Zebrafish embryo microinjection

Ribonucleoprotein delivery using the Alt-R™ CRISPR-Cas9 System

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The methods presented here are provided by a customer who has 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.

Methods

1. Resuspend Alt-R crRNA and tracrRNA in Nuclease-Free IDTE Buffer to final concentrations of 100 µM each.
2. Mix the following components to create a 3 µM gRNA solution:

Component	Amount
100 µM Alt-R™ CRISPR-Cas9 crRNA	3 µL
100 µM Alt-R™ CRISPR-Cas9 tracrRNA	3 µL
Nuclease-Free Duplex Buffer (IDT)	94 µL
Final volume	**100 µL**

3. Heat at 95°C for 5 min.
4. Remove from heat, and allow to cool to room temperature (15–25°C) on your bench top.

   Note: the final concentration for the crRNA is 36 ng/µL and for the tracrRNA is 67 ng/µL.
5. Dilute Cas9 protein to a working concentration of 0.5 µg/µL:

Component	Amount
10 µg/µL Cas9 protein	0.5 µL
Cas9 working buffer (20 mM HEPES; 150 mM KCl, pH 7.5)	9.5 µL
Final volume	**10 µL**

6. Assemble the RNP complexes, for each injection:
   a. Combine 3 µL of gRNA (from step 4) with 3 µL of diluted Cas9 protein (from step 5).
   b. Incubate at 37°C for 10 min.
   c. Allow to cool to room temperature.

7. Collect embryos at the 1-cell stage and inject 3 nL of RNP complex (from step 6).

   Note: Dr Essner typically injects at least 15 embryos for each target and includes 5 uninjected embryos as controls.

8. Check injected fish for obvious toxicity at the following time points after fertilization:
   - 8 hr
   - 1 day
   - 2 days
   - 4 days

9. Isolate genomic DNA at day 4 after injection using the NaOH method.

   Note: Pool 5 fish into 1 tube. Include 3 tubes for each injection and 1 tube of uninjected fish for a control.

10. Run PCR specific for your targeted region.

11. Analyze PCR products on 2% acrylamide gels to estimate mutation efficiency.

    Note: Alternatively, sequence bands to determine accurate mutation frequency, or use a T7EI mismatch cleavage assay to estimate mutation efficiency.

IDT recommends using Alt-R™ S.p. Cas9 Nuclease 3NLS combined with the Alt-R CRISPR-Cas9 crRNA and tracrRNA to generate a ribonucleoprotein editing complex for high editing efficiency across most target sites. View the *Alt-R CRISPR-Cas9 User Guide for ribonucleoprotein transfection* of mammalian cell lines (available at www.idtdna.com/CRISPR).