Multi-seq methods

* ESC maintenance and differentiation
  + GFP-Brychyury mESC cells (Gadue et al. 2006) were maintained and differentiated through day 2 of the directed differentiation protocol according to Spangler et al. (Spangler et al. 2018). At day 2, four different primitive-streak induction treatments were established by adding growth factors Wnt3a (25ng/mL) and Activin A (WA; 9ng/mL) along with one of the following additional growth factors: Noggin (150ng/mL), Gsk inhibitor (10mM), or BMP4 (0.5ng/mL). After a 48-hour primitive streak induction, embryoid bodies (EBs) were dissociated to a single cell suspension by incubating them in TrypLE and straining through a 40uM cell strainer. The GFP+ population was isolated by fluorescence-activated cell sorting (FACS) and subsequently re-aggregated to form EBs in SFD with bFGF (10ng/mL) for 48 hours. At day 6, EBs were dissociated and plated as a monolayer on 0.1% gelatin-coated tissue culture plates in SFD with bFGF (10ng/mL) and Gdf5 (30ng/mL) for 48 hours. Separate experiments were started in a staggered manner such that on the day of sequencing we had samples representing every day of the experiment from day 0 to day 8. We had samples representing four different primitive streak inductions conditions as well as samples that were not sorted for GFP at day 4. On the day of sequencing, EBs and monolayers were dissociated to a single cell suspension through the use of TrypLE and 40uM cell strainers.
* Multi-Seq protocol
  + We utilized the MULTI-seq sample barcoding and library preparation protocol from the McGinnis lab (McGinnis et al. 2019) in order to sequence 27 samples in two 10x capture runs. In short, the protocol involved tagging cell membranes with sample specific barcodes using a lipid-modified oligonucleotide (LMO). The LMOs (reagents obtained from the McGinnis lab) anchor into the cell membrane and allow for the attachment of a normal ssDNA oligonucleotide (MULTI-Seq barcode). A unique MULTI-seq barcode was added to each sample after which, all samples were pooled and sequenced as if they were one sample. An equal number of cells from each sample were combined to make up the pooled samples and ensure equal representation of each sample after down-stream sequencing. MULTI-Seq barcodes were used down-stream to identify which cells were from which samples.
  + Include multi-seq barcode sequences we used in supplement. We used barcodes 1-16.
  + Attach multi-seq protocol as supplemental methods?
* Library Preparation and Sequencing
  + After cells were successfully tagged and pooled, they were submitted to the sequencing core facility for 10x capture and library preparation. A Truseq library preparation was performed with the necessary adjustments made to accommodate the MULTI-Seq platform (outlined in supplemental methods). Libraries were sequenced on Illumina NovaSeq.
* Samples (make figure?)
  + Round 1
    - d0
    - d1
    - d2
    - d3WA
    - d3WAB
    - d3WAN
    - d3WAG
    - d4WA
    - d4WAB
    - d4WAN
    - d4WAG
  + Round 2
    - d4g164WAG unsorted
    - d4g164WAB unsorted
    - d4g164WAG sorted
    - d4g164WAB sorted
    - d5g163WAG unsorted
    - d5g163WAB unsorted
    - d5g163WAG sorted
    - d5g163WAB sorted
    - d6g162WAG unsorted
    - d6g162WAB unsorted
    - d6g162WAB sorted
    - d7g161WAG unsorted
    - d7g161WAB unsorted
    - d7g161WAB sorted
    - d8g160WAG unsorted
    - d8g160WAB unsorted

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