# Delivery of ribonucleoprotein complexes into *P. falciparum*-infected erythrocytes by electroporation

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The method presented here is provided by customers who have used the Alt-R CRISPR-Cas9 System. This method 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.

## Consumables and equipment

### Consumables from IDT

| Item | Catalog # |
|---|---|
| Alt-R CRISPR-Cas9 crRNA* | IDT predesigned and custom crRNA*: www.idtdna.com/CRISPR-Cas9 |
| Alt-R CRISPR-Cas9 tracrRNA | 1072532, 1072533, 1072534 |
| Alt-R S.p. Cas9 Nuclease V3† | 1081058, 1081059 |
| Alternatives: | |
| Alt-R S.p. HiFi Cas9 Nuclease V3 | 1081060, 1081061 |
| Alt-R S.p. Cas9 D10A Nickase V3 | 1081062, 1081063 |
| Alt-R S.p. Cas9 H840A Nickase V3 | 1081064, 1081065 |
| Nuclease-Free IDTE Buffer, pH 7.5 (1X TE solution) | 11-01-02-02 |

* We guarantee the performance of our predesigned gRNAs targeting human, mouse, rat, zebrafish, or nematode genes. For other species, use our proprietary algorithms to design custom gRNAs. If you have spacer designs of your own or from publications, use our design checker tool to assess their on- and off-targeting potential before ordering gRNAs that are synthesized using our Alt-R gRNA modifications. For details about the predesigned gRNA guarantee, see www.idtdna.com/CRISPR-Cas9.

† Alt-R S.p. Cas9 Nuclease V3 (wild-type) is suitable for most genome editing studies. However, some experiments may benefit from use of Alt-R S.p. HiFi Cas9 Nuclease V3, which has been engineered to reduce off-target effects, while retaining the on-target potency of wild type Cas9. Alt-R Cas9 nickases create single-stranded breaks. When a nickase variant is used with 2 gRNAs, off-target effects are reduced, and homology-directed repair is promoted.

## Consumables from other suppliers

| Item | Supplier | Catalog # |
|---|---|---|
| Absolute ethanol | General laboratory supplier | Varies |
| Cytomix transfection buffer | Custom:<br>120 mM KCl, 0.15 mM $CaCl_2$, 2 mM EGTA,<br>5 mM $MgCl_2$, 10 mM $K_2HPO_4/KH_2PO_4$, pH 7.6,<br>25 mM HEPES, pH 7.6 | N/A |
| Delivery/Rescue plasmid | Custom (context-dependent) | N/A |
| Fresh O+ Blood | Red Cross / Donation | N/A |
| RPMI 1640 GlutaMAX™ media | Thermo Fisher Scientific | 72400-047 |
| 10% AlbuMAX™ II Lipid-Rich BSA (w/v) | Thermo Fisher Scientific | 11021045 |
| Hypoxanthine | Sigma/Merck | H9377 |
| Gentamycin sulfate | Sigma/Merck | G1914 |
| Sodium acetate, 3M | Sigma/Merck | S2889 |

## Equipment

| Item | Supplier | Catalog # |
|---|---|---|
| Gene Pulser Xcell™ Electroporation System | Bio-Rad | 1652660 |
| Electroporation Cuvette Plus (400 µL – 2 mm) | BTX | 45-0125 |

# Protocol

## Culture *P. falciparum*-infected erythrocytes

1. Maintain *P. falciparum*-infected erythrocyte cultures under standard culturing conditions and routinely synchronize with 5% sorbitol.
2. Perform RNP transfections on ring-stage cultures at 6–10% parasitemia (4% haematocrit).

## Prepare RNA

1. Resuspend the crRNA and tracrRNA in Nuclease-Free IDTE Buffer:
   - Alt-R CRISPR-Cas9 crRNA, 2 nmol in 20 µL (100 µM)
   - Alt-R CRISPR-Cas9 tracrRNA, 5 nmol in 50 µL (100 µM)
2. Mix the crRNA and tracrRNA oligos in equimolar concentrations (1 µL each, per transfection) in a sterile microcentrifuge tube to a final duplex concentration of 50 µM.
3. Incubate at 95°C for 5 minutes and then let cool to room temperature on the bench.

## Form RNP complex

1. Mix 1.4 µL of the RNA solution with 1 µL of Cas9 enzyme per transfection (provided as a 61 µM suspension, 10 µg/µL).
2. Incubate at room temperature for 20 minutes.

## Prepare rescue plasmid/RNP complex

1. Linearize 50 µg of user-provided plasmid (per transfection) using two compatible restriction enzymes and incubate overnight.

   **Note:** The rescue plasmid is dependent on your desired modification; however, linearizing the plasmid prevents the need for drug cycling or other methods for plasmid elimination following transfection/parasite recovery.

2. Precipitate the linearized rescue template by adding 3M sodium acetate to a final concentration of 10% (v/v), then add ethanol to a final concentration of 70% ethanol (v/v).
3. Incubate at –20°C for at least 4 hours.
4. Centrifuge at 14,000 x *g* for 15 minutes (at 4°C).
5. Wash the DNA pellet with ice-cold 70% ethanol and centrifuge again.
6. Using a fresh pipette tip, remove the supernatant.

7. Dry the linearized rescue template for 3 minutes in a sterile environment, then resuspend in 400 µL of cytomix.
8. Mix 2 µL (up to 8 µL) of RNP complex with the rescue template suspension.

   **Tip:** Typically, 2 µL is sufficient to recover integrated parasites; however, you may need up to 8 µL of RNP complex for a standard transfection.

## Transfect cells

1. Prepare complete RPMI 1640 media by adding 10% AlbuMAX II (w/v) to a final concentration of 0.5%, hypoxanthine to 200 µM, and gentamycin sulfate to 20 mg/L.
2. Transfer 1.5 mL of ring-stage *P. falciparum*-infected erythrocyte culture (4% haematocrit, 6–10% parasitemia) to a 10 mL tube.
3. Centrifuge the cell suspension for 5 minutes at 500 x *g*.
4. Aspirate the supernatant and resuspend the cell pellet with the RNP-Rescue plasmid solution (400 µL).
5. Transfer the cell suspension to an electroporation cuvette.
6. Start electroporation with the following parameters:
   - Voltage: 310 V
   - Capacitance: 950 µF
   - Resistance: ∞

   **Note:** Run electroporation for approximately 8–12 msec.
7. Immediately transfer the cell suspension to a fresh 10 mL tube with complete RPMI 1640.
8. Centrifuge the cell suspensions for 5 minutes at 500 x *g*.
9. Using a fresh pipette tip, aspirate the supernatant.
10. Add 10 mL of RPMI and 200 µL of fresh O+ blood.
11. Store the cultures overnight under standard culturing conditions.
12. Apply the appropriate drug selection the following day.
13. Monitor the cultures by Giemsa smear, then replace the media every other day until *P. falciparum* is detectable (typically 2–3 weeks).

Because *P. falciparum* does not contain NHEJ, no indels form at the PAM site, thus explains why you will always have to provide a rescue template for DNA recombination. Now you are ready to perform a PCR to confirm integration.

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## Technical support:

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