2022/06/06

Reddy Lab PolyA plus RNA-seq protocol: Glucocorticoid receptor agonist project

Transfection

A549 cells (first obtained at passage 87) were expanded under standard culture conditions using Ham's F-12K (Kaighn's) Medium, 10% v/v FBS, 1% v/v penicillin-streptomycin for a total of seven passages. Cells were collected by trypsinization, pelleted, and resuspended in supplemented Lonza SF electroporation buffer. The whole genome STARR-seq library was electroporated into approximately 10^9 cells using the Lonza 4D-Nucleofector LV unit and the CM-130 program. Electroporated cells were incubated at room temperature for ten minutes before adding warm antibiotic-free media. Fifty million transfected cells were plated into eleven 500 cm^2 plates and allowed to recover in the incubator under standard conditions. Two hours post-electroporation, 20 μl of treatment compound was added to a plate for a final concentration of 1 μM. Four hours post-treatment, cells were rinsed with PBS (pH 7.4), and lysed with 10 mL of RLT buffer (QIAGEN) supplemented with 2-mercaptoethanol (Sigma). Lysates were harvested from plates and passed through a 18-gauge needle twenty times and stored at -80°C. The transfection protocol was repeated four times. Treatments from each transfection are considered biological replicates.

PolyA plus RNA-seq library construction

Total RNA was purified and used as templates for poly-A plus RNA-seq library construction according to the supplemental PolyA plus RNA-seq library construction protocol.