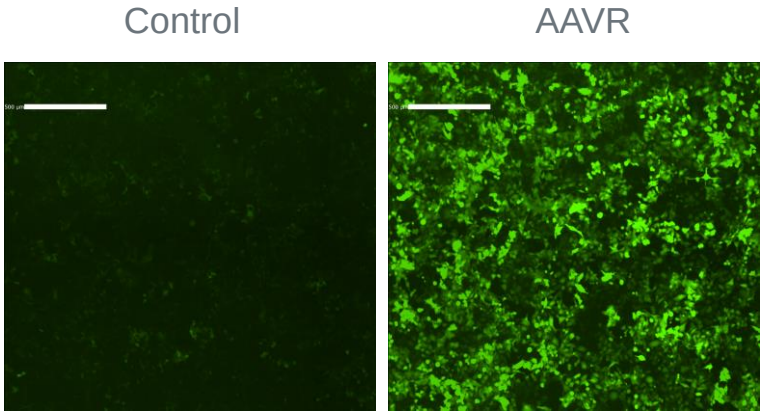
AAV2-HBKO = R585A + R588A

Can AAVR cells still enhance transduction for AAV2-HBKO capsid?

AAVR cells: AAVR and heparan sulfate receptor

Enhanced transduction by AAV2-HBKO mutant





AAV2-HBKO-CBA-eGFP MOI 1E6vg/cell, 72hpi

→ R585 and R588 are not required for AAVR binding to AAV2

Cell line engineering summary

Module 1:

Choose cell

Epithelia

Liver

Muscle

Neuron

Bone

Retina

Module 2:

Choose assay

Promoter

Luciferase

Module 3:

Engineer vector

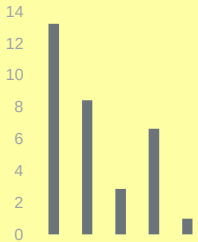
Piggybac

Lentivirus

CRISPR

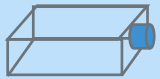
Module 4:

Test system



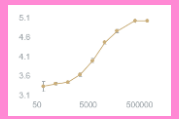
Module 5:

Develop Cell Line

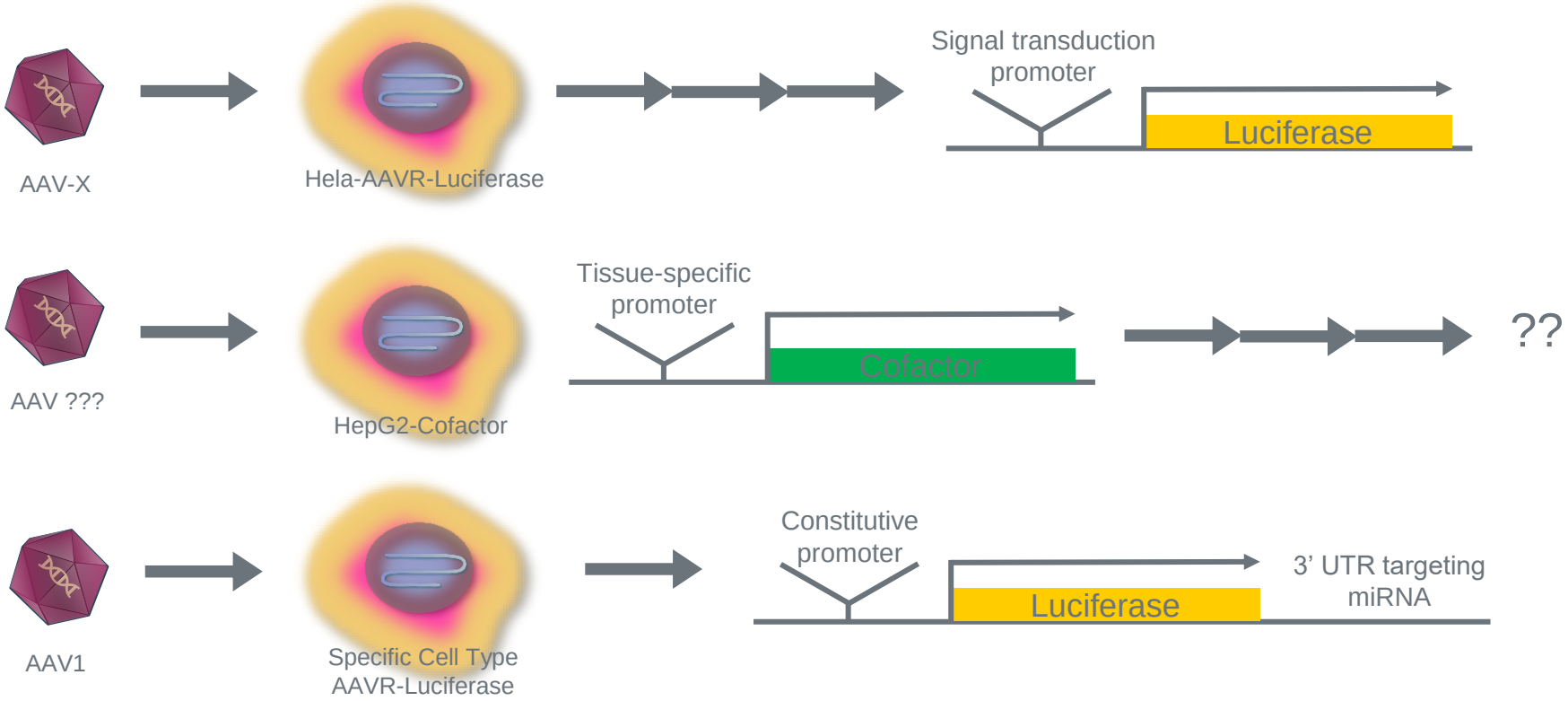


Module 6:

Develop Assay



Bioassay cell engineering examples



Reporters offer improved assays

AAV-miRNA potency assay

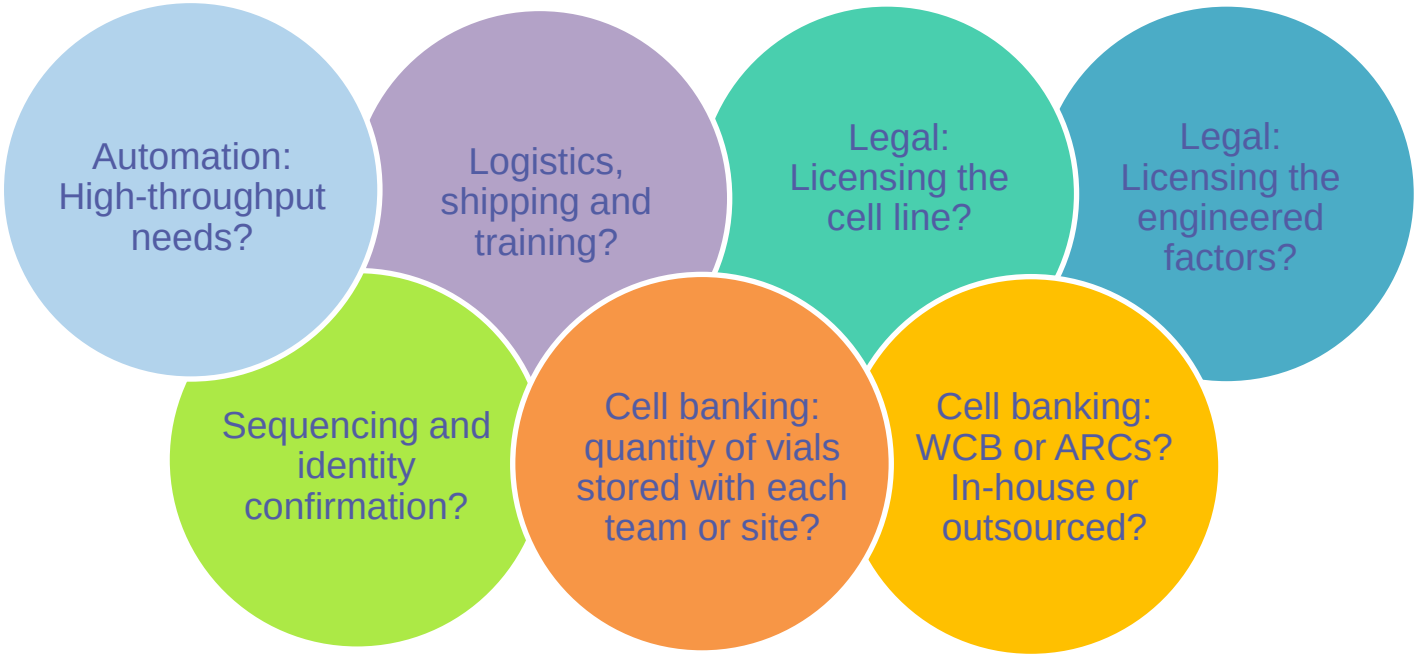
qPCR

- ~ 7 hours, four days
- Pipetting errors
  - Cell seeding
  - AAV infection
  - RNA extraction lysis buffer
  - RNA extraction polyT beads
  - RNA extraction wash buffers
  - RNA extraction elution buffer
  - Reverse transcription
  - qPCR

Luciferase reporter

- ~ 2.5 hours, four days
- Pipetting errors
  - Cell seeding
  - AAV infection
  - Luciferase reagent
- Improved assay dynamic range?
- Improved precision and linearity?

Bioassay cell line engineering considerations



Potency assay development timeline and strategy

~2 years | ~1-2 years | ~1-2 years

CMC	CLD / Tox/Cer / GMP/FAM		CTM	
Analytical Development	Early method development	Method Qualification, **Cell line engineering**	Method Validation, Transfer	
Analytical Strategy *	Release: Expression	Release: Expression Char: MOA potency	Release: MOA potency and expression	Release: MOA potency

THANK YOU

Analytical Development
Sonia Connaughton
Arkadi Manukyan
Alex Depalma
Jarrod Dean
Ying Xu
Aisleen McColl-Carboni
Hannah Maheno
Claire Davies
Francis Poulin

Characterization
Lin Liu
Xiaoying Jin
Qiyu Wang

CLD
Victor Cairns
Jason Vitko

Research
Amy Frederick
Jen Sullivan
Cate O'Riordan
Shelley Nass
Denise Woodcock

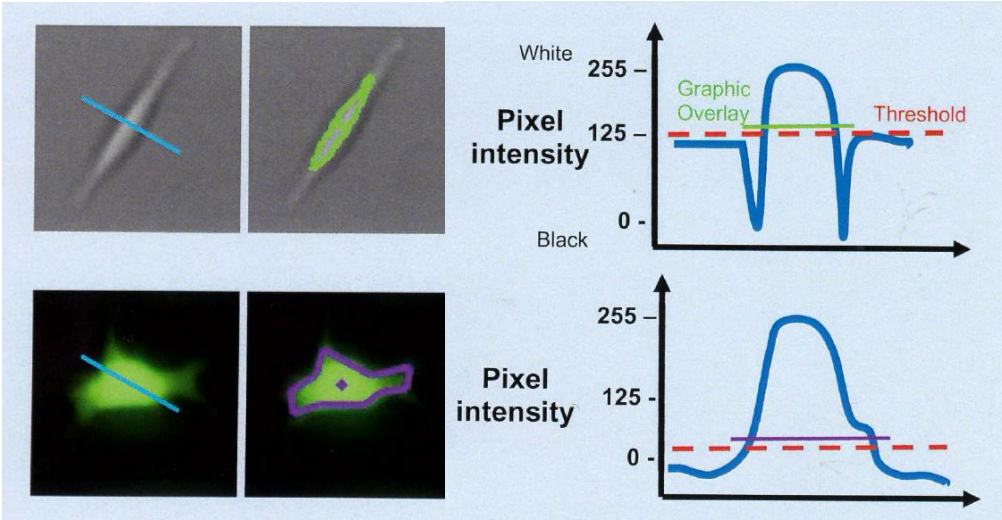
Legal
Anne Collins
Ludovic Villeger

SANOFI

AAVR cells: Assay development

Celigo Cell Imager

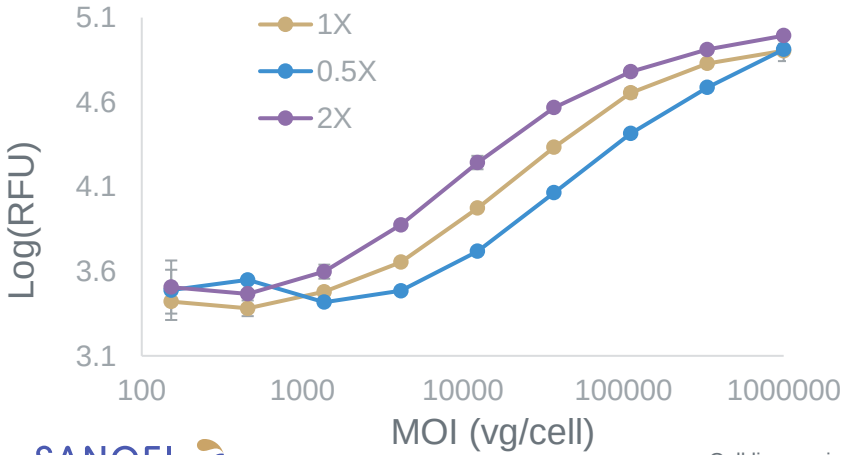
- Plates or flasks imaged in up to 5 channels: Brightfield and 4 fluorescent colors
- Images and counts every cell in each well
- Fast scanning acquires images with minimal plate movement

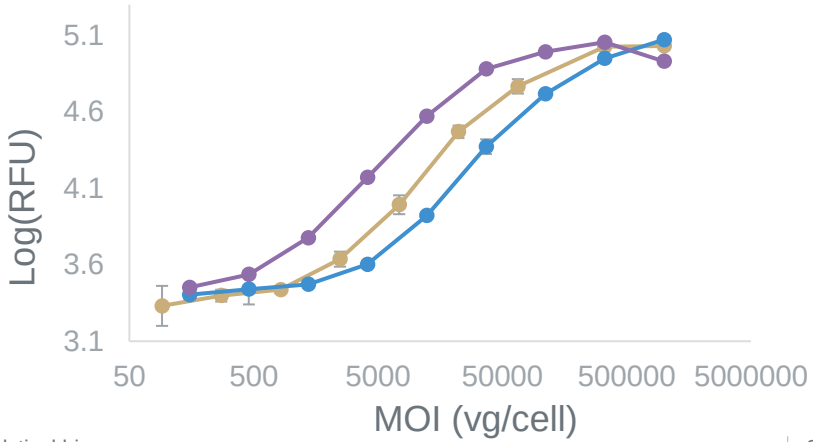


AAVR cells: Assay development

Potency mimics

	AAV5				AAVX			
	Potency Mimic	**Potency (%CV)**	**% Recovery**	**F test p value**	**Adjust R^2**	**Potency (%CV)**	**% Recovery**	**F test p value**	**Adjust R^2**
AAVR	0.5X	43.5% (8%)	87%	0.406	0.993	49.5% (6%)	99%	0.278	0.997
	2X	224.3% (5%)	112.2%	0.332	0.993	276.1% (6%)	138.1%	0.312	0.996
	1X	92.7% (7%)	92.7%	0.770	0.996	96.2% (13%)	96.2%	0.806	0.982





Reporters offer improved assays