

Authenticating Samples X

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1. Overview

1.1. Specimen Description

Species: patient-derived xenograft (PDX) tumor sample dissected from an immunodeficient mouse. Number of Specimens: 1

1.2. Services Performed

Service: Cell Line and Model Authentication - Comprehensive

Service items:

- Sample identification by matching to standard PDX and cell line databases
- Human-mouse interspecies contamination quantification
- Sample genetic heterogeneity quantification

- Sample contamination check and contaminant identification (if the contaminant is already in the standard PDX and cell line databases)
- Mouse syngeneic model identification (if applicable)
- Mouse strain (if applicable)
- Viral infection detection
- Mycoplasma contamination detection
- Gender identification for human

2. Result Summary

(See Table below)

Table 1. 1. Specimen Description

| QC-ID | Sample ID | Species |
|----------------|------------------------------|---------|
| 20200819-02699 | GA9273-R1P2-20191119-R-37790 | Human |

Table 2. Result Summary

| QC Item | Results |
|--------------------------------------|---------------------|
| Mouse ratio | 28.9% |
| Matched sample | GA9273 |
| Heterogeneity ratio* | 1.91% |
| Contaminant detected | None |
| Mouse syngeneic model | NA |
| Mouse strain | NA |
| Viral infection | EBV |
| Mycoplasma contamination | Negative |
| Gender | Male |
| Genetic admixture (CEU: CHB: YRI) ** | 0.002: 0.002: 0.996 |

*: See definition in Reference 1.

** : The three reference populations are: Han Chinese (CHB), Nigeria Yoruba (YRI) and Utah residents with Northern and Western European ancestry from the CEPH collection (CEU).



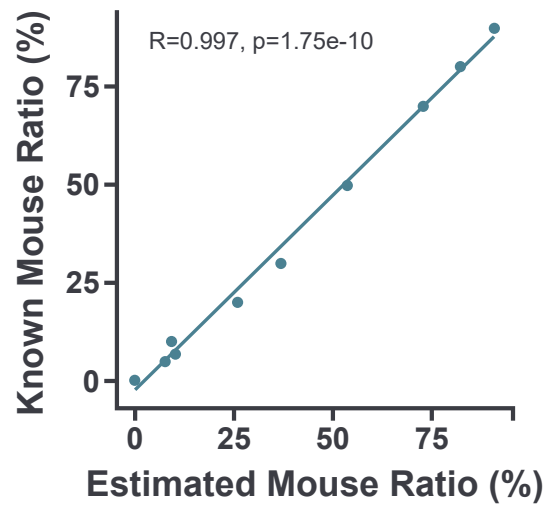
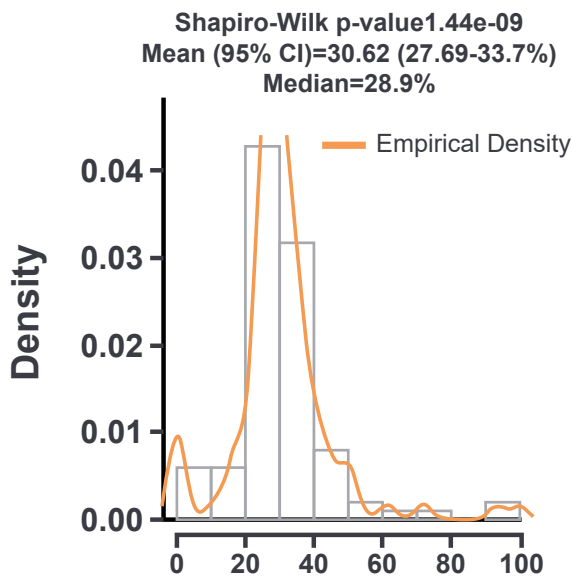
3. Results

3.1. Mouse Ratio

Comment: Mouse ratio is estimated based on 108 100-300bp human-mouse homologous segments that share identical flanking sequences and are amplified by same primers.

Conclusion: The mouse ratio in Sample GA9273-R1P2-20191119-R-37790 is 28.9%.

4A below, adopted from Reference [1]), and can reliably detect mouse ratio at ~0.1% [1].

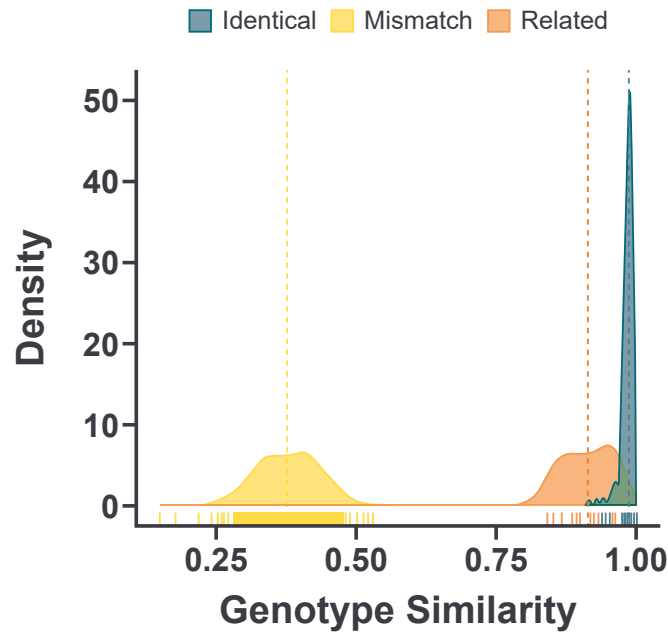


3.2. Matched Sample

Conclusion: Sample GA9273-R1P2-20191119-R-37790 is GA9273 based on its high identity to GA9273P00—the standard reference sample of PDX model GA9273

Technical note: Genotype similarities calculated from >200 SNPs are always >90% between identical samples even at the presence

of minor contaminant. In contrast, genotype similarities between unrelated samples are almost always below 50%. If two samples are related, for example by deriving from same parental sample, their genotype similarity can range from about 80% to close to 100%, depending on their genetic divergence. Below: Figure 1A from Reference [1].



| Reference | # Matched SNPs | # Nonmatched SNPs | Total SNPs | Genotype similarity (%) |
|------------|----------------|-------------------|------------|-------------------------|
| GA9273P00 | 222 | 5 | 227 | 97.80 |
| CR5076P00 | 111 | 98 | 209 | 53.11 |
| LU9342P00 | 115 | 103 | 218 | 52.75 |
| CL01123 | 111 | 100 | 211 | 52.61 |
| LU11885P00 | 109 | 99 | 208 | 52.40 |



3.3. Heterogeneity Ratio

Conclusion: Sample GA9273-R1P2-20191119-R-37790 has a heterogeneity ratio 1.91% with the above distribution of heterogeneity ratios for 227 SNPs. This heterogeneity ratio is within the standard value of PDX models with ~30% mouse ratios (see Figure 1C of Reference 1).

Technical note: Heterogeneity ratio is a quantitative measure for genetic heterogeneity of a sample by using the >200 SNPs and is defined in Table 1 of Reference [1]. Heterogeneity ratio comes from genetic heterogeneity and contamination. High heterogeneity ratio, as well as a two/three-modal distribution, usually indicates possible contamination. See Reference [1] for details.

3.4. Contaminant Detection

Conclusion: No contaminant is detected for sample GA9273-R1P2-20191119-R-37790.

Technical note: In this quantile-quantile plot, each dot is a reference cell line; theoretical and sample quantiles were calculated from a beta distribution fitted to genotype similarities between the test sample and all reference samples. The 99% confidence band is shaded. A reference sample was denoted as contaminant if (1) it had the highest genotype similarities, (2) its genotype similarity was above the 99% confidence upper bound in the quantile-quantile graph and (3) its P-value was $<1.0E-6$ in the fitted beta distribution. An example for contaminant is shown below (Figure 3D from Reference [1]).

Figure 3.3

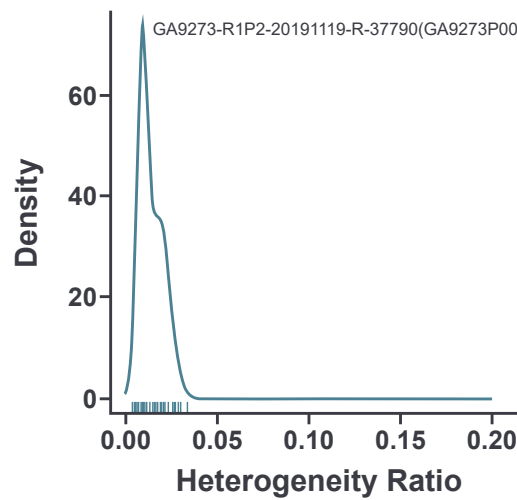
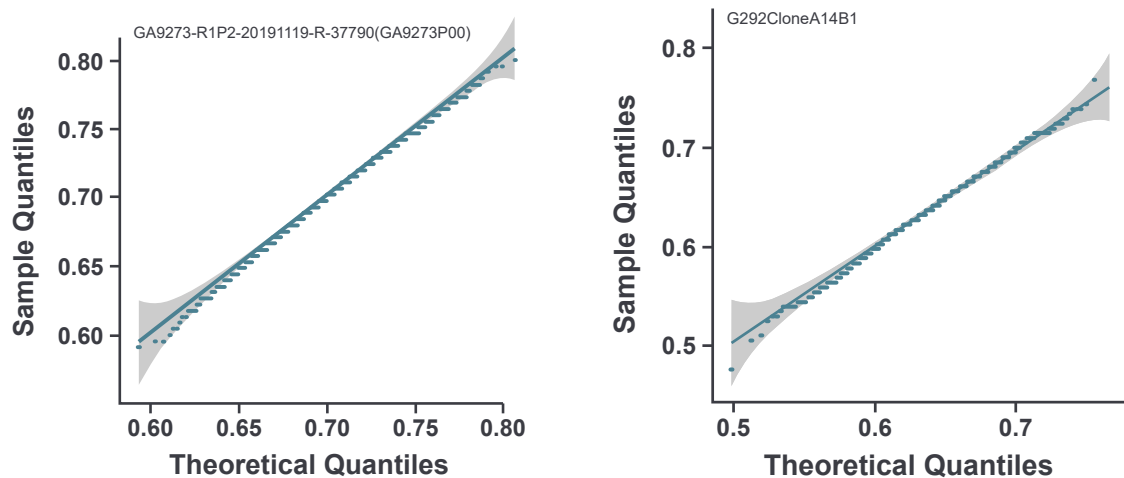


Figure 3.4



3.5. Viral Infection & Mycoplasma Contamination

Comment: If the read depth is > 1000, a base is accounted as a high coverage one.

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

HBV: Hepatitis B virus

HIV: Human immunodeficiency virus

HPV: Human papillomavirus

Conclusion: Sample GA9273-R1P2-20191119-R-37790 is EBV positive, and CMV/EBV/HIV/HPV16/HPV18/Mycoplasma negative.

Technical note: Multiple pairs of primers were used to detect each virus, and one pair of universal primers were used to detect all mycoplasma species. See Reference [1] for details.

3.6. Gender Identification

Comments: (1) If the sum of read depths of the 3 Y-chromosome SNPs >1000, the sample is identified as "Male"; If the sum if <300, the gender is predicted as "Female"; If the estimated mouse ratio is over 95%, or the sum of depths is less than 1000 and more than 300, the gender is not assigned. (2) Because Y chromosome is frequently lost in cancer genomes, it is advised to use microscope- based observation to confirm the gender prediction.

Conclusion: Sample GA9273-R1P2-20191119-R-37790 is male.

3.7. Genetic Admixture Analysis

Based on the genotyping data of 143 SNPs, the genetic admixture of this sample is 0.2% CHB, 0.2% CEU, and 99.6% YRI, where CHB stands for Han Chinese, CEU for Utah residents with Northern and Western European ancestry from the CEPH collection, YRI for Nigeria Yoruba.

Conclusion: Sample GA9273-R1P2-20191119-R-37790 is predominantly of YRI origin.

Table 3.5.

| Virus & Mycoplasma | Number of high coverage bases |
|--------------------|-------------------------------|
| CMV | 0 |
| EBV | 428 |
| HBV | 0 |
| HIV | 0 |
| HPV16 | 0 |
| HPV18 | 0 |
| Mycoplasma | 0 |

Table 3.5.

| SNP-anchored Y chromosome segment | Read depth |
|-----------------------------------|------------|
| hg19_chrY_14832620 | 2713 |
| hg19_chrY_15467824 | 3723 |
| hg19_chrY_15591537 | 4342 |



4. Signatures

4.1. Sponsor Approval

Sponsor Representative

Date

4.2. Crown Bioscience (Suzhou) Approval

Crown Bioscience Representative

Date

5. References

- ¹ Chen,X., Qian,W., Song,Z., Li,Q. and Guo,S. (2020) Authentication, characterization and contamination detection of cell lines, xenografts and organoids by barcode deep NGS sequencing. *NAR Genomics and Bioinformatics*, 2, 3. <https://academic.oup.com/nargab/article-abstract/2/3/lqaa060/5893932>

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