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Transposition of CHO-K1 Cells With TcBuster-M™

Using the Neon™ Electroporation System

Introduction

This protocol describes how to genetically modify CHO-K1 cells using the TcBuster-M transposon system. This is accomplished using the Neon electroporation system with the 100 µL kit. TcBuster-M is a commercially available hyperactive transposase that efficiently integrates large and/or multi-cistronic cargo into many different cell types while consistently providing robust integration and high editing efficiency.

Notice: When working with transposons, the end user should always titrate the transposon to determine optimal electroporation conditions for the construct. Continued optimization in the end user's experimental environment is recommended. If you have any technical questions about the TcBuster DNA transposon system or would like to design a custom transposon, please contact our technical support team at techsupport@bio-techne.com for assistance.

TABLE // 01

Materials Required

Material	Catalog Number
TcBuster-M Compatible DNA Transposon (Plasmid)	TCBP001-100 TCBP002-100 Custom, if applicable
TcBuster-M 001.1 mRNA	TCB-001.1-100
Media, CD CHO, Liquid	ThermoFisher
GlutaMAX Supplement	
Anti-Clumping Agent	
100 µL Neon NxT Transfection Kit	
Neon NxT Electroporation System	
DPBS	Multiple vendors
Nuclease-Free Water	
1.5mL Eppendorf Tubes	
6-Well Plate	
Polycarbonate 125 mL Shaker Flask	
Orbital Plate Shaker	
Cell Counter	

General Guidelines

- Maintain sterile technique, wearing gloves, using nuclease-free reagents and sterile pipette filter tips for best results.
- All reagents should be stored according to the manufacturer's recommendations.
- Avoid multiple freeze-thaws of the TcBuster-M mRNA and store as single-use aliquots.
- If diluting plasmid or TcBuster-M mRNA, do so in sterile nuclease-free water. The total volume of nucleic acids added should not exceed 10% of the total reaction volume.
- Work quickly and carefully; avoid leaving CHO-K1 cells in the R buffer for more than 30 minutes as this leads to reduced transposition efficiency and viability.

TABLE // 02

Suggested Controls

Control	Description	Purpose
No Electroporation	Standard grow out	Wild type cells for comparison to experimental samples. Used as control for toxicity from TcBuster-M and/or plasmid, cell death from electroporation, and viability/growth after genome modification.
Electroporation Only	No TcBuster-M or plasmid	Used as control to anticipate cell death caused by electroporation alone.
Plasmid + Electroporation Only	No TcBuster-M mRNA added to reaction, only plasmid + electroporation	Used as control to anticipate background signal caused by episomal expression of the plasmid. This signal is typically transient and is expected to disappear after one week of culture.
Positive Control	Transposon plasmid that has validated high editing efficiency	Ensures that all reactions, protocol, and equipment are properly functioning.

TABLE // 03

Timeline

Preparation	Transposition	Selection
Day 0 • Prepare media • Passage CHO-K1 cells 2-3 days before electroporation	**Day 2** • Mix and count cells • Prepare reagents and reaction tubes • Transpose cells • Incubate (2 days)	**Day 4** • Check transposition efficiency and cell growth progress • If applicable, begin selection

Protocol

Preparation

Day 0

Prepare Media

1. Mix and sterile filter complete media:
   a. CD CHO Medium
   b. GlutaMAX (6 mM)
   c. Anti-Clumping Agent (0.5%)

Passage CHO-K1 Cells

2. Passage cells to 0.2-0.3 x 10^6 cells/mL every 3-4 days.
    a. Do not allow cells to exceed 5-6 x 10^6 cell/mL.

3. It is recommended to passage CHO-K1 cells 2-3 days before electroporation.
    a. If starting from frozen cells, expand cells for at least 5 days from time of thaw before performing the electroporation.

Transposition

Day 2

Mix and Count Cells

1. Gently mix the cell complexes to break them apart, approximately 4–5 times, and transfer entire volume to a 50 mL conical tube.

2. Count resuspended cells and set aside while preparing other reagents.

Prepare Reagents and Reaction Tubes

3. Prepare and pre-warm 5 mL of complete media per electroporation reaction, including controls.

4. Add 5 mL of complete media per well of a pre-labeled 6-well plate. Place the plate in an incubator at 37 °C, 5% CO_2 until ready to use.

5. Prepare reactions in 1.5 mL tubes according to the guidelines given in Table 4 for your specific plasmid size.
    a. Try not to pre-mix mRNA and DNA in 1.5 mL tube at this step as DNA could contain RNases. Place DNA and mRNA on separate sides on the bottom of the tube.
    b. These concentrations are a starting point; parameters may need to be optimized for your specific applications.

6. Prepare the Neon NxT electroporation system in a way that maintains sterility throughout the procedure (preferably within a biosafety cabinet).
    a. Insert the electroporation tube within the pipette station until a click is heard.
    b. Add 2 mL of room temperature E100 buffer.

TABLE // 04

TcBuster-M mRNA Transposase and DNA Transposon Concentration Guide

Component	Concentration
TcBuster-M 001.1 mRNA	1.2 µg / 120 µL reaction
Small/Medium Plasmid (4 kb – 6.5 kb)	8 µg / 120 µL reaction
Large Plasmid (6.5 kb - 12 kb)	10 µg / 120 µL reaction
Custom Plasmid	Titrate plasmid to determine

Transpose Cells

7. Centrifuge cells at 300 x g for 5 minutes.

8. Aspirate supernatant and resuspend the cell pellet in 10 mL DPBS.

9. Centrifuge cells at 300 x g for 5 minutes.

10. Aspirate supernatant and resuspend cells in the appropriate volume of R buffer at a concentration of 25 x 10^6 cells/mL. Be sure to account for cell pellet volume during resuspension. Follow the example given below for proper resuspension technique.
    a. *Example*: For an experiment using 5 reactions:
        i. Final volume desired: 600 µL
        ii. Resuspend cell pellet with half that volume: 300 µL
        iii. New volume after resuspension: 350 µL
        iv. Add R buffer to reach 600 µL final volume: 250 µL

11. Move prepared 6-well plate with complete media to biosafety cabinet.

12. Only preparing one reaction at a time, mix 120 µL of cells in R buffer with the prepared plasmid and TcBuster-M mRNA in the 1.5 mL reaction tube. Mix 3-5 times without introducing bubbles.

13. Insert a Neon 100 µL tip into the Neon pipette. Ensure that the clamp of the Neon pipette fully grasps the mount stem of the 100 µL tip.

14. Depress the Neon pipette to the first stop, immerse the tip into the prepared reaction mixture, slowly release the Neon pipette to aspirate the reaction mixture into the tip.
    a. It is important to ensure that no bubbles are present within the Neon 100 µL tip when electroporating as this will cause arcing. We recommend including an extra 20 µL in each reaction to decrease bubbles in the pipette tip.
    b. It has been observed that the Neon tip may aspirate slowly. It is recommended to keep the pipette tip submerged in the reaction mixture until certain the tip has been filled.

15. Insert the Neon pipette into the Neon tube on the pipette station until a click is heard.
    a. Ensure that air has not entered the 100 µL tip after placing into the E100 buffer.

16. Electroporate this reaction mixture using the Neon system with the following program: **1550 V, 30 ms, 1 pulse**.
    a. This program is a starting point; parameters may need to be optimized depending on plasmid construct.

17. Immediately after electroporation, gently dispense cells into the prepared 6-well plate containing 5 mL of complete media.
    a. Do not mix cells once plated.
    b. Do not place cells against the vessel walls.
    c. Do not incorporate Penicillin/Streptomycin into the complete media during recovery post electroporation.

18. Repeat this process for each reaction.

19. Place the 6-well plate in the incubator at 37 °C, 5% CO_2 on an orbital shaker with speed between 90-120 rpm for 2 days.

Selection

Day 4

Check Transposition Efficiency and Cell Growth

1. When checking transposition efficiency, it is recommended to do this at least 2 days after electroporation and not to handle electroporated cells prior to this.

2. Mix cells thoroughly and sample each well for cell counts and for desired flow cytometry applications.

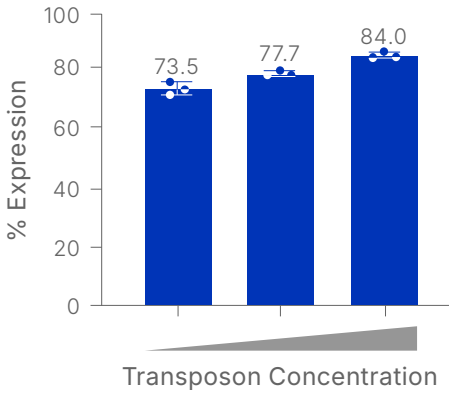
3. If applicable, begin selection at this time.

TABLE // 05

Troubleshooting

Problem	Possible Cause	Solution
Low Transposition Efficiency	Increase plasmid concentration	Titrate plasmid up to 2 µg higher than suggested concentration above
	Increase program intensity	Try increasing the voltage by 50 and/or the duration by 2
	Not enough cells in transposition reaction	Ensure 2.5 x 10^6 ± 1 x 10^6 cells are used per reaction
No Transposition	Not all reagents were added to reaction tube	Ensure TcBuster-M mRNA and plasmid are both added to reaction
	Arcing occurred during electroporation	Ensure there are no pockets of air within tip
Low Viability and/or Low Fold Expansion	Not enough cells plated in the 6-well plate	Refer to table above for minimum cells plated
	Cell growth exceeded maximum capacity of the 6-well plate	Plate cells in shaker flasks with additional media
	Arcing occurred during electroporation	Ensure there are no pockets of air within tip

Representative Data



CHO-K1 cells modified with TcBuster on the Neon electroporation system achieve transposon gene expression > 70%. CHO-K1 cells were thawed and cultured in suspension for at least 5 days before electroporation. Cells were electroporated using the Neon electroporation system at the 100 µL scale. 2.5 × 10^6 cells were electroporated per sample with TcBuster-M mRNA transposase and increasing concentrations of medium-sized DNA transposon. TcBuster achieved > 70% transposition efficiency with different transposon concentrations with a maximum of 84% efficiency. Data points represent the average of technical triplicates ± SD.