Zebrafish embryo microinjection

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

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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.

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

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

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.

This protocol was developed using a discontinued version of Cas9 protein (Alt-R *S.p.* Cas9 Nuclease 3NLS). The currently available product (Alt-R Cas9 Nuclease V3) has an improved NLS and should be directly substituted into this protocol at the same volumes and concentrations. IDT recommends using Alt-R™ *S.p.* Cas9 Nuclease V3 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).

Revision history

Version	Release date	Description of changes
2	July 2022	Corrected document for internal MAPSS compliance
1	October 2016	Initital release

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