Stem Cell Research 17 (2016) 93–96

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource

A human *MIXL1* green fluorescent protein reporter embryonic stem cell line engineered using TALEN-based genome editing

Vera Alexeeva [a,b], Sunita L. D'Souza [a,b,c,*], Christoph Schaniel [a,b,d,e,*]

[a] Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States
[b] Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States
[c] Experimental Therapeutics Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States
[d] Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States
[e] Mount Sinai Institute for Systems Biomedicine, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States

ARTICLE INFO

Article history:
Received 16 May 2016
Accepted 20 May 2016
Available online 26 May 2016

ABSTRACT

We have generated a *MIXL1*-eGFP reporter human embryonic stem cell (hESC) line using TALEN-based genome engineering. This line accurately traces endogenous MIXL1 expression via an eGFP reporter to mesendodermal precursor cells. The utility of the *MIXL1*-eGFP reporter hESC line lies in the prospective isolation, lineage tracing, and developmental and mechanistic studies of MIXL1^{+} cell populations.

Resource table: *MIXL1*-eGFP hESC.

Name of Stem Cell construct	*MIXL1*-eGFP reporter human embryonic stem cell line
Institution	Icahn School of Medicine at Mount Sinai
Person who created resource	Vera Alexeeva, Sunita L. D'Souza, Christoph Schaniel
Contact person and email	Sunita L. D'Souza, sunita.d'souza@mssm.edu; Christoph Schaniel, christoph.schaniel@mssm.edu
Date archived/stock date	May 22, 2013
Origin	Human embryonic stem cell line WA09 (H9; NIH registration number 0062)
Type of resource	Biological reagent; human embryonic stem cell line; genetically modified
Sub-type	Cell line
Key transcription factors	MIXL1
Authentication	Identity and purity of cell line confirmed (Fig. 1F)
Link to related literature (direct URL links and full references)	N/A
Information in public databases	N/A

1. Resource details

A *MIXL1*-eGFP reporter human embryonic stem cell (hESC; clones 4–21) line was generated using TALEN-based genome targeting of WA09 (H9, NIH registration number 0062) hESCs replacing the stop codon of endogenous *MIXL1* with a 2A-eGFP cassette, thus, creating a *MIXL1-2A-eGFP* allele, (Fig. 1A).

Correct integration was confimed by sequencing of PRC products obtained from genomic DNA using specific primers (see Table 1) as well as Southern blot analysis (Fig. 1B).

Prior to deciding to use WA09 to create the MIXL1 reporter line, we sent the line for G-banded karyotype analysis. Initial results showed a normal karyotype of parental WA09 (Fig. 1C, left panel). The reporter was created and then both the reporter and the parental line were resent for karyotype analysis. However, upon high resolution chromosomal analysis of the *MIXL1*-eGFP hESC line as well as the parental WA09 hESCs, a clonal abnormality, an interstitial duplication, resulting in partial trisomy of the long (q) arm of chromosome 1 was identified in both lines, which is a recurrent acquired abnormality in human pluripotent stem cell cultures (Na et al. 2014) (Fig. 1C, right panels). In addition, the long (q) arm of chromosome 1 is partially duplicated and translocated to the end of the short arm of chromosome X in the *MIXL1*-eGFP hESC line (10 of 20 cells examined). Seven of 20 *MIXL1*-eGFP hESCs were found to have no abnormality. Short tandem repeat analysis confirmed the WA09 hESC origin of the generated *MIXL1*-eGFP reporter hESC line (Fig. 1D).

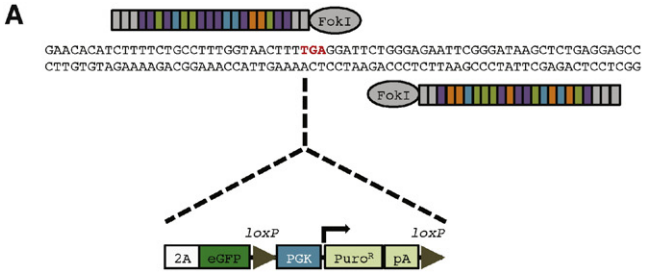
To confirm the pluripotency of the *MIXL1*-eGFP hESCs, the expression of several pluripotency markers was analyzed by quantitative real-time PCR as well as immunocytochemsitry. Endogenous expression

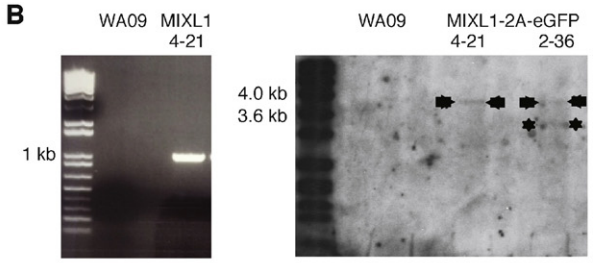
* Corresponding authors at: Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1496, New York, NY 10029, United States.
E-mail addresses: sunita.d'souza@mssm.edu (S.L. D'Souza), christoph.schaniel@mssm.edu (C. Schaniel).

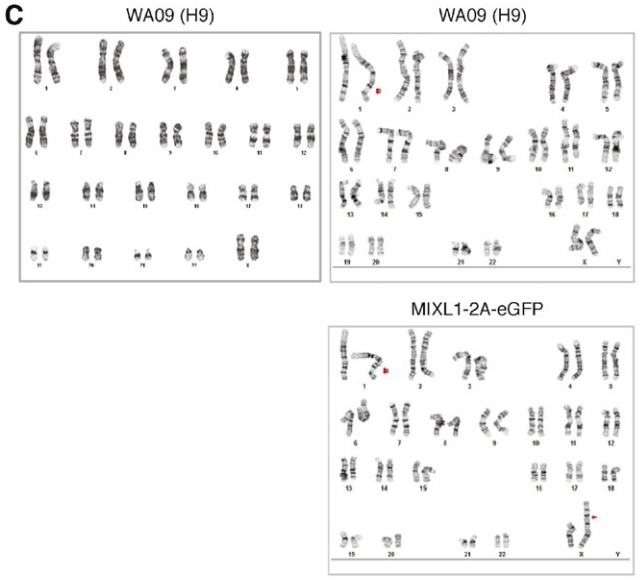
http://dx.doi.org/10.1016/j.scr.2016.05.010

of NANOG, OCT4 and SOX2 was determined at the mRNA level by real-time PCR (Fig. 1C). Protein expression of OCT4, NANOG, and SOX2 was assayed by immunocytochemsitry (Fig. 1D).

Three germ-layer differentiation ability was demonstrated by spontaneous in vitro differentiation of embryoid bodies with subsequent replating and immunocytochemical detection of smooth muscle actin







D

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	26,28
TPOX	6-13	10,11
D8S1179	7-18	8,14
vWA	10-22	17,17
Amelogenin	X,Y	X,X
Penta_D	2.2, 3.2, 5, 7-17	9,13
CSF1PO	6-15	11,11
D16S539	5, 8-15	12,13
D7S820	6-14	9,11
D13S317	7-15	9,9
D5S818	7-16	11,12
Penta_E	5-24	11,14
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	13,13
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32.32.2,33,33.2, 34,34.2,35,35.2,36-38	30,30
TH01	4-9,9.3,10-11,13.3	9.3,9.3
D3S1358	12-20	13,16