



# Assay development within the field of AAV therapies; engineering *iLite*<sup>®</sup> cell lines for specific and analytical applications

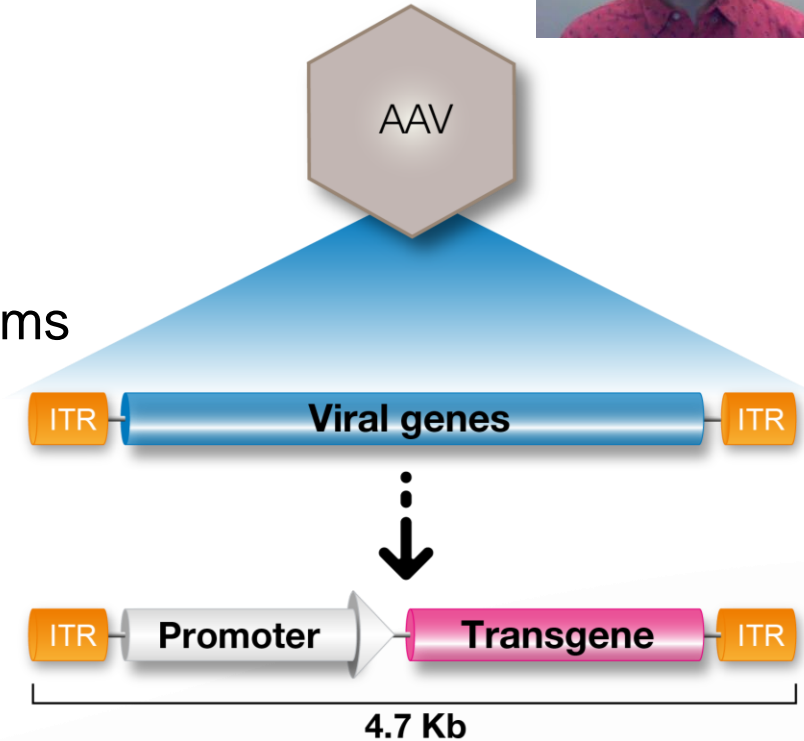
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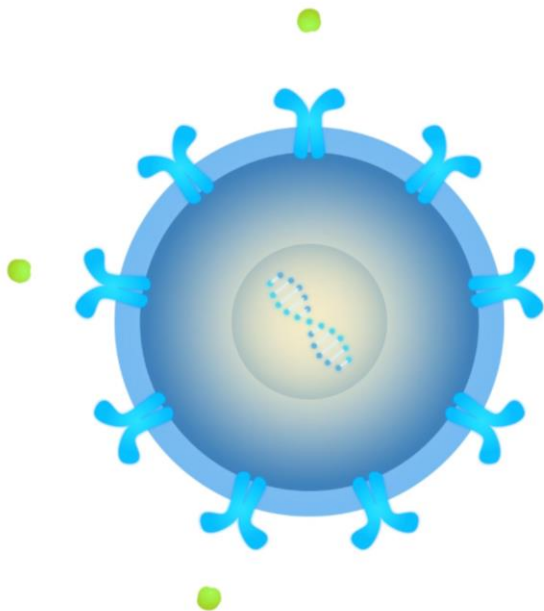
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# Adeno-associated viral vectors (AAV)

- Non-pathogenic
- Transduction of dividing/quiescent cells
- Long-term expression
- Variety of serotypes with different tropisms
- High titer preps can be obtained
- Low cloning capacity (4.7 Kb)



# *iLite*<sup>®</sup> Technology



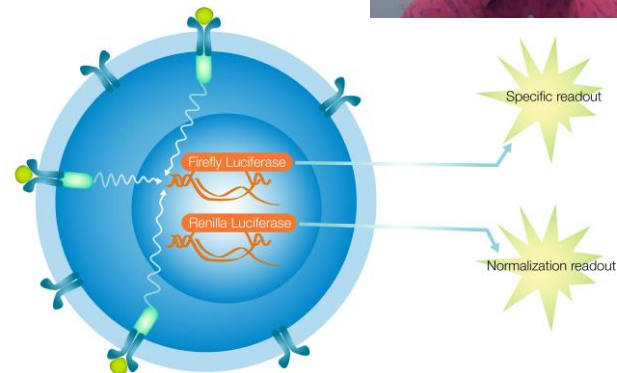
- A cleverly engineered cell-based reporter gene system
- Functionality of the compound can be determined, not just binding interaction
- Reflects the Mechanism of Action (MoA) of the gene therapy product
- Can be design & customized for specific uses/targets and therapies

# iLite<sup>®</sup> Assay Ready Cells

Cells are provided in an "Thaw & Use" assay-ready format for a rapid and convenient workflow

The Assay Ready format

- **decreases timelines and workload**
- gives **superior performance.**
- renders the assay more robust due to **less inherent variability –all cells harvested and frozen at same passage number**



# Strategies for C&GT assay development



**Specific  
Therapies  
or Vectors**

**Analytical &  
Characterising  
Applications**

# Strategies for C&GT assay development



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# Strategies for C&GT assay development



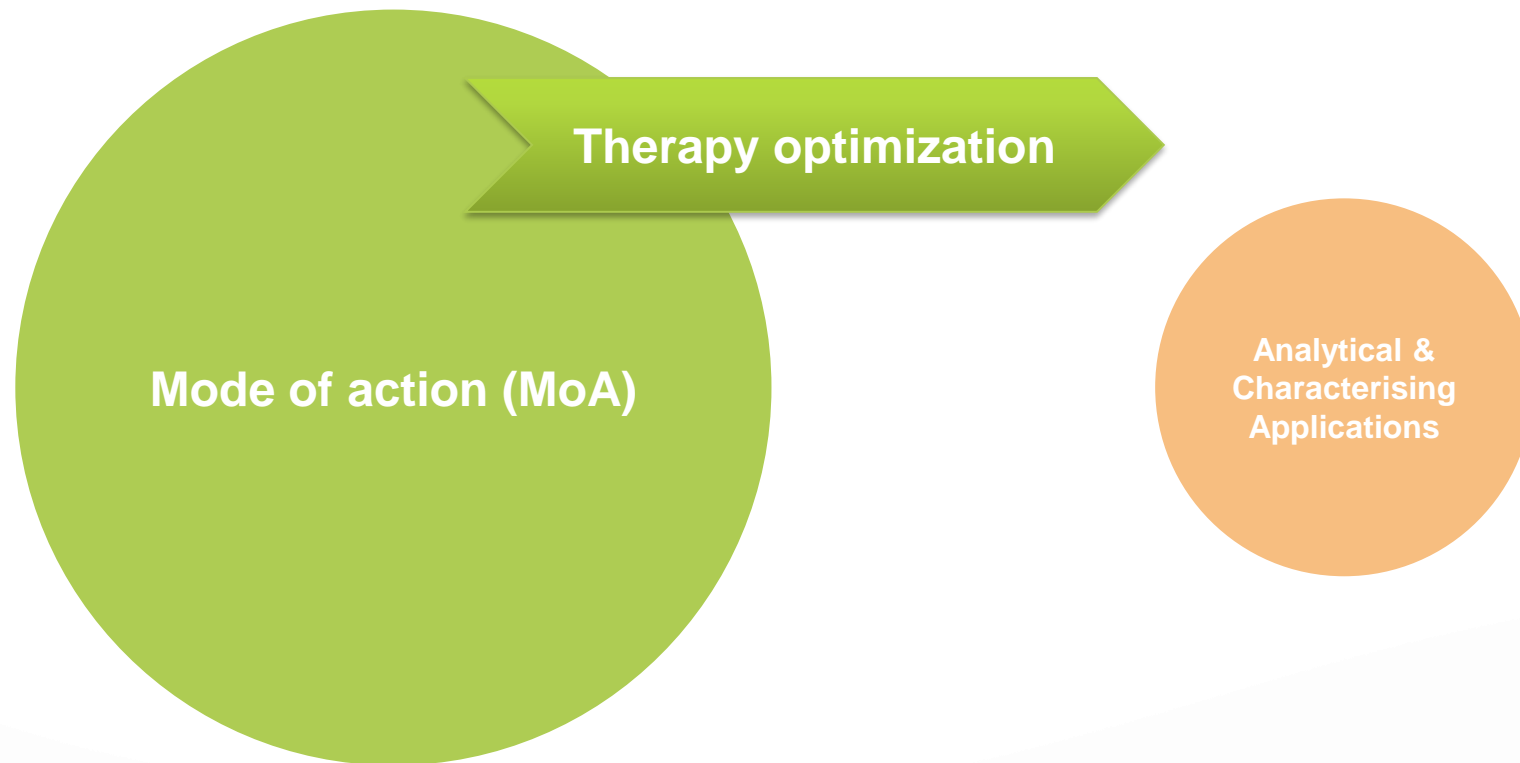
**Specific  
Therapies  
or Vectors**

-

**Mode of action  
(MoA)**

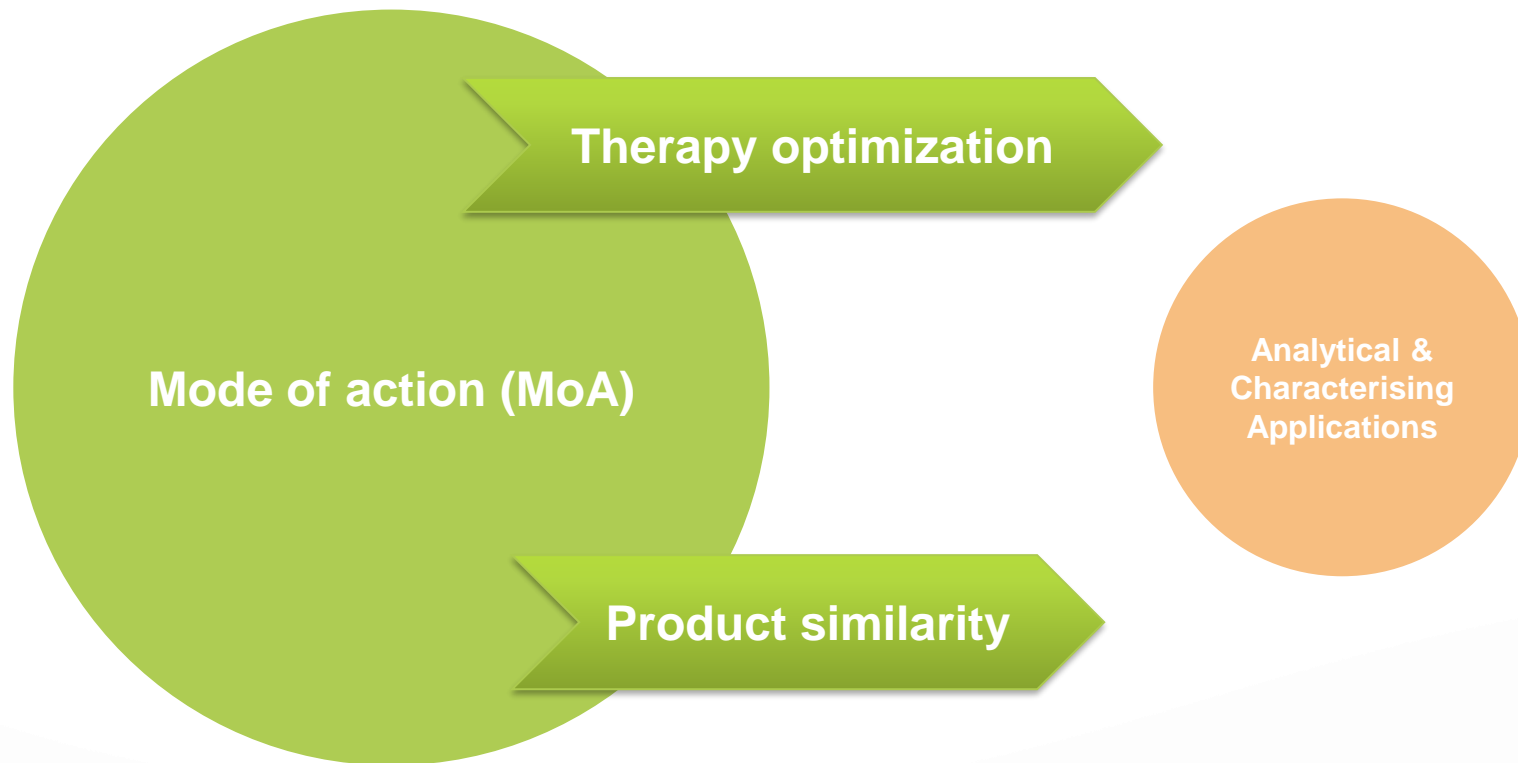
**Analytical &  
Characterising  
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# Strategies for C&GT assay development

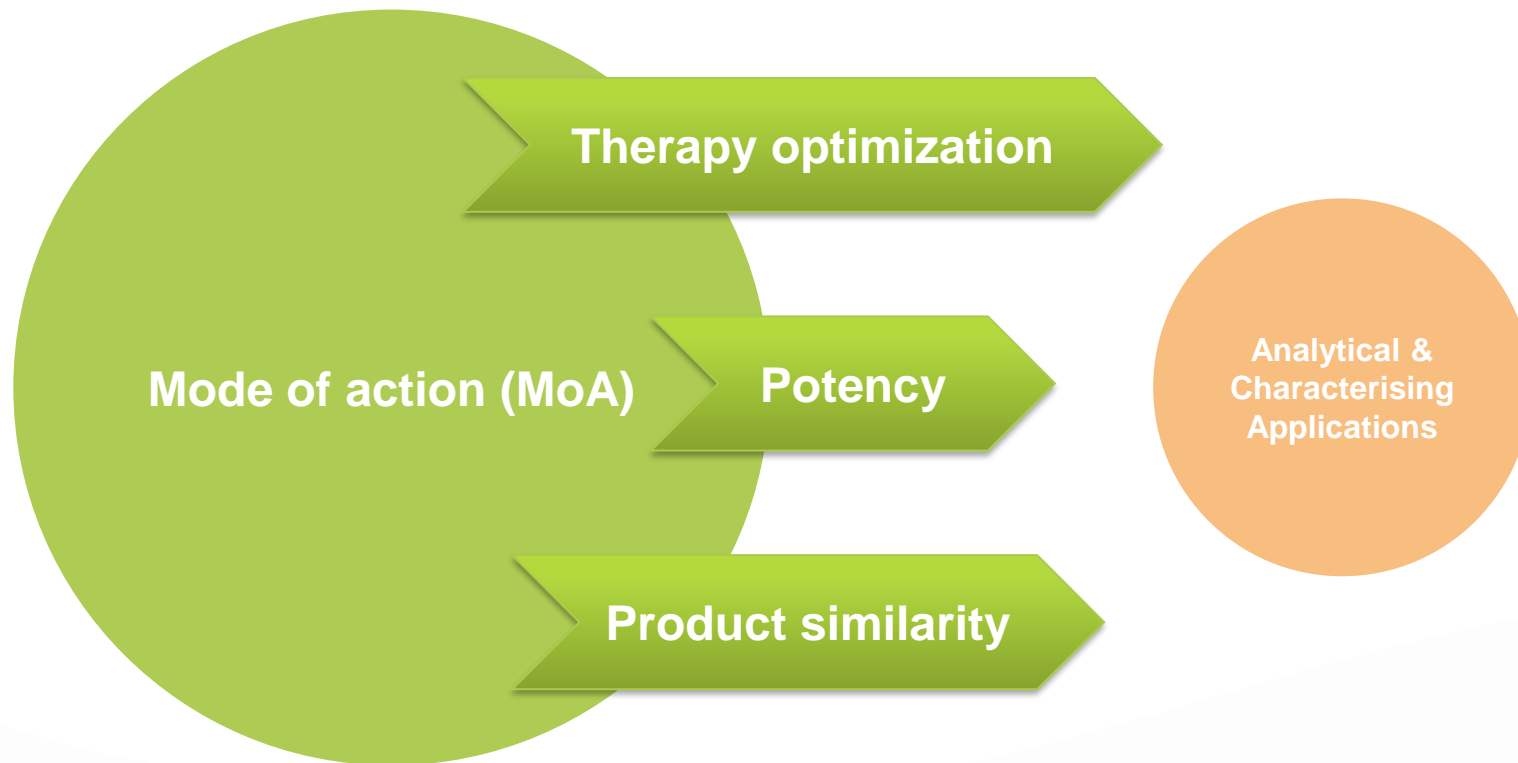




# Strategies for C&GT assay development



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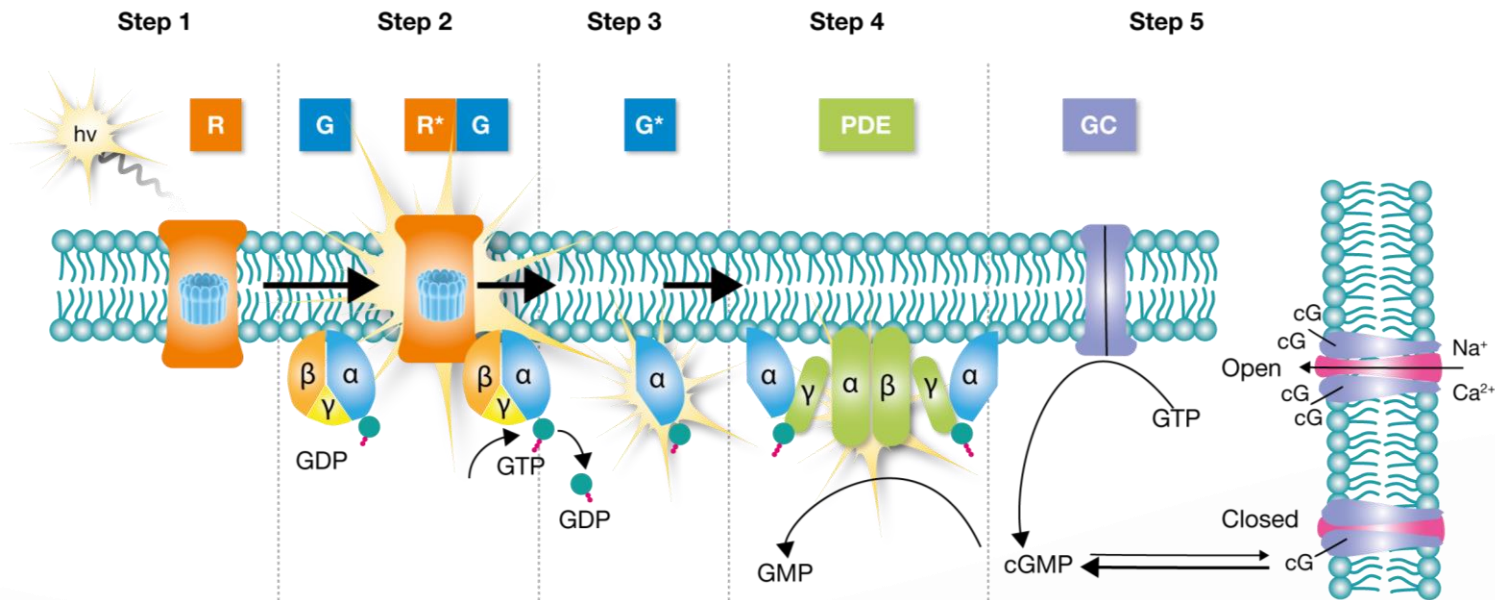
# Gene therapy for the eye

- Most boosted clinical development
  - Market authorization of Luxturna
- Eye advantages:
  - Immune privileged; Inhibitory unique microenvironment
  - Physical barriers (e.g. the blood-retinal barrier)
  - Prevent vectors from disseminating systemically
  - Retinal cells do not proliferate after birth
  - Small size
  - Animal models and technologies



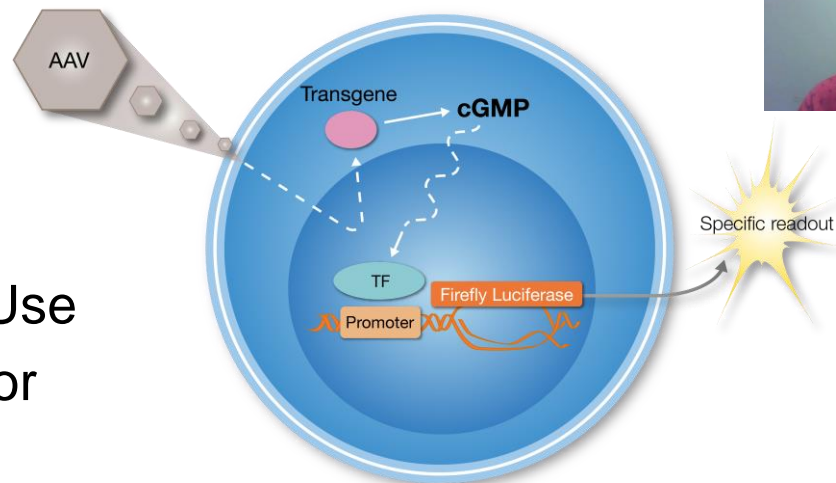
# Gene therapy for the eye

- Retinosis pigmentaria - Photoreceptor phosphodiesterase (PDE)
- Leber congenital amaurosis - Guanylate cyclase (GC)



# *iLite*<sup>®</sup> cGMP

- Human retinal cells
- Mimic the MoA of the vector
- Assay ready format: Thaw & Use
- Relevant information the vector
  - Improve therapy optimization
  - Product similarity
  - Potency of the vector



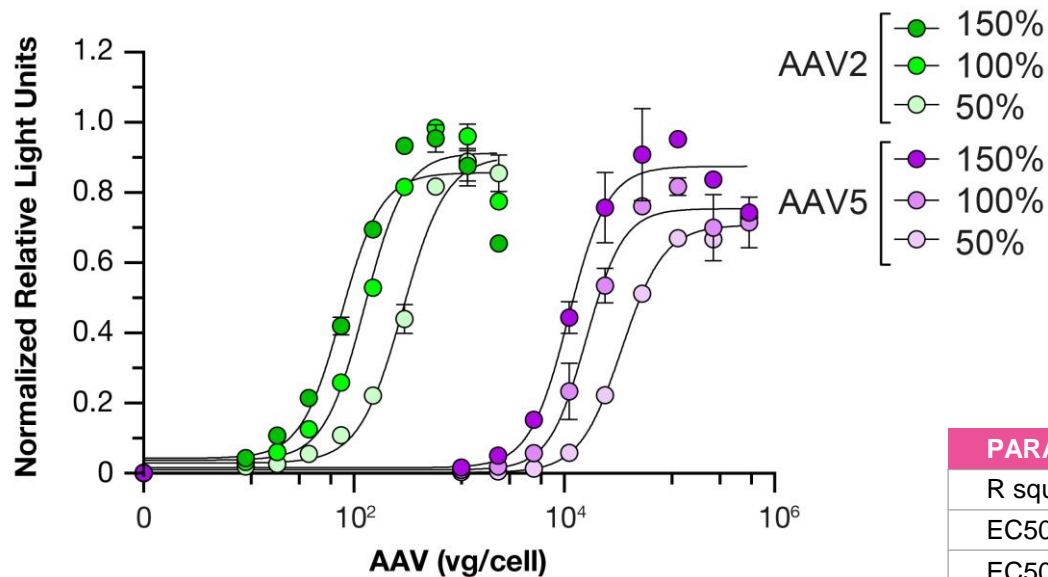
Incubation of *iLite*<sup>®</sup> cGMP/GC cells with variable amounts AAV vg/cell: AAVs transduce the *iLite*<sup>®</sup> cells



**72 hours**

Firefly Luciferase expression proportional to AAV transduction

# *iLite*<sup>®</sup> cGMP – Vector Potency



This technology can be then used in different situations such as the comparison of different AAV lots, different AAV manufacturing methods or even the optimization of the capsid or construct design.

PARAMETER	AAV2			AAV5		
	150%	100%	50%	150%	100%	50%
R squared	0.94	0.98	0.99	0.97	0.98	0.99
EC50 (measured)	76	127	292	11010	16190	35310
EC50 (expected)	85		253	10790		32380
Rel. Potency (measured)	168%		43%	147%		46%
Accuracy	89%		115%	102%		109%

# Strategies for C&GT assay development



**Specific  
Therapies  
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# Strategies for C&GT assay development

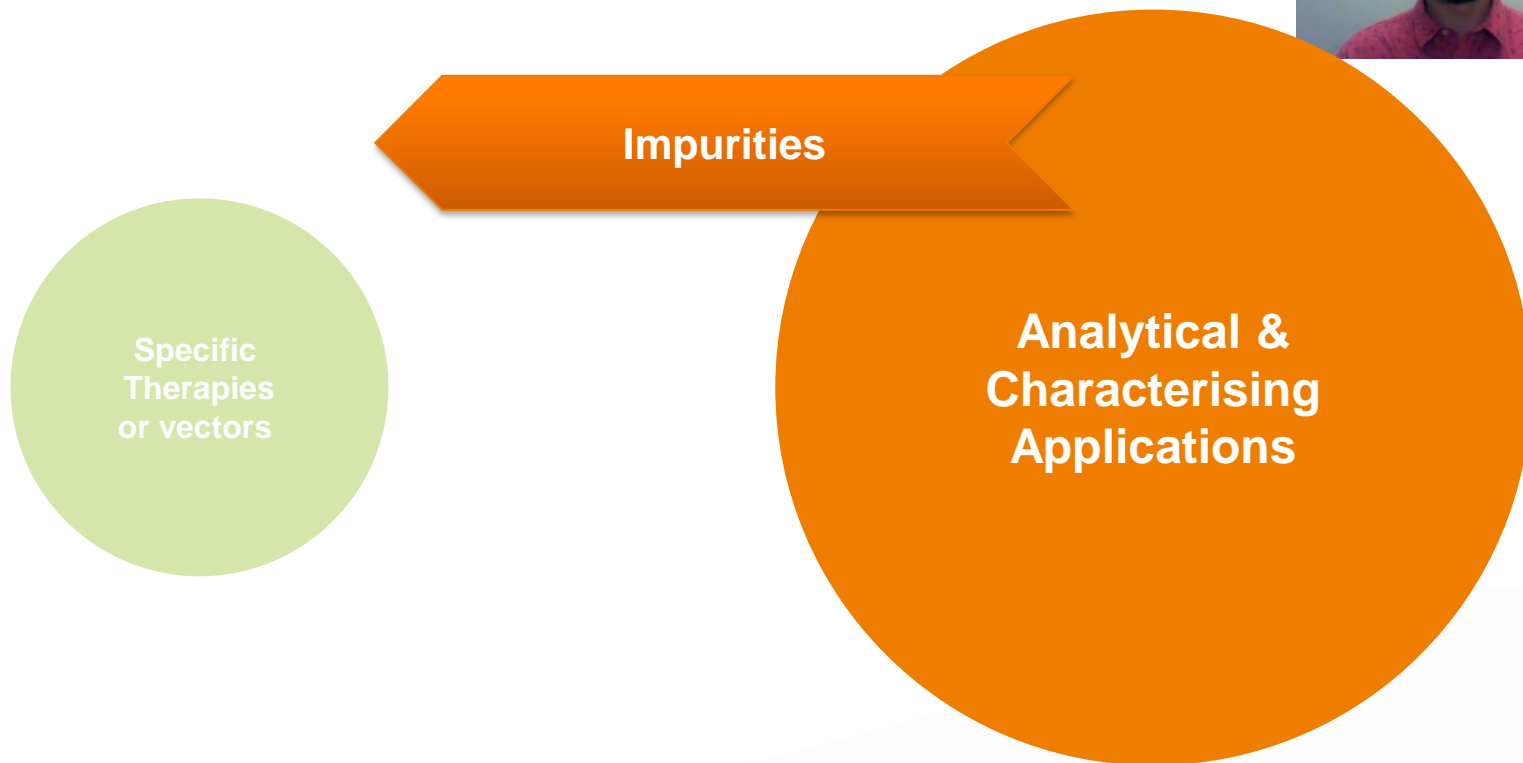


Specific  
therapies  
or vectors

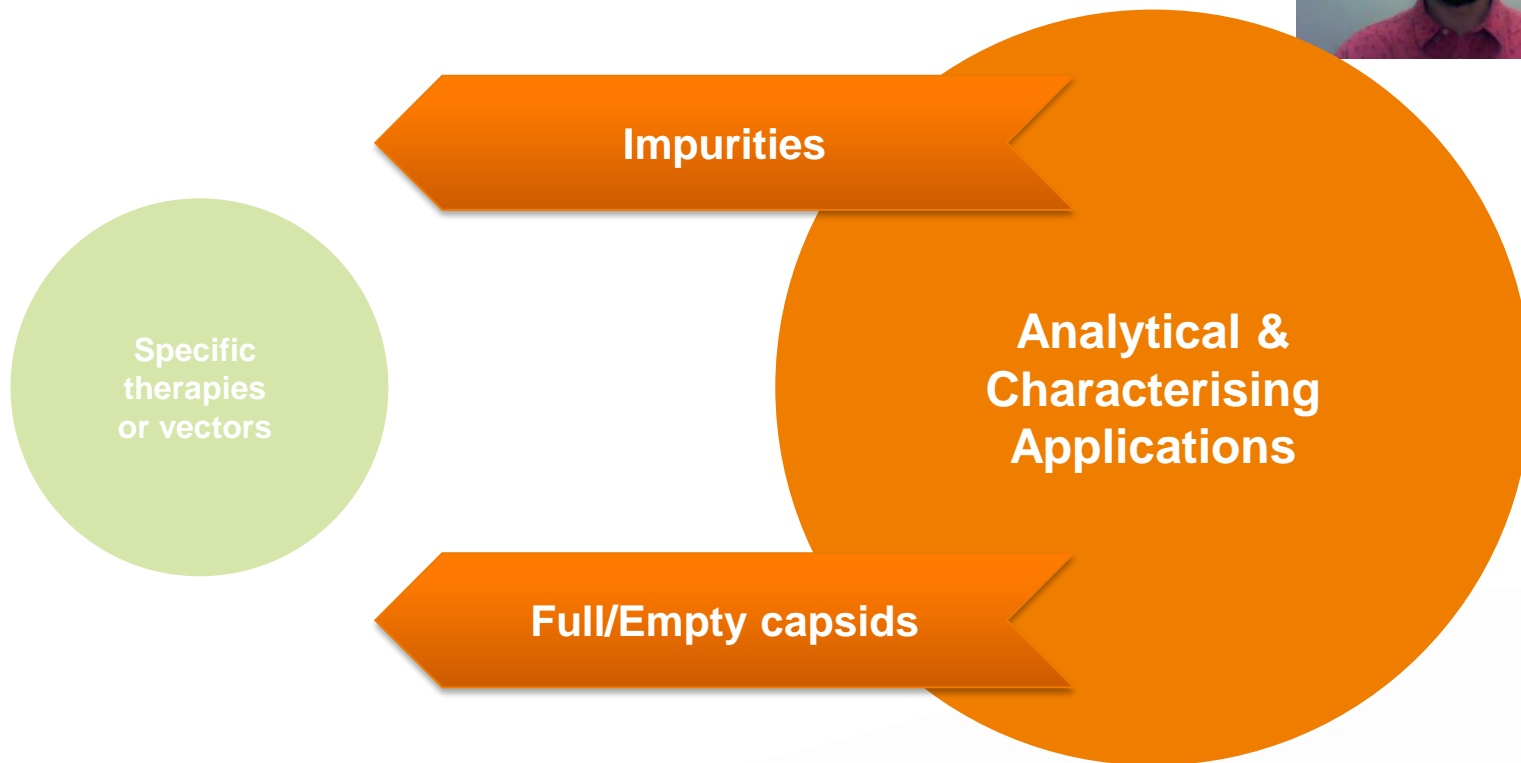
**Analytical &  
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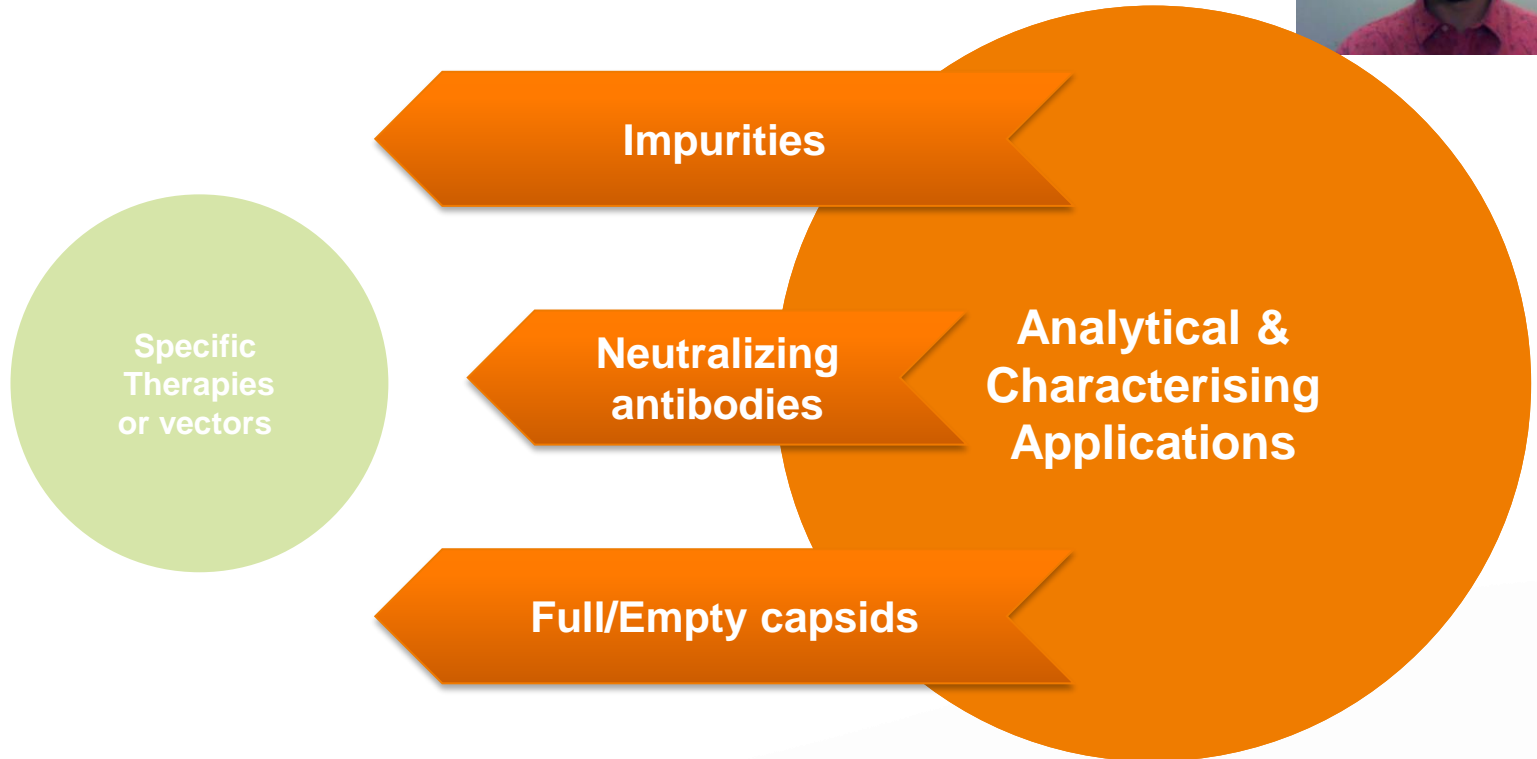
# Strategies for C&GT assay development



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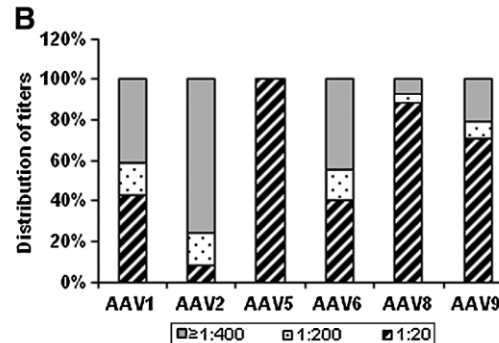
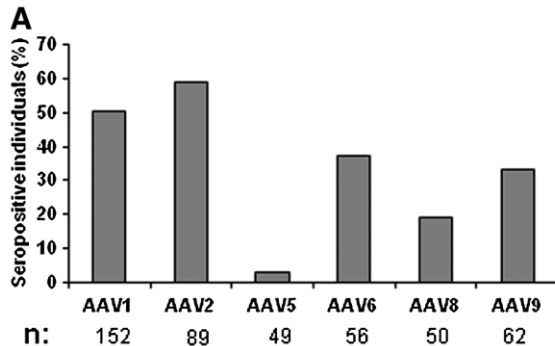
# Strategies for C&GT assay development



# Neutralizing antibodies



- Previous exposure to wt AAV virus occurs in up to 90% of the human population, often during early childhood.



Sera were judged positive for neutralizing capacity when a 1:20 dilution of serum inhibited vector transduction by 50% or more.

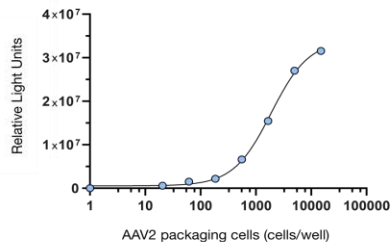
Boutin, S. *et al. Hum Gene Ther* 21, 704–712 (2010).

- The assessment of neutralizing AAV-antibodies is beneficial and required before, during and after treatment with AAV-mediated gene therapies.

# iLite<sup>®</sup> AAV-Neutralizing Platform



## Activation Assay



Incubation of AAV responsive reporter cells with variable cell number of AAV packaging cells: produced AAV transduce reporter cells

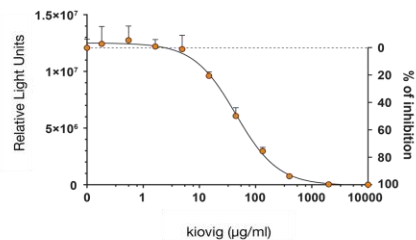


**Overnight**

Firefly Luciferase expression proportional to AAV transduction

Calculation of the optimal number of packaging cells

## Neutralizing Assay



Incubation of AAV responsive reporter cells with optimal AAV packaging cell number and NAB standards/serum samples: free AAV transduce reporter cells

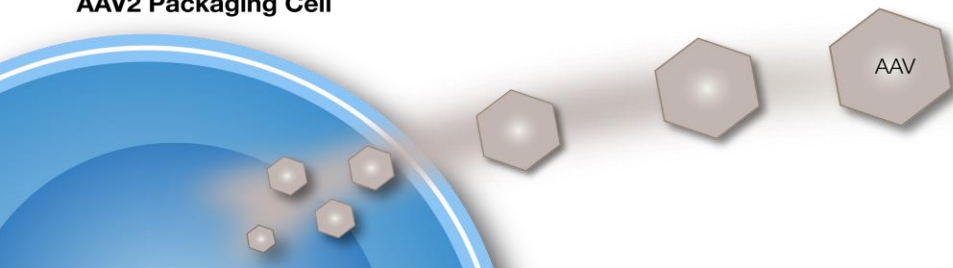


**Overnight**

Firefly Luciferase expression proportional to AAV transduction

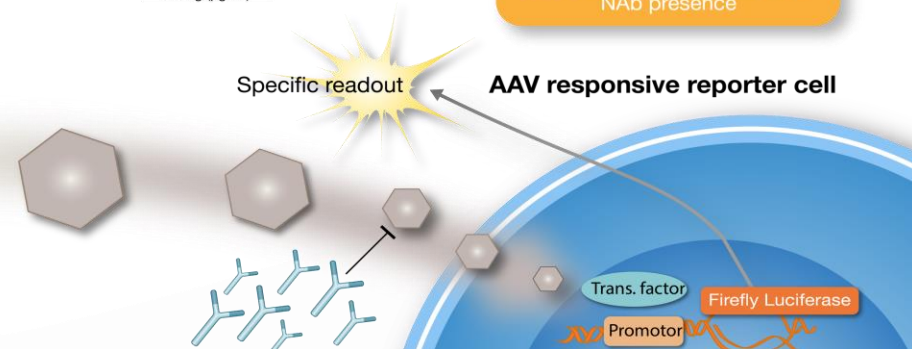
Calculation of inhibition of AAV transduction - detection of AAV NAB presence

## AAV2 Packaging Cell



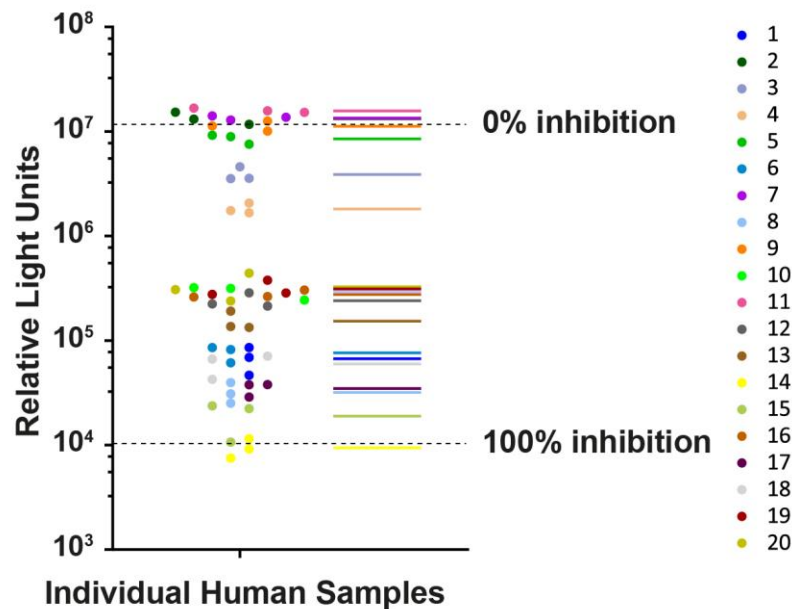
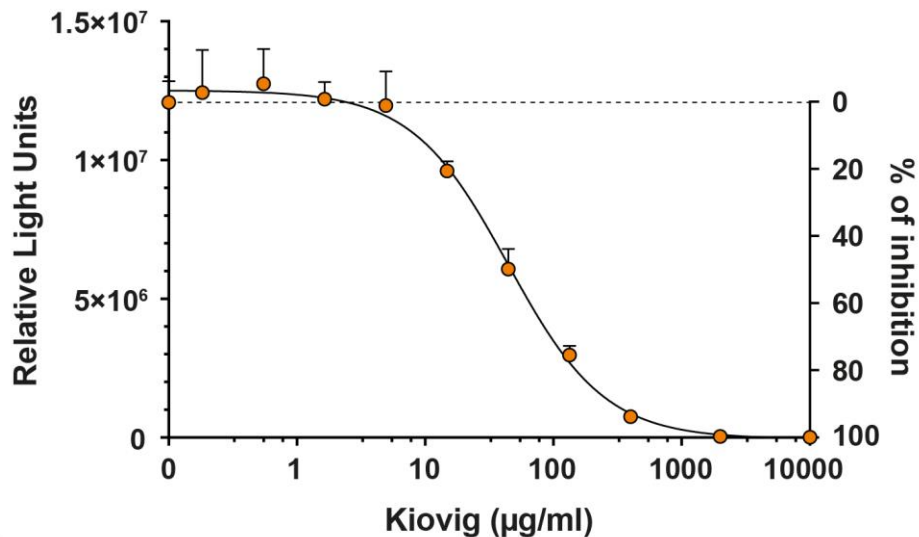
## Specific readout

## AAV responsive reporter cell



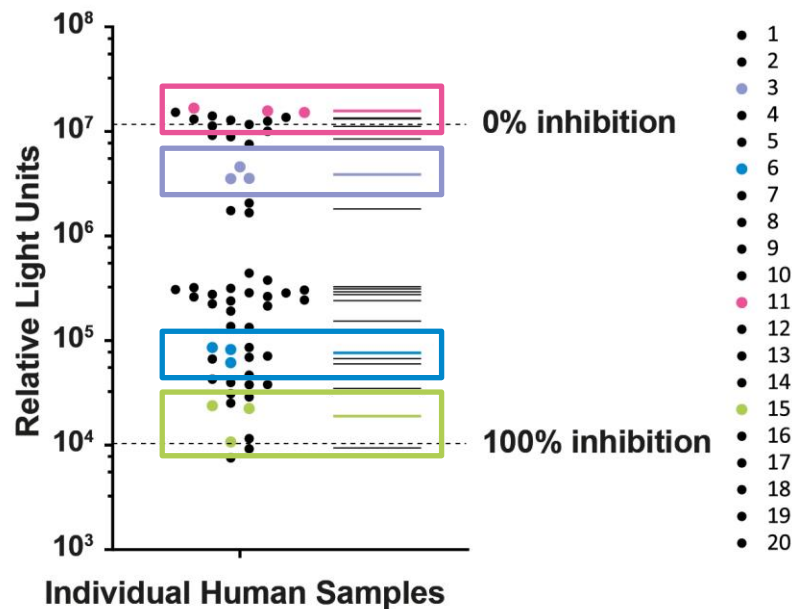
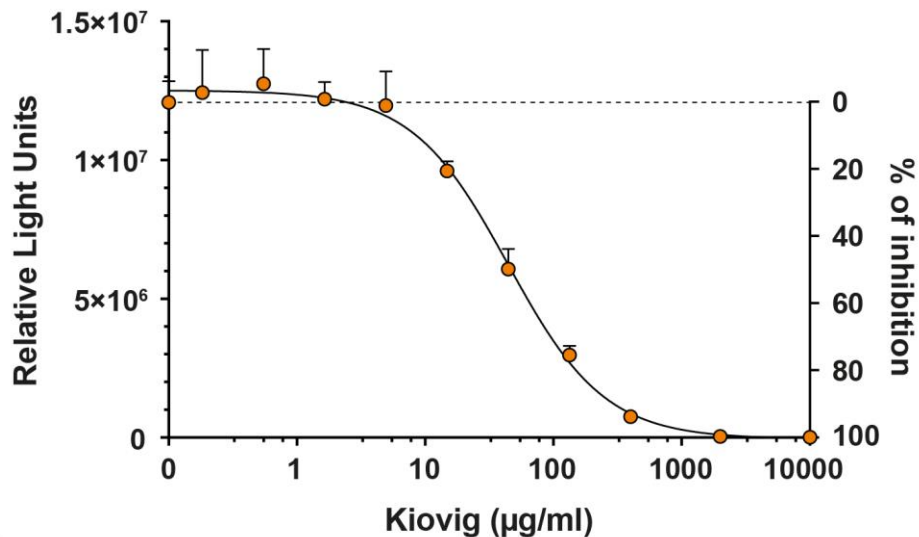
# *iLite*<sup>®</sup> AAV-Neutralizing Platform

## Determining NABs



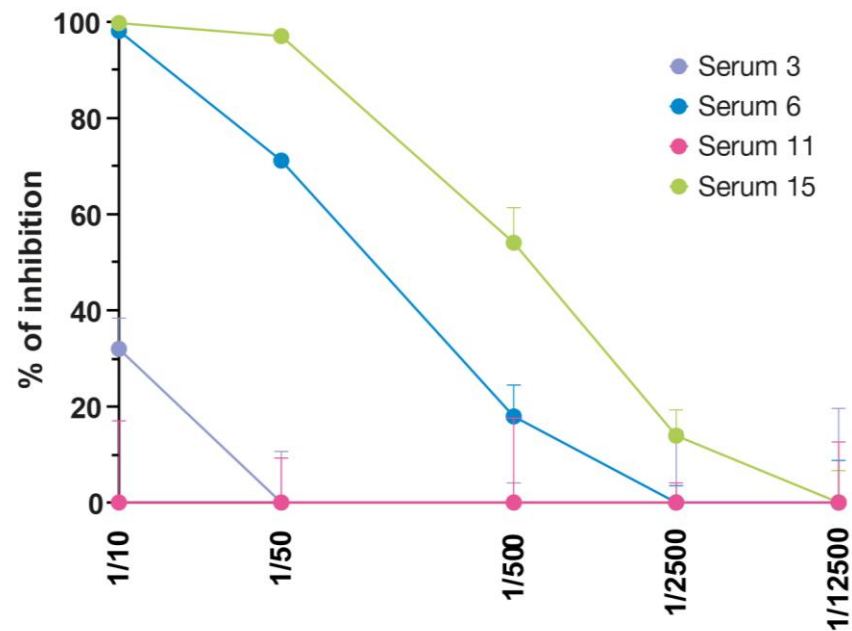
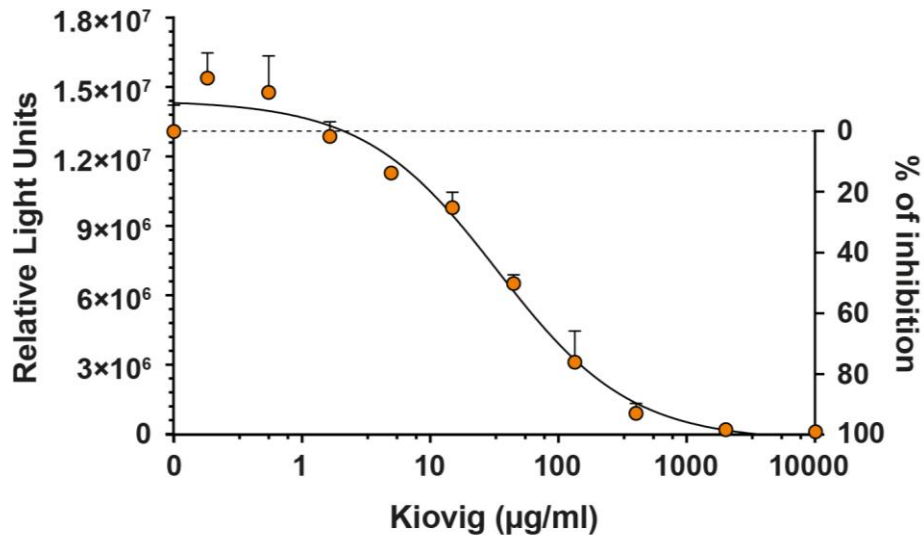
# *iLite*<sup>®</sup> AAV-Neutralizing Platform

## Determining NABs



# *iLite*<sup>®</sup> AAV-Neutralizing Platform

## *Titering NABs*



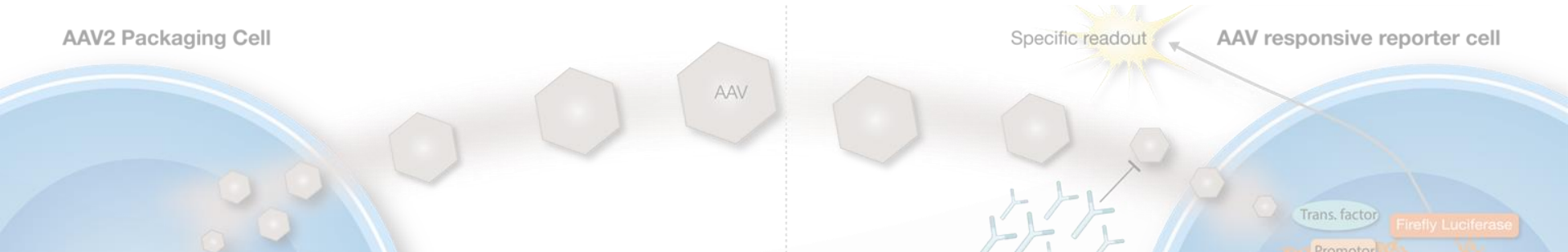


# *iLite*<sup>®</sup> AAV-Neutralizing Platform



- Can be used to screen sera for neutralizing antibodies to recombinant AAV vectors
- Serotype-specific detection of NAbs (AAV2 available; AAV5, 8 and 9 to be available soon)
- Modular system enables custom solution for any hybrid-/chimeric capsids
- Eliminates need to purify vector
- Cells are available in frozen, thaw & ready to use format

AAV2 Packaging Cell



# Conclusion / Take home message



- The development of bioassays for the cell and gene therapy field highly depend on the outcome we want to assess – *Specific therapy*
  - Important to mimic characteristics – *iLite*<sup>®</sup> cGMP
    - **Cell type** – Human retinal cell line
    - **MoA Reflective** - The *iLite*<sup>®</sup> Technology mimics the MoA by using the cGMP metabolism, allowing the assessment and quantification of the level of gene therapy product produced as a result of a successful AAV transduction and subsequent protein transcription.
  - Can be then used for assessing potency, improving vector, comparing manufacturing methods.



# Conclusion / Take home message



- The development of bioassays for the cell and gene therapy field highly depend on the outcome we want to assess - *Analytical Applications*
  - Important to use a well-define bioassay prepared for a variety of scenarios – *iLite*<sup>®</sup> AAV-Neutralizing Platform
    - **Vector detached** - The *iLite*<sup>®</sup> Technology is able to determine NAbS against the AAV without requiring any special AAV production or specific characteristic.
    - **Flexibility bioassay** - Proof of concepts data has been presented for detection of anti-AAV2 NAbS and serotypes (AAV5, 8 and 9) are under development.
  - The modular setup of this platform can also be utilized to design tailored solutions for NAb assessment with next generation vectors (e.g. hybrid, chimeric, or semi-synthetic capsids)



**Thank you for your attention!**

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