GENDX

NGStrack: Chimerism monitoring by means of next-generation sequencing

Post-transplant monitoring of chimerism levels is essential for the early detection of relapse after HSCT. Currently, monitoring is mainly performed through STR or qPCR testing. Disadvantages of STR lie in its laborious nature and low sensitivity. While qPCR is considerably less laborious and results in higher sensitivity, each monitoring experiment requires additional recipient pre-transplant material. Performing chimerism monitoring by next-generation sequencing (NGS) allows for highly sensitive detection of a plethora of markers, without requiring pre-transplant DNA for each monitoring experiment. Here, we present NGStrack, a novel method to facilitate chimerism monitoring by NGS, using a streamlined workflow that allows for direct sequencing of PCR products.

Targeted markers and direct indexing amplification workflow

Primers were designed targeting 31 hypervariable biallelic marker sites that are also targeted in the existing GenDx AlleleSEQR, KMRtype® and KMRtrack® qPCR product lines (Table 1). An amplification protocol that includes simultaneous indexing was developed for this assay (Figure 1). Seven multiplex mixes incorporating three to six markers each were designed to allow for testing of specific marker groups, making efficient use of the NGS flow cell. Amplicons were then subjected to a single SPRI bead clean-up step prior to Illumina MiSeq sequencing. The total time from DNA to ready-to-sequence library was under four hours with a hands-on time of approximately 2 hours.

Measurements are both accurate and sensitive

To ensure sample variation did not affect initial results, both homo- and heterozygous samples (Coriell Institute) were used to demonstrate proof-of-principle. Artificial chimeric samples (DNA in DNA) were then created to test assay sensitivity across a range of dilutions. A minor fraction of 0.5% could be reliably detected by the prototype assay (Figure 2). The absolute deviation between expected chimerism percentages and observed chimerism measurements was, on average, 1.27% (0.4% - 3.4%). This implies accurate detection of chimerism percentages at both high and low levels of chimerism.

Proficiency study results

NGStrack was tested on five United Kingdom National External Quality Assessment service (UK NEQAS) studies. Each study consists of a pre-donor, pre-recipient and two post-transplant samples. Assays are deemed adequate when results are within 2.5 standard deviations of an average calculated based on all participating laboratories. Pre-transplant samples were tested to identify informative markers. Post-transplant samples were then tested to determine chimerism levels. For all ten post-sample measurements, NGStrack results were within “satisfactory” limits as specified by UK NEQAS, as shown in Figure 3.

Conclusion

The assay design described here enables monitoring of post-transplant chimerism levels by NGS in a highly efficient workflow. Sensitive, accurate and reproducible results are produced, providing an NGS based, easy-to-use, alternative to traditional STR and qPCR testing of chimeric status.

Table 1: Markers included in NGStrack

NGStrack marker	NGStrack mix	Part of KMR	Part of AlleleSEQR	Chromosome and arm
TRK001	TRK mix 001	Extended 2*	Yes	8q
TRK002	TRK mix 002	Extended 2*	Yes	14q
TRK003	TRK mix 003	Extended 2*	Yes	18q
TRK004	TRK mix 001	Extended	Yes	18q
TRK005	TRK mix 006	-	Yes	13q
TRK006	TRK mix 005	-	Yes	1p
TRK008	TRK mix 002	-	Yes	7p
TRK009	TRK mix 001	Core	Yes	17p
TRK010	TRK mix 007	Extended	Yes	5q
TRK011	TRK mix 003	Core	Yes	Xp
TRK013	TRK mix 003	Core	Yes	6q
TRK014	TRK mix 003	Extended	Yes	12q
TRK015	TRK mix 006	-	Yes	5q
TRK016	TRK mix 007	Core	Yes	17q
TRK017	TRK mix 001	Extended	Yes	Yp + Xp
TRK018	TRK mix 007	Extended 2*	Yes	11q
TRK019	TRK mix 002	Core	Yes	20q
TRK020	TRK mix 006	Extended	Yes	1p
TRK024	TRK mix 005	-	Yes	3q
TRK025	TRK mix 002	Extended 2*	Yes	5q
TRK029	TRK mix 005	Extended	Yes	2q
TRK030	TRK mix 002	Core	Yes	9q
TRK031	TRK mix 004	Extended	Yes	11p
TRK033	TRK mix 005	Extended	Yes	12q
TRK035	TRK mix 004	Core	-	2q
TRK038	TRK mix 004	Core	-	5q
TRK039	TRK mix 004	Core	-	17p
TRK044	TRK mix 004	Core	-	Xq
TRK045	TRK mix 001	Core	-	10q
TRK052	TRK mix 007	Core	-	10q
TRK055	TRK mix 001	Core	-	11q

Figure 1: Direct indexing amplification strategy

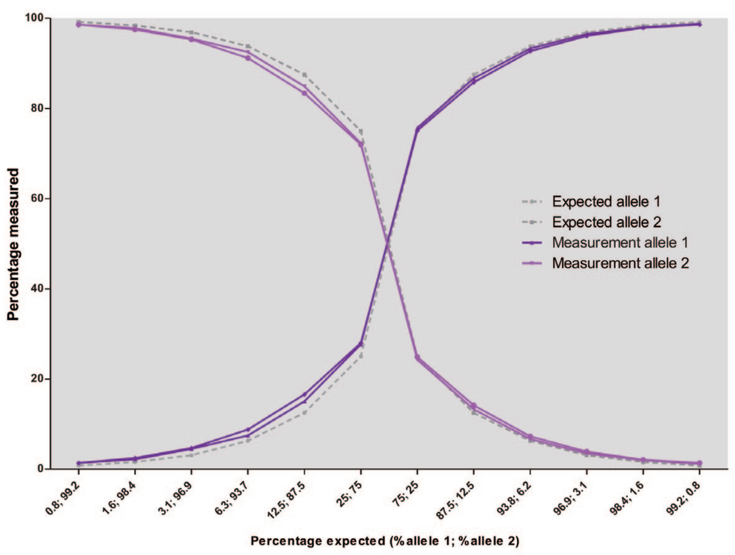

Figure 2: Chimerism measurements in artificial mixture of two homozygous samples. The same marker (TRK018) was tested in duplicate

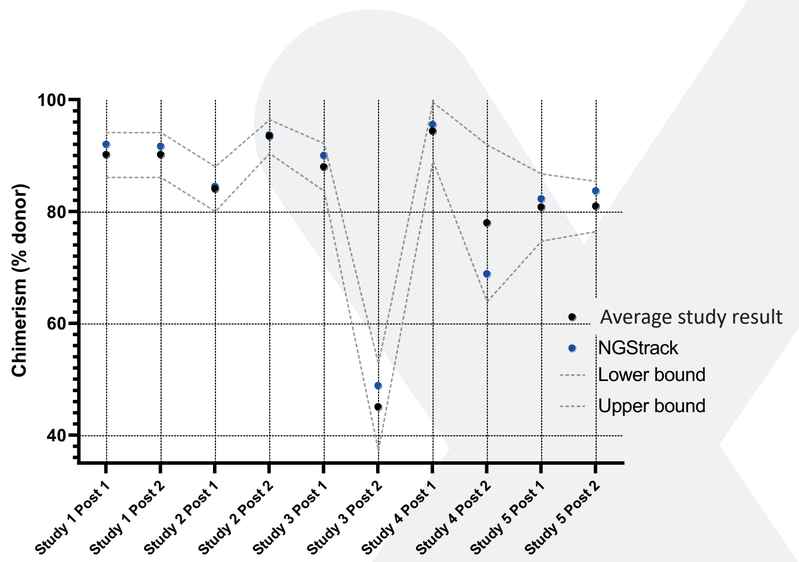

Figure 3: Results from five proficiency studies. Lower and upper bounds indicate 2.5 standard deviation difference from average shown by black dots. Blue dots show results obtained with NGStrack.

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Pascal van der Weele[1], Robert Welleweerd[1], Jannetje Kooij[1], Maarten T. Penning[1], Erik H. Rozemuller[1]

1 GenDx, Utrecht, the Netherlands

experts in transplant diagnostics