APPLICATION NOTE

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Optimization of reverse transfection of Dharmacon™ Edit-R™ synthetic crRNA and tracrRNA components with DharmaFECT™ transfection reagent in a Cas9-expressing cell line

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Introduction

Dharmacon™ Edit-R™ predesigned crRNA libraries enable rapid, high-throughput analysis of hundreds of genes with multiple target sites per gene. An arrayed library format permits single-well phenotypic analysis, including high-content assays and other morphological or reporter assays. Identification of relevant hits and successful screening outcomes from these types of assays depend on high transfection efficiency.

To obtain the highest transfection efficiency of the Edit-R synthetic crRNA:tracrRNA components with minimal negative effects on cell viability, it is recommended to carefully optimize transfection conditions for each cell line using positive control crRNAs. A reverse transfection method can be used to increase the throughput and reproducibility of a screen, and to more easily implement automated systems to carry out transfections.

Here we present an example of transfection optimization in reverse transfection format using a recombinant U2OS reporter cell line stably expressing Cas9 nuclease under the CAG promotor (Ubi[G76V]-EGFP-Cas9 cells). An un-cleavable ubiquitin moiety (Gly76Val) fused to EGFP allows constitutive degradation of the EGFP protein (and low basal fluorescence), while disruption of the proteasome components by functional protein knockout leads to accumulation of EGFP and detectable fluorescence.

Reverse transfection optimization

A full protocol for reverse transfection of synthetic crRNA is available here (https://dharmacon.horizondiscovery.com/uploadedFiles/Resources/edit-r-reverse-transfect-array-crrna-plates-protocol.pdf). A transfection optimization experiment should include two to three cell densities and a range of DharmaFECT™ transfection reagent volumes. In this example, U2OS Ubi[G76V]-EGFP-Cas9 cells were trypsinized, then diluted to 5000, 10000 or 20000 cells per 80 µL in growth medium. Edit-R PPIB synthetic crRNA Control (Cat #UK-007050-01) was used as a positive control for gene editing and Edit-R crRNA targeting a proteasome gene *PSMD7* was used as a positive control for the phenotypic assay. The crRNAs were co-transfected with an equimolar amount of synthetic tracrRNA at a final concentration of 25 nM, using a range of DharmaFECT 4 transfection reagent from 0.02-0.3 µL/well. After incubation of the crRNA:tracrRNA with the transfection regent for 20 minutes at room temperature, the transfection mixture was transferred to triplicate wells of tissue culture plates and the suspended cells were added on top of the transfection complex. Transfected cells were incubated in a 37 °C, 5% CO_2 incubator for 72 hours. A resazurin viability assay and a DNA mismatch assay using T7EI endonuclease were performed 72 hours post-transfection, and EGFP fluorescence was read on the Envision plate reader (Perkin Elmer) for the *PSMD7* crRNA phenotypic assay control.

Results

Transfection conditions previously determined for delivery of siRNAs in a cell line of interest can be used as a starting point for transfection optimization with Edit-R crRNA and tracrRNA. Here we used DharmaFECT 4 transfection reagent that was previously determined to provide the highest siRNA transfection efficiency for these U2OS cells. A range of DharmaFECT 4 transfection reagent concentrations was assessed to deliver synthetic crRNA:tracrRNA targeting *PPIB* for the highest gene editing efficiency and lowest impact on cell viability (Figure 1). The optimal transfection reagent concentration depended on cell density (0.02, 0.04 or 0.12 µL/well of DharmaFECT 4 for 5000, 10000 or 20000 cells per well, respectively), leading to high gene editing efficiency and little effect on cell viability.

A range of cell densities and DharmaFECT 4 transfection reagent concentrations were also assessed with synthetic crRNA:tracrRNA targeting *PSMD7* for gene knockout using the EGFP fluorescence intensity and cell viability (Figure 2). As expected, a strong correlation was observed between the optimal conditions for *PSMD7* and *PPIB* crRNAs. The use of 20000 cell per well in 96-well format with 0.12 µL/well of DharmaFECT 4 transfection reagent resulted in the most robust conditions with high editing efficiency (> 6 fold increase of EGFP fluorescence compared to untreated cells) as well as excellent cell viability (> 80%).

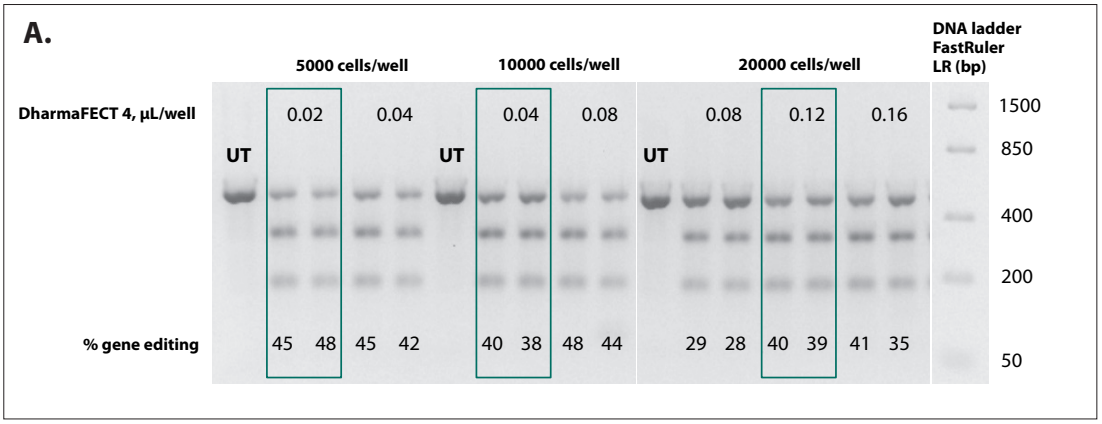
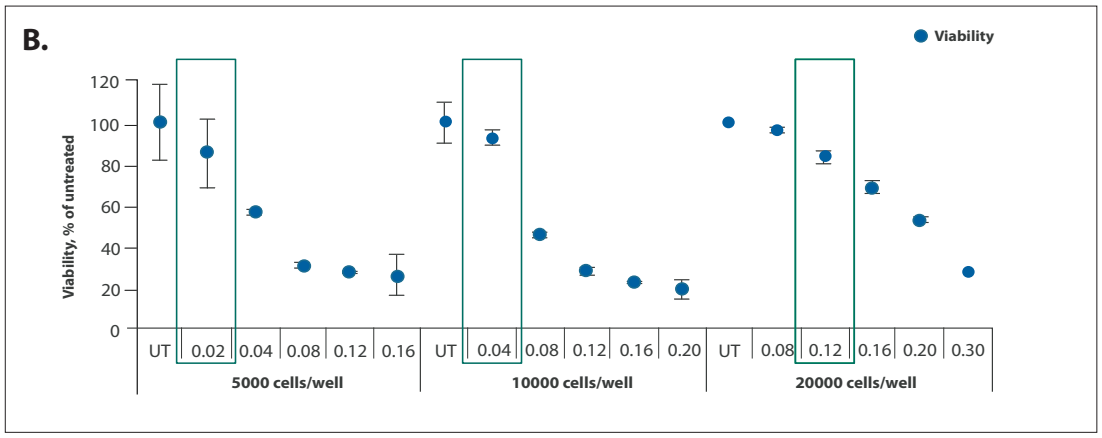


Figure 1. Identification of optimal reverse transfection conditions using PPIB crRNA positive control. **A.** *PPIB* gene editing efficiency assessed with DNA mismatch assay using T7EI 72 hours post-transfection (duplicate wells are shown). The amount of transfection reagent required for successful gene editing varies with cell density. FastRuler™ Low Range DNA Ladder (Thermo Scientific, Cat #SM1103). **B.** Cell viability measured with resazurin assay 72 hours post-transfection. Optimal amount of transfection reagent per well will result in cell viability of 80% or higher. Best conditions based on gene editing efficiency and viability are boxed in green. UT = untreated control, 0.02, 0.04, 0.08, 0.12, 0.16, 0.20, 0.30 = µL/well of DharmaFECT 4 transfection reagent.



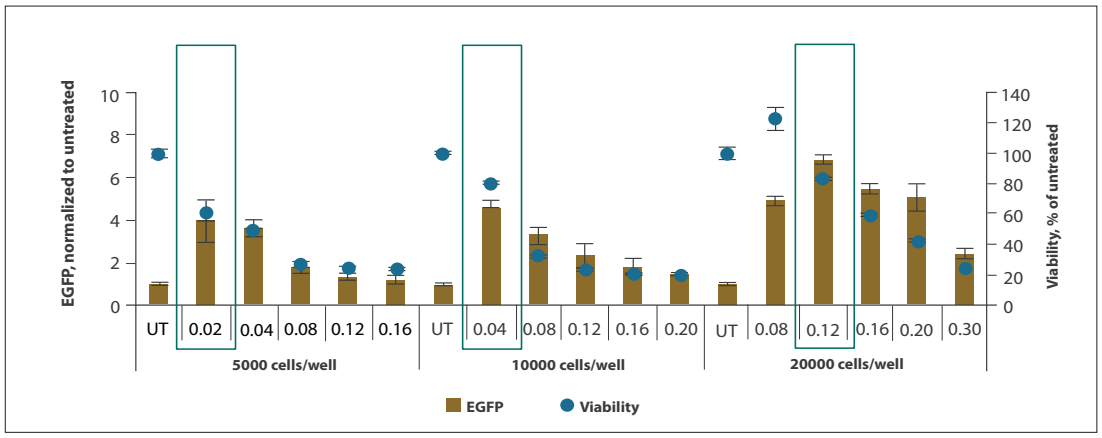


Figure 2. Phenotypic EGFP readout of PSMD7 functional gene knockout. For all cell densities, the optimal transfection conditions determined with *PPIB* crRNA (Figure 1) correlate with the best functional gene knockout, as indicated by EGFP readout. The best conditions for each cell density based on functional gene knockout efficiency and viability are boxed in green. UT = untreated control, 0.02, 0.04, 0.08, 0.12, 0.16, 0.20, 0.30 = µL/well of DharmaFECT 4 transfection reagent.

Conclusion

Arrayed crRNA libraries allow for functional genetic screens using a wide range of phenotypic readouts, including high-content imaging, therefore further facilitating the discovery of genes with roles in different biological processes.

A reverse transfection method for delivery of arrayed synthetic crRNA:tracrRNA libraries allows for functional gene knockout screening in an automated, high throughput manner. Determining the optimal transfection conditions to obtain the highest editing efficiency with minimal effects on cell viability is of utmost importance for the success of the screen.

Transfection conditions need to be optimized for every cell line keeping in mind the assay time-point and phenotypic assay requirements, as different cell densities and growth characteristics affect the transfection efficiency. Transfection conditions that induce changes in cell viability and/or provide insufficient delivery of the targeting agent can mislead researchers toward false interpretations of data, which in the long run, are time consuming and costly.

If you have any questions, contact

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