Microinjection of *Bactrocera tryoni* (Queensland fruit fly) embryos

How to prepare Alt-R® CRISPR-Cas9 ribonucleoprotein complexes for microinjection

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The methods presented in this document have been provided by an IDT customer who has 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 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.

Consumables

Kits and reagents	Ordering information
Enzyme dilution reagents:	
Option 1: HEPES and KCl	General laboratory supplier
Option 2: 1X Phosphate buffered saline (PBS)	General laboratory supplier
Option 3: Opti-MEM® media	Thermo Fisher (Cat # 51985091)
Alt-R CRISPR-Cas9 crRNA	IDT predesigned and custom crRNA: www.idtdna.com/CRISPR-Cas9
Alt-R CRISPR-Cas9 tracrRNA or Alt-R CRISPR-Cas9 tracrRNA – ATTO™ 550	IDT (cat # 1072532, 1072533, 1072534) IDT (cat # 1075927, 1075928)
Alt-R *S.p.* Cas9 Nuclease 3NLS	Discontinued; substitute with Alt-R *S.p.* Cas9 Nuclease V3 (cat #1081058, 1081059, 10000735)
Nuclease-Free Duplex Buffer	IDT (cat # 11-01-03-01)
Reagents for 10X Injection buffer:	
Sodium phosphate	General laboratory supplier
KCl	General laboratory supplier
Nuclease-Free Water	IDT (cat # 11-05-01-14)
HDR template:	
Ultramer® DNA Oligonucleotides	IDT (www.idtdna.com/Ultramer)

Methods

A. Prepare buffers and enzyme

1. Prepare the Cas9 enzyme working buffer as described in the following table:

Component	Amount	Final concentration
1 M HEPES, pH 7.5	200 µL	20 mM
1 M KCl	1.5 mL	150 mM
Nuclease-Free Water	~6.5 mL*	—
Total volume	**10 mL**	**—**

* Add 6.5 mL of water, verify pH 7.5, and add more water to reach final volume.

Note: 1X PBS or Opti-MEM media can be used in place of Cas9 enzyme working buffer.

2. Before use, thoroughly mix the stock Alt-R S.p. Cas9 enzyme by inverting the tube several times and briefly centrifuge the tube.

3. Dilute Alt-R S.p. Cas9 enzyme to a working concentration (for example, 1 µg/µL).

Note: The molecular weight of the enzyme is 163,700 g/mol. All Alt-R S.p. Cas9 enzymes are provided at a stock concentration of 61 µM (10 µg/µL).

Example dilution for 1 µg/µL:

Component	Amount (µL)
Cas9 dilution buffer (step A1)	9
Alt-R Cas9 enzyme	1
Total volume	**10**

4. Prepare the 10X injection buffer as described in the following table:

Component	Amount	Final concentration
0.1 M Sodium phosphate buffer, pH 6.8*	0.5 mL	1 mM
1 M KCl	2.5 mL	50 mM
Nuclease-Free Water	47 mL	—
Total volume	**50 mL†**	**—**

* 51 mL of 0.2 M NaH_2PO_4 + 49 mL of 0.2 M Na_2HPO_4 + 100 mL of Nuclease-Free Water, then verify pH 6.8.

† Store 1 mL aliquots of the 10X injection buffer at –20°C.

B. Prepare Alt-R CRISPR-Cas9 guide RNA

1. Prepare stock solutions of Alt-R CRISPR-Cas9 crRNAs and tracrRNA.
   a. Spin down RNA pellet using a microcentrifuge.
   b. Resuspend each RNA oligo in Nuclease-Free Duplex Buffer to 100 µM final concentrations as described in the following table:

Normalized amount delivered (nmol)*	Volume of resuspension buffer (µL)
2	20
5	50
10	100
20	200
100	1000

* Prepare positive and negative controls using the same methods as for the experimental complexes—ideally using the same lots of buffers.

Note: To calculate your own dilutions, use the IDT Resuspension Calculator at www.idtdna.com/SciTools.

   c. Store stock solutions at –20°C when not in use.
2. Mix crRNA and tracrRNA in equimolar concentrations in a sterile microcentrifuge tube to create a final duplex concentration of 40 µM:

Component	Amount (µL)	Final concentration
100 µM Alt-R CRISPR-Cas9 crRNA (step B1)	1	~472 ng/µL
100 µM Alt-R CRISPR-Cas9 tracrRNA (step B1)	1	~887 ng/µL
Nuclease-Free Duplex Buffer	0.5	—
Total volume	**2.5**	**40 µM (duplex RNA)**

3. Heat at 95°C for 5 min.
4. Remove from heat and allow to cool to room temperature (20–25°C).

C. Assemble ribonucleoprotein (RNP) complex

1. Mix the following to assemble RNPs for injection:

   a. With no donor template for knockout experiments:

Component	Amount (µL)	Final concentration
crRNA:tracrRNA duplex (40 µM) (step B4)	2.5	10 µM*
Diluted Cas9 enzyme (1 µg/µL) (step A3)	3	300 ng/µL
10X Injection buffer (step A4)	1	1X
Nuclease-Free Water	3.5	—
Total volume	**10**	**—**

   b. With donor template for homology-directed repair (HDR) experiments:

Component	Amount (µL)	Final concentration
crRNA:tracrRNA duplex (40 µM) (step B4)	2.5	10 µM*
Diluted Cas9 enzyme (1 µg/µL) (step A3)	3	300 ng/µL
ssODN (1 µg/µL)†	2	200 ng/µL
10X Injection buffer (step A4)	1	1X
Nuclease-Free Water	1.5	—
Total volume	**10**	**—**

   * The final concentration of the crRNA is ~118 ng/µL and of the tracrRNA is ~222 ng/µL.

   † 4 nmol of Alt-R HDR Donor Oligo can be used as the single-stranded deoxyoligonucleotide (ssODN) donor template.

   Note: 10 µL of injection mix can be used to inject ~300–400 embryos.

2. Incubate at room temperature for 5 min to assemble the RNP complexes.

   Note: The injection mix can now be used for embryo microinjections.

Revision history

Version	Release date	Description of changes
2	July 2022	Updated Cas9 NLS Nuclease to our newer Cas9 Nuclease V3; updated associated part numbers
1	May 2018	Initital release.

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