HIGH THROUGHPUT AMENABLE GENE EDITING TOOLS FOR FUNCTIONAL GENOMICS AND ENGINEERED CELL LINE DEVELOPMENT

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ABSTRACT AND INTRODUCTION

Latest advancements in genome editing technologies have revolutionized gene therapy and opened up new opportunities for treating diseases related to perturbations in protein expression and mutations in the genome. With editing tools like CRISPR-Cas9 researchers are able to efficiently knock out genes of interest and study its functions or knock in a specific change into the genome and correct a disease relevant mutation. However there is a significant need for easy-to-use and efficient high throughput (HTP) gene editing workflows which will enhance the capability of these tools in challenging and disease cell types including primary T-Cells and stem cells. The work described here includes:

1. HTP amenable tools and optimized workflows including multiplexed gene editing using pre-complexed CRISPR-Cas9 ribonucleoprotein (RNP) and
2. Application using genome-wide functional knockout screening tools such as CRISPR and siRNA libraries.

To date, we have observed up to 80% editing efficiency with multiplexed RNP complex targeted to simultaneously edit six different genes in T-Cells. Data also shows that the pre-complexed CRISPR-Cas9 RNP complex described here is stable for at least six months without compromising the editing efficiency when tested in T-Cells giving the user the flexibility to reuse the mix for subsequent experiments. The HTP tools and protocols developed through this work will expand the toolbox capability for disease modeling and drug discovery by enhancing overall productivity thereby accelerating biotherapeutic research.

MATERIALS AND METHODS

All experiments described here were performed using

Figure 1: Multiplexed gene editing workflow using CRISPR-Cas9 RNP

Figure 2: CRISPR-Cas9 RNP dosage optimization

Figure 3: Ready-to-use CRISPR-Cas9 RNP mix is stable up to six months

Figure 4: Feasibility test of ready-to-use CRISPR-Cas9 RNP mix with HDR donor DNA

REFERENCES


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TRADEMARKS/LICENSING

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