

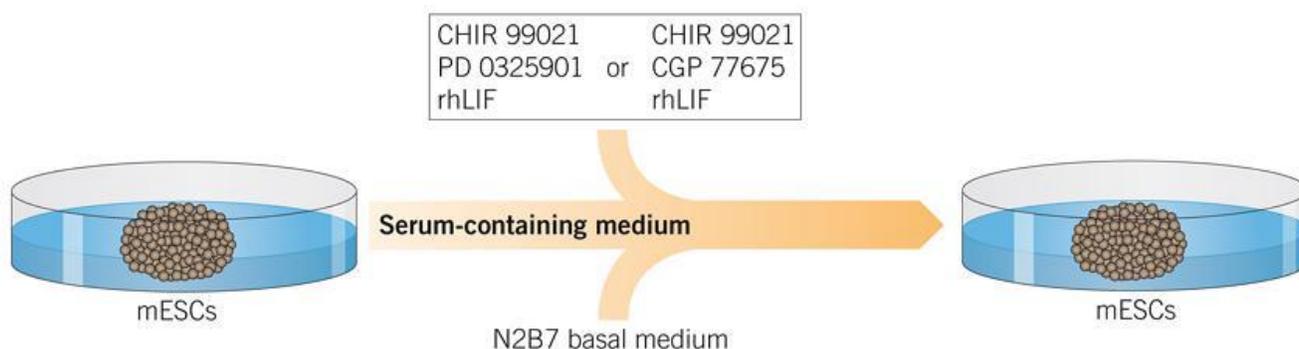
Culturing Transcriptomically Distinct Pluripotent mESCs

This is intended as a guide only; for full experimental details please read the reference provided.

In Brief

Kolodziejczyk *et al.* describe a protocol that uses two different cocktails of small molecules to maintain heterogeneous populations of mESCs with different metabolisms. STO feeder cells were used for mESC maintenance.

The use of 2i medium results in a heterogeneous population of blastocyst-like cells with a stable or 'ground state' of pluripotency. Alternative 2i (a2i) medium also results in a heterogeneous population of blastocyst-like cells with a stable or 'ground state' of pluripotency, which is transcriptomically distinct from that obtained with 2i medium. A subpopulation of 2C-like cells is present with 2i medium. No differentiating cells are observed with either 2i or a2i.



Cocktails

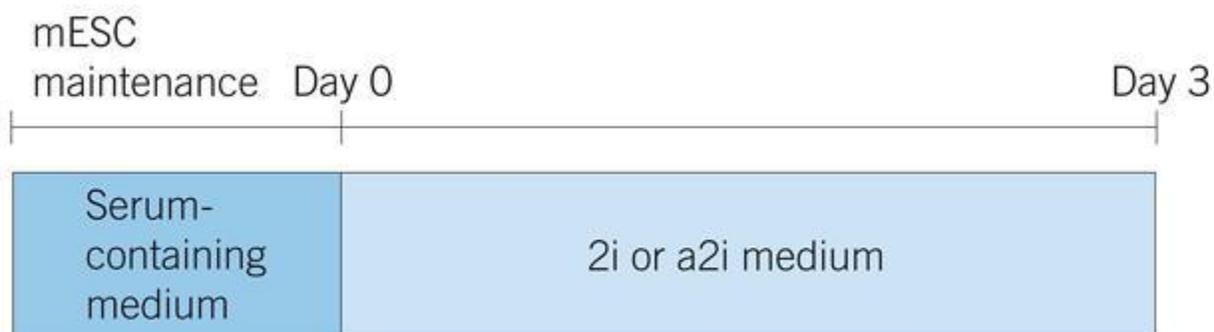
Serum-containing Medium		2i Medium		a2i Medium	
DMEM		N2B27		N2B27	
1X Pen/Strep		CHIR 99021 (Cat.No. 4423)	3 µM	CHIR 99021 (Cat.No. 4423)	3 µM
15% FBS		PD 0325901 (Cat.No. 4192)	1 µM	CGP 77675	
Beta-Mercaptoethanol	0.1 mM	rhLIF	100 U/ml	rhLIF	100 U/ml

Serum-containing Medium

2i Medium

a2i Medium

rhLIF 100 U/ml



Reference

Kolodziejczyk *et al.* (2015) Single cell RNA-sequencing of pluripotent states unlocks modular transcriptional variation. *Cell Stem Cell* 17471. PMID: [26431182](https://pubmed.ncbi.nlm.nih.gov/26431182/)