Transfection Optimization Kit (Multi-guide)

Thank you for choosing the Transfection Optimization Kit to optimize your knockout experiment! Synthego's Transfection Optimization Kit (Multi-guide) is a positive control kit that is meant to be used in conjunction with Synthego's Gene Knockout Kit v2.

The inclusion of a positive control is vital to understanding the effectiveness of your CRISPR-Cas9 experiment. Positive controls are used for a number of reasons, mainly to optimize transfection for your specific cell type and to troubleshoot issues. Synthego recommends using this kit to optimize your transfection conditions prior to using the target-specific sgRNA supplied in the Gene Knockout Kit v2.

Like the Gene Knockout Kit v2, this kit uses a multi-guide strategy, in which multiple sgRNAs are designed to jointly knockout a single gene. When co-transfected, the sgRNAs induce a 21+ bp fragment deletion in the *TRAC* locus, resulting in a robust knockout (Fig 1).

Materials Provided

Quantity	Name	Description	Storage
1.5 nmol	Human *TRAC* multi-guide sgRNA, modified	sgRNA 1: 5'-CUCUCAGCUGGUACACGGCA-3' sgRNA 2: 5'-GAGAAUCAAAAUCGGUGAAU-3' sgRNA 3: 5'-ACAAAACUGUGCUAGACAUG-3'	-20˚C for up to 12 months
300 pmol	Cas9 2NLS nuclease	Wild type Cas9 from *S. pyogenes* (20 µM , 162 ug/nmol)	-20˚C until expiration date
1.5 ml	Nuclease-free Tris-EDTA Buffer (1X TE buffer)	10 mM Tris, 1 mM EDTA (pH 8.0)	Room temperature
1.5 ml	Nuclease-free water	-	Room temperature
20 µl	Human *TRAC* PCR primer mix	Forward: 5'- TCAGGTTTCCTTGAGTGGCAGG - 3' Reverse: 5'- TAAGGCCGAGACCACCAATCAG - 3' (10 µM)	-20˚C for up to 12 months
20 µl	Sequencing primer	5'-CTGGCCGTGAACGTTCACTGAAATCATGGC-3' (10 µM)	-20˚C for up to 12 months

Note: The PCR band size for human TRAC is: 667 bp

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Representative Data

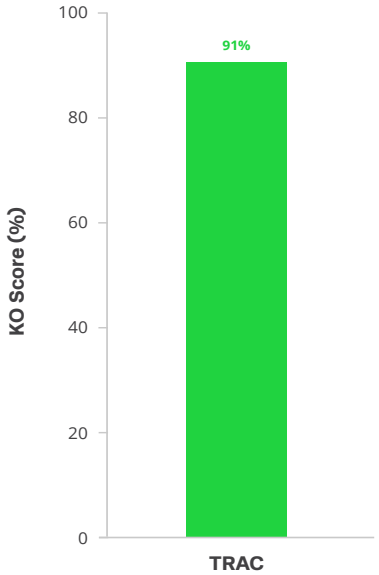

Figure 1. Synthego's chemically modified multi-guide sgRNA (human TRAC) included in the Transfection Optimization Kit (Multi-guide) generates high knockout efficiency (91%), as demonstrated in HEK293 cells. Multi-guide sgRNA targeting TRAC was complexed with spCas9 and transfected via nucleofection. Genomic DNA was PCR-amplified and analyzed using ICE to evaluate knockout efficiency. Knockout (KO) Score refers to the percentage of sequences that result in a putative knockout (frameshift-inducing indels and 21+ bp fragment deletions).

Step 1: Rehydrate the Multi–guide sgRNA

Synthego's multi-guide sgRNA ships dry at ambient temperature and remains stable for several weeks at room temperature. Dried sgRNA may be stored at -20°C for long-term storage (up to 6 months).

Be sure to work in an RNase-free environment.

Note: The quantity of material present (printed on the tube) is measured by UV absorbance spectroscopy at a wavelength of 260 nm prior to dehydration.

1. Briefly centrifuge the tube containing dried multi-guide sgRNA to ensure the pellet is collected at the bottom.

2. For cell lines and primary cells: carefully rehydrate 1.5 nmol multi-guide sgRNA (1-3 sgRNAs/tube) in 15 µl nuclease-free buffer (1X TE buffer*; provided) and pulse vortex for 30 seconds to ensure complete mixing. The final concentration of the sgRNA will be 100 µM (100 pmol/µl)

   * TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

   For microinjection: It is critical to only hydrate and dilute sgRNA in a nuclease-free 1X microinjection buffer (e.g., 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0; not provided).

3. Rehydrated sgRNA should be stored at -20°C. Under these conditions, the sgRNA will be stable for up to 12 months.

Step 2: Dilute the Multi–guide sgRNA

1. Depending on the application, the multi-guide sgRNA may be used directly at the rehydration concentration

in 1X TE buffer or diluted to a working stock using nuclease-free water in a sterile microcentrifuge tube. Synthego's nucleofection protocol requires a concentration of 30 µM multi-guide sgRNA (see Example Dilution below). For Synthego's lipofection protocol, a working concentration of 3 µM is needed.

Example Dilution: Add 6 µl of 100 µM multi-guide sgRNA to 14 µl of nuclease-free water to make a total volume of 20 µl of 30 µM multi-guide sgRNA (30 pmol/µl).

2. Use the diluted multi-guide sgRNA immediately or store at -20°C for up to 3 months (or 6 months if not repeatedly thawed).

Step 3: Transfect Cells

Choose either Synthego's nucleofection or lipofection to be used with this kit and the Gene Knockout Kit v2. All protocols are available at Synthego.com/resources.

Note: The positive control multi-guide sgRNA provided in this kit is sufficient for about eight transfections when using Synthego's nucleofection protocol and about 384 transfections when using Synthego's lipofection protocol.

Step 4: Analyze Knockout Efficiency

Synthego's Inference of CRISPR Edits (ICE) is a free online tool that provides an easy quantitative assessment of genome editing using Sanger sequencing data. The software compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited pools of cells.

For instructions on how to analyze the editing efficiency using Synthego's Inference of CRISPR Edits (ICE) tool, please see Synthego's Knockout Analysis protocol. To generate knockout clones, see our Clonal Expansion protocol. All protocols are available at Synthego.com/resources.

Additional Information

For details on Synthego's money-back guarantee, see: Synthego.com/legal/money-back-guarantees

For an up-to-date list of all Synthego protocols and other resources, please visit Synthego.com/resources

For technical assistance, contact our Scientific Support Team:
Ph: 888.611.6883
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About Synthego

Synthego is the leading genome engineering innovation company. The company's automated, full-stack genome engineering platform enables broader access to CRISPR to accelerate basic scientific discovery, uncover cures for diseases, and develop novel synthetic biology applications. Headquartered in Silicon Valley, Synthego is used by scientists from the largest global biotechnology companies and global biology universities to unlock the potential of gene editing.