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Chimerism Monitoring by Next-Generation Sequencing: a Multiplatform Comparison

Introduction

Accurate monitoring of the chimeric status after stem cell transplantation is essential for early detection of relapse, and at the moment mainly performed by STR or qPCR. The major disadvantages of STR testing are the laborious data analysis and poor sensitivity. The qPCR technique enables a much quicker workflow and better sensitivity. However, a disadvantage is the need of a pre-transplant sample for each monitoring event. Performing chimerism monitoring by NGS will eliminate these limitations while allowing for a multiplexed setup, reducing the amount of needed lab work and DNA. Additionally, the workflow could be combined with routine NGS HLA typing.

Materials & Methods

A set of three biallelic assays based on an insertion/deletion (NGS-KMR005, NGS-KMR015 and NGS-KMR018) were selected to be tested in an artificial chimeric range, mixing a DNA that is homozygous positive for the insertion with a DNA that is homozygous positive for the deletion. Two DNA samples, NA12560 and NA17201, were selected from the Coriell sample panel (Coriell Institute for Medical Research). Table 1 shows the typing of the selected assays and the two DNA samples, with S indicating a deletion and L an insertion (A) as well as the artificial chimeric range that was applied (B).

After a singleplex amplification, amplicons were visualized on a 1.5% agarose gel including the negative control (NC) and 100 bp ladder (NEB), and showed strong bands for all assays in each condition from 1-13 (Figure 1). These amplicons were subsequently used in the NGSgo library preparation workflow (GenDx) at 100 ng input to be run on the Illumina MiSeq and Ion Torrent S5. Additionally, the SQK-LSK108 ligation kit was applied to run samples on the MinION system (Oxford Nanopore). Due to barcode limitations only one assay (NGS-KMR015) was tested on the MinION.

MinION data was basecalled and demultiplexed applying the Albacore 2.0 software package. NGS data from all three platforms was analyzed with customized analysis tools designed to quantitate the two variants, the presence of an insertion or deletion, of each assay.

Table 1. A. Typing of two DNA samples for three assays (with S indicating a deletion and L an insertion). B. Artificial chimeric range of the two DNA samples that was applied.

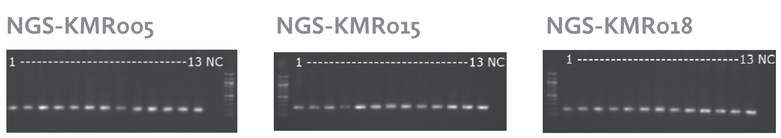
A

	NA12560	NA17201
NGS-KMR005	SS	LL
NGS-KMR015	LL	SS
NGS-KMR018	SS	LL

B

Condition	1	2	3	4	5	6	7	8	9	10	11	12	13
NA12560	0%	0.01%	0.1%	0.3%	1%	10%	50%	90%	99%	99.7%	99.9%	99.99%	100%
NA17201	100%	99.99%	99.9%	99.7%	99%	90%	50%	10%	1%	0.3%	0.1%	0.01%	0%

Figure 1. Amplicons on a 1.5% agarose gel for each assay and each condition (1 to 13), also including the negative control (NC) and 100 bp ladder.



Results

The theoretical percentages of each condition/sample (as explained in Table 1) were compared with the experimental percentages obtained on the respective platforms. These percentages were plotted against each other as represented in Figure 2 for the complete dataset and Figure 3 for the lower range of 0-20%. Results are based on an average read depth of 49,681, 49,117 and 19,242 for Illumina MiSeq, Ion Torrent S5 and MinION respectively. Both figures show that with the Illumina MiSeq the results are closest to the expected values, represented by the blue dots that are closely located on or near the theoretical (dashed) line. As an example, detailed results for the three assays tested with NA12560 on the Illumina MiSeq can be found in Table 2. It can be observed that experimental percentages are a close match with the theoretical percentages, with the results for NGS-KMR015 slightly better in comparison to NGS-KMR005. Examining the number of reads obtained for each condition, NGS-KMR015 shows an increase in reads 2 to 19 fold. This shows that with a higher read depth, a more accurate result might be possible and further investigations on required read depth for the best achievable sensitivity and accuracy should be performed.

For the Ion Torrent S5 platform, represented with the purple dots in Figure 2 and 3, a percentage could not always be obtained for each condition/sample and purple dots were located above the dotted (theoretical) line consistently. Additionally, on average 79% (varying between 16-99%) of the retrieved data contained short reads with an average length of 50 bp that could not be mapped to a specific assay. The current assay design makes use of amplicons with an average size of 250 bp, which is a preferential size for the Illumina workflow in terms of chip capacity.

For the Ion Torrent, this amplicon size is difficult to amplify and sequence clonally. A larger amplicon of at least 400 bp may be preferred. For the moment the type of data obtained and its accuracy is not sufficient for chimerism monitoring.

The MinION data, represented by the grey dots in Figure 2 and 3, shows the most adverse results of the three platforms. The insertion/deletion of NGS-KMR015 has a length of 6 bp, this short length may be more difficult for a MinION system to call correctly in a quantitative manner. The correct calling of insertions/deletions is something that Oxford Nanopore is working on to improve, so the compatibility of the MinION platform with this type of assay may change in the future.

Finally, the amplifications that were performed for this study were done in a singleplex fashion. Despite this setup, data from for example NGS-KMR005 could be detected in a sample with NGS-KMR015 (and all other possible combinations) (results not shown). On average this represented 0.15% and 2.23% of the data for Illumina MiSeq and Ion Torrent S5 respectively. When aiming for a sensitivity of 0.05% (equal to the current sensitivity of the qPCR workflow) or lower, this level of contaminating background is not advised and should be investigated further.

Conclusions

The results demonstrate that NGS-based chimerism monitoring could be feasible in a wide dynamic range for the Illumina MiSeq platform. Although data looks promising for the Ion Torrent S5, this workflow can be improved by optimization of the amplicon sizes. Chimerism monitoring on the MinION might be improved by analyzing larger insertions/deletions. This demonstrates that each of the tested NGS platforms will have its own unique characteristics for which an optimal workflow needs to be designed.

Figure 2. Theoretical percentage (dashed line) compared to experimental percentage (bullets) for each platform. Assay and DNA sample names on top of each graph. Range 0-100%.

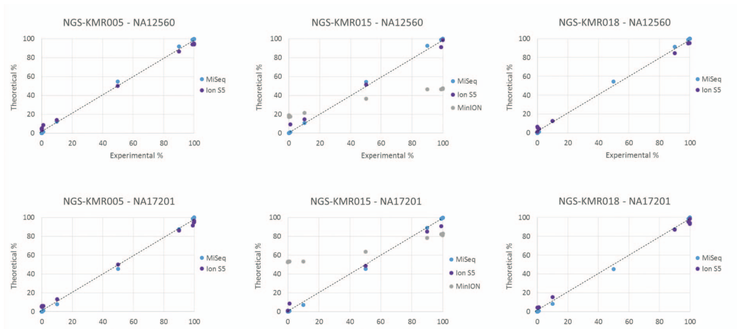


Figure 3. Theoretical percentage (dashed line) compared to experimental percentage (bullets) for each platform. Assay and DNA sample names on top of each graph. Range 0-20%.

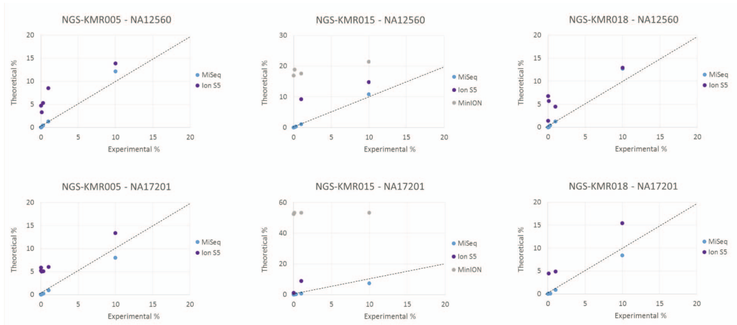


Table 2. Theoretical percentage of sample NA12560 compared to the experimental percentage and total number of reads for assays NGS-KMR005, NGS-KMR015, and NGS-KMR018 (Illumina MiSeq).

	NGS-KMR005		NGS-KMR015		NGS-KMR018	
Theoretical %	Experimental %	Total reads	Experimental %	Total reads	Experimental %	Total reads
0	0.032	6,277	0.027	120,603	0.006	17,082
0.01	0.031	9,656	0.027	141,462	0.016	18,681
0.1	0.17	7,759	0.15	73,856	0.079	25,253
0.3	0.43	10,475	0.37	113,712	0.38	25,749
1	1.3	10,956	1.1	135,489	1.3	10,026
10	12.2	16,832	10.8	161,663	12.7	10,770
50	54.7	25,928	54.4	152,875	54.6	14,276
90	92.0	17,771	92.6	113,481	91.6	16,353
99	99.1	17,637	99.2	135,135	99.1	20,151
99.7	99.8	17,153	99.8	113,197	99.9	26,293
99.9	99.9	33,557	99.9	50,991	99.9	17,832
99.99	100.0	29,038	99.9	72,959	100.0	25,018
100	100.0	34,520	100.0	75,556	100.0	11,564

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