Cell Line Authentication Record

Short Tandem Repeat Profiling, Mycoplasma Screening & Provenance Verification

Overall Determination

AUTHENTICATED

100% STR concordance · mycoplasma negative

Record No.: CLA-2026-0417
Cell Line: VXO-114 (Vertex-114)
Species / Tissue: *Homo sapiens*, mammary gland, adenocarcinoma
Submitted By: Dr. Priya Anand, Vertex Oncology Research Institute
Testing Facility: Nimbus Genomics Core Facility, Authentication Services
Report Date: 14 March 2026

This record documents the identity verification of cell line VXO-114, maintained under Nimbus Genomics Core Facility SOP NGC-QA-014 (*Cell Line Authentication and Contamination Screening*). Testing was performed on a cryopreserved working bank vial thawed and expanded to passage 5 for analysis, in accordance with the ANSI/ATCC ASN-0002-2011 standard for human cell line authentication by short tandem repeat (STR) profiling [1]. Results, provenance documentation, and passage history are summarised in the sections below.

1 Sample Identification

Cell Line Details

Designation: VXO-114
Common Name: Vertex-114
Origin: Human, female, age 54
Tissue: Mammary gland, invasive ductal carcinoma
Morphology: Epithelial, adherent
Biosafety Level: BSL-2

Sample Under Test

Vial ID: VXO114-WB-0037
Bank Tier: Working cell bank
Passage at Freeze: P4
Passage at Testing: P5
Cryovial Count Remaining: 22 of 24
Storage: Vapour-phase LN_2, Tank 3, Rack B, Box 7

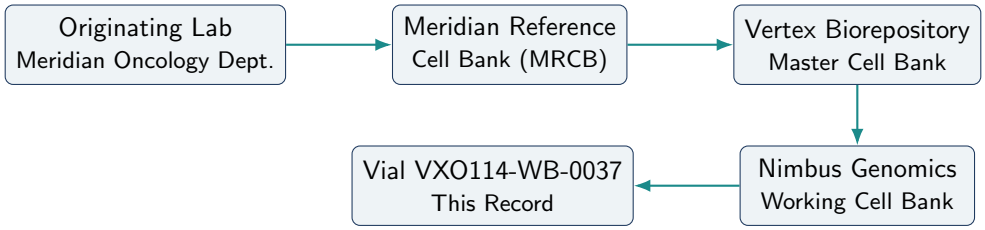
1.1 Culture Conditions at Time of Sampling

Cells were harvested at 80–85% confluence from a T-75 flask, and a 2×10^6-cell pellet was submitted for genomic DNA extraction. A duplicate flask was retained under quarantine culture pending release of this report, per SOP NGC-QA-014 §4.2.

2 Provenance Chain

The diagram below traces custody of VXO-114 from its originating deposit through to the working-bank vial analysed in this record.

Parameter	Value
Growth medium	DMEM/F-12 (1:1), 10% fetal bovine serum, 1% penicillin–streptomycin
Incubation	37 °C, 5% CO_2, humidified atmosphere
Passage method	TrypLE dissociation, 0.25% for 4 min, split ratio 1:4
Doubling time (P5)	27.6 h (mean of 3 confluency intervals)
Mycoplasma status at collection	Negative (see Section 4)
Sampling technician	R. Osei, Nimbus Genomics Core Facility



2.1 Chain-of-Custody Log

Date	From → To	Purpose	Init.
2021-06-02	Meridian Oncology Dept. → MRCB	Original deposit, seed stock (P0)	J.K.
2021-09-14	MRCB → Vertex Biorepository	Licensed transfer, MTA VX-2021-118	S.T.
2021-09-30	Vertex Biorepository → Master Cell Bank	Expansion to master bank (P1–P3)	S.T.
2025-11-04	Master Cell Bank → Nimbus Genomics	Shipped on dry ice, receipt confirmed intact	R.O.
2025-11-05	Nimbus Genomics → Working Cell Bank	Expansion and cryopreservation (P3–P4)	R.O.
2026-03-02	Working Cell Bank → Authentication Lab	Vial WB-0037 thawed for this record	A.M.

No deviations from the documented chain were identified. The material transfer agreement (MTA VX-2021-118) and original deposit certificate are held on file at Vertex Biorepository and were cross-referenced against the vial label and internal barcode (`VXO114-WB-0037`) prior to testing.

3 Short Tandem Repeat (STR) Profiling

Genomic DNA was extracted from the submitted vial (QIAamp DNA Mini Kit) and amplified using a nine-locus multiplex covering the eight ANSI/ATCC core loci plus Amelogenin for sex determination [1]. Fragment sizing was performed by capillary electrophoresis (3500 Genetic Analyzer, 0.2 repeat-unit resolution) and alleles were called against an internal allelic ladder. The resulting profile was compared against the reference profile held by Meridian Reference Cell Bank (MRCB) for VXO-114, deposited at the time of original banking.

Locus	Sample A1	Sample A2	MRCB Ref. A1	MRCB Ref. A2	Match
Amelogenin	X	X	X	X	✓
CSF1PO	10	12	10	12	✓
D13S317	8	12	8	12	✓
D16S539	11	12	11	12	✓
D5S818	11	12	11	12	✓
D7S820	8	11	8	11	✓
TH01	7	9.3	7	9.3	✓
TPOX	8	11	8	11	✓
vWA	16	18	16	18	✓

3.1 Locus-by-Locus Comparison

Concordance: 9/9 loci (100%) **Interpretation:** Authentic match to reference profile (threshold for authentic identity: $\geq 80\%$ concordance, per ANSI/ATCC ASN-0002-2011 §6.3 [1, 2])

3.2 Cross-Contamination Screen

The profile was additionally screened against Nimbus Genomics' internal database of 214 actively cultured lines to rule out inter-culture cross-contamination. No matching or partially matching profile (>80% concordance) was identified outside of VXO-114's own banked records, consistent with the findings of large-scale misidentification surveys [3].

No off-ladder alleles, stutter artefacts requiring re-call, or evidence of mixed-cell population (tri-allelic patterns) were observed at any locus.

4 Mycoplasma Contamination Screening

Spent culture medium (collected 24–48 h post-feed, prior to antibiotic exposure) was tested by quantitative PCR using the Nimbus MycoScreen qPCR Kit, which targets the conserved 16S rRNA region shared across the *Mycoplasma*, *Acholeplasma*, and *Spiroplasma* genera most commonly implicated in cell culture contamination [4].

Sample	Ct (16S target)	Ct (IAC)	Result
VXO-114 supernatant, P5	Undetermined (>40)	24.1	**Negative**
Positive control (*M. orale* standard)	18.3	24.4	Positive (expected)
Negative control (nuclease-free water)	Undetermined (>40)	23.9	Negative (expected)
No-template control	Undetermined (>40)	Undetermined	Valid (no amplification)

IAC = internal amplification control, confirming absence of PCR inhibition.

Result: NEGATIVE for mycoplasma contamination
Assay run 2026-03-04, operator A. Mensah, instrument QuantStudio-5 #QS5-02

Assay validity criteria were met: the positive control amplified within the expected Ct window (16–21), both negative controls remained undetermined, and the internal amplification control confirmed the absence of PCR-inhibiting substances in the test sample. Per SOP NGC-QA-014, mycoplasma screening is repeated at every fifth passage and immediately prior to any cryopreservation event.

5 Passage Number Tracking

Passage history is recorded from the point VXO-114 entered Nimbus Genomics custody (P3, November 2025). Earlier passages (P0–P3) were established and expanded externally by the originating laboratory and Vertex Biorepository; those records are retained on file per the provenance log in Section 2 but are not independently re-verified here.

Passage	Date	Viability	Doubling (h)	Split	Notes	Init.
P0–P3	2021-06 to 2021-09	n/a	n/a	n/a	Established at Meridian Oncology Dept.; expanded to master bank at Vertex Biorepository. External records on file, not re-verified.	—
P3	2025-11-05	91%	29.2	1:3	Received ex Vertex Biorepository, thawed under quarantine culture.	R.O.
P3	2025-11-09	95%	27.8	1:4	First passage post-thaw; normal adherent epithelial monolayer.	R.O.
P4	2025-11-14	96%	27.1	1:4	Confluent monolayer, no morphological drift noted.	R.O.
P4	2025-11-19	97%	26.9	—	Cryopreserved: 24 vials, 1×10^6 cells/vial, Tank 3 Rack B Box 7.	R.O.
P4→P5	2026-03-02	89%	30.4	1:3	Vial WB-0037 thawed for this authentication record.	A.M.
P5	2026-03-06	94%	27.4	1:4	Expansion split; morphology consistent with historical reference images.	A.M.
P5	2026-03-09	95%	27.6	—	Harvested for STR extraction and mycoplasma sampling (this record).	A.M.

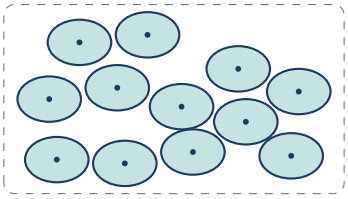
Passage Limit Policy

Per SOP NGC-QA-014 §5.1, VXO-114 is approved for experimental use through **passage 20** from the working-bank freeze point (P4), beyond which senescence-associated doubling time drift (>15% increase from baseline) requires re-authentication before further use. Doubling time at P5 (27.4–27.6 h) remains within 3% of the P4 baseline.

6 Morphological and Cytogenetic Notes

6.1 Phase-Contrast Morphology

Brightfield imaging at P5 (40×, phase contrast) showed a uniform adherent epithelial monolayer with cobblestone packing, consistent with reference images captured at banking (P1, Vertex Biorepository archive). No spindle-shaped outgrowths, vacuolation, or granular debris suggestive of a differing cell population were observed.



Schematic: representative monolayer field, P5

6.2 Modal Karyotype

A rapid metaphase spread count ($n = 20$ spreads, Giemsa banding) was performed as a supplementary check against gross chromosomal contamination or cross-species mix-up.

Metric	Observation
Modal chromosome number	58 (hypertriploid range)
Spreads within ± 2 of mode	17 / 20 (85%)
Marker chromosomes noted	2 stable markers, consistent with archived P1 karyotype
Species confirmation	Human (no non-human centromeric banding pattern detected)

The modal count and marker chromosomes are consistent with the karyotype recorded at original banking, providing corroborating (non-definitive) support for the STR-based identity determination in Section 3.

7 Conclusion and Certification

Based on the STR profiling, mycoplasma screening, and provenance review documented in this record, cell line VXO-114 (vial VXO114-WB-0037, passage 5) is determined to be **authentic** and **free of mycoplasma contamination** as of the testing date.

- ✔ STR profile: 9/9 core loci concordant with MRCB reference (100%)
- ✔ No cross-contamination detected against 214-line internal database
- ✔ Mycoplasma qPCR: negative, all assay controls valid
- ✔ Provenance chain: complete and unbroken, MTA on file
- ✔ Karyotype and morphology: consistent with archived reference

Next scheduled re-authentication: at or before passage 20, or sooner if morphology, doubling time, or mycoplasma surveillance flags a deviation, per SOP NGC-QA-014 §5.1.

7.1 Approval

Aditi Mensah
Senior Scientist, Authentication Services
Nimbus Genomics Core Facility
Date: 14 March 2026

Dr. Owen Kavanagh
Quality Manager
Nimbus Genomics Core Facility
Date: 14 March 2026

This record is retained for the life of the cell bank plus 10 years, per Nimbus Genomics document retention policy NGC-QMS-002. Distribution: submitter, quality file, working-bank archive.

References

[1] Yvonne A. Reid, Douglas R. Storts, Terry Riss, and Lisa Minor. Authentication of human cell lines by STR DNA profiling analysis. *In Vitro Cellular & Developmental Biology – Animal*, 49(10):780–782, 2013. Summarizes ANSI/ATCC ASN-0002-2011 standard for human cell line authentication.

[2] Amanda Capes-Davis, Yvonne A. Reid, Margaret C. Kline, Douglas R. Storts, Eugene Strauss, Wilhelm G. Dirks, Hans G. Drexler, R. A. F. MacLeod, Glyn Sykes, Arihiro Kohara, Yukio Nakamura, Eugene Elmore, Raymond W. Nims, Christine Alston-Roberts, Ray Barallon, Georg V. Los, Roland M. Nardone, Paul J. Price, Amanda Steuer, Jane Thomson, and John R. Masters. Match criteria for human cell line authentication: Where do we draw the line? *International Journal of Cancer*, 132(11):2510–2519, 2013.

[3] Min Yu, Sridar K. Selvaraj, Marie M. Y. Liang-Chu, Shirin Aghajani, Meghan Busse, Jing Yuan, Gary Lee, Frank Peale, Christiaan Klijn, Richard Bourgon, Joshua S. Kaminker, and Richard M. Neve. A resource for cell line authentication, annotation and quality control. *Nature*, 520(7547):307–311, 2015.

[4] Cord C. Uphoff and Hans G. Drexler. Detecting mycoplasma contamination in cell cultures by polymerase chain reaction. In Ian A. Cree, editor, *Cancer Cell Culture: Methods and Protocols*, volume 731 of *Methods in Molecular Biology*, pages 93–103. Humana Press, 2011.