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Evaluating AAV Transduction of iCell® Cardiomyocytes²

iCell Lab Note

Introduction.

The differentiation of human induced pluripotent stem cells (iPSC) into specialized cell types has enabled the development of biologically-relevant in vitro models. These systems are compatible with gene delivery technologies, including adeno-associated viral (AAV) vectors widely used in gene therapy research. AAV transduction is often assessed using GFP detected by imaging or flow cytometry; however, these methods can lack sensitivity, require long incubation times, and offer limited quantitative precision. To this end, Promega has developed the AAV NanoLuc®-HaloTag® Dual Reporter System – a sensitive and versatile platform enabling rapid luminescent measurements and complementary imaging-based analysis. This Lab Note uses iCell® Cardiomyocytes² to highlight key features of the technology and demonstrate how transduction of human iPSC-derived cell types can be used to evaluate AAV serotype selectivity and support analytical characterization of AAV gene therapies.

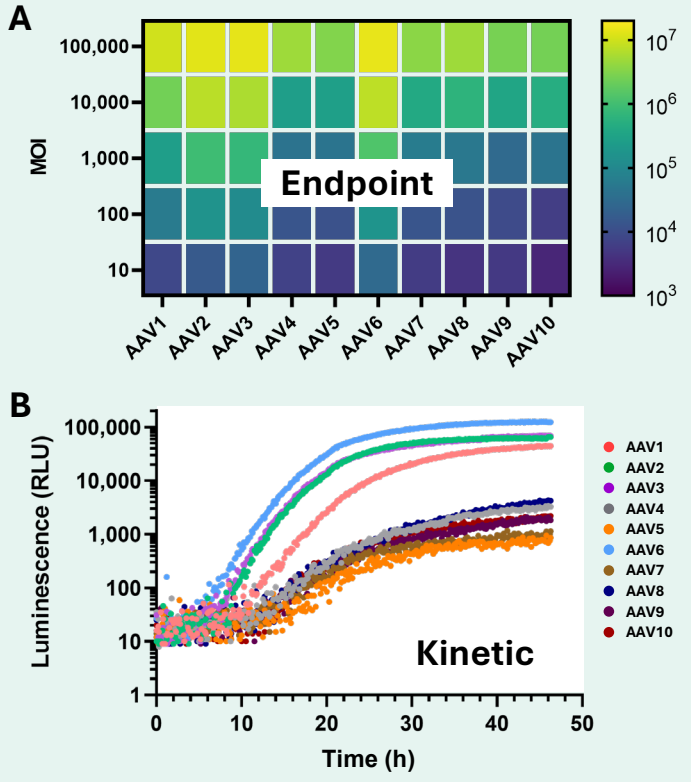

Figure 1. Monitoring AAV transduction via NanoLuc reporter. iCell Cardiomyocytes² were transduced on Day 7 with 10 different AAV constructs. **(A)** For endpoint analysis, a range of MOI (10→100,000) were used and incubated with luciferase substrate 48 h later. NanoLuc signal intensity was proportional to transduction efficiency. **(B)** For kinetic analysis, cells were transduced with AAV (MOI 100,000 for each) and incubated on a CLARIOstar Plus plate reader (BMG Labtech) equipped with an ACU to run environmentally-controlled experiments. AAV6 (light blue) was observed to drive highest expression in iPSC-derived cardiomyocytes. Luminescence signal peaks about ~24 h post-transduction.

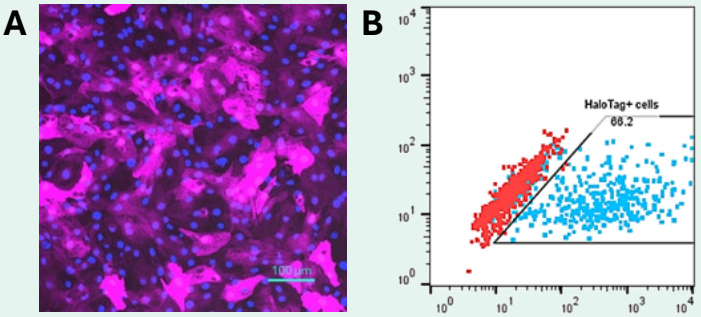

Figure 2. Evaluating AAV transduction with HaloTag. **(A)** Live-cell image of iCell Cardiomyocytes² labeled with Janella Fluor® HaloTag 646 ligand 24 h post-AAV6 transduction (MOI of 100K). iCell Cardiomyocytes² are in magenta; nuclei in blue; scale baris 100 µm. **(B)** Flow cytometry plot provides a more quantitative expression of HaloTag-labeled cells under the same transduction conditions.

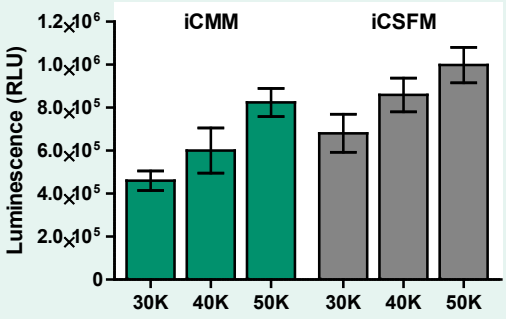

Figure 3. Serum-free media improves transduction efficiency. iCell Cardiomyocytes² were plated at various densities and cultured until Day 7. Cells were kept in iCMM or switched into serum-free medium (iCSFM) during AAV6 transduction (MOI of 100,000 for 24 h). Use of iCSFM improved the transduction efficiency as indicated by a higher luminescence signal.

Methods.

- Day 0: Coat 96-well plate with 0.1% gelatin for 1 h at 37 °C.
- Thaw and plate iCell Cardiomyocytes² in iCell Cardiomyocytes Plating Medium (iCPM) per the Quick Guide.
  - Use viable number of cells/vial on CoA for cell counts.
  - Recommended plating density is ~50K viable cells/well in 200 µl.
- Day 1: Exchange 100% of media to iCell Cardiomyocytes Maintenance Medium (iCPM → iCMM).
- Days 2-7: Perform 100% iCMM media change every 2 days.
- Day 7: On day of assay, change 100% of media to iCell Cardiomyocytes Serum-free Assay Medium (iCMM → iCSFM).
  - Dilute AAV in iCSFM to the desired MOI (ranging from 10 to 10^6).
  - Transduce cells with AAV subtype(s) and incubate for 24-48 h.
    - For kinetic analysis, add Endurazine substrate to transduced cells and read RLU every 5-10 min for 48 h.
- Day 8+: Evaluate transduction efficiency via endpoint assay:
  - Add 2X Nano-Glo Live Cell Substrate and read RLU signal on a compatible plate reader (e.g., GloMax® instrument).
  - Label cells with HaloTag 646 ligand for imaging or flow cytometry.

- For more information on the use of Promega technologies, please visit: www.promega.com/applications/gene-therapy-tools/adeno-associated-virus-solutions

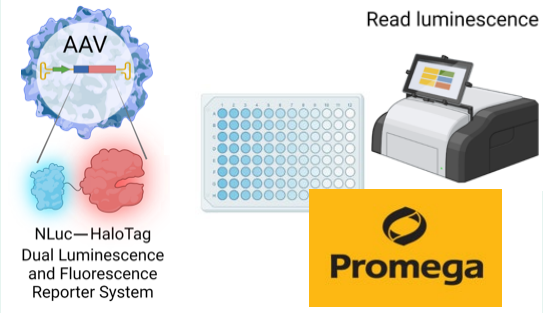

Figure 4. Schematic of Promega technologies.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Cardiomyocytes², 01434 kit	FCDI	R1017
▪ iCell Cardiomyocytes Plating Medium	(incl. in kit)	M1001
▪ iCell Cardiomyocytes Maintenance Medium	(incl. in kit)	M1003
iCell Serum-free Assay Medium	FCDI	M1038
0.1% Gelatin Solution	STEMCELL Technologies	07903
96-well White TC-treated Microplates	Corning	3610
Nano-Glo® Live Cell Substrate	Promega	N205B
Nano-Glo® Endurazine™ Substrate	Promega	N2570
Janelia Fluor HaloTag 646 Ligand	Promega	HT106A
AAV Vector + Virus Manufacturing	Various	N/A

Summary.

iCell Cardiomyocytes² offer a robust and physiologically-relevant platform for studying human cardiac biology, making them an ideal cell model for evaluating AAV transduction efficiency in the development of AAV-based gene therapies (see Wu et al.). We partnered with Promega to leverage the NanoLuc-HaloTag Dual Reporter System for a comprehensive, quantitative assessment of AAV serotype transduction. This approach enabled both real-time monitoring of transgene expression and endpoint analyses. Our findings demonstrate that AAV6 is the most efficient serotype for transduction of iPSC-CM under our experimental conditions, and that iCell Cardiomyocytes Serum-Free Assay Medium further enhances transduction efficiency. Together, these advanced technologies provide a scalable and highly controlled experimental framework for investigating cardiac-specific disease models, supporting preclinical research, and potentially serving as a platform for potency assays in therapeutic development.

References.

Wu, I., Zeng, A., Greer-Short, A. *et al. Commun. Med.* **2024** 4(38): 1. https://doi.org/10.1038/s43856-024-00450-w

Highlights.

NanoLuc-HaloTag Dual Reporter Technology is a versatile approach for high-throughput assessment of AAV transduction.

AAV6 proved to be the best serotype for the transduction of iCell Cardiomyocytes², which matches with other reports in the literature.

iCell Cardiomyocytes² recapitulate many key features of human cardiac cells, offering a powerful preclinical model for evaluating AAV-based gene therapies.

Scan here to download the iCell Cardiomyocytes² Quick Guide.

Contact **Technical Support** for more protocol details and supportive data.
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