OPTIMISING HEK293T CULTURE FOR THE IMPROVED MANUFACTURE OF GENE THERAPIES

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The development of gene therapies into effective tools for molecular medicine can be contributed to the successful outcomes of clinical trials followed by therapeutic product approval. Despite this, there are still many challenges to product development, including the scalability of the process and translating laboratory research into viable clinical applications. Cell expansion is currently labour and time intensive, and the processes lack the reproducibility and standardisation for regulatory and commercial compliant production.

This research aims to improve current gene therapy manufacturing processes by developing a standardised process for manufacturing to a clinical grade standard. One avenue to achieve this is by increasing the overall cell yield and lentivirus titre. Initial experiments showed that implementing standardisation techniques and set seeding densities produced more favourable results than generic 1 in 3 splits. Overall applying the set seeding densities led to stable growth and routine passages, thereby reducing the reliance on visual confluency estimations. This resulted in a more predictable and reliable process that also had set culture periods, consistent harvest densities and fold expansions that were maintained over long culture periods, ranging between $3x10^5$ and $4x10^5$ cells/cm^2 and 15-20 fold expansions per passage. Thereby creating a more suitable clinical grade manufacturing process which could be applied to both gene therapy and CAR-T cell therapy vector production.

Future work aims investigate what factors influence transfection, discern what differences in approach are required for the different types of gene therapy and if a standard methodology can be developed for each approach.