



Information on provided CRISPR/Cas9-edited human isogenic iPSC line

Author : M. Bouma

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Print date :

Revision date: 27-01-2020

Receiving scientist: T. Buck, J. Wijnholds

Provided human iPSC clones date/name: 22-07-2020

isoXXLUMC0116iCRB09

iso: isogenic line

XX (two digit code): identification number of isogenic subclone

LUMC: institute where hiPSC were generated;

four digit code: identification number hiPSC lines LUMC hiPSC core facility;

i: induced pluripotent stem cells;

two digit code: identification number of hiPSC clone.

To guarantee correct referral to the provided hiPSC lines in the future, please use this nomenclature in presentations, publications, etc.

If this line has not been published before please make sure researchers of the LUMC hiPSC are included as coauthor or at least acknowledged in the first publication describing this line.

Patient/hiPSC line information:

hiPSC line name prior to gene editing: LUMC0116iCRB09

hiPSC line passage nr. prior to gene editing: p18

CRISPR/Cas9-edited gene: CRB1

Mutation before editing (DNA): c.3122T>C heterozygous homozygous

Mutation after editing (isogenic):

Heterozygous repaired clones: c.3120C>G & c.3122T>C (both heterozygous),

Homozygous repaired clone: c.3120C>G (homozygous)

Method of reprogramming:

Polycistronic LV: see Wahrlich et al. Mol Ther (2011)

Episomal vectors with p53 knockdown ("Y4") or without p53 knockdown ("Y3"): see Okita et al. Nat Met (2011)

Sendai virus (SeV): see Nishimura et al. JBC (2011)

Genome editing method:

Cas9 nuclease

Cas9D10A nickase



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Sequence of crRNA(s) used:

CTGGGACAGTGGGTCTGTCG

Sequence of ssODN used:

ACAAGTTTGCAGTCAGTGAATGATGGCACATGGCACGAAGTGACCCTTTCGATGACAGAC
CCTACTGTCCCAGACCTCCAGGTGGCAAATGGAAGTGGACA

Repair was verified by sequence analysis.

General note:

Make sure that this line is cultured under local safety regulations.



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Information on provided isogenic subclone(s): 02 (homozygous repair)

Clone number/passage number: iso02LUMC0116iCRB09 p23 (2x), p25 (3x), p26 (1x)

Culture method: TeSR-E8/Vitronectin

Cells provided as: live culture; last split:
 cryovial (6x)

Karyotyping (g-banding); P:

Result:

Expression of pluripotency markers by flow cytometry:

- ✓ NANOG: 93.4%
- ✓ SSEA4: 89.9%
- ✓ OCT3/4: 90.5%

Immunofluorescent staining after trilineage *in vitro* differentiation (STEMdiff Trilineage differentiation kit, Stem Cell Technologies) showed expression of the following proteins:

- ✓ Nestin (ectoderm)
- ✓ PAX6 (ectoderm)
- ✓ FABP7 (ectoderm)
- ✓ Eomes (endoderm)
- ✓ FOXA2 (endoderm)
- ✓ GATA4 (endoderm)
- ✓ Vimentin (mesoderm)
- ✓ CDX2 (mesoderm)
- ✓ Brachyury (mesoderm)



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Information on provided isogenic subclone(s): 03 (heterozygous repair)

Clone number/passage number: iso03LUMC0116iCRB09 p23 (2x), p25 (3x), p26 (1x)

Culture method: TeSR-E8/Vitronectin

Cells provided as: live culture; last split:
 cryovial (6x)

Karyotyping (g-banding); P:

Result:

Expression of pluripotency markers by flow cytometry:

- ✓ NANOG: 91.6%
- ✓ SSEA4: 95.5%
- ✓ OCT3/4: 88.0%

Immunofluorescent staining after trilineage *in vitro* differentiation (STEMdiff Trilineage differentiation kit, Stem Cell Technologies) showed expression of the following proteins:

- ✓ Nestin (ectoderm)
- ✓ PAX6 (ectoderm)
- ✓ FABP7 (ectoderm)
- ✓ Eomes (endoderm)
- ✓ FOXA2 (endoderm)
- ✓ GATA4 (endoderm)
- ✓ Vimentin (mesoderm)
- ✓ CDX2 (mesoderm)
- ✓ Brachyury (mesoderm)



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Information on provided isogenic subclone(s): 04 (Heterozygous repair)

Clone number/passage number: iso02LUMC0116iCRB09 p26 (2x), p27 (2x)

Culture method: TeSR-E8/Vitronectin

Cells provided as: live culture; last split:
 cryovial (4x)

Karyotyping (g-banding); P:

Result:

Expression of pluripotency markers by flow cytometry:

- ✓ NANOG: 97.3%
- ✓ SSEA4: 86.9%
- ✓ OCT3/4: 99.5%

Immunofluorescent staining after trilineage *in vitro* differentiation (STEMdiff Trilineage differentiation kit, Stem Cell Technologies) showed expression of the following proteins:

- ✓ Nestin (ectoderm)
- ✓ PAX6 (ectoderm)
- ✓ FABP7 (ectoderm)
- ✓ Eomes (endoderm)
- ✓ FOXA2 (endoderm)
- ✓ GATA4 (endoderm)
- ✓ Vimentin (mesoderm)
- ✓ CDX2 (mesoderm)
- ✓ Brachyury (mesoderm)



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Contact information LUMC iPS Core Facility:

Dr. Harald Mikkers
Dept. of Cell and Chemical Biology
LUMC iPSC core facility
Leiden University Medical Center
P.O. Box 9600, Post zone S1-P
2300 RC Leiden
The Netherlands
Phone: (31)715269668
E-mail: H.Mikkers@lumc.nl

Dr. Christian Freund
Dept. of Anatomy and Embryology
LUMC iPSC core facility
Leiden University Medical Center
P.O. Box 9600, Post zone S1-P
2300 RC Leiden
The Netherlands
Phone: (31) 715269351/68897
E-mail: C.M.A.H.Freund@lumc.nl

Website: <https://www.lumc.nl/research/facilities/hips-core-facility/>