iPSC Line Certificate of Analysis

The Jackson Laboratory

Product Description	JAX human iPSC line (parental iPSC line for generation of iNDI panel)
Parental Cell Line	KOLF2-C1 (Genome Research Limited)[1]
Cell Type	Induced pluripotent stem cell
Species	Homo sapiens
Common Name	Human
Passage Number of Distribution LOTs	P3[2]

Vial Volume	0.5 ml
Cells per Vial	0.5 x 10^6 cells
Recovery	See JAX standard protocol
Storage:	-196°C (liquid or vapor phase of liquid nitrogen)

Characterization and Validation of Clone used for generating Distribution LOTs[3]

Test Description	Method	Specification	Result
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 KOLF2.1J.*
Copy Number Variation	Analysis of genomic DNA on Illumina Infinium Global Diversity Arrays (GDA) either with added NeuroBooster (NBA) [Ref1][4] or with Cytogenetics-8 (GDA-Cyto).	**NBA:** B-allele frequencies and Log R ratio values were downloaded from Illumina GenomeStudio. Abnormal patterns were observed by visual inspection and plotted using R (v3.6.1) with the GWASTools package [Ref 2][4]. Genotyping data was compared to KOLF2.1J WGS data to identify large patterns of mismatching SNPs. **GDA-Cyto:** VIA™ (Bionano Genomics) software was used to process .gtc files, call CNVs, and visualize B-allele frequency and Log R ratio data. CNVs identified were cross-referenced with known variations in the parental KOLF2.1J line [Ref 3, 4][4]. Edited clone array data was compared to KOLF2.1J array data to identify novel variations that were not intended. Particular attention is paid to potential on-target anomalies.	Pass *See downloadable "Array Data" file in QC Data section of KOLF2.1J.*

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**
Human bloodborne pathogens	h-IMPACT Panel (IDEXX), PCR	**NEGATIVE FOR** Hepatitis B Hepatitis C HIV1 HIV2	 HTLV 1 HTLV 2 Treponema pallidum	**Pass**
Non-viral Agent Panel Testing	qPCR	**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	 SV PVM Rotavirus Reovirus 3	**Pass**
Yeast and Fungal Testing	Inoculation and aerobic culture for 10 days at 25°C.	**NEGATIVE FOR growth**		**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**

micetech@jax.org
1-800-422-6423 (USA, Canada, Puerto Rico)
1-207-288-5845 (International)

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Footnotes

1. Please view the publication containing the details of the KOLF2.1J iPS 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 in 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. Cell Stem Cell. 2022 Dec 1;29(12):1685-1702.e22. doi: 10.1016/j.stem.2022.11.004. PMID: 36459969.
    2. Gogarten SM, Bhangale T, Conomos MP, Laurie CA, McHugh CP, Painter I, Zheng X, Crosslin DR, Levine D, Lumley T, et al. GWASTools: an R/ Bioconductor package for quality control and analysis of genome-wide association studies. Bioinformatics. 2012, Dec 15;28(24), 3329-3331. doi: 10.1093/bioinformatics/bts610. PMID: 23052040
    3. Gracia-Diaz C, Perdomo JE, Khan ME, Roule T, Disanza BL, Cajka GG, Lei S, Gagne AL, Maguire JA, Shalem O, Bhoj EJ, Ahrens-Nicklas RC, French DL, Goldberg EM, Wang K, Glessner JT, Akizu N. KOLF2.1J iPSCs carry CNVs associated with neurodevelopmental disorders. Cell Stem Cell. 2024 Mar 7;31(3):288-289. doi: 10.1016/j.stem.2024.02.007. PMID: 38458176.
    4. Ryan M, McDonough JA, Ward ME, Cookson MR, Skarnes WC, Merkle FT. Large structural variants in KOLF2.1J are unlikely to compromise neurological disease modeling. Cell Stem Cell. 2024 Mar 7;31(3):290-291. doi: 10.1016/j.stem.2024.02.006. PMID: 38458177.
5. Validated clone is expanded and 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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