

Snapshot: Strategies for generating the highest quality isogenic iPSC lines

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If you would like to learn more about our services, you can speak to one of our experts by e-mailing:

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Background

Therapeutic uses for stem cells range from cell transplantation to in vitro human disease modelling and are more relevant than current animal models. One of the main challenges facing researchers is the need to generate matched controls for the investigation of genetic mutations. However, editing induced Pluripotent stem cells (iPSCs) is difficult as they are difficult to transfect, do not grow well as single cells and possess different DNA repair mechanisms.

Sampled and CRISPR gene editing

In order to provide the highest quality iPSCs for researchers to gain crucial insights from, Sampled employs CRISPR gene editing tools to reprogram cells and grow edited clones. However, this is only half of the process, it is imperative that edited cell lines are validated thoroughly so that the iPSCs which are then delivered to the client are of the highest quality.

Cells transfected with the gRNA and Cas9 protein are sorted into single cells by flow assisted cell sorting (FACS) to ensure single cell expansion of the edited clones. Sanger Sequencing is then employed to verify if the edited clones were correctly edited. Through next generation sequencing, sections of DNA from parental cells are compared to that of the CRISPR edited cells to ensure that the entire culture is uniformly edited. Potential off target sites are then identified and Sanger sequenced to ensure no unintended edits are present within the edited iPSC line. SNP Trace is then performed to ensure the edited cell line matches the source cell.

Additional Quality Control for iPSCs

Sampled also offers several additional quality control tests for testing iPSC quality such as:

- G band karyotyping to ensure genetic stability
- Identification of stemness markers
- Differentiation of iPSCs into all three germ layers

Sampled hosts NINDS and NIMH repositories

Sampled also hosts the NIMH Repository and the NINDS Human Cell and Data Repository, both of which contain iPSC and source cells that can be requested by scientist's worldwide (both not for profit and for profit). We are able to provide "off the shelf" cells/ cell lines as well as create bespoke isogenic pairs tailored to fit your research requirements. If you would like to contact one of our scientists to discuss your iPSC needs, you can email us at contact@sampled.com