



# GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM AMNIOTIC EPITHELIAL CELLS

Blake, William L.<sup>1</sup>, Hansel, Marc<sup>2</sup>, Strom, Stephen C.<sup>3</sup>, Davila, Julio C.<sup>4</sup>, Engle, Sandra J.<sup>1</sup>

<sup>1</sup>Genetically Modified Models CoE, Pfizer, Groton, CT USA, <sup>2</sup>Dept. of Pathology, University of Pittsburgh, Pittsburgh, PA, USA, <sup>3</sup>Dept. of Pathology and McGowan Institute for Regenerative Medicine, Pittsburgh, PA, USA, <sup>4</sup>Pfizer, Chesterfield-St. Louis, MO, USA

## Introduction

Human amniotic epithelial (hAE) cells, isolated from term placenta, a discarded and relatively non-controversial tissue source, exhibit characteristics of human embryonic stem (hES) cells, including expression of cell surface markers SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. In addition, hAE cells express Oct4 and Nanog, genes involved in the maintenance of pluripotency. Also, in vitro differentiation of hAE cells has demonstrated their potential to form cell types of all three germ layers (endoderm, mesoderm, and ectoderm). Unlike hES cells, hAE cells do not express telomerase, and they are subject to senescence. Since AE cells possess some of the characteristics of hES cells we posited that hAE cells may reprogram with relative ease to induced pluripotent stem (iPS) cells.

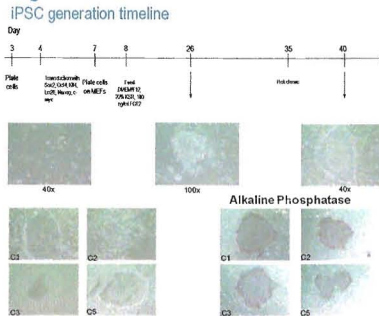
## Objective

To generate iPS cells from hAE cells utilizing lentiviral transduction approach

## Methods

Freshly plated AE cells were transduced with lentiviruses harboring Sox2, Oct4, KLF4, c-Myc, Lin28 and Nanog DNAs (ThermoFisher Scientific, Waltham, MA) and iPS cells were generated according to the timeline listed below in Fig 1.

## Fig. 1.

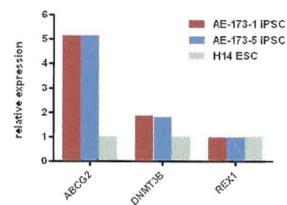


## Results

Putative iPS clones were alkaline phosphatase positive (Fig. 1) and expressed ABCG2, DNMT3B, and Rex1 (Fig. 2, below), genes associated with fully reprogrammed iPS.

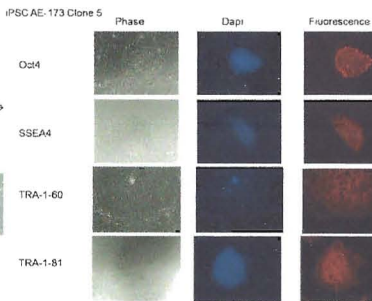
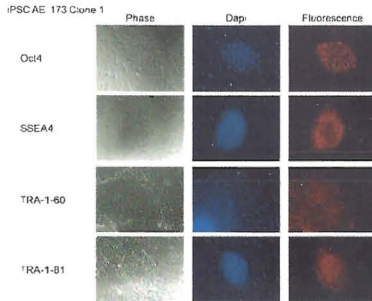
## Fig. 2.

AE-iPSC express genes associated with full reprogramming



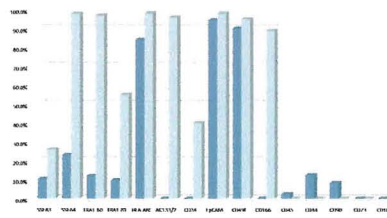
## Fig. 3

Immunocytochemistry analysis revealed that putative AE-iPSC clones expressed cell surface markers, SSEA-4, TRA1-60, TRA-1-81, in addition to Oct4 protein.



## Fig. 4.

FACS analysis showing differential surface antigen expression of AE-iPS vs. parent AE cells



## Fig. 5

AE-iPSC clones (1,5) exhibited normal 40XY karyotype.



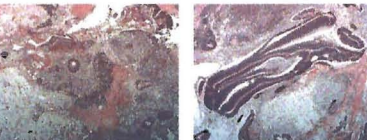
## Fig. 6

Teratomas formed by subcutaneous injection of AE-iPSC clones.



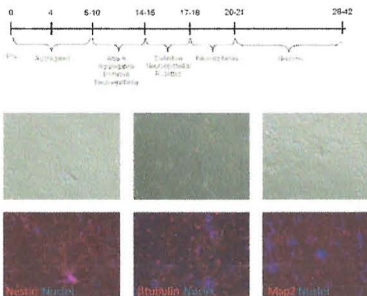
## Fig. 7

H&E stain of teratoma sections



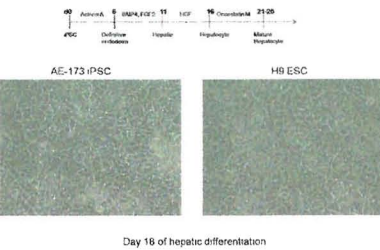
## Fig. 8

In vitro differentiation of AE-iPSC to neuronal phenotype



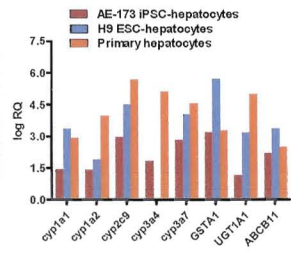
## Fig. 9

In vitro differentiation of AE-iPSC to hepatocytes



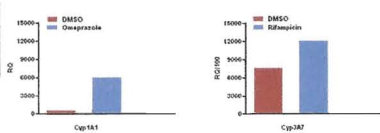
## Fig. 10

Phase 1, 2 and 3 gene expression in AE-iPSC-derived hepatocytes



## Fig. 11

Induction of Cyp expression in AE-iPSC-derived hepatocytes



## Fig. 12

Albumin and  $\alpha$ -Fetoprotein production in AE-derived hepatocytes

Hepatic protein	AE-173 iPSC-hepatocytes	H9 ESC-hepatocytes	Primary hepatocytes
$\alpha$ -Fetoprotein, $\mu$ g/ml	34.7 $\pm$ 4.0	17.1 $\pm$ 8.0	not Detected
Albumin, ng/ml	58.3 $\pm$ 42.4	325.8 $\pm$ 330.0	680.5 $\pm$ 65.2

## Conclusion

Data support the hypothesis that AE cells can be reprogrammed to iPS cells and thus may provide a non-controversial, genetically diverse source from which iPS cell lines can be generated for experimental study