HLA GENOTYPING BY NEXT GENERATION SEQUENCING AT PATHOQUEST

PathoQuest

PathoQuest is the leading expert in providing NGS services to the biopharmaceutical industry. We offer a range of GMP and non-regulated validated services for cell and gene therapies, including HLA genotyping for novel and emerging cell therapy applications. HLA genotyping can also be used to detect intra-species cellular contamination within presumed clonal human cell lines.

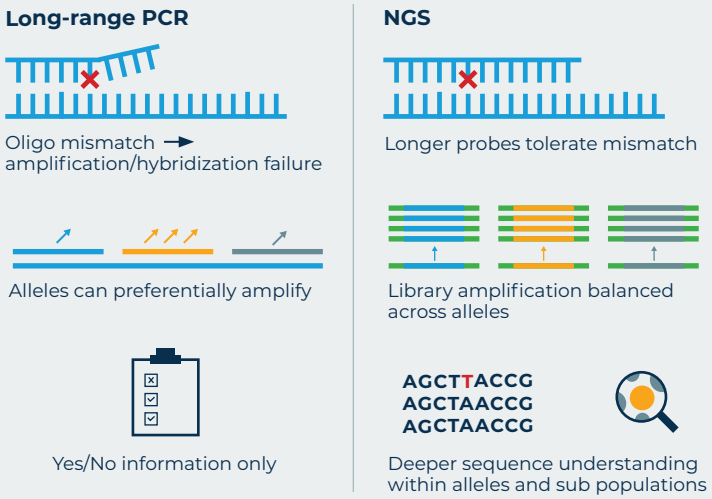
MODALITIES TESTED

- Allogeneic cell therapies
- Gene modified cell therapies, e.g. CAR-T
- Stem cell therapies, including mesenchymal stem cells
- Donor tissue / patient samples
- Cell lines used across biologic manufacturing
- Cell bank identity testing

BENEFITS OF NGS FOR HLA GENOTYPING

- ✓ Validated method for HLA genotyping
- ✓ Fast results – within 1 week (including bioinformatics)
- ✓ More reliable and robust than long-range PCR
- ✓ Allele level resolution
- ✓ Coverage across 17 loci, including all exons
- ✓ Contamination detection of human cell lines

PCR VS NGS FOR HLA TESTING



Human leukocyte antigen (HLA) genotype testing has traditionally been used to identify donor-recipient matches in organ transplantation. However, HLA genotyping is also useful to developers of allogeneic cell therapies such as mesenchymal stem cells and other "off the shelf" gene-modified cell therapies such as CAR-T to maximize efficacy by ensuring limited recipient response from the patient [1].

There are several different methods for HLA genotyping, ranging from PCR hybridization based, to sequencing of the alleles by Sanger and NGS. A common method is long-range PCR followed by probe hybridization to a sequence specific oligo (SSO method) to identify HLA specific alleles. The SSO method is fast and highly scalable, however can suffer from PCR artefacts and dropouts caused by hybridization failure, leading to HLA ambiguity [2]. Direct sequencing by NGS of the long range PCR amplicons mitigates issues with probe hybridization, as well as providing base level resolution of the HLA alleles. However, potential issues with PCR remain, such as introducing errors into the target sequence [3].

To mitigate these issues, PathoQuest uses DNA indexing followed by hybrid capture for targeted enrichment to generate the libraries ready for sequencing. Over 1000 probes targeting highly conserved intron regions provide coverage for all known and novel HLA alleles across 17 loci of the HLA genes†.

Depending upon the application and sensitivity required, our HLA genotyping can also identify the presence of contaminating human cells within a human cell line to 5% sensitivity**. Intra-species contamination detection can prove difficult with other tests, such as barcode sequencing of cytochrome oxidase subunit 1 (COI) which identifies and discriminates separate species [4].

SAMPLE REQUIREMENTS	SHIPMENT & STORAGE	STANDARD TURNAROUND TIME	FASTTRACK TURNAROUND TIME	SENSITIVITY OF CONTAMINATION DETECTION**	OUTPUT
Minimum $1x10^6$ cells (or equivalent) Backup sample required BSL1 or 2*	Dry Ice / -80°C	2 weeks	4 days	Detection contaminating human cells validated to 5% abundance.	HLA Genotype Report† Reporting of contaminating human cells ≥5%

1. Kot, M., Baj-Krzyworzeka, M., Szatanek, R., Musiał-Wysocka, A., Suda-Szczurek, M., & Majka, M. (2019). International Journal of Molecular Sciences, 20(22), 5680.
2. Erlich, H. (2012). Tissue Antigens, 80(1), 1-11.
3. Brown, N. K., Merkens, H., Rozemuller, E. H., Bell, D., Bui, T. M., & Kearns, J. (2021). Human Immunology, 82(4), 296-301.
4. Pentinsaari, M., Salmela, H., Mutanen, M., & Roslin, T. (2016). Scientific reports, 6(1), 1-12.

* Biosafety level classifications can vary between regulatory authorities – contact PathoQuest to discuss. Contact PathoQuest for patient or tissue samples.
** NGS should be considered as semi-quantitative in this application. Abundance figures are provided at the occurrence rate of the contaminating allele as it appears within the read data. Detection of contaminants may be possible below the validated 5% level. Please discuss with our experts if this is required.
† Genotyping of genes: HLA-A/-B/-C/-E/-F/-G/-H, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, DPB1, MICA and MICB

Find out more: **www.pathoquest.com/index.php/HLA**
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