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Addressing HEK293 cell lineage diversity through basal media optimization

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ABSTRACT

Adeno-associated virus (AAV) has become an attractive vector for gene therapy; however, generally low titer yield limits its viability as a therapeutic. Various methods have been employed to achieve increased titer, including cell engineering, expression platform optimization, transfection optimization, and media development.

One of the preferred methods to produce recombinant AAV (rAAV) is helper-free triple transfection using HEK293 cells. In this method AAV is produced by transfection of three plasmids: a transfer construct encoding the therapeutic gene, a RepCap construct encoding the replication and capsid genes, and a helper construct encoding adenovirus genes into HEK293. We aimed to develop media that address the lower titer of the HEK293 system, but the high diversity of HEK293 cells and processes used for gene therapy would make it difficult to develop one medium that suites all uses. Therefore, we developed a panel of media to support AAV production by helper-free triple transfection using HEK293 cells with increased viral titers agnostic of client manufacturing processes or cell lineage.

MATERIALS AND METHODS

Cells Culture and Adaptation: Internal 293F clones, 293F-1 and 293F-2, were cryorecovered in an adaptation medium for three passages and directly adapted to Gibco™ FreeStyle™ F17 Expression Medium (F17) and the Gibco™ Viral Vector HEK Media Panel (HEK Panel). Shake flask cultures were cultivated in a humidified incubator at 37° C with 8% CO_2 and agitated at 125 RPM. Cultures were passaged every three to four days inoculated at 0.6 x 10^6 or 0.3 x 10^6 cells/mL, respectively.

The 293S clone, 293SF-3F6 provided by the National Research Council of Canada, was cryorecovered for three passages and subsequently sequentially adapted to the same adaptation medium as the 293F clones and F17 followed by direct adaptation to the HEK Panel media. Cultures were cultivated similar to 293F except with 5% CO_2 and at 140 RPM.

Media: Adaptation medium, F17, and HEK Panel media were supplemented with 8mM (293F-1) or 4mM (293F-2) Thermo Scientific™ GlutaMAX™ Supplement, or 4 mM Glutamine (293S).

Transfection and AAV2 Production: Cultures were triple transfected in shake flasks to produce AAV2 encoding GFP. On the day of transfection 293F cells were inoculated at 3 x 10^6 cells/mL. Cultures were transfected with a total of 1.5 µg/mL of DNA using PEIpro™ transfection reagent (PolyPlus) at a 1:1 PEI:DNA ratio (w/w) for the HEK Panel media for 293F-2. For 293F-1 HEK Panel medium 2 and F17 used 2:1 PEI:DNA. DNA and PEI were complexed for 10 minutes at 10% of the culture volume in Gibco OptiMEM I. Triple transfections consisted of plasmids pAAV-GFP, pRC2, and pHelper (CellBiolabs) at 1:3.03:1.44 and 1:3:1 (w/w) ratios for 293F-1 and 293F-2, respectively. Cultures were fed glucose back up to 6 g/L 24 hours post transfection and harvested 72 hours post transfection. 293S were transfected similar to 293F-1 except they were inoculated at 1 x 10^6 cells/mL and transfected with 1 µg/mL of DNA and a plasmid ratio of 1:1:1.

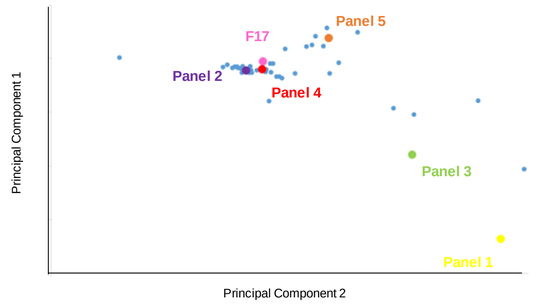
Culture Lysis and Titer Quantitation by qPCR: Culture harvests were thawed and lysed by sonication (293F-1, 293S) or a proprietary lysis buffer (293F-2). Lysates were diluted in DNase I Buffer (Invitrogen™ reagent) and treated with 0.01 U/µL Exonuclease I and 5 U/µL DNase I (Thermo Scientific). Subsequently, lysates were incubated with 0.5 µg/µL Proteinase K (Invitrogen). Titer was quantified by qPCR using a TaqMan® assay targeting GFP. Linearized pAAV-GFP was used for the standard curve.

Transfection Efficiency: Transfection efficiency was determined by quantifying the percent GFP positive cells by flow cytometry using a Gallios Flow Cytometer (Beckman Coulter) or an Attune NxT Flow Cytometer (Thermo Scientific).

Principal Component Analysis: PCA was performed on 58 media in R version 3.5.3 (2019-03-11).

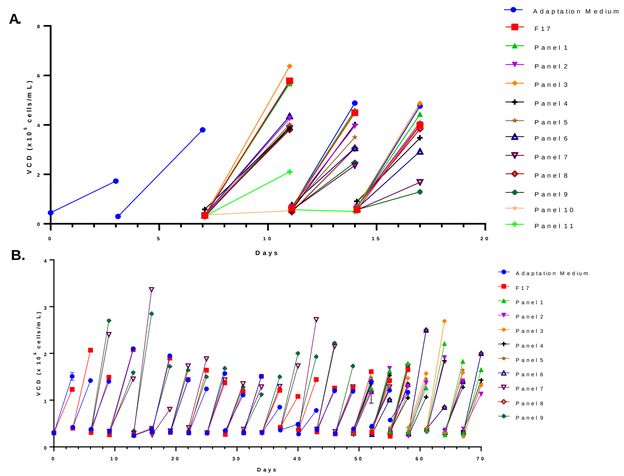
RESULTS

Figure 1. Principal Component Analysis



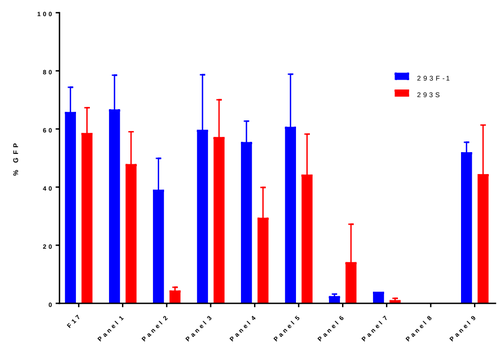
Fifty-eight media from an internal formulation library were assessed for diversity by PCA. Seventeen media were selected for further screening based on their diversity among 61 principal components. The media are plotted against the two main principal components. F17 and the five HEK Panel media are highlighted.

Figure 2. Panel Medium Adaptation



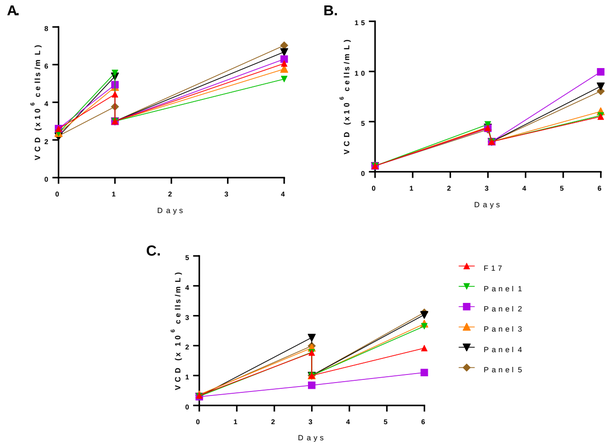
293F-1 (A) were directly adapted to 17 panel media, 11 of which supported growth. 293S (B) required sequential adaptation into either adaptation medium or F17 before direct adaptation to 11 panel media, 9 of which supported growth. 293F-2 (not shown) directly adapted to the final five HEK Panel media.

Figure 3. Transfection Efficiency



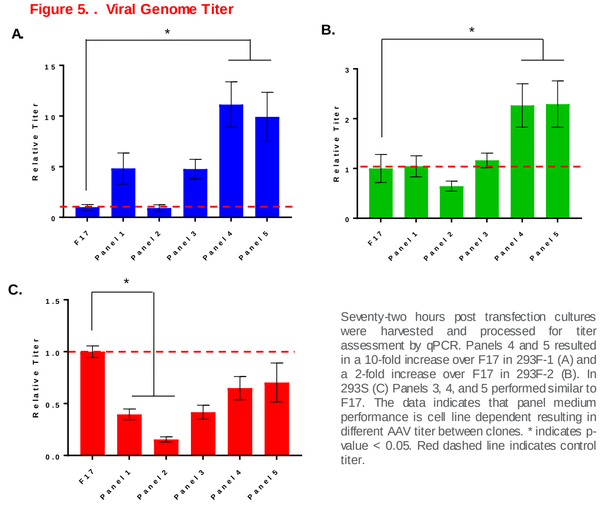
Transfection efficiency was determined by assessing the percent GFP positive cells 72 hours post transfection by flow cytometry. Panels 6, 7, and 8 transfected poorly in both cell lines, while Panel 9 resulted in low titer and were eliminated from the panel.

Figure 4. Transfection VCD



On the day of transfection 293F-1 (A) and 293F-2 (B) were diluted to 3 x 10^6 cells/mL and transfected as described in methods. VCD for both 293F clones in each of the five panel media was similar to the F17 control. 293S (C) were transfected similarly except they were diluted to 1 x 10^6 cells/mL prior to transfection. The VCD was similar to F17 for 293S in the panel media except for Panel 2. Overall, the panel media had similar growth to F17.

Figure 5. . Viral Genome Titer



Seventy-two hours post transfection cultures were harvested and processed for titer assessment by qPCR. Panels 4 and 5 resulted in a 10-fold increase over F17 in 293F-1 (A) and a 2-fold increase over F17 in 293F-2 (B). In 293S (C) Panels 3, 4, and 5 performed similar to F17. The data indicates that panel medium performance is cell line dependent resulting in different AAV titer between clones. * indicates p-value < 0.05. Red dashed line indicates control titer.

CONCLUSIONS

The data indicates that the diverse components and concentrations in the developed media panel formulations address the diversity of HEK293 cell lines and AAV production processes. Regardless of clone or process the Panel media does not alter cell growth compared to the F17. However, the Panel media has a differential effect on AAV production depending on the clone ranging from a 10-fold increase over F17 in 293F-1 for Panels 4 and 5 and down to similar titer to F17 in 293S for the same media

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TRADEMARKS/LICENSING

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