Information on provided human iPSC line

Receiving scientist: Dr. J. Wijnholds, Ophthalmology
Project description:
Generate “CRB1 mutant retina in a dish” and use these CRB1 iPS cells to generate CRB1 iPS cells with heterozygous or homozygous mutations in the CRB2 gene by CRISPR/CAS9 gene editing. The CRB1 and CRB1-CRB2 mutant iPS cells will be used for:
a) mechanistic studies to study the development of RP or LCA
b) to search for potential modifiers of the CRB1 gene
c) to study the potential for AAV gene therapy vectors containing CRB1, CRB2 and associated genes to rescue or modify the “RP or LCA retina in a dish” phenotype
d) to test CRISPR/CAS9 gene editing of the CRB1 gene in iPS cells to rescue the RP or LCA phenotype
e) to test the capacity of human rod and cone photoreceptors and Müller glial cells, obtained from iPS derived “retina in a dish”, in retinal transplantation studies
Provided human iPSC clones date/name:

LUMC0117iCRB

LUMC: institute where hiPSC were generated;
four digit code: identification number hiPSC lines LUMC hiPSC core facility;
i: induced pluripotent stem cells;
CRB: disease designator;
two digit code at the end: identification of hiPSC clone.

This nomenclature is according to international standards (Luong et al. Cell Stem Cell 2011); To guarantee correct referral of the iPSC lines in the future, please refer to this nomenclature in presentations, publications, etc.

Please acknowledge the LUMC hiPSC core facility in publications.

Patient information:

Gender:	Male
Sample date:	16-01-2017
Year of Birth:	1985
Reprogrammed cell type:	Skin fibroblasts
WK number somatic cells:	WK12854
Affected gene:	CRB1
Mutation:	CRB1 mutation heterozygous: c.2911G>T (p.(Glu971*)) and c.1892A>G (p.Tyr631Cys))

Important: reconfirm mutation upon receipt of hiPSC lines!

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)

General note:
Please check local safety regulations before starting to culture provided hiPSCs in your lab.

The provided lines □ have been karyotyped at passage / ✓ have **not** been karyotyped. We strongly recommend karyotype analysis upon initiation of the culture and afterwards on a regular basis using common karyotyping methods. Result in case of karyotyping: n/a

hiPSCs generated with SeV: Provided hiPSCs were tested by the LUMC hiPSC core facility for the absence of SeV by RT-PCR and immunofluorescent staining at the passage indicated. SeV was detectable/undetectable by these assays. Nevertheless we cannot guarantee that hiPSCs are 100% free of SeV, and receiving researchers take full responsibility for culturing the cells at appropriate safety levels. The protocol for detection of SeV is available upon request.

hiPSCs generated with episomal vectors: Reprogramming exploiting additional p53 knockdown increases the risk for aneuploidy in hiPSCs. hiPSCs users are strongly advised to perform karyotype testing prior to further analysis of the provided hiPSCs. Although in principle episomal vectors are non-integrating they can have a relatively high(partially) integration frequency according to some of the leading stem cell researchers. The LUMC hiPSC core facility has not screened the provided hiPSC clones for (partial) integration but recommends to do this.

Information on provided clone(s):

Clone number/passage number; **Clone 01** ; P5

Culture method: Vitronectin XF™ (STEMCELL Technologies),
TeSR™-E8™ (STEMCELL Technologies)

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

SeV absence tested by RT-PCR and IF in clone at indicated passage:
✓ Not applicable □ No □ Yes Results:

			Positive	Negative
Clone:	Passage at time of RT-PCR:	Result:	☐	☐
Clone:	Passage at time of IF staining:	Result:	☐	☐

Pluripotency marker expression by immunofluorescent staining:
- ✓ NANOG
- ✓ SSEA4
- ✓ OCT4

Spontaneous in vitro differentiation showed expression of
- ✓ β3-tubulin (ectoderm),
- ✓ AFP (endoderm)
- ✓ CD31 (mesoderm)

Information on provided human iPSC line

Clone number/passage number; **Clone 07** ; P5

Culture method: Vitronectin XF™ (STEMCELL Technologies),
TeSR™-E8™ (STEMCELL Technologies)

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

SeV absence tested by RT-PCR and IF in clone at indicated passage:
✓ Not applicable □ No □ Yes Results:

			Positive	Negative
Clone:	Passage at time of RT-PCR:	Result:	☐	☐
Clone:	Passage at time of IF staining:	Result:	☐	☐

Pluripotency marker expression by immunofluorescent staining:
- ✓ NANOG
- ✓ SSEA4
- ✓ OCT4

Spontaneous in vitro differentiation showed expression of
- ✓ β3-tubulin (ectoderm),
- ✓ AFP (endoderm)
- ✓ CD31 (mesoderm)

Information on provided human iPSC line

Clone number/passage number; **Clone 08**; P5

Culture method: Vitronectin XF™ (STEMCELL Technologies),
TeSR™-E8™ (STEMCELL Technologies)

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

SeV absence tested by RT-PCR and IF in clone at indicated passage:
✓ Not applicable □ No □ Yes Results:

			Positive	Negative
Clone:	Passage at time of RT-PCR:	Result:	☐	☐
Clone:	Passage at time of IF staining:	Result:	☐	☐

Pluripotency marker expression by immunofluorescent staining:
- ✓ NANOG
- ✓ SSEA4
- ✓ OCT4

Spontaneous in vitro differentiation showed expression of
- ✓ β3-tubulin (ectoderm),
- ✓ AFP (endoderm)
- ✓ CD31 (mesoderm)

Additional information/protocols provided:

iPS_SOP_0032.2, iPS_SOP_0045.2, iPS_SOP_0046.1 and iPS_SOP_0050.2

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