Direct vs indirect indexing for NGS-Based Chimerism Monitoring

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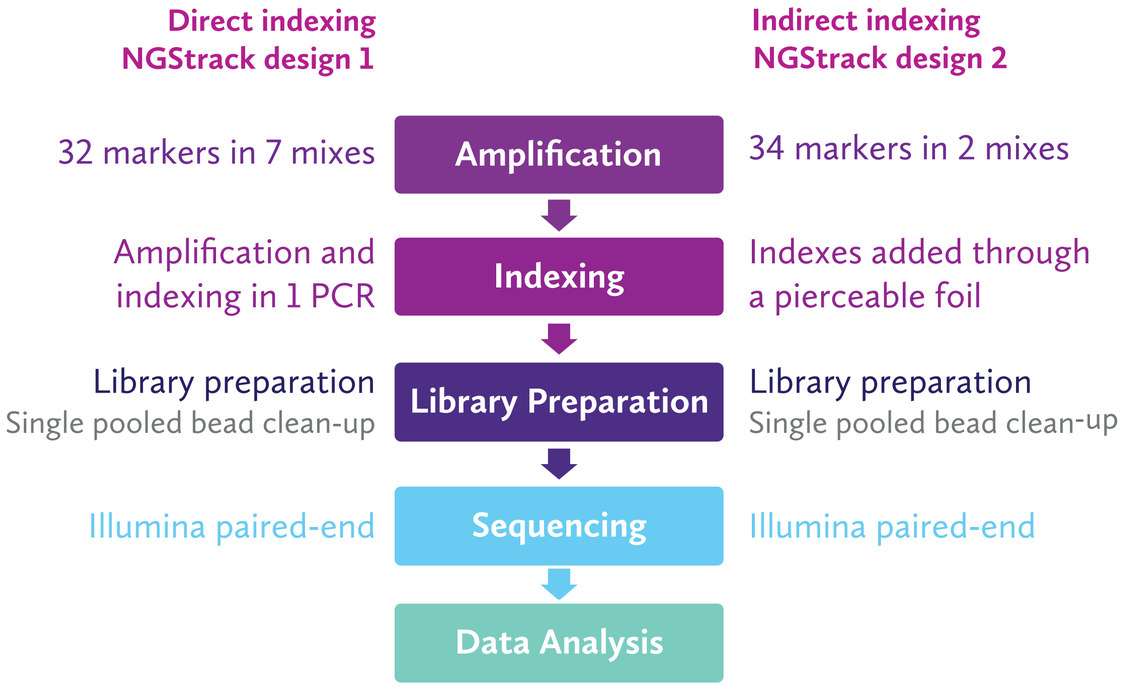
Introduction

Chimerism monitoring is usually performed using qPCR or STR-based methods. New methods are being developed using Next Generation Sequencing (NGS). NGStrack is a NGS-based method for chimerism monitoring, using hypervariable biallelic indel markers, spread across 17 chromosomes. Amplicons are generated and indexed using PCR. This can be through direct indexing, where amplicons are generated and simultaneously indexed in a single PCR step. Alternatively, indirect indexing can be used, where first amplicons are generated and indexing is performed in a separate PCR step. Here, we compare the direct and indirect indexing approaches for NGS-based chimerism monitoring.

Method

External quality assessment samples from 5 UK NEQAS studies of the Leucocyte Immunophenotyping Programme (including in 2 genotyping and 2 monitoring samples per study), were tested with NGStrack design 1 (direct indexing) and design 2 (indirect indexing). For direct indexing, the samples were amplified and indexed simultaneously, employing 7 multiplex mixes targeting 32 indel markers. For indirect indexing, amplicon generation and indexing were performed in 2 PCRs, using 2 multiplex mixes of 34 indel markers. Library preparation was identical for both methods: one pool for genotyping and one pool for monitoring samples was prepared. Subsequently, a single bead-based clean-up was performed. Libraries were sequenced on an Illumina MiSeq and data was analyzed using TRKengine. To compare both methods, we evaluated monitoring accuracy (average chimerism percentage of all informative markers compared to UK NEQAS average), data quality and ease of use (pipetting steps and assay time). Data quality was judged based on mappability (percentage valid read of total number of reads generated) and noise levels (based on (non-informative) homozygous typings).



Results and conclusion

Both direct and indirect indexing approaches, where 32 and 34 indel markers are used, result in high quality results. The measured chimerism percentages are accurate as they meet the standards of five different UK NEQAS proficiency studies. For monitoring samples, the number of informative markers for each donor-recipient pair ranged from 17 to 23. With a small increase in mappability and lower noise levels, the data quality of the indirect indexing approach was slightly higher. As only two amplification mixes are used in the indirect indexing protocol, number of pipetting steps is reduced in this method. Direct indexing provides the least hands-on time, but requires additional DNA due to having seven mixes. Indirect indexing requires slightly more hands-on time, but has the same total duration and requires less DNA due to having fewer mixes. Especially for high-throughput scenarios and when DNA quantities are limited, the indirect indexing approach is an interesting alternative.

	Direct indexing (design 1)				Indirect indexing (design 2)			
	Informative for				Informative for			
Study	Recipient	Donor	Recipient +donor	Total	Recipient	Donor	Recipient +donor	Total
1	6	9	3	**18**	6	10	3	**19**
2	7	7	3	**17**	7	8	3	**18**
3	7	9	7	**23**	7	10	6	**23**
4	5	9	4	**18**	5	10	3	**18**
5	9	9	2	**20**	8	10	3	**21**