Mouse zygote microinjection

Alt-R™ CRISPR-Cas9 System ribonucleoprotein delivery

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The methods presented in this document has been provided by an IDT customer(s) who have used the Alt-R CRISPR-Cas9 System in their experiments. This document may serve as a starting point for using the Alt-R CRISPR-Cas9 System in similar model organisms but may not be fully optimized for your gene or application. IDT does not guarantee methods or any performance of such methods. IDT Application Specialists can only provide general technical support and troubleshooting support in relation to the methods outlined in this document.

Guidelines

- The Alt-R CRISPR-Cas9 System has two CRISPR RNA components (crRNA and tracrRNA). These two RNAs must be annealed at 1:1 molar concentration to generate active guide RNAs. Note that, for calculating and making dilutions of individual oligos, the crRNA is 36 bases, and the tracrRNA is 67 bases.
- The complete guide RNA complex (annealed crRNA and tracrRNA) must then be incubated with Alt-R S.p. Cas9 Nuclease 3NLS to obtain active ribonucleoprotein (RNP) complexes.
- The Mouse Genome Engineering Core Facility typically uses 20 ng/µL of guide RNA and 20 ng/µL of Cas9 protein for mouse zygote microinjections. Other concentrations may also work.
- Use embryo-grade Injection Buffer (1 mM Tris HCl, pH 7.5; 0.1 mM EDTA) to reconstitute RNAs and for all subsequent dilutions.
- Use filter tips for pipetting.

Methods

Resuspend crRNA and tracrRNA

1. Add Injection Buffer (1 mM Tris HCl, pH 7.5; 0.1 mM EDTA) to the dry crRNA and tracrRNA (e.g., final concentration of 1 µg/µL).

   Note: Use the IDT Resuspension Calculator to calculate the buffer volume. Like many transgenic labs, our core facility follows the mass (µg/µL) system for the Injection Mix. Alternatively, use molar concentrations.

Prepare guide RNA

1. Mix 5 µg of crRNA and 10 µg of tracrRNA (e.g., 5 µL of 1 µg/µL crRNA and 10 µL of 1 µg/µL tracrRNA) to create the guide RNA.
2. Anneal in a thermocycler (95°C for 5 min, then ramp down to 25°C at 5°C/min). Alternatively, incubate the tube in a beaker containing nuclease-free water at 95°C, and let cool to room temperature.

Prepare RNP Injection Mix

1. Calculate the volumes of guide RNA and Cas9 protein needed for 100 µL of Injection Mix.

   Note: Our core facility uses final concentrations of 20 ng/µL for both guide RNA (from Step 2) and Cas9 protein.

2. Dilute the guide RNA in 80 µL of Injection Buffer.
3. Add Alt-R S.p. Cas9 Nuclease 3NLS to a final concentration of 20 ng/µL.

   Note: Since Alt-R S.p. Cas9 Nuclease 3NLS is supplied at high concentrations, our core facility will sometimes make intermediary dilutions (e.g., 200 ng/µL) and then dilute it to the final concentration (20 ng/µL).

   Note: If donor DNA is not used, adjust the volume to 100 µL using Injection Buffer. If donor DNA is included, proceed to step 4, and do not adjust the volume until Add donor DNA.

4. Incubate at room temperature for 10–15 min to allow formation of RNP complexes.

Add donor DNA (optional)

1. Add donor DNA to the Injection Mix. Adjust the final volume to 100 µL using injection buffer.

   Note: The suggested final concentration of donor DNA is 5–20 ng/µL.

Centrifuge and filter the Injection Mix

1. Centrifuge the Injection Mix at 13,000 rpm for 5–10 min at room temperature.
2. Pass 80 µL of supernatant through a Millipore filter (UFC30VV25).

   Note: This additional, precautionary step eliminates any solid particles and prevents clogging of the microinjection needles.

Perform microinjection

1. Load the Injection Mix into needles and follow microinjection procedures as described [1].

References

1. Harms DW, Quadros RM, et al. (2014) Mouse genome editing using CRISPR/Cas system. Curr Protoc Hum Genet, 83:15.17.11–15.17.27.

Revision history

Version	Release date	Description of changes
2	July 2022	Updated document to internal MAPSS compliance
1	July 2017	Initital release

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