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Target enrichment of 10X Genomics scRNA-seq gene expression libraries

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Disclaimer

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

Abstract

This protocol describes target enrichment of 10X Genomics scRNA-seq gene expression libraries of neuron mono-culture or co-culture with mouse primary astrocytes.

Target enrichment of 10X Genomics scRNA-seq gene expression libraries

1 Enrichment DNA probe panel for target enrichment for scRNA-seq gene expression libraries using a hybridization-based method (Twist Bioscience, PN 101001, 104445, 100856, and 101262) was designed by Twist Bioscience for the 5′ UTR and first ~400bp of the coding sequence of all transcripts annotated in Gencode v41 of the 222 transcription factor genes of interest along with 4 positive control genes (TFRC, UBQLN2, GRN, and CDH2) as well as 7 housekeeping genes (DPM1, KRIT1, BAD, ARF5, POLDIP2, HCCS, TSR3).

2 Target enrichment is performed on the 10X Genomics 5′ HT v2 gene expression sequencing libraries of the putative regulatory element (pRE) Perturb-seq screens, according to the manufacturer's recommended protocol Standard Hybridization v2 Protocol (Twist Bioscience, DOC-001273 REV 3.0)

3 The enriched libraries were amplified using the 10X Genomics Amp Mix (10X Genomics, PN 2000047) and purified using Twist Purification Beads.

4 The target-enriched gene expression libraries were sequenced using Illumina Nextseq 2000, NovaSeq 6000 or NovaSeq X Plus sequencing to target depth of ~5 × 10^3 reads/cell.