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# Mitochondrial DNA base editing in HEK293T cells

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#### Manuscript citation:

Lake NJ, et al. Quantifying constraint in the human mitochondrial genome. bioRxiv (2023). https://doi.org/10.1101/2022.12.16.520778

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Protocol status: Working

We use this protocol and it's working

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Keywords: DdCBE, Base Editor, Mitochondria, Transfection, HEK293, mitochondrial dna base editing, cytosine base editor,

derived cytosine base editor, ddcbe half, enrichment of cell, ddcbe, dna, right plasmid, dual plasmid system

#### **Abstract**

This protocol is for the transfection of mitochondrial-targeted DddA-derived cytosine base editors (DdCBE), and their subsequent selection, in HEK293T cells. This uses a dual plasmid system, where a 'left' and 'right' DdCBE are needed for editing. Enrichment of cells with both DdCBE halves is achieved by separate drug selection for the left and right plasmids.

### **Materials**

HEK293 cells (ATCC, CRL-3216)

HEK media

- DMEM DMEM (Gibco, 11965092)
- 10% FBS (R&D Systems S11150)
- No antibiotics

**DdCBE** plasmids:

Left/left-dead (blastR) and Right (PuroR)

Lipofectamine 3000 reagents (ThermoFisher, L3000008)

Opti-Mem I reduced serum medium (ThermoFisher, 31985070)

12-well tissue culture plates (Corning, 353043)

Blasticidin (ThermoFisher, A1113903)

Puromycin (ThermoFisher, A1113803)



### **Protocol materials**

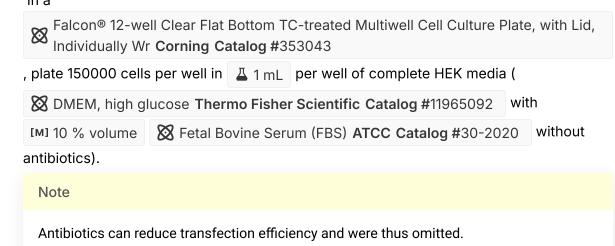
- HEK293T ATCC Catalog #CRL-3216
- Blasticidin S HCI (10 mg/mL) Thermo Fisher Catalog #A1113903
- Puromycin Dihydrochloride **Thermo Fisher Catalog** #A1113803
- X Lipofectamine™ 3000 Transfection Reagent Thermo Fisher Scientific Catalog #L3000008
- Blasticidin S HCI (10 mg/mL) Thermo Fisher Catalog #A1113903
- Puromycin Dihydrochloride **Thermo Fisher Catalog** #A1113803
- DMEM, high glucose **Thermo Fisher Scientific Catalog** #11965092
- X Fetal Bovine Serum (FBS) ATCC Catalog #30-2020
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- X Fetal Bovine Serum (FBS) ATCC Catalog #30-2020
- X Lipofectamine™ 3000 Transfection Reagent Thermo Fisher Scientific Catalog #L3000008
- X Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Scientific Catalog #**31985070
- X Fetal Bovine Serum (FBS) ATCC Catalog #30-2020
- , Falcon® 12-well Clear Flat Bottom TC-treated Multiwell Cell Culture Plate, with Lid, Individually Wr Corning Catalog #353043
- MEM, high glucose **Thermo Fisher Scientific Catalog #**11965092
- Opti-MEM™ I Reduced Serum Medium Thermo Fisher Scientific Catalog #31985070
- Right DdCBE Plasmid addgene Catalog #179686
- X Lipofectamine™ 3000 Transfection Reagent **Thermo Fisher Scientific Catalog** #L3000008
- Left DdCBE Plasmid addgene Catalog #179682
- Left Dead (inactive) DdCBE Plasmid addgene Catalog #179683

## Troubleshooting



## Plating of the HEK293T Cells

- 1 Plating of HEK293T ATCC Catalog #CRL-3216
- 1.1 The day before transfection, trypsinize and count the cells.
- 1.2 In a



1.3 Wait for cells to attach Overnight at 37 °C in a 5% CO<sub>2</sub> tissue culture incubator.

## Transfection of DdCBE Plasmids

- 2 Transfection of DdCBE Plasmids
- - Elipofectamine™ 3000 Transfection Reagent Thermo Fisher Scientific Catalog #L3000008

into  $\perp$  50  $\mu$ L (total volume) of



Opti-MEM™ I Reduced Serum Medium **Thermo Fisher** Scientific Catalog #31985070 and mix well.

2.2 In a separate tube, for each well of cells to be transfected, dilute Δ 2 μg of each DdCBE plasmid Left DdCBE Plasmid addgene Catalog #179682 or Left Dead (inactive) DdCBE Plasmid addgene Catalog #179683 with Right DdCBE Plasmid addgene Catalog #179686 that had been modified to include PuroR marker (left and right, for total of Δ 4 μg plasmid DNA (pDNA))

#### Note

The DdCBE plasmids used were obtained from Addgene, which included left (Addgene #179682) and right (Addgene #179686) DdCBE plasmids for editing, and a left dead (i.e. inactive) DdCBE plasmid (Addgene #179683) used with the right as a control. For this protocol, the right DdCBE plasmid was modified by replacing the *BSD* gene with *PuroR* to enable dual selection. Please see the associated publication for more plasmid details.

into ♣ 50 µL (total volume) of

Opti-MEM™ I Reduced Serum Medium Thermo Fisher
Scientific Catalog #31985070

. Then add 🚣 8 μL P3000 Reagent from

Eipofectamine™ 3000 Transfection Reagent Thermo Fisher Scientific Catalog #L3000008

(a 2:1 ratio to DNA) directly to the diluted pDNA. Mix well.

2.3 Add the diluted pDNA solution in P3000 reagent (from 2.2) to diluted

Lipofectamine™ 3000 Transfection Reagent Thermo Fisher
Scientific Catalog #L3000008

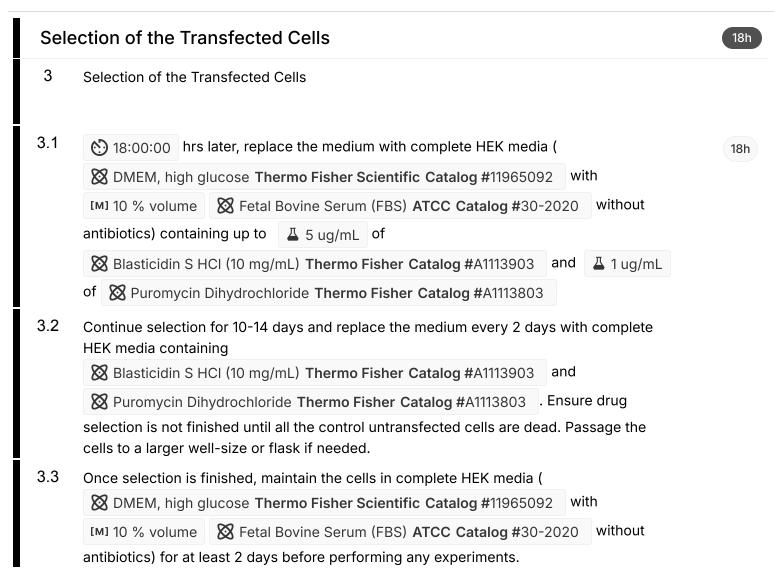
(from 2.1), mix, and incubate for 50 00:15:00 min at room temperature.

Discard the old medium in the well. Add ☐ 1 mL complete HEK media ( ☐ DMEM, high glucose Thermo Fisher Scientific Catalog #11965092 with

IMI 10 % volume ☐ Fetal Bovine Serum (FBS) ATCC Catalog #30-2020 without antibiotics) to each tube, mix well, and add to the corresponding well. Do this step well by well. Incubate the cells at ☐ 37 °C in a 5% CO₂ tissue culture incubator.

15m





### Protocol references

Protocol adapted from Mok, B.Y., et al. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 583, 631–637 (2020), and Mok, B.Y., et al. CRISPR-free base editors with enhanced activity and expanded targeting scope in mitochondrial and nuclear DNA. Nat Biotechnol 40, 1378–1387 (2022).