abcam

High throughput platform for generation of physiological relevant and highly validated edited cell lines

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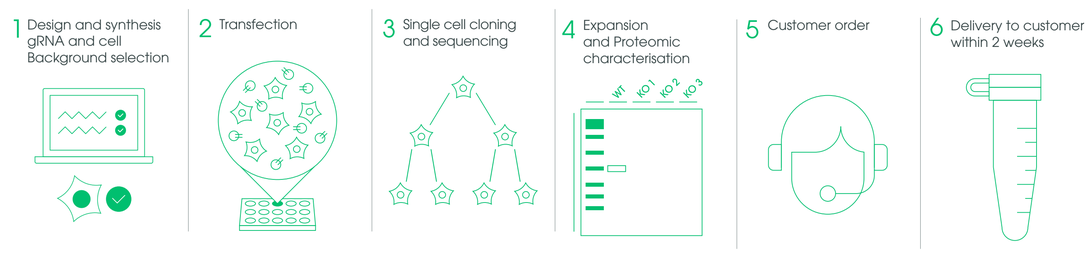
Introduction

The recent advancement in gene-editing technologies and the emergence of CRISPR-Cas9 has revolutionized our understanding of the genetic basis of disease through better modelling and drug target discovery, ultimately opening new doors for the development of gene-targeted therapies. CRISPR-Cas9 is widely used as a tool to understand gene function in the context of disease models.

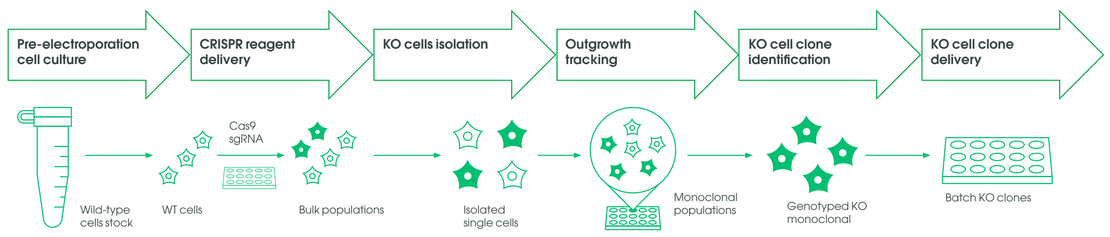
Despite its massive impact, CRISPR-Cas9 is still a lengthy, cumbersome and tedious process, and requires further investigation towards its improvement by making it simple, fast and easy to use. To address this, Abcam launched one of the largest portfolios of physiologically relevant and highly validated edited cell lines.

We've first successfully established a high throughput cell-editing platform that enabled us to subsequently generate over 600 CRISPR Knock-out cell lines in less than three months. We then further expanded our capabilities by generating bespoke CRISPR-Cas9 engineered Knock-in cell lines overexpressing key target proteins and markers. These engineered cell lines will not only be pivotal towards establishing novel cell-based assays for potential drug candidates but will also serve as a focal point in eliminating the current issues of high cost, time, feasibility and reproducibility associated with the current approaches.

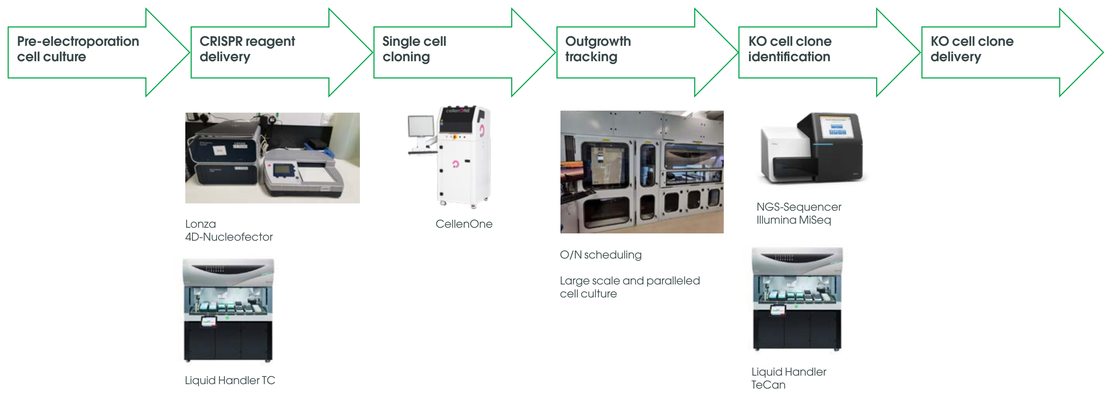
Technical and Pipeline Overview



The HTP platform is a 6 step process:

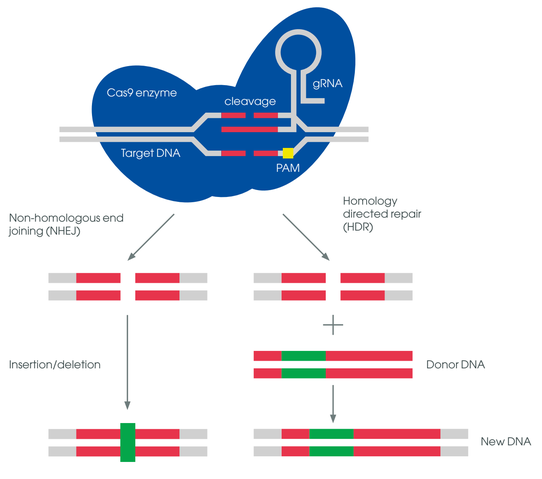


Instrument Integration in the HTP Platform



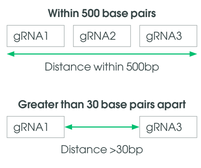
CRISPR overview: Guide strategy and Transfection

CRISPR: Gene editing mechanism



KO design

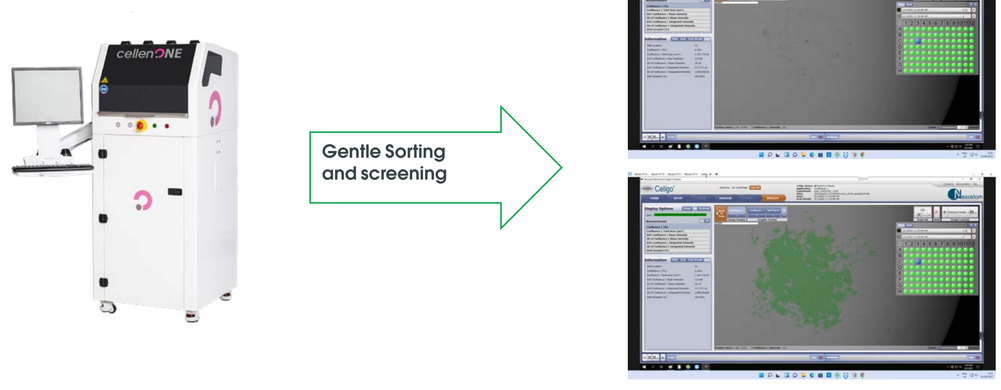
5 success criteria levels
Outsourced
200–300 targets per month
sgRNA and sequencing primers



Electroporation

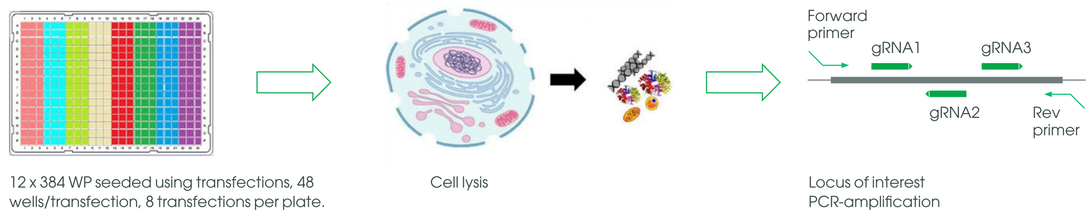
96 well plate format : 92 target per cell line.
Compatible with most cell line.

Single cell seeding: Assured monoclonality and high viability



Representative images of the DAXX edited cells

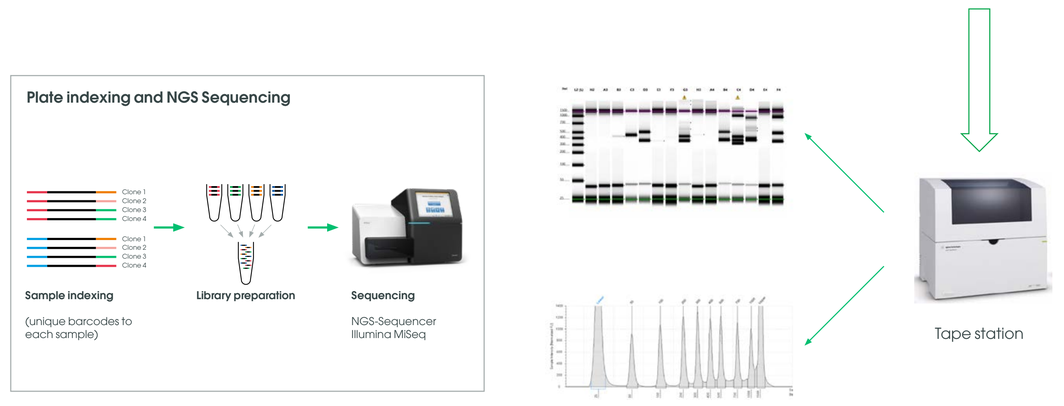
Amplicon Specific Library Preparation



12 x 384 WP seeded using transfections, 48 wells/transfection, 8 transfections per plate.

Cell lysis

Locus of interest PCR-amplification



Miseq Sequence Analysis: Target gene DAXX

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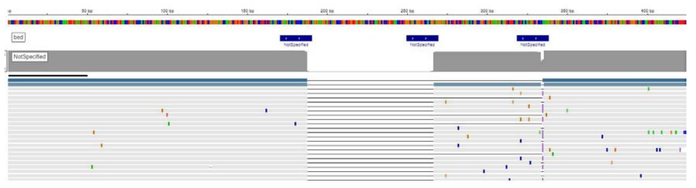
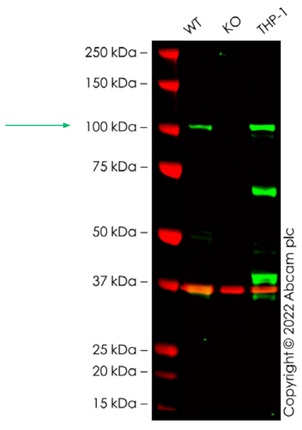


Edited amplicon sequence of DAXX aligned with CDS to confirm out of frame translation



Conclusion

HTP Platform has enabled us to:

- Offer one of the largest and comprehensive catalogue of highly validated physiologically relevant edited cell models
- Diverse selection of ready-to-use CRISPR/cas9 edited KO cell lines validated in key applications, KO cell lysates, iPSC-derived primary neurons and myocytes, and custom gene editing services (PM, KI, stable cell lines)