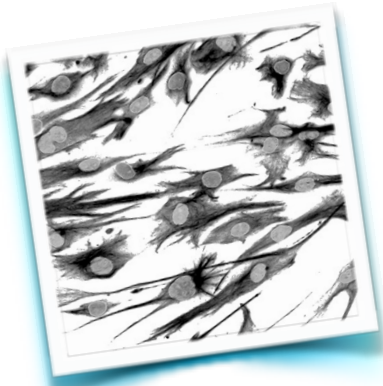


Definitive endoderm from hPSCs

Mark J. Tomishima, SKI Stem Cell Research Facility
<http://stemcells.mskcc.org>, tomishim@mskcc.org



1. Expand an appropriate population of human ES cells. Cells can be on feeders or feeder free. Allow human ES cells to expand for 2 or 3 days in hES media before starting differentiation.
2. Aspire media and wash with PBS. Initiate differentiation with Endoderm Induction Medium.
3. Feed cells every other day, and assay on day 6.

Endoderm Induction Medium

RPMI (Mediatech)

Glutamax

0.5% HyClone FBS

100 ng/ml activin A (R&D Systems)

According to D'Amour et al. 2005, ~80% of the population at day 4-5 are definitive endoderm, defined by SOX17/FOXA2 double positive cells and the absence of SOX7 and AFP. Cells can be further purified by CXCR4 flow cytometry.

D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. 2005. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol.* 12:1534-1541.