LeXPress Hybrid Capture Reagents

Background

Liquid-phase hybridization capture sequencing is, based on the principle of complementary base pairing, to design and synthesize nucleic acid probes for regions of interest, so that DNA libraries can be enriched and sequenced using liquid-phase hybridization in target regions, so as to achieve detection on target regions at lower costs. Thanks to the ability of the probes for tolerating certain differences between target regions and the ability for jointly capturing surrounding regions, liquid-phase hybridization capture can accurately detect a variety of mutations such as SNV, InDel, CNV, SV, and gene fusions. Due to its wide application in supporting disease exploration, the detection of new pathogens or pathogenic genes has been greatly improved. As a cost-effective, reproducible and stable research strategy, it is also of great significance for personalized treatment of specific individual genetic backgrounds.

More rapid and stable experimental workflows are urgently needed for the detection of both tumor and pathogenic microorganism infections, so as to further streamline the process. Overnight hybridization (~16 hr) and redundant elution in traditional liquid-phase hybridization capture have undoubtedly affect the detection process. Therefore, LexigenBio has launched **LeXPress Hybrid Capture Reagent**, which can be compatible with rapid hybridization capture of different probes.

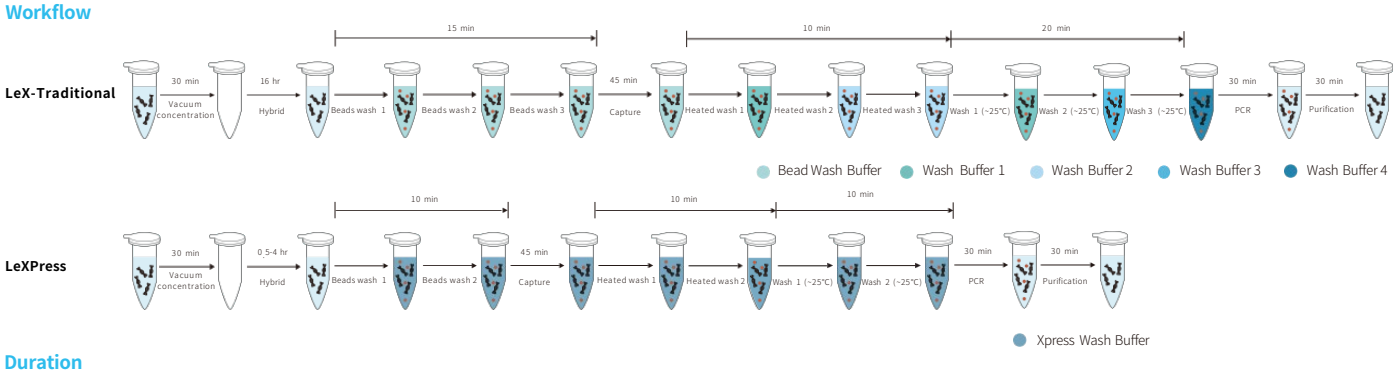
Introduction

LeXPress Hybrid Capture Reagents is a kit optimized for the targeted capture with rapid hybridization elution steps of Panels/Probes developed by LexigenBio . The complete liquid-phase hybridization capture system is composed of LeXPrep Blockers series and 120 nt Panels/Probes series, and supports flexible selection between 0.5-4 hr rapid hybridization and overnight hybridization by adjusting the hybridization time to optimize the capture performance for panels of different sizes.

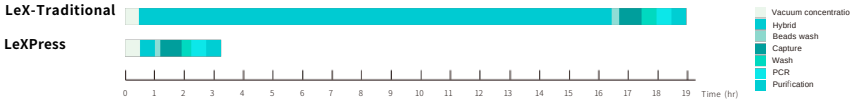
Feature

- Simplify the types of elution buffer into one to reduce the possibility of human errors during operation.
- Simplify the experimental process, make it more convenient and user-friendly for operation to facilitate the development of automatic workstation.
- Support flexible selection between rapid hybridization for 0.5-4 hr or overnight hybridization for 16 hr.
- Support the adjustment of hybridization time to optimize capture performance.

Workflow



Duration



Note: LeX-Traditional indicates LeXPrep Hybrid Capture Reagents; LeXPress indicates LeXPress Hybrid Capture Reagents.

Performance

Capture performance with various hybridization time

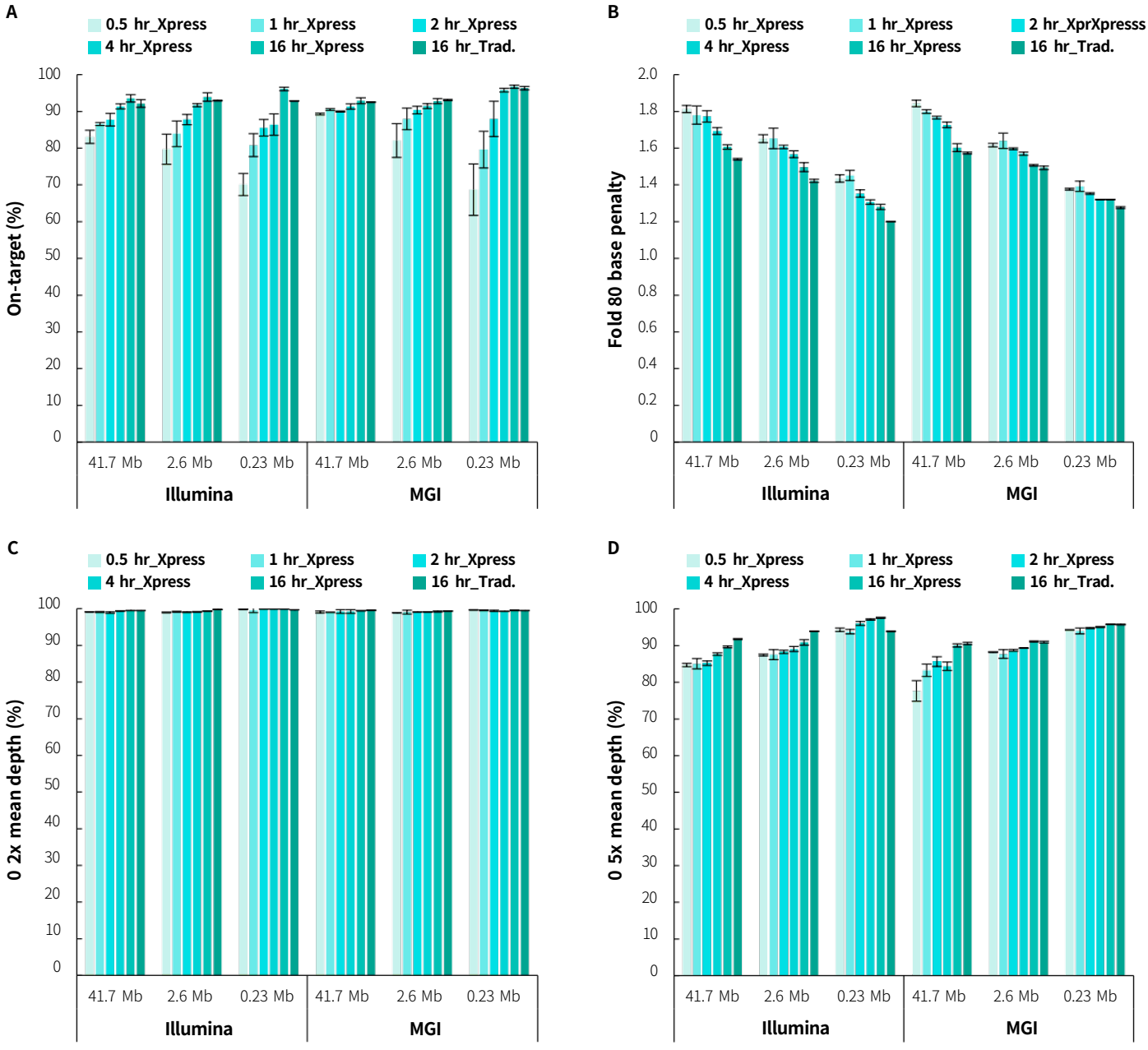


Figure 1. Capture performance of LeXPress Hybrid Capture Reagents with various hybridization time. 100 ng of Human Genomic DNA (Promega, G1471) was used for pre-library preparation with the LeXPrep EZ DNA Library Preparation Kit v2, with 25 min of enzymatic digestion at 25°C; 500 ng/pre-library (1-plex) input was used, following the user manual of hybrid capture of LeXPress Hybrid Capture Reagents (0.5/1/2/4/16 hr_Xpress) and LeXPrep Hybrid Capture Reagents (16 hr_Trad). Sequencing was performed using Illumina Novaseq 6000, PE 150 and DNBSEQ-T7, PE 150. Appropriate amount of data was selected for subsequent analysis. The BWA was used for alignment to the reference genome hg38 and On-target rate was calculated by the number of reads. **A.** On-target rate; **B.** Fold 80 base penalty; **C.** 0.2x mean depth; **D.** 0.5x mean depth.

Note: 41.7 Mb, 2.6 Mb and 0.23 Mb in the assay are LeXome Core Panel, LeXOnco Plus Panel v2.0 and LeXLungCancer Panel v1.0, respectively.

Capture performance for panels of different sizes

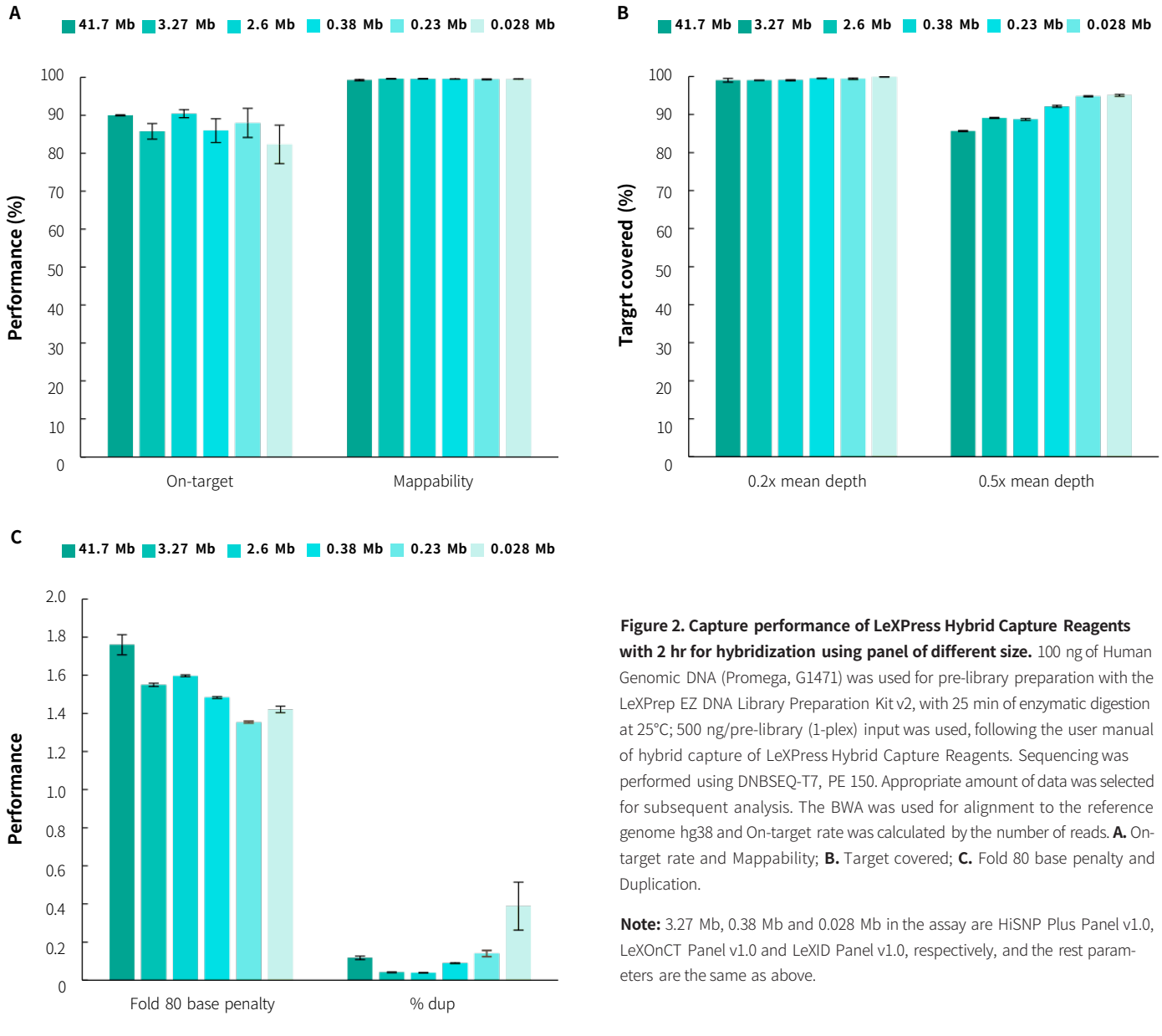


Figure 2. Capture performance of LeXPress Hybrid Capture Reagents with 2 hr for hybridization using panel of different size. 100 ng of Human Genomic DNA (Promega, G1471) was used for pre-library preparation with the LeXPrep EZ DNA Library Preparation Kit v2, with 25 min of enzymatic digestion at 25°C; 500 ng/pre-library (1-plex) input was used, following the user manual of hybrid capture of LeXPress Hybrid Capture Reagents. Sequencing was performed using DNBSEQ-T7, PE 150. Appropriate amount of data was selected for subsequent analysis. The BWA was used for alignment to the reference genome hg38 and On-target rate was calculated by the number of reads. **A.** On-target rate and Mappability; **B.** Target covered; **C.** Fold 80 base penalty and Duplication.

Note: 3.27 Mb, 0.38 Mb and 0.028 Mb in the assay are HiSNP Plus Panel v1.0, LeXOnCT Panel v1.0 and LeXID Panel v1.0, respectively, and the rest parameters are the same as above.

Capture performance on different sequencing platforms

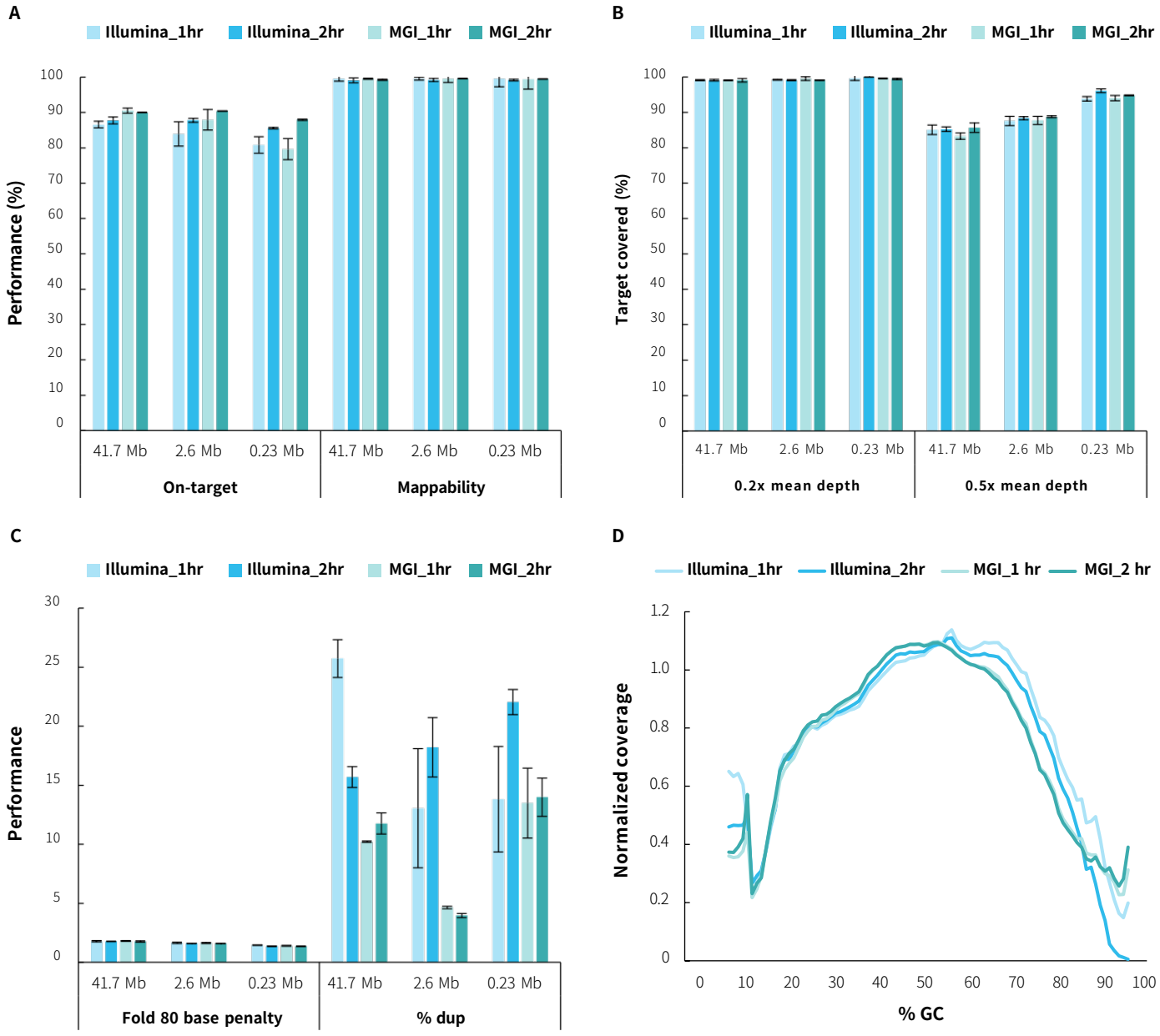


Figure 3. Capture performance of LeXPress Hybrid Capture Reagents on different platforms. 100 ng of Human Genomic DNA (Promega, G1471) was used for pre-library preparation with the LeXPrep EZ DNA Library Preparation Kit v2, with 25 min of enzymatic digestion at 25°C; 500 ng/pre-library (1-plex) input was used, following the user manual of hybrid capture of LeXPress Hybrid Capture Reagents. Sequencing was performed using Illumina Novaseq 6000, PE 150 and DNBSEQ-T7, PE 150. Appropriate amount of data was selected for subsequent analysis. The BWA was used for alignment to the reference genome hg38 and On-target rate was calculated by the number of reads. **A.** On-target rate and Mappability; **B.** Target covered; **C.** Fold 80 base penalty and Duplication; **D.** The unbiased of GC coverage.

Note: The unbiased of GC coverage is only presented as 2.6 Mb Panel.

Multiple variants analysis

Type	Variant	Reference	Observed allele frequency (%)/copy number			
			16 hr_Trad.		1 hr_Xpress	
			Illumina	MGI	Illumina	MGI
SNV	EGFR L858R	1%	1.09%	0.85%	1.01%	0.96%
SNV	KRAS A146T	1%	1.42%	0.79%	1%	1.26%
SNV	NRAS Q61K	1%	1.06%	1.56%	1.14%	0.72%
SNV	EGFR T790M	2%	2.18%	2.32%	1.77%	1.72%
Deletion	EGFR ΔE746_A750	2%	1.46%	1.26%	1.43%	1.27%
SNV	KRAS G12D	2%	2.36%	2.41%	2.19%	2.03%
Insertion	EGFR V769_D770 insASV	3%	2.19%	2.71%	3.21%	2.65%
SNV	EGFR G719S	4%	3.95%	4.17%	3.03%	3.63%
SNV	KRAS G13D	4%	4.49%	3.53%	3.70%	4.46%
Fusion	EML4-ALK Fusion V3	5%	6.08%	4.20%	5.37%	4.35%
Fusion	CD74-ROS1 Fusion	6%	6.73%	6.12%	6.51%	5.65%
SNV	BRAF V600E	7%	4.48%	5.83%	6.14%	5.60%
SNV	PIK3CA H1047R	7%	5.20%	7.39%	5.52%	6.96%
CNV	ERBB2 Amplification	5 copies	5.05	4.77	4.77	4.73

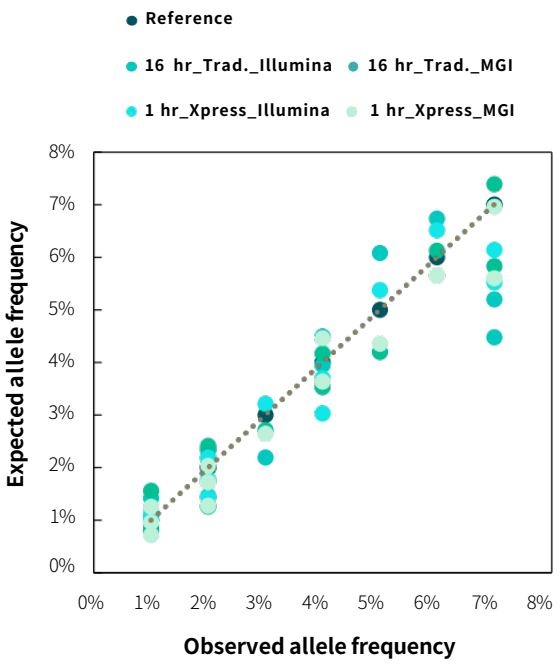


Figure 4. Correlation between expected allele frequency and observed allele frequency. 50 ng of PancancerLight 800 gDNA Reference Standard (Genewell, GW-OGTM800) was used for pre-library preparation with the LeXPrep EZ DNA Library Preparation Kit v2, with 25 min of enzymatic digestion at 25°C; 500 ng/pre-library (1-plex) input was used, following the user manual of hybrid capture of LeXPress Hybrid Capture Reagents and LeXPrep Hybrid Capture Reagents, using the 0.23 Mb panel for 1 hr rapid hybridization (1 hr_Xpress) and 16 hr overnight hybridization (16 hr_Trad.). Sequencing was performed using Illumina Novaseq 6000, PE 150 and DNBSEQ-T7, PE 150. Appropriate amount of data was selected for variant analysis using Vardict.

Ordering Information

Product	#Catalog
LeXPress Hybrid Capture Reagents, 16 rxn	LX05402
LeXPress Hybrid Capture Reagents, 96 rxn	LX05401

Statement

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