PLATEMASTER® and EXTRACTMAN®: Rapid Enrichment and Clonal Selection of CRISPR Modified Cells

TECHNICAL NOTE TN205

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The directed manipulation of DNA in an organism's genome provides the ability to study the functional relationship between genotype and phenotype. Advances in genome editing tools, such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology have made this process faster, more reliable, and less expensive. This technology has been used to create useful animal models for studying complex diseases, as well as correcting or inducing disease states within a cell. It has also been used for modifying the genomes of organisms such as fungus, parasites, crops, and livestock, demonstrating the commercial and therapeutic utility of the technology.

Figure 1
EXTRACTMAN®

CRISPR allows researchers to modify a genome by removing, inserting, or mutating the targeted DNA. CRISPR works by introducing guide RNA (gRNA) and the Cas9 protein into the host. The gRNA directs the Cas9 protein to a specific sequence of DNA, whereupon Cas9 breaks both strands of the DNA, which induces DNA repair in the host. If additional DNA is incorporated into the Cas9 complex, that specific DNA will then be inserted into the doublestrand break before the cell begins the repair process.

CRISPR is changing the face of how scientists are exploring new drug targets and uncovering how diseases work at the genetic level. However, as with any biological system, CRISPR is not 100% efficient, requiring screening to identify and isolate the cell or strain carrying the desired modification. The introduced constructs often include screening or selection markers such as fluorescence, antibiotic resistance, or antigens. These markers aid researches in enriching their starting population. While antibiotic resistance and fluorescence are commonly used, they also require a great deal of time, weeks for antibiotic selection, and resources, cell sorting with a flow cytometer, to be effective. EXTRACTMAN® enables rapid isolation of proteins that bind to an antibody-coated paramagnetic particle, and is a fast and efficient tool that can be used to enrich populations of CRISPR modified cells when an antigen based selection marker (such as CD4) is used.

Figure 2
PLATEMASTER®

Following enrichment, the modification of interest is verified by screening single cell clones. PLATEMASTER can be used in the biological safety cabinet to plate cells, perform growth medium exchanges, and expand cultures fast and efficiently by pipetting liquid to 96 wells in a single step.

GILSON®

CRISPR WORKFLOW WITH EXTRACTMAN AND PLATEMASTER

01 Deliver Cas9 Guide RNA Complex

02 Enrich Cell Population

- CD4 Enrichment with Dynabeads

EXTRACTMAN enables rapid and ergonomic enrichment of the starting populations when a CD4 selection marker is used.

03 Isolate Single Cell Clones

- Plate Cell Suspension

PLATEMASTER allows fast, accurate and easy plating of the enriched populations to create single cell clones.

04 Cell Line Maintenance

- Medium Exchanges
- Passaging

PLATEMASTER increases the efficiency of maintaining cultures through medium exchanges and creating duplicate plates for expansion and screening.

05 Screening Clones for Target of Interest

- DNA Isolation
- PCR Screening

PLATEMASTER reduces cross contamination when screening the clones through DNA isolation and minimizes errors when dispensing master mix for PCR. .

06 Expand Clones and Perform Experiments

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