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

Receiving scientist: J. Wijnholds & T. Buck. Opthamology
Provided human iPSC clones date/name: 22-07-2020

isoXXLUMC0128iCRB01

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 acknowledgd in the first publication describing this line.

Patient/hiPSC line information:
hiPSC line name prior to gene editing: LUMC0128iCRB01
hiPSC line passage nr. prior to gene editing: 13
CRISPR/Cas9-edited gene: CRB1
Mutation before editing (DNA): c.3122T>C ✓ heterozygous □ homozygous
Mutation after editing (isogenic): c.3120C>G ✓ heterozygous □ 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

Sequence of crRNA(s) used:
CTGGGACAGTGGGTCTGTCG

Sequence of ssODN used:
ACAAGTTTGCAGTCAGTGAATGATGGCACATGGCACGAAGTGACCCTTTCGATGACAGAC
CCACTGTCCCAGACCTCCAGGTGGCAAATGGAAGTGGACA

Repair was verified by sequence analysis.

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

Information on provided isogenic subclone(s):

Clone number/passage number; iso01LUMC0128iCRB01 p18(2x) p20(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: 94.0%
- ✓ SSEA4: 99.2%
- ✓ OCT3/4: 96.5%

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

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

Clone number/passage number; iso02LUMC0128iCRB01 p18(2x), p21(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: 83.5%
- ✓ SSEA4: 85.8%
- ✓ OCT3/4: 82.3%

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

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

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