Stem Cell Production Facility
iPS Cell Line Derivation Report Form
Document No: VCCRI_SCPF_JD1
Effective Date: 26 Nov 2025
Client Name: Jonathan Du/Thomas Grewal
Institute: USyd

Certificate of Analysis (CoA)

Cell Line Name	GM18453 (453)
hPSCreg ID and QR code	VCCRIi043-A
Clones Provided	3.1; 6.1; 7.2
Passage Number	P10

This cell line was tested for the following specifications:

Test Description	Test Specification	Result
Mycoplasma	No contamination detected	Pass
Karyotyping	No aneuploidies detected	Pass
Pluripotency		
Immunocytochemistry (FC)	OCT3/4, TRA-1-60, TRA-1-81, SSEA4	Pass
Differentiation		
Tri-lineage differeniation	Successful differentiation to endoderm, mesoderm, and ectoderm	Pass
Morphology	Normal cell morphology observed	Pass

Reprogramming Information:

iPSC Line Name: 453
Vial ID(s): 453 hiPSC (clone name)
Starting Cell Type: ☑ Fibroblast ☐ PBMC ☐ Other
Reprogramming Factors: ☑ OCT3/4 ☑ SOX2 ☑ KLF4 ☑ c-Myc
Reprogramming Method: ☑ Sendai Virus (CytoTune™-iPS 2.0 Sendai Reprogramming Kit) ☐ Episomal

Culturing information:

Storage: LN2 Vapour Phase

Thawing of frozen vials:

- Upon receipt of the frozen vials, it is recommended to thaw the cells and initiate the culture immediately to retain the highest cell viability.
- Before recovering cells, prepare coated Matrigel®plates, following manufacturer's instructions.
- To thaw the cells, put the vial in a 37°C water bath with gentle agitation for ~1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
- Pipette the cells into a 15 mL conical tube using a 5 mL Stripette, add 5 mL warm mTeSR™ Plus media dropwise. This culture is performed in a feeder-free system.
- Centrifuge at 300 x *g* for 5 minutes at room temperature.
- Remove the supernatant and re-suspend the cells gently (using a 5 mL stripette) in culture medium supplemented with 10 µM Y27632. Resuspend very gently.
- Seed the cells on a warm culture dish, pre-coated with Matrigel®.
- Recommended thawing in 2 wells of a 6-well plate/vial.
- Incubate in a 37°C, 5% CO_2 incubator overnight.
- The next day, change media without Y27632. Daily media changes are performed until 70% confluent.

Passaging:

- On the day of passage, gently wash the wells with DPBS once.
- Add 1 mL of ReleSR™ – swirl around to ensure even coverage of the well. Discard ReleSR™ after 30-45 seconds. Incubate plate in 37°C, 5% CO_2 incubator for 4 minutes.
- Add 2 mL warm mTeSR™ Plus after 4 minutes, gently tap the sides of the plate to dissociate the cells.
- Collect dissociated clumps of cells in a 15 mL tube, using a 5 mL stripette.
- Passage the cells at the recommended ratio, in fresh Matrigel® coated plates with warm media.
- For more details and information, please refer to the Maintenance of hPSCs in mTeSR Handbook

Recommended passaging ratio:	1:20
Passaging frequency (Based on recommended ratio):	5-6 days
Rate of differentiation:	☐ *High (>50%)* ☐ *Moderate (20-40%)* ☑ *Low (<10%)*
Freezing Medium:	Cryostore CS10

Characterisation of provided clones:

Molecular Karyotyping

Performed by:	Victorian Clinical Genetics Services, Parkville, VIC
At passage number:	P8
Analysis and Result:	arr(X,Y)x1,(1-22)x2
Inference:	NO ANEUPLOIDIES DETECTED
Comments:	All three clones karyotypically normal

Immunocytochemistry:

Pluripotency Markers:

SSEA-4	TRA-1-60	TRA-1-81	OCT3/4
☑	☑	☑	☑

At passage number:	P10
Percentage of positive population:	>90% positive population recorded.
Comments:	

Characterisation of differentiation potential:

All clones provided for this cell line were assessed for their differentiation potential towards three germ layers using STEMdiff™ Trilineage Differentiation Kit

Differentiation potential result: ☑ Endoderm (SOX17) ☑ Mesoderm (T/BRY) ☑ Ectoderm (PAX6)

At passage number	P10
Comments:	All clones differentiated to three lineages successfully.

Additional Information:

Disclaimer: *VCCRI Innovation Centre - Stem Cell Production facility (SCPF) will not be responsible for degradation, spontaneous differentiation, contamination, and/or cell death in the case of any changes made to the reagents and methods by the client. SCPF will store up to three backup vials for each supplied clone and any additional reprogrammed material, for up to six months. This material will be destroyed after six months, or shipped to the client at their own expense. Clients should contact SCPF staff for any questions regarding the product.*

All iPSCs to be used for research purposes only.

Pallavi Srivastava

Digitally signed by Pallavi Srivastava
Date: 2025.11.27 10:13:10 +11'00'

Research Scientist
Victor Chang Cardiac Research Institute
Innovation Centre

Contact Information

Stem Cell Production Facility
Innovation Centre, VCCRI

Pallavi Srivastava, MSc, PhD (Research Scientist)
Michael Bullen, BSc, PhD (Research Scientist)

Email: ***SCPFenquiries@victorchang.edu.au***

https://www.victorchang.edu.au/innovation-centre

1.1 Immunocytochemistry: FACS analysis

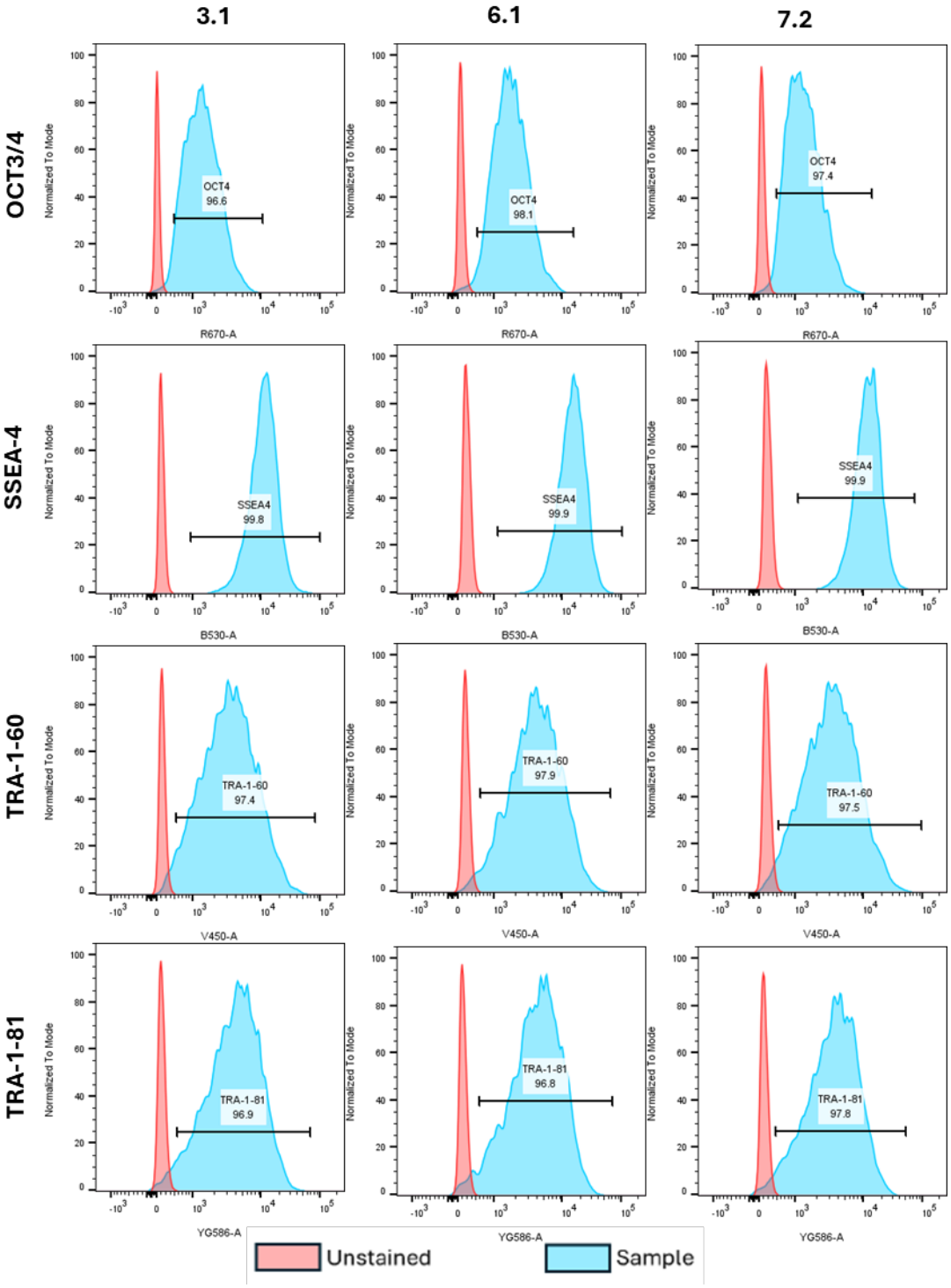

Figure 1: FACS plots for pluripotency markers tested for three clones of hiPSCs from sample number 453(GM18453). All clones show >90% of cells positive for the selected markers. Range based on reference material. (1, 2)

1.2 Cell Morphology: Brightfield images

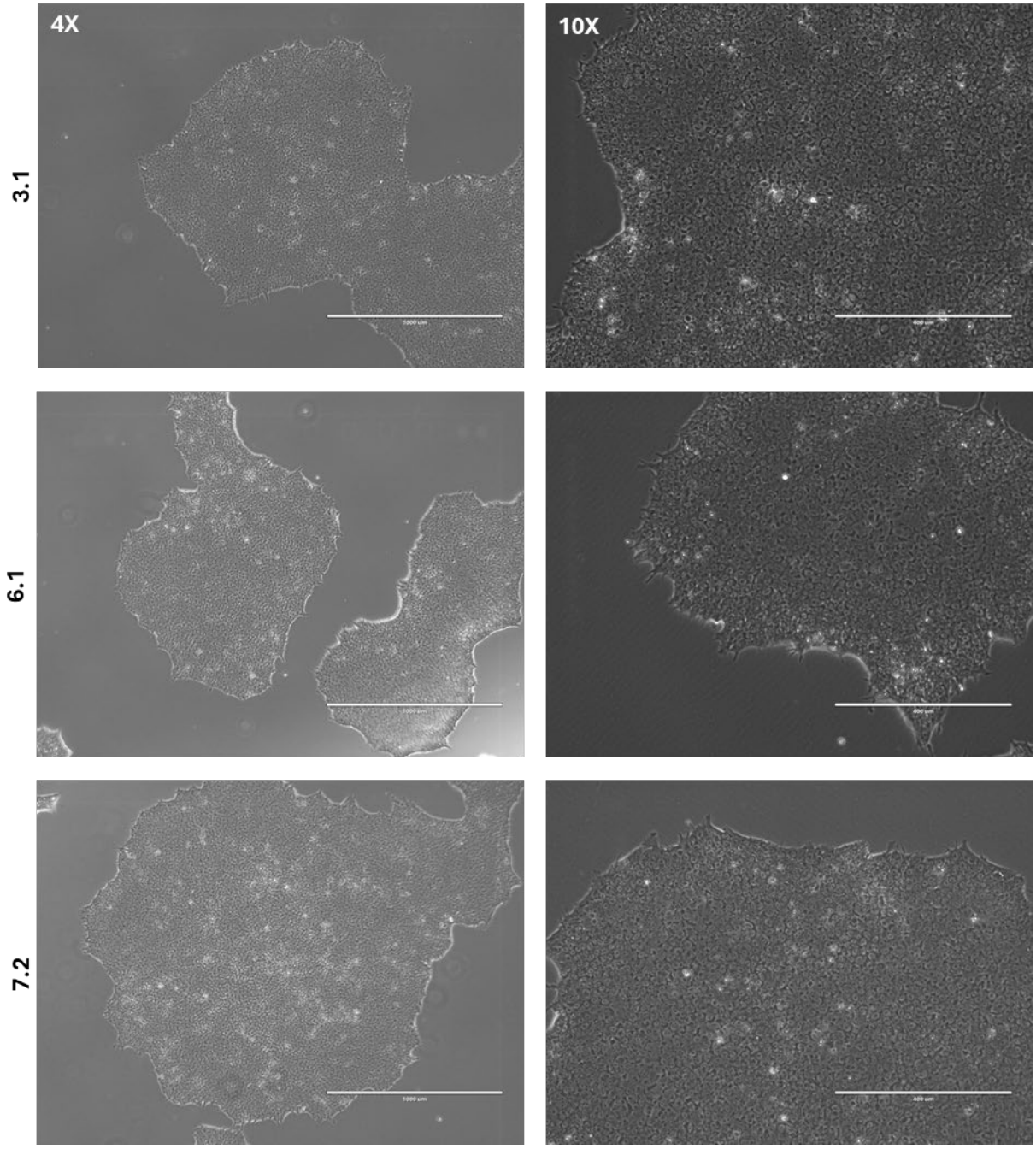

Figure 2: Brightfield images for three clones of hiPSCs from sample number 453(GM18453). Images taken at D4 after passaging at 4X and 10X.

1.3 Immunostaining: Tri- lineage differentiation

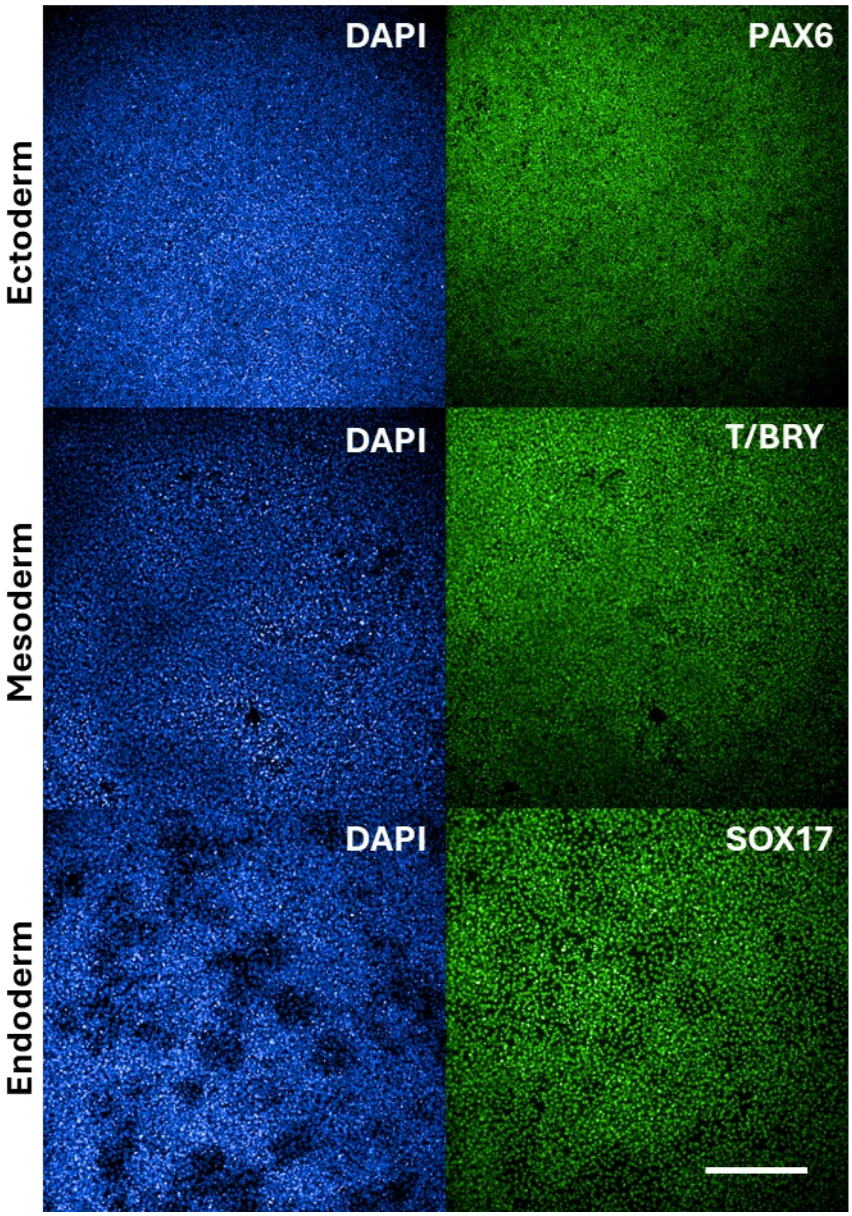

Figure 3: Representative fluorescent images from one clone (3.1) of hiPSCs from sample number 453(GM18453). Staining performed after 7 days of directed differentiation. Scale bar – 100 µm.

1. O'Shea O, Steeg R, Chapman C, Mackintosh P, Stacey GN. Development and implementation of large-scale quality control for the European bank for induced Pluripotent Stem Cells. Stem Cell Research. 2020;45:101773.
2. Baghbaderani BA, Syama A, Sivapatham R, Pei Y, Mukherjee O, Fellner T, et al. Detailed Characterization of Human Induced Pluripotent Stem Cells Manufactured for Therapeutic Applications. Stem Cell Reviews and Reports. 2016;12(4):394-420.

Victorian Clinical Genetics Services
Murdoch Children's Research Institute
Flemington Road, Parkville, VIC 3052 Australia
P +61 1300 118247 F +61 3 8341 6366
W vcgs.org.au ABN 51 007 032 760

LIMS Report #: 1356270

Patient: 453 3.1 453 3.1

Dr. Pallavi Srivastava
Victor Chang Cardiac Research Institute
405 Liverpool Street
DARLINGHURST NSW 2010

Phone: 0292958600

DOB: Unknown
Sex: Unknown
VCGS sample ID: 25C113410
Date requested: 09-Oct-2025
Date collected: 09-Oct-2025
Date received: 13-Oct-2025
Date reported: 03-Nov-2025
Source: DNA - unspecified
Ext. sample ID:
Ext. patient ID:

Cytogenetics Laboratory

Clinical details	Cell line
Specimen source	DNA - unspecified

Molecular karyotype

Array type	Illumina Infinium GSA-24 v3.0
Resolution	0.50Mb
Reference genome	GRCh38 / hg38 (Dec 2013)
Molecular karyotype	arr (X,Y)x1,(1-22)x2
Result	NO ANEUPLOIDIES DETECTED

Interpretation

Male molecular karyotype. No aneuploidies were detected in this sample.

Molecular karyotyping is limited in its ability to detect low grade mosaicism and genomic copy number changes below the resolution stated. Balanced rearrangements and Robertsonian translocations will not be detected. This test does not exclude single gene disorders caused by sequence mutations or trinucleotide repeat expansions (such as fragile X syndrome, Huntington disease, some spinocerebellar ataxias, Friedreich ataxia and myotonic dystrophy). Testing for fragile X syndrome should be considered in individuals with developmental delay/ intellectual disability. Further genomic based testing may be considered if there remains a high suspicion of a monogenic disorder. Copy number variants that do not contain genes, are well established polymorphisms, or are assessed as being of unlikely clinical significance (based on "ACMG Technical Standards for the Interpretation and Reporting of Constitutional Copy Number Variants"), will not be reported. The classification is based on the current scientific evidence available at the time of reporting. Reporting of regions of homozygosity (>5Mb) is dependent on referral setting and clinical indication. CNVs that contain autosomal recessive genes will not be reported unless there is specific clinical relevance, high carrier population frequency or a history of consanguinity. Please contact the laboratory if there is a family history of a known recessive disorder or a clinically suspected recessive condition. This testing was performed on a standard SNP microarray platform which may not have sufficient probe coverage to detect clinically relevant CNVs related to this patient's specific clinical features. Please contact the laboratory if a higher resolution microarray may be required for a specific gene or genetic condition. This test does not exclude the possibility of tissue limited mosaicism and further testing of an alternative tissue may be considered if clinically indicated. Interpretation is based on the UCSC GRCh38/hg38 human reference sequence.

Validated: 03-Nov-2025 by David Francis

Enquiries: +61 1300 11 8247

END OF TEST REPORT

FINAL REPORT

PATHOLOGY REPORT

Victorian Clinical Genetics Services
Murdoch Children's Research Institute
Flemington Road, Parkville, VIC 3052 Australia
P +61 1300 118247 F +61 3 8341 6366
W vcgs.org.au ABN 51 007 032 760

LIMS Report #: 1356273

Patient: 453 6.1 453 6.1

Dr. Pallavi Srivastava
Victor Chang Cardiac Research Institute
405 Liverpool Street
DARLINGHURST NSW 2010

Phone: 0292958600

DOB: Unknown
Sex: Unknown
VCGS sample ID: 25C113411
Date requested: 09-Oct-2025
Date collected: 09-Oct-2025
Date received: 13-Oct-2025
Date reported: 03-Nov-2025
Source: DNA - unspecified
Ext. sample ID:
Ext. patient ID:

Cytogenetics Laboratory

Clinical details	Cell line
Specimen source	DNA - unspecified

Molecular karyotype

Array type	Illumina Infinium GSA-24 v3.0
Resolution	0.50Mb
Reference genome	GRCh38 / hg38 (Dec 2013)
Molecular karyotype	arr (X,Y)x1,(1-22)x2
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Validated: 03-Nov-2025 by David Francis

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END OF TEST REPORT

FINAL REPORT

PATHOLOGY REPORT

Victorian Clinical Genetics Services
Murdoch Children's Research Institute
Flemington Road, Parkville, VIC 3052 Australia
P +61 1300 118247 F +61 3 8341 6366
W vcgs.org.au ABN 51 007 032 760

LIMS Report #: 1356274

Patient: 453 7.2 453 7.2

Dr. Pallavi Srivastava
Victor Chang Cardiac Research Institute
405 Liverpool Street
DARLINGHURST NSW 2010

Phone: 0292958600

DOB: Unknown
Sex: Unknown
VCGS sample ID: 25C113412
Date requested: 09-Oct-2025
Date collected: 09-Oct-2025
Date received: 13-Oct-2025
Date reported: 03-Nov-2025
Source: DNA - unspecified
Ext. sample ID:
Ext. patient ID:

Cytogenetics Laboratory

Clinical details	Cell line
Specimen source	DNA - unspecified

Molecular karyotype

Array type	Illumina Infinium GSA-24 v3.0
Resolution	0.50Mb
Reference genome	GRCh38 / hg38 (Dec 2013)
Molecular karyotype	arr (X,Y)x1,(1-22)x2
Result	NO ANEUPLOIDIES DETECTED

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Validated: 03-Nov-2025 by David Francis

Enquiries: +61 1300 11 8247

END OF TEST REPORT

FINAL REPORT

PATHOLOGY REPORT

Garvan Molecular Genetics Services
Mycoplasma Test Results

Sender

Name	Pallavi Srivastava
Address	Victorchang Institute 405 Liverpool Street Darlinghurst 2010
email	p.srivastava@victorchang.edu.au

Cell Line Sample

Sample Name:	453 3.1
Garvan Sample ID:	GKC377
Sample Material:	Cells
Reception Date:	30.10.2025

Mycoplasma Testing Performed

The results included here relate only to the sample tested and the results apply to the sample as received. If the sample acceptance criteria of the sample submission guidelines have not been met but a continuation of the testing procedure was consented, the result may be compromised. This test report shall not be reproduced except in full, without full written approval of the testing laboratory.

All samples have been tested as received by the methods of CLIS_1, CLIS_2, CLIS_3
For this test the extracted DNA was amplified in a multiplex PCR with primers specific for Mycoplama 23S rRNA and 16S rRNA. These primers detect the following species: Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma hominis, Mycoplasma argininis, Mycoplasma salivarium, Mycoplasma pirum, Mycoplasma fermentans, Acholeplasma laidlawii, Mycoplasma pneumoniae, Mycoplasma gallisepticum, Mycoplasma genitalium, Mycoplasma penetrans, Mycoplasma synoviae Mycoplasma bovis, Mycoplasma hypopneumoniae, Ureaplasma urealyticum & Spiroplasma citri. The PCR was run with positive, negative, contamination check & efficiency check controls.

PCR Results

PCR 1	Negative
PCR 2	Negative
PCR 3	Negative
PCR 4	Negative

Final Result

Test result for Mycoplasma	Negative

Authorisation

Reviewed by:	Seena Bhalla
Reviewed Date:	31.10.2025
Authorised by:	Pavel Bitter
Authorised Date:	03.11.2025

Kind Regards
Pavel Bitter
(Laboratory Manager)

Garvan Institute of Medical Research
384 Victoria Street
Darlinghurst, 2010, NSW
http://www.garvan.org.au/research/capabilities/molecular-genetics

Ph: 02 92958384
Email: gmg@garvan.org.au

Garvan Molecular Genetics Services
Mycoplasma Test Results

Sender

Name	Pallavi Srivastava
Address	Victorchang Institute 405 Liverpool Street Darlinghurst 2010
email	p.srivastava@victorchang.edu.au

Cell Line Sample

Sample Name:	453 6.1
Garvan Sample ID:	GKC378
Sample Material:	Cells
Reception Date:	30.10.2025

Mycoplasma Testing Performed

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PCR Results

PCR 1	Negative
PCR 2	Negative
PCR 3	Negative
PCR 4	Negative

Final Result

Test result for Mycoplasma	Negative

Authorisation

Reviewed by:	Seena Bhalla
Reviewed Date:	31.10.2025
Authorised by:	Pavel Bitter
Authorised Date:	03.11.2025

Kind Regards
Pavel Bitter
(Laboratory Manager)

Garvan Institute of Medical Research
384 Victoria Street
Darlinghurst, 2010, NSW
http://www.garvan.org.au/research/capabilities/molecular-genetics

Ph: 02 92958384
Email: gmg@garvan.org.au

Garvan Molecular Genetics Services
Mycoplasma Test Results

Sender

Name	Pallavi Srivastava
Address	Victorchang Institute 405 Liverpool Street Darlinghurst 2010
email	p.srivastava@victorchang.edu.au

Cell Line Sample

Sample Name:	453 7.2
Garvan Sample ID:	GKC379
Sample Material:	Cells
Reception Date:	30.10.2025

Mycoplasma Testing Performed

The results included here relate only to the sample tested and the results apply to the sample as received. If the sample acceptance criteria of the sample submission guidelines have not been met but a continuation of the testing procedure was consented, the result may be compromised. This test report shall not be reproduced except in full, without full written approval of the testing laboratory.

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PCR Results

PCR 1	Negative
PCR 2	Negative
PCR 3	Negative
PCR 4	Negative

Final Result

Test result for Mycoplasma	Negative

Authorisation

Reviewed by:	Seena Bhalla
Reviewed Date:	31.10.2025
Authorised by:	Pavel Bitter
Authorised Date:	03.11.2025

Kind Regards
Pavel Bitter
(Laboratory Manager)

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