Non viral method of Gene Editing using CRISPR-Cas9 System

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The Advantages of solid phase CRISPR/Cas9 system

HIGHER GENE EDITING EFFICIENCY

Editing efficiency = Transfection efficiency × Potency of CRISPR/Cas9 system

Types and concentration of transfection reagents
Concentration of nucleic acid,
Conditions of solid phase formation

CAS9、gRNA、HDR donor expression,
Construct, Sequence of gRNA

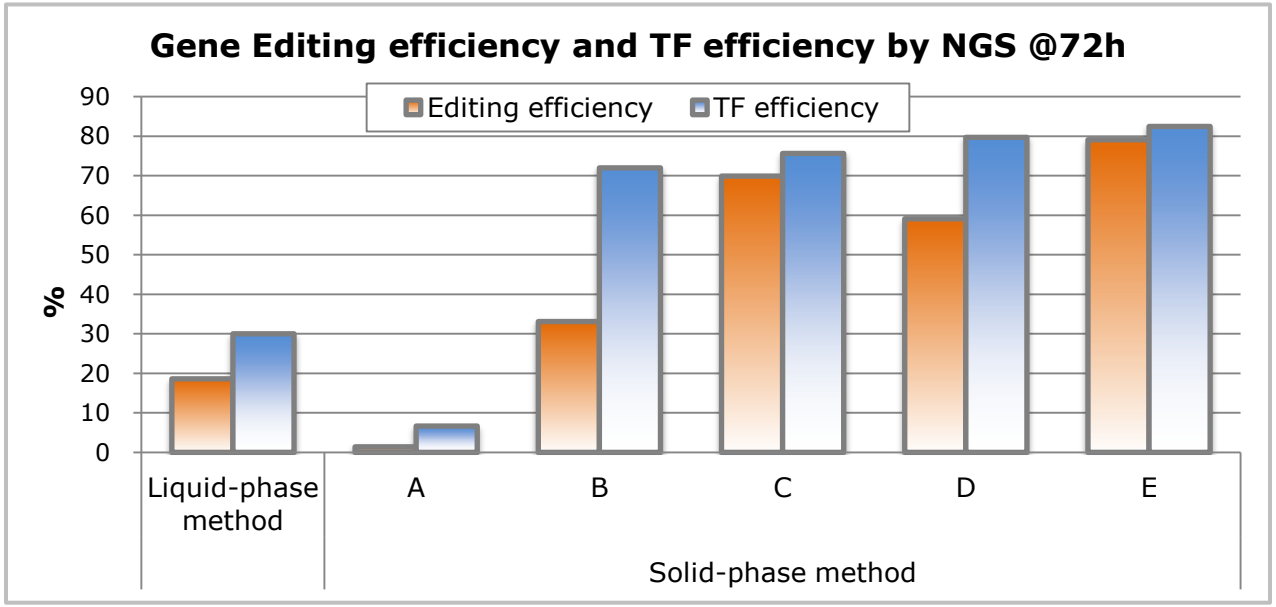
- Optimum transfection condition selected by the optimization plate
- Screening of sequence of gRNA and knockout screening will become available by multi-plates

ADVANTAGE OF THIS METHOD

- Simple operation ; just seed cells onto the plate without exchanging medium culture
- High transfection efficiency & reproducibility due to the accelerator
- Manufacture under strict quality control
- Plates can be stored for up to 12months at -20℃

Comparison between liquid/solid phase①

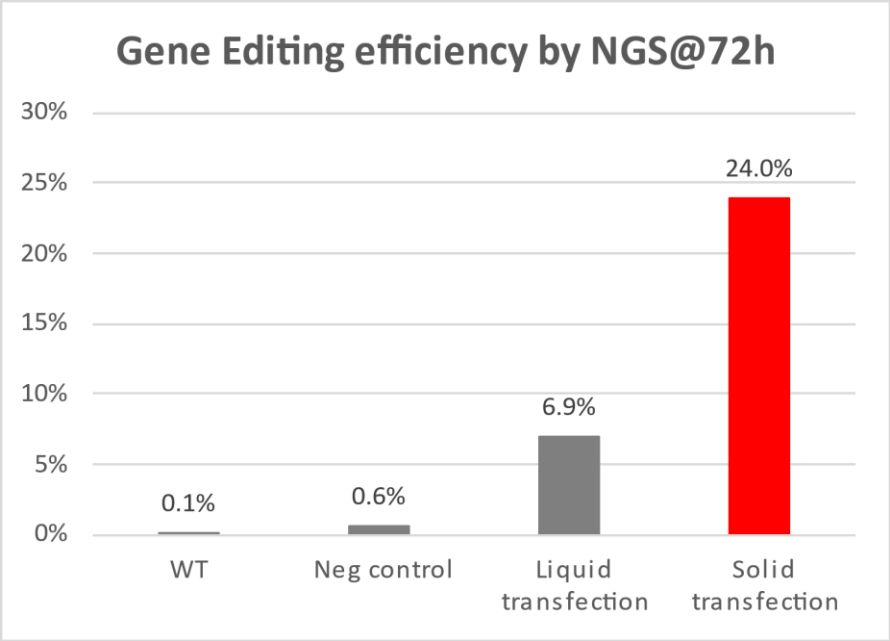
- Cell under study; HEK293T
- Cas9; Dharmacon Edit-R Cas9 Nuclease Expression plasmid (Anti Puromycin)
- gRNA; Dharmacon Edit-R PPIB Synthetic crRNA Control
- Comparison between /Cas9 Plasmid+gRNA by liquid phase transfection method (using selected reagent) and Cas9 Plasmid by liquid phase + gRNA by solid phase transfection method (under selected conditions using the optimization Plate)
- Puromycin added 24 hrs after transfection, cells cultured for a further 24 hrs
- Significantly greater editing efficiency is seen with the solid phase method



Density of gRNA; almost ¼ compared with actual method

Comparison between liquid/solid phase②

- Cell under study; mouse stem cells
- Cas9; Stable expression strain
- gRNA; Dharmacon Edit-R PPIB Synthetic crRNA Control
- Comparison between /gRNA by liquid phase transfection method (using selected reagent) and gRNA by solid phase transfection method (under selected conditions using the optimization Plate)
- Significantly greater editing efficiency is seen with the solid phase method



Type of solid phase CRISPR/Cas9 method

■ **Types of solid-phase CRISPR / Cas9 plates (including under development)**

Cas9	gRNA	
	Plasmid	**RNA**
Cas9-introduced cells	Kits for plasmid	Kits for RNA
plasmid	Kits for plasmid	under development
mRNA	under development	under development

Development of technology for introducing gRNA by expressing Cas-9 into difficult-to-introduce cells with messenger RNA