BIOMEDICUM
STEM CELL CENTER

IPS cell certification

Date: Sep 19, 2024

Cell Line: HEL350 clones HEL350.3 and HEL350.4

iPSC induction: Date of induction 19.02.2024. Fibroblasts were reprogramed using the CRISPRa method (PMID: 35063129)
Cell line origin: 106 cd6
Investigator: *Tapanainen Juha/Khatun Masuma*

Culture Description: The iPSC lines were reprogrammed and cultured under feeder-free conditions. They were passaged using 0.5 mM EDTA on Matrigel-coated plates and maintained in E8 medium for over 10 passages.

Characterization:
i) iPSC morphology assessment: Monitoring typical iPSC colony morphology.
ii) Culture maintenance: Assessing the stability and survival of iPSC clones over ten passages.
iii) RT-PCR: Used to confirm the removal of transgene vectors.
iv) Mycoplasma test: Used to confirm the absence of mycoplasma.

Results:
i) All iPSC lines maintained typical human pluripotent stem cell morphology during extended culture.
ii) All clones survived and retained their morphological characteristics over multiple passages.
iii) HEL350.3 tested negative for the episomal vector. HEL350.4 tested positive for the episomal vector (faint band).
iv) Mycoplasma test was negative for all lines.

Interpretation:
Based on the analysis, both HEL350 clones (HEL350.3 and HEL350.4) meet the common criteria for iPSCs.

Recommendations:
- Use a Rho-kinase inhibitor (Y-27632) 24 hours after thawing to enhance cell survival.
- Fingerprinting has not been performed; it is important to match the produced iPSC lines to their corresponding donor cell lines.
- It is advisable to analyze the karyotype of the iPSC lines before using them in any experiments.
- A faint episomal vector band was detected by PCR in line HEL350.4. This should disappear from the cells with further passaging. It is important to monitor for the presence of transgene vectors.
- Ensure compliance with the ISSCR Guidelines for Stem Cell Research and Clinical Translation (ISSCR Guidelines) for required characterizations, including morphology assessment, pluripotency verification, and genetic stability.

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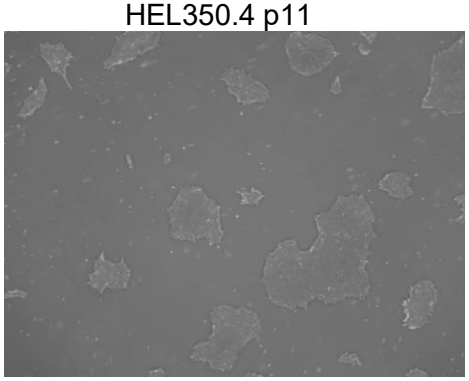
A) Morphology assessment

HEL350.4 p11



B) OriP RT-PCR