Microsynth

User Guide

CRISPR Gene Editing with Optimized crRNA and tracrRNA from Microsynth

Overview

Microsynth offers two chemically optimized ready-to-use guide RNAs (crRNA and tracrRNA) which – together with the Cas endonuclease – can directly be used in your experiment without any cloning steps. This user guide gives you some guidance on how to use our guide RNAs and what addtional reagents you need for a successful CRISPR experiment.

Delivered by Microsynth

crRNA

Chemically optimized and customized to your needs

tracrRNA

Chemically optimized and universally usable for your CRISPR experiments

Additional Reagents Needed

Cas9 nuclease

e.g. TrueCut Cas9 Protein v2 from Thermo Fisher (Catalog No. A36496) or EnGen® Cas9 NLS, S. pyogenes from NEB (Catalog No. M0646T) or equivalent.

Nuclease-free water

e.g. from Thermo Fisher (Catalog No. AM9932) or equivalent.

Annealing buffer

e.g. 30 mM HEPES, pH 7.5, 100 mM potassium acetate.

Lipofection agent

e.g. Lipofectamine RNAiMAX Transfection Reagent from Thermo Fisher (Catalog No. 13778030) or equivalent

Protocol

1. Dissolve your crRNA and tracrRNA in the recommended volume of nuclease-free water to yield a 100 µM stock solution.
2. Dilute an aliquote 10 fold in your favourite annealing buffer e.g. 30 mM HEPES, pH 7.5, 100 mM potassium acetate to get your working concentration which is 10 µM.

Annealling

3. Mix crRNA and tracrRNA equimolar to yield 5 µM crRNA/tracrRNA complex.
4. Heat the sample to 92 °C for 2 min and cool slowly to 20 °C to form the crRNA/tracrRNA complex. This process must take at least 15 min.
5. Aliquote the cooled solution and further dilute the aliquots depending on the experiments you are going to perform (e.g. 1:5 to yield 1 µM complex).

Incubation

6. Prepare your crRNA/tracrRNA/Cas9 (RNP) complex using 1x Cas9 buffer. Add the annealed crRNA/tracrRNA and Cas9 enzyme in a molar ratio of 1:1. Incubate for 10 min at RT (e.g. mix 2 µl crRNA/tracrRNA complex, 2 µl Cas9 (1 µM stock solution), 2 µl Cas9 buffer 10x and 14 µl nuclease-free water to yield 20 µl of a 100 nM RNP complex -> sufficient for transfections in the 200 µl scale)

Transfection

7. Standard concentrations for your transfections are 10 nM crRNA/tracrRNA
8. Use your lipofection agent of choice or alternatively use electroporation to transfect your cells.

Contact

Do you have any questions? Please get in contact with our product expert Christian Winiger (oligo.support@microsynth.ch)

Software

Microsynth can recommend the following two free online CRISPR/Cas tools:
https://crispr.cos.uni-heidelberg.de/index.html
http://chopchop.cbu.uib.no/

Further Info

Additional useful information about CRISPR/Cas can be found at:
https://www.microsynth.ch/crispr-cas.html
https://www.microsynth.ch/crispr-cas9-sequencing.html

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