



Feb 02, 2026

Duke cell-thawing protocol for TF Perturb-seq Benchmarking WTC11 iPSC

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvrz3gmk/v1>

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Protocol Citation: Boxun Li, Charles Gersbach 2026. Duke cell-thawing protocol for TF Perturb-seq Benchmarking WTC11 iPSC. [protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvrz3gmk/v1](https://dx.doi.org/10.17504/protocols.io.6qpvrz3gmk/v1)

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Protocol status: Working

We use this protocol and it's working

Created: January 30, 2026

Last Modified: February 02, 2026

Protocol Integer ID: 242358

Keywords: WTC11, TF Perturb-seq, cell-thawing, seq benchmarking of wtc11 ipsc, thawing protocol, seq benchmarking wtc11, protocol for tf perturb, wtc11 ipsc, thawing at duke university, duke cell, seq benchmarking, protocol, tf perturb, thawing, cell, duke university

Funders Acknowledgements:

NIH

Grant ID: HG012053

Disclaimer

This protocol is adapted from the work of Boxun Li in the Gersbach lab at Duke University.

Abstract

This protocol describes the process used for cell-thawing at Duke University for TF Perturb-seq Benchmarking of WTC11 iPSC.

Materials

Suspension Medium: mTeSR Plus media + 1% BSA + 10 μ M ROCKi

- 45mL mTeSR Plus (StemCell Tech, 100-0276) + 5mL 10% BSA solution (Sigma-Aldrich A1595) + 50 μ L 10mM ROCKi (Stemcell Tech, 72304)

10X Genomics reagents

- 5' HT v2 reagents (including 5' CRIPSR kit) (PNs 1000374, 1000375, 1000451, and 1000215)
- GEM-X 5' v3 reagents (including Feature Barcoding kit) (PNs 1000698, 1000699, 1000703, 1000215)
- Keep these two sets of reagents in two separate ice buckets and tube racks. Put their respective gel beads near the reagents to prevent mix-ups.

Ice buckets with ice (2)

Centrifuge set at 4C and 250 xg

Procedure

- 1 Quickly thaw the frozen transduced WTC11 iPS cells by swirling the vials (1 vial of biorep 1, 1 vial of biorep 2) in 37C water bath.
- 2 Transfer the thawed cells to a 15 ml cone tube (one tube per vial thawed). Slowly add 5 ml ice-cold Suspension Medium dropwise to cells.
 - a. Keep cells in media and on ice whenever possible.
- 3 Pellet cells at 250 xg at **4 degrees** for 5 min with the appropriate centrifuge brake setting to prevent cells from stirring up.
- 4 Wash cells once by aspirating the supernatant, adding 5 ml ice-cold Suspension Medium, and pelleting cells at 250 xg at **4 degrees** for 5 min.
- 5 Wash cells a second time with 5 ml ice-cold Suspension Medium.
- 6 Wash cells a third time with 5 ml ice-cold Suspension Medium.
- 7 Resuspend each cell pellet in 300µL ice-cold Suspension Medium (aiming for a concentration of 5 ~ 6 M cells/ml).
- 8 Take 10µL of cell suspension, dilute it 1:5 with Suspension Medium, and count them with Countess 3 (mixed 1:1 with Trypan Blue). Take two technical replicates per cell suspension and take the average concentration and viability of the two replicates. Note the concentrations and viability. We got 92% and 89%, respectively.
- 9 Optional: If cell clumps exist, filter the cell suspension with 30 um Pre-Separation filters (Miltenyi Biotec).
- 10 According to the concentration, dilute each cell suspension to 1500 cells/µL with Suspension Medium.
- 11 Count the cells again. Cell viability should be >80%.
- 12 Load 10X chips for targeted recovery of 20k cells per lane. Run two lanes per biosample per chemistry. This means 2 lanes x 2 biosamples x 2 chemistries = 8 lanes in total.
 - a. First, load Chip N for 5' HT v2 (with 5' CRISPR). 4 lanes in total.



b. Second, load GEM-X v3 chip for GEM-X 5' v3 (with Feature Barcoding). 4 lanes in total.

13 Proceed to reverse transcription step and prepare gene expression and sgRNA sequencing libraries following manufacturer's instructions.