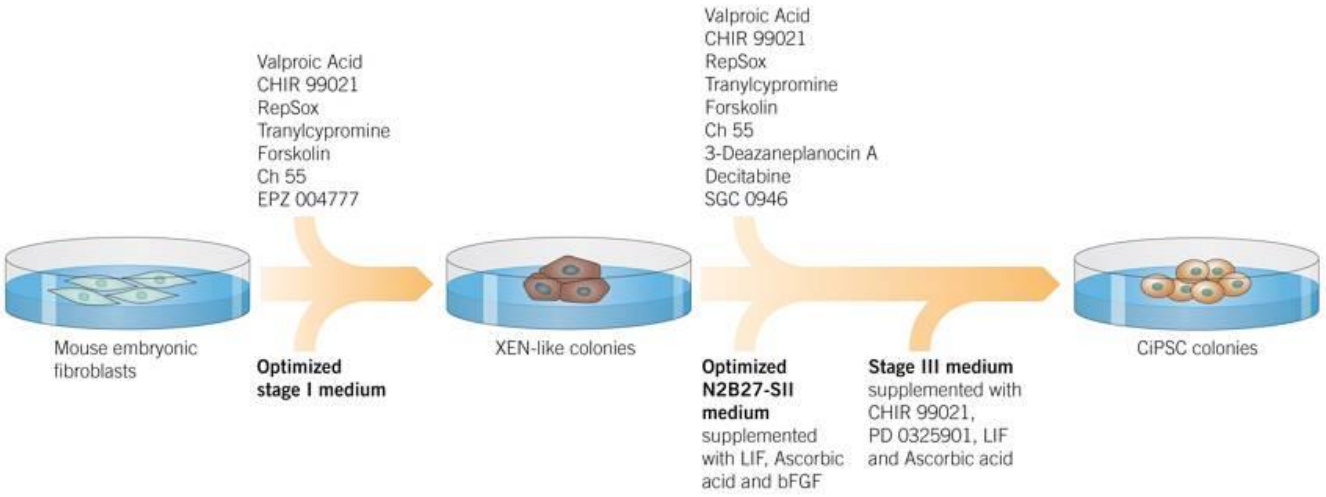
# Highly Efficient Generation of CiPSCs from MEFs

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

## In Brief

Zhao et al. describe a protocol for the highly efficient reprogramming of mouse embryonic fibroblasts (MEFs) to chemically-induced pluripotent stem cells (CiPSCs) in 16-20 days using a small molecule cocktail.

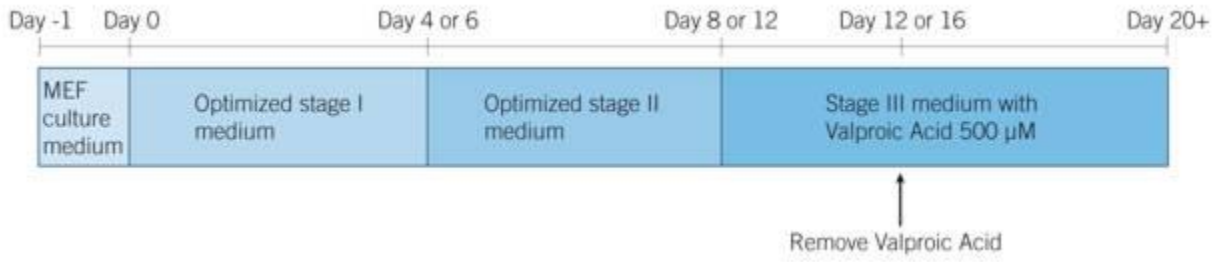
Briefly, MEFs were grown in MEF culture medium. On day 0 the medium was changed to optimized stage I medium. On day 4-6 small extraembryonic endoderm (XEN)-like colonies appeared and stage I medium was exchanged for optimized N2B27-SII medium. Cells were cultured for a further 4-6 days, then on day 8 or 12 N2B27-SII medium was exchanged for stage III medium containing 500 µM Valproic Acid (VPA). VPA was removed on day 12 or 16, and cells were cultured for a further 4-10 days in stage III medium, when CiPSC colonies emerged.




## Cocktails

| Optimized Stage I Medium (FBS/KSR-based medium) | | Optimized N2B27-SII Medium (N2B27-based medium ) | | Stage III Medium (N2B27-based medium) | |
|---|---|---|---|---|---|
| Valproic acid (Cat.No. 2815) | 100 µM | Valproic acid (Cat.No. 2815) | 1 mM | CHIR 99021 (Cat.No. 4423) | 3 µM |
| CHIR 99021 (Cat.No. 4423) | 40 µM | CHIR 99021 (Cat.No. 4423) | 10 µM | PD 0325901 (Cat.No. 4192) | 1 µM |

| Optimized Stage I Medium (FBS/KSR-based medium) | | Optimized N2B27-SII Medium (N2B27-based medium ) | | Stage III Medium (N2B27-based medium) | |
|---|---|---|---|---|---|
| RepSox (Cat.No. 3742) | 10 µM | RepSox (Cat.No. 3742) | 10 µM | LIF | 10 ng/ml |
| Tranylcypromine (Cat.No. 3852) | 5 µM | Tranylcypromine (Cat.No. 3852) | 5 µM | L-Ascorbic Acid (Cat.No. 4055) | 50 µg/ml |
| Forskolin (Cat.No. 1099) | 10 µM | Forskolin (Cat.No. 1099) | 10 µM | | |
| Ch 55 (Cat.No. 2020) | 1 µM | Ch 55 (Cat.No. 2020) | 1 µM | | |
| EPZ 004777 (Cat.No. 5567) | 5 µM | 3-Deazaneplanocin A (Cat.No. 4703) | 0.05 µM | | |
| | | Decitabine (Cat.No. 2624) | 0.5 µM | | |
| | | SGC 0946 (Cat.No. 4541) | 5 µM | | |
| | | L-Ascorbic Acid (Cat.No. 4055) | 50 µg/ml | | |
| | | LIF | 10 ng/ml | | |
| | | bFGF | 25 ng/ml | | |




## Reference

**Zhao** *et al.* (2018) Single-cell RNA-seq reveals dynamic early embryonic-like programs during chemical reprogramming. Cell Stem Cell. ***23*** 31 PMID: 29937202