iPSC Certificate of Analysis

Product Description	JAX human iPSC line (iNDI panel) containing a genetically engineered mutation
Parental Cell Line	KOLF2.1J (JAX Product# JIPSC1000)[1]
Cell Type	Induced pluripotent stem cell
Species	Homo sapiens
Common Name	Human
Passage Number Range of Distribution LOTs	p4 through p6[2]
CRISPR-mediated Gene Variant	See product code's specific webpage for details
Gene Editing Strategy	See Product Code's specific webpage for comprehensive design strategy (Sequence Map files)
Vial Volume	0.5 ml
Recovery	See JAX standard protocol
Storage:	-196°C (liquid or vapor phase of liquid nitrogen)

Gene-edited Clone Characterization and Validation[3]

Test Description	Method	Specification	Result
Gene-edited Variant Sequence Confirmation	PCR and Sanger sequencing of wild type and variant alleles.	Sanger sequence matches predicted design strategy. Heterozygous variants have ~ equal base signal in Sanger traces.	Pass *See downloadable Sanger Sequence file in QC data section of cell line webpage.*
Karyotype	G-banding with trypsin treatment and Giemsa stain (GTG-banding) of metaphase chromosomes.	> 90% of cells are euploid and exhibit proper G-band pattern.	Pass *See downloadable Karyotype file in QC Data section of specific cell line webpage.*
Copy Number Variation	Analysis of genomic DNA on Infinium Global Diversity Arrays (GDA) with added NeuroBooster [Ref 1].[4]	Genomic integrity of iPS lines, we investigated the B-allele frequency and Log R ratio values of each iPS cell line which were downloaded from Illumina GenomeStudio. Abnormal patterns were observed by visual inspection and were plotted using R (v3.6.1) with the GWASTools package [Ref 2]. Additionally genotyped cell line was compared to the KOLF2.1J WGS data and investigated for large patterns of mismatching SNPs which imply genomic integrity issue and lead to failure to pass.	Pass *See downloadable "Array Data" file in QC Data section of specific cell line webpage.*

Distribution LOT Testing[5]			
Test Description	Method	Specification	Result
Cell Viability	Thaw and expansion using JAX Standard Protocol, which can be found on the iPSC webpage. Cell viability was assessed using Trypan Blue staining method and counted on a hemocytometer	>80% viability	**Pass**
Non-viral Agent Panel Testing	qPCRh	**NEGATIVE FOR** Mycoplasma (Genus) Segmented filamentous bacterium (SFB) Corynebacterium bovis	**Pass**
Viral (DNA) Panel Testing	qPCR	**NEGATIVE FOR** Minute virus of mice (MVM) Mouse Parvovirus (MPV) Mouse Kidney Parvovirus (MKPV) Ectromelia virus (ECTA) Murine Adenovirus (MAdV) Murine Cytomegalovirus (MCMV) Mouse Thymic Virus (MTV) Mouse Polyomavirus (MPyV)	**Pass**
Viral (RNA) Panel Testing	qPCR	**NEGATIVE FOR** Mouse Hepatitis Virus (MHV) Murine Norovirus (MNV) TMEV LDEV LCMV LCMV SV PVM Rotavirus Reovirus 3	**Pass**
Bacterial Testing (Gram-negative)	Inoculation and aerobic culture for 48 hours.	**NEGATIVE FOR (species level)** Citrobacter Escherichia Enterobacter Klebsiella Proteus Salmonella Serratia Shigella Acinetobacter Pseudomonas Pasteurella Stenotrophomonas Campylobacter Flavobacterium Streptobacillus	**Pass**
Bacterial Testing (Gram-positive)	Inoculation and aerobic culture for 48 hours.	**NEGATIVE FOR (species level)** Enterococcus Streptococcus Staphylococcus Listeria Corynebacterium	**Pass**
Gene-edited Variant Sequence Confirmation	PCR and Sanger sequencing of wild type and variant alleles from distribution LOTs.	Sequence trace profile matches the sequence profile of the original, gene-edited clone.	**Pass**

Footnotes

1 Please view the pre-publication containing the details of the KOLF2.1J cell line's development

2 Passage number of distribution LOT definition: p1 is defined as the passage number of the cells when the clone was initially characterized/validated by Sequencing, Karyotyping and genome-wide copy number array.

3 Characterization/validation tests performed on the clones arising from the gene editing design specified n the Sequence Map files and the cell line's web product page details. Only clones that pass these processes were expanded in culture for the production of distribution LOT vials.

4 References:

1. Pantazis C, Yang A, Lara E, McDonough JA, Blauwendraat C, Peng L, et al. A reference induced pluripotent stem cell line for large-scale collaborative studies. https://www.biorxiv.org/content/10.1101/2021.12.15.472643v4
2. Gogarten SM, Bhangale T, Conomos MP, Laurie CA, McHugh CP, Painter I, Zheng X, Crosslin DR, Levine D, Lumley T, et al. (2012). GWASTools: an R/Bioconductor package for quality control and analysis of genome-wide association studies. Bioinformatics 28, 3329–3331.

5 Expansion of validated gene-edited clones are frozen in large scale LOTs. Each LOT has one random vial thawed for viability, growth, pathogen testing and genome variant sequence confirmation.

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