Mouse zygote microinjection

Alt-R™ CRISPR-Cas9 System ribonucleoprotein delivery

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The methods presented here are provided by customers who have used the Alt-R CRISPR-Cas9 System. This can 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 these methods, and application specialists at IDT can only provide general guidance with limited troubleshooting and support.

General notes:

1. 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.
2. 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.
3. 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.
4. Use embryo-grade injection buffer (1 mM Tris HCl, pH 7.5; 0.1 mM EDTA) to reconstitute RNAs and for all subsequent dilutions.
5. Use filter tips for pipetting.

Methods: RNP preparation

1. **Resuspend crRNA and tracrRNA.** 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 at www.idtdna.com/Calc/resuspension to calculate the buffer volume. Like many transgenic labs, the core facility follows the mass (μg/μL) system for the injection mix. Alternatively, use molar concentrations.

2. **Prepare guide RNA.**
   a. 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).
   b. Anneal in a thermocycler (95°C for 5 min and 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.

3. **Prepare RNP injection mix.**
   a. Calculate the volumes of guide RNA and Cas9 protein needed for 100 µL of injection mix.

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

   b. Dilute guide RNA in 80 µL of injection buffer.
   c. 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, the core facility occasionally makes intermediary dilutions (e.g., 200 ng/µL) and then dilutes it to the final concentration (20 ng/µL).

   d. If donor DNA is not used, adjust the volume to 100 µL using injection buffer. If donor DNA is included, proceed to step 3e, and do not adjust the volume until step 4.
   e. Incubate at room temperature for 10–15 min to allow formation of RNP complexes.

4. **(Optional) Add donor DNA.**
   a. Add donor DNA to the injection mix.

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

   b. Adjust the final volume to 100 µL using injection buffer.

5. **Centrifuge and filter the injection mix.**
   a. Centrifuge the injection mix at 13,000 rpm for 5–10 min at room temperature.
   b. 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.

6. **Perform microinjection.** Load the injection mix into needles and follow microinjection procedures as described [1].

Reference

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.

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