MINERVA BIOTECHNOLOGIES

Novel stem cell growth factor NME7$_{AB}$ dramatically improves iPSC generation, expansion and differentiation

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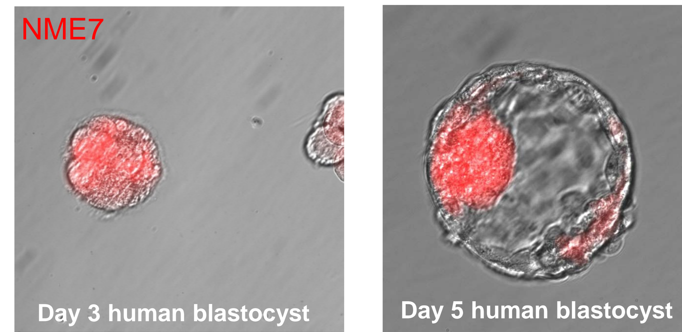
OVERVIEW

Despite the 10 years that have passed since the Nobel Prize winning discovery of iPSCs, their generation and expansion are still routinely done in bFGF-based media on feeder cells or extracellular matrix substrates. These methods are inefficient, labor-intensive and relatively non-scalable.

We discovered a growth factor NME7$_{AB}$ that is expressed in the inner cell mass of human blastocysts. iPSC generation using the Yamanaka factors in serum-free, FGF-free, NME7$_{AB}$ media over an antibody adhesion layer increased feeder-free reprogramming efficiency by 1-2 orders of magnitude.



The NME7$_{AB}$ system enables single cell passaging and large-scale expansion in high-capacity flasks: 10-fold expansion in four days. Resultant cells express pluripotency markers, as well as naïve markers, have two active X chromosomes if generated from female cells and differentiate down all three germlines. Directed differentiation is superior to that of FGF grown cells in terms of quality, quantity and purity with no clonal differentiation bias.

METHODS

Reprogramming

We generated hiPSCs from human foreskin fibroblasts in a serum-free, feeder-free, FGF2-free media containing NME7$_{AB}$. We transduced fibroblasts with OCT4, SOX2, KLF4, and c-MYC (OSKM) in NME7$_{AB}$ minimal media using Sendai virus. (100K human fibroblasts per well). Images 21-days post transduction

Expansion

We expanded the hiPSCs in MN-C3 coated T75 & T75 Flasks. Starting with two (2) 6-well plates, we expanded to 1.14 billion cells in 17 days

Differentiation

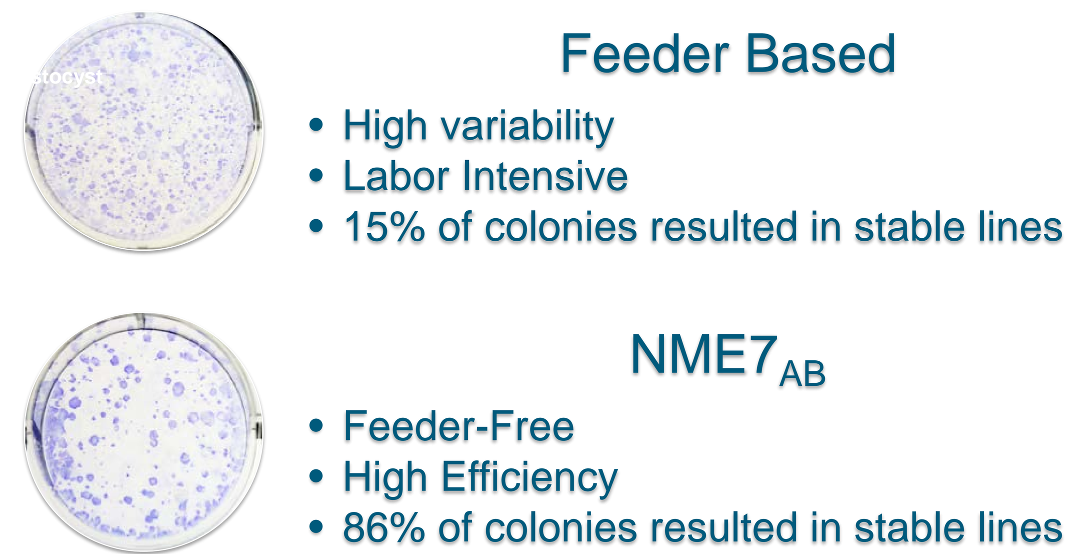
We formed EBs from the hiPSC lines and differentiated the cells into all three germ lineages

Karyotype Stability

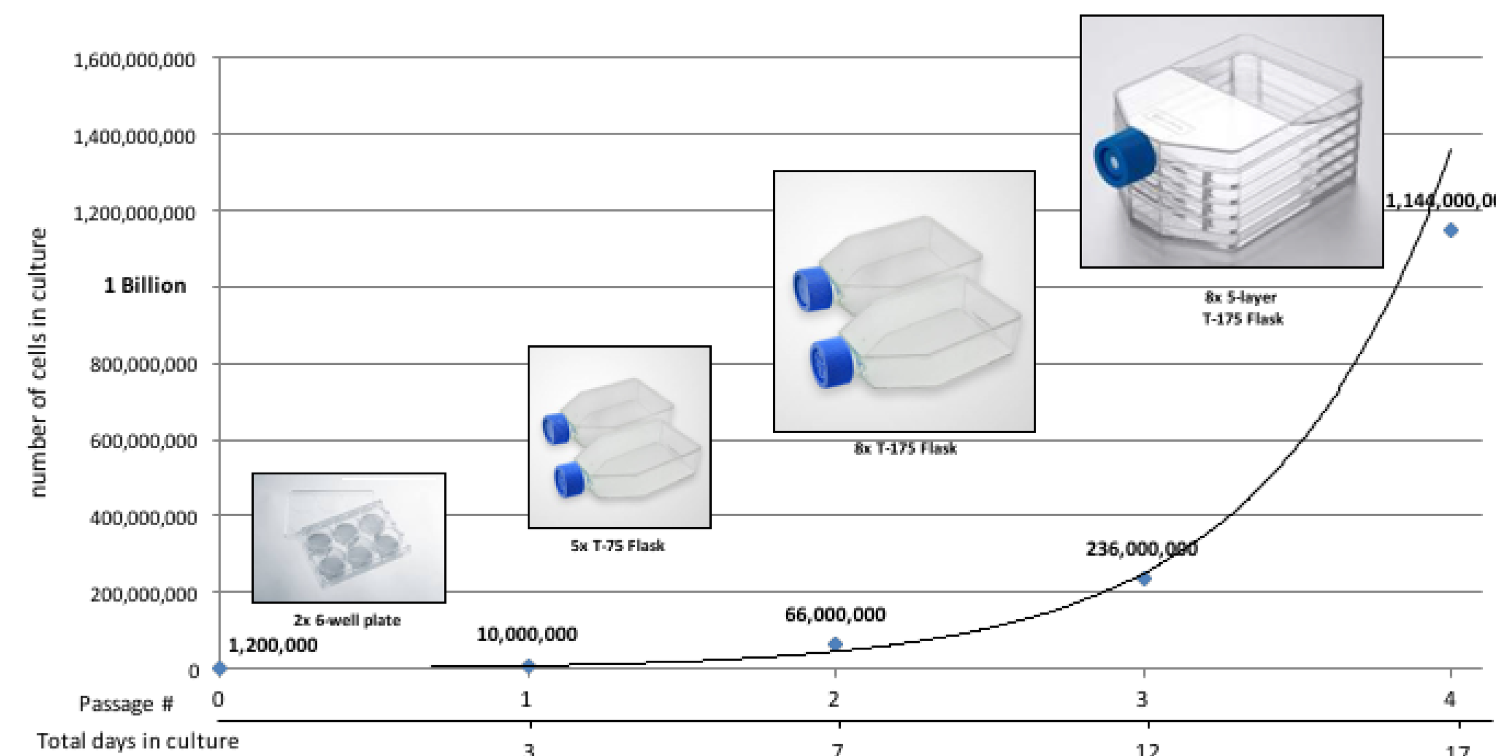
The cells were confirmed to have a normal karyotype by cytogenetic analysis performed on twenty G-banded metaphase cells

RESULTS

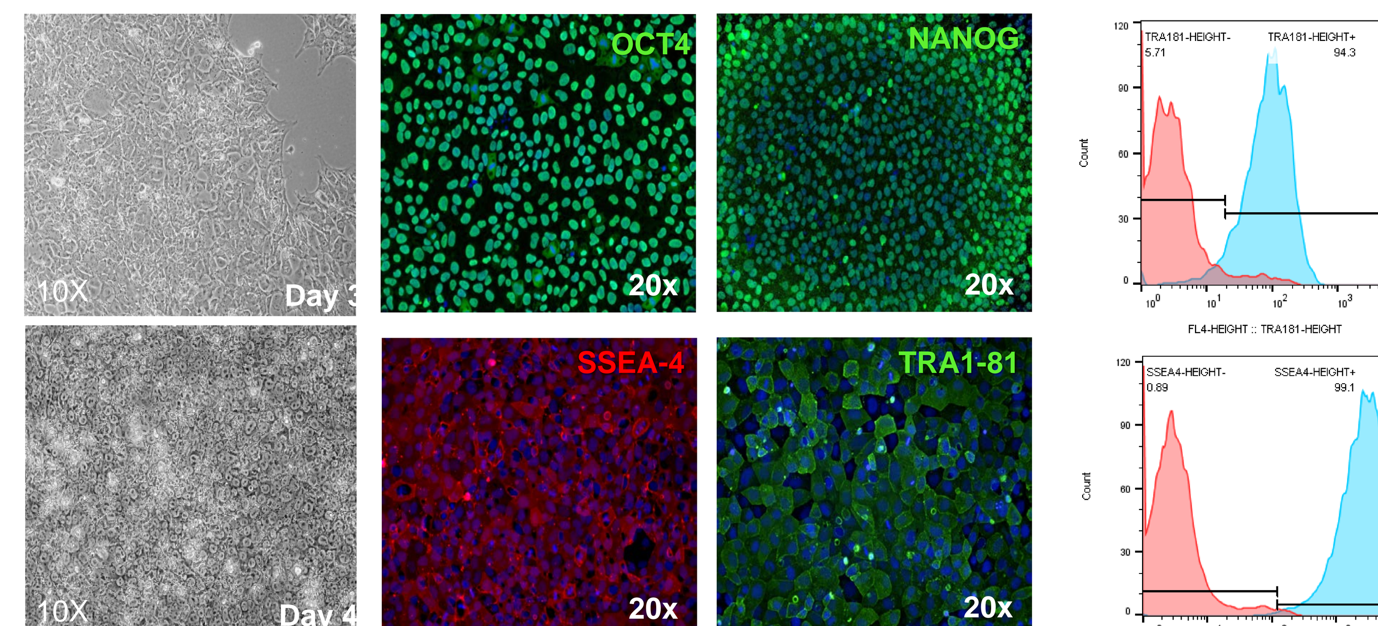
Despite the desire to move away from the variability and labor associated with MEFs, scientists believe they need feeders in order to achieve high efficiency reprogramming. The NME7$_{AB}$ System provides high efficiency feeder-free reprogramming with improved cell line survival



Large-scale production of hiPSCs has been one of the major challenges for translational applications of hiPSC technology. Here we demonstrate a scalable, robust and economic method that yields over 1 billion high quality cells in 17 days.

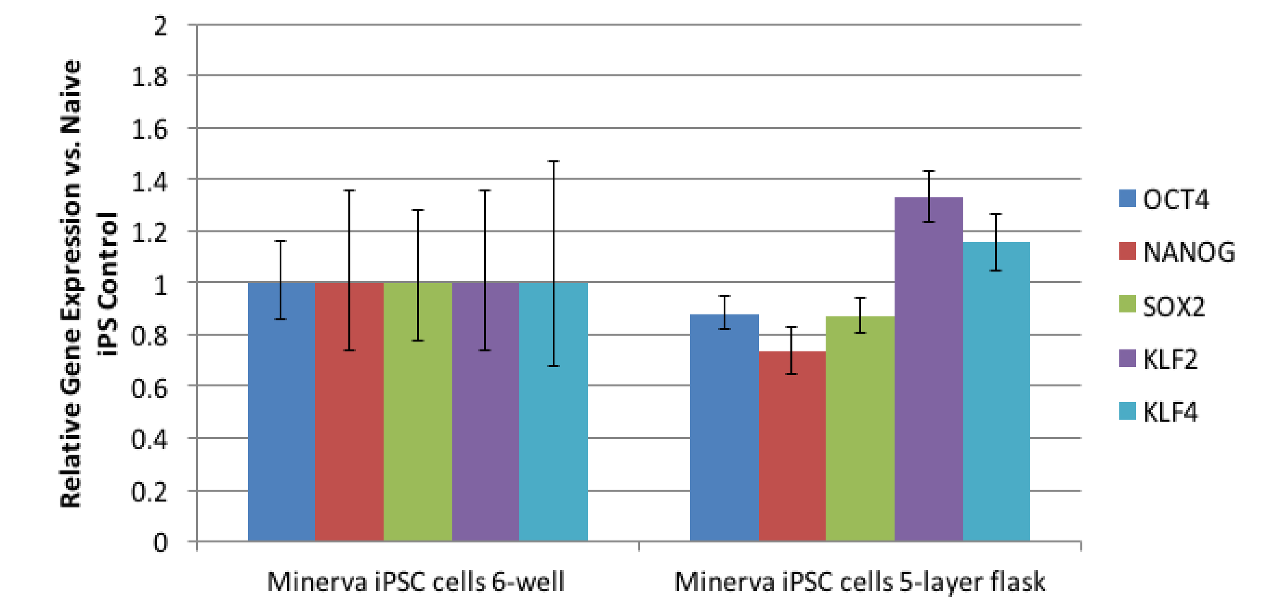


Cells expanded in the NME7$_{AB}$ System maintain expression of pluripotency markers.

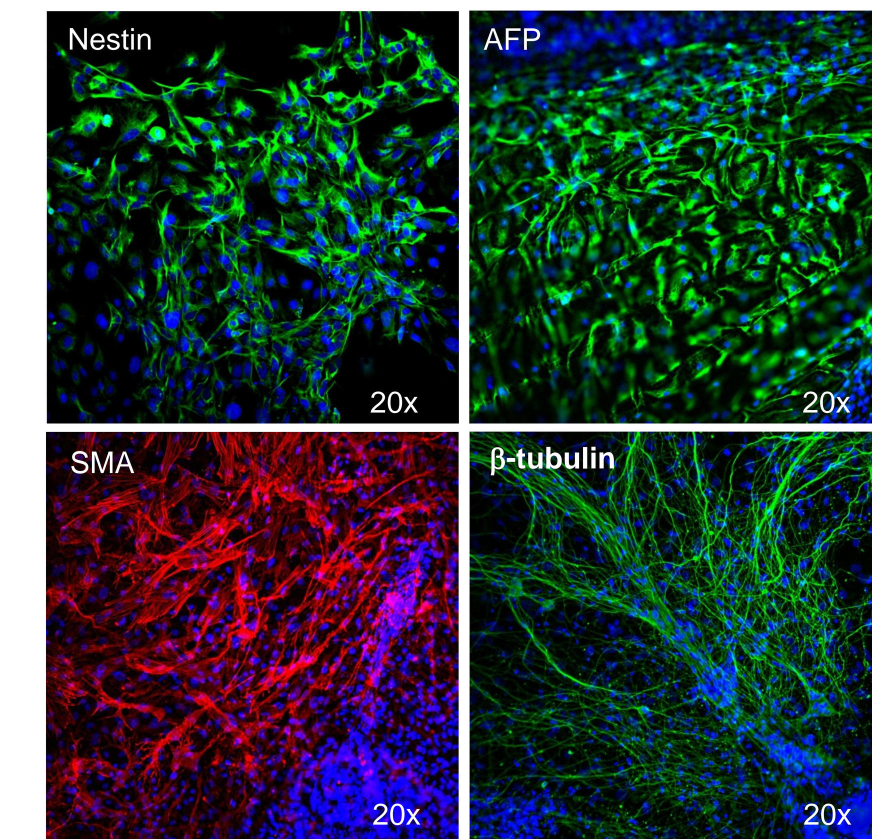


RESULTS

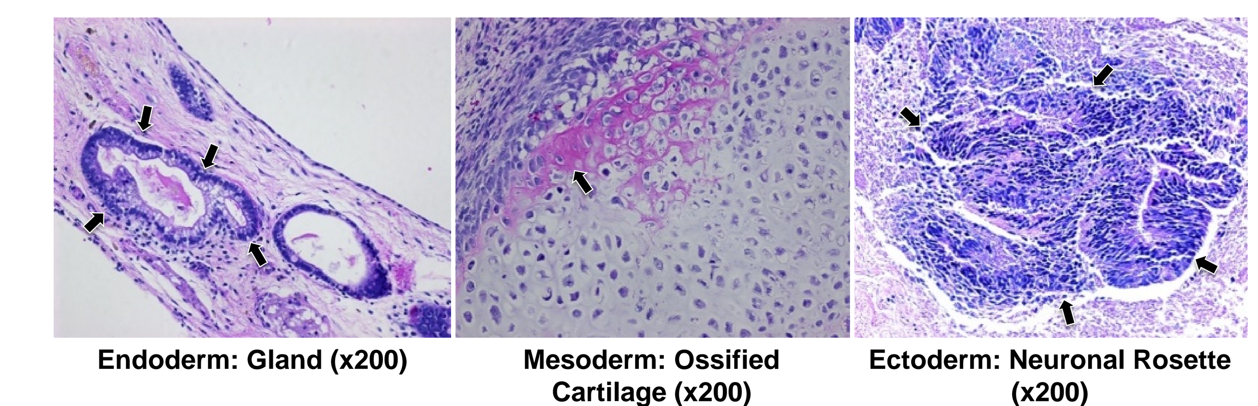
Pluripotency of the cells is also maintained in both 6-well and flask formats.



These expanded NME7$_{AB}$-grown iPSCs were able to form embryoid bodies containing cells from all three germ layers.



NME7$_{AB}$-grown iPSCs form teratomas that generate all three germ lines.



Endoderm: Gland (x200) Mesoderm: Ossified Cartilage (x200) Ectoderm: Neuronal Rosette (x200)

CONCLUSIONS

This work demonstrates that NME7$_{AB}$ is sufficient for long-term culture of human pluripotent stem cells in the absence of serum, feeder cells, FGF2 or other growth factors. iPSC generation in NME7$_{AB}$ is more efficient, without the need for feeders, and have stable karyotypes. Human ES and iPS cells can be rapidly expanded in this culture system for high-efficiency directed differentiation.

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