Detecting Cancer Burden in Liquid Biopsies

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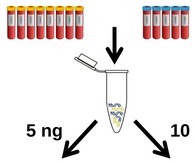
Abstract

Liquid biopsy is a non-invasive sample source that can be utilized to assess cancer burden by measuring the tumor-derived fraction of circulating, cell-free DNA (cfDNA) from plasma. We evaluated two assays to monitor cancer burden using cfDNA: whole genome bisulfite sequencing (WGBS) and targeted amplicon sequencing for 56 oncology-related genes. Genome-wide hypomethylation is a surrogate biomarker for cancer that can be detected independently of tumor genotype. Amplicon-based detection of tumor-specific mutations provides a window into tumorigenesis and potential therapeutic resistance. We tested samples with both assays to characterize their efficacy across a broad spectrum of cancer types, stages, and treatment regimens. cfDNA was extracted from tumor-bearing patients and normal controls. To monitor methylation density, WGBS was performed using 5 ng of bisulfite-converted cfDNA with the Accel-NGS® Methyl-Seq DNA Library Kit. To detect tumor-specific mutations, 10 ng of cfDNA was used for the Accel-Amplicon™ 56G Oncology Panel. Six out of eight cancer samples demonstrated significant hypomethylation in cfDNA, ranging from 2-40% when compared to healthy controls. The 56 gene amplicon panel identified point mutations in the cfDNA of only three samples, all of which had the highest observed hypomethylation (18-40%). For all but two cancer samples, corresponding mutations were also found in the primary tumor at allele frequencies significantly higher than in the cfDNA fraction (e.g., 22% in tumor vs. 5% in cfDNA). The three cancer samples that had primary tumor mutations that were not detected in cfDNA also had the lowest observed hypomethylation. Therefore, a correlation between hypomethylation and detection of tumor mutations in the cfDNA fraction may exist. Further studies will elucidate which assay is more sensitive at detecting tumor burden in cfDNA: hypomethylation using the Accel-NGS Methyl-Seq kit or the Accel-Amplicon 56 gene targeted sequencing assay with a limit of detection at 1% allele frequency.

Experimental Design

Tumor bearing blood, n = 8
(Streck Cell-free DNA BCT®)



Healthy control blood, n = 5
(Streck Cell-free DNA BCT)

Isolate cfDNA with QIAamp® Circulating Nucleic Acid kit

5 ng

10 ng

Accel-NGS Methyl-Seq

Bisulfite conversion and Accel-NGS Methyl-Seq library construction

↓

Calculate hypomethylation status of cancer samples compared to healthy controls from 10M Illumina MiSeq® reads using Methypipe.

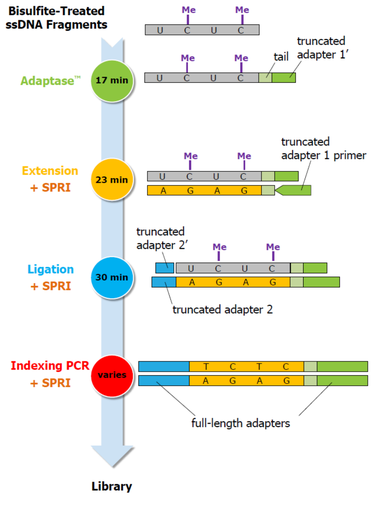
Accel-Amplicon 56G Oncology Panel

Accel-Amplicon 56G library construction

↓

LoFreq and GATK variant calling from 5000X coverage was compared to corresponding tumor FFPE and normal adjacent tissues.

Accel-NGS Methyl-Seq

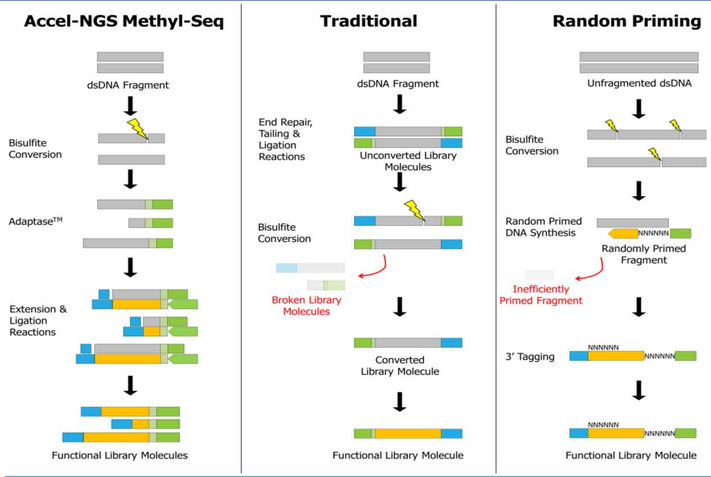


- ✓ 2-hour library prep workflow
- ✓ Post-bisulfite prep uses efficient Adaptase ssDNA library technology
- ✓ Supports input range of 100 pg to 100 ng with minimal PCR cycles
- ✓ Retains high sequence complexity for comprehensive and uniform methylome coverage

WGBS from 10 ng Coriell NA12878	
Total Reads	183.5 M
Aligned	86.4%
Genome Coverage	8.9X
Duplication	7.9%
Estimated Library Size	1.4B
CpG Uncovered	2.2%
CpG ≥ 1X	97.8%
CpG ≥ 5X	96.4%

Paired-end sequencing was performed on a HiSeq® with V4 chemistry with 125 bp PE. Analysis was performed using BSMap and Picard tools.

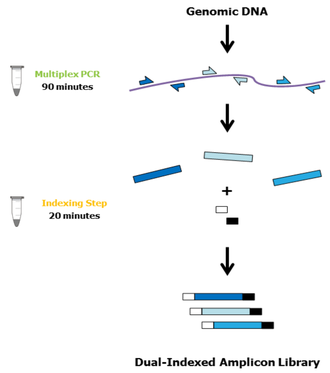
Superior Methyl-Seq Performance



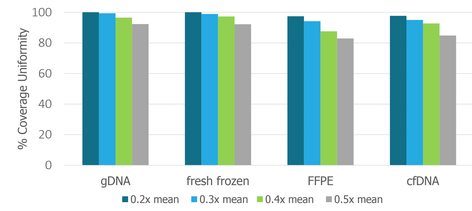
1 ng Arabidopsis DNA Input	Swift Methyl-Seq	Traditional	Random Priming
% Reads Aligned	83.3%	80.7%	73.4%
Avg. Genome Coverage	18X	10X	12X
% Genome Covered ≥10x	77%	17%	31%
% Duplicate Reads	18%	62%	46%
Estimated Library Size	38 M	6 M	12 M

Paired-end sequencing was performed on a HiSeq with V4 chemistry with 125 bp PE. Analysis performed using BSMap and Picard tools using 30M reads for each method for direct comparison.

Accel-Amplicon 56G Panel



- ✓ Single-tube amplification for hundreds of primer pairs, including overlapping amplicons
- ✓ Supports inputs of 10-25 ng
- ✓ For Illumina and Ion Torrent™
- ✓ cfDNA and FFPE compatible (120-160 bp amplicons)



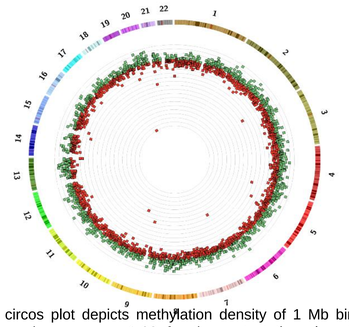
- ✓ > 95% uniformity (> 20% mean coverage) and > 95% reads on target for multiple sample types

56G Panel: Hotspot and Comprehensive Coverage of 56 Oncology-related Genes

56G panel genes and number of amplicons per gene. Hotspot loci (white), contiguous, overlapping coverage (blue) and comprehensive coding exon coverage for TP53 (darker blue).

ABL1	5	*CSF1R*	2	*FBXW7*	6	*GNAS*	2	*KIT*	14	*NPM1*	1	*STK11*	5
AKT1	2	*CTNNB1*	1	*FGFR1*	2	*HNF1A*	4	*KRAS*	3	*NRAS*	3	*SMAD4*	10
ALK	2	*DDR2*	1	*FGFR2*	4	*HRAS*	2	*MAP2K1*	5	*PDGFRA*	4	*SMARCB1*	4
APC	9	*DNMT3A*	1	*FGFR3*	6	*IDH1*	1	*MET*	6	*PIK3CA*	11	*SMO*	5
ATM	19	*EGFR*	9	*FLT3*	4	*IDH2*	2	*MLH1*	1	*PTEN*	14	*SRC*	1
BRAF	2	*ERBB2*	4	*FOXL2*	1	*JAK2*	2	*MPL*	1	*PTPN11*	2	*TP53*	21
CDH1	3	*ERBB4*	8	*GNA11*	2	*JAK3*	3	*MSH6*	4	*RB1*	12	*TSC1*	1
CDKN2A	2	*EZH2*	1	*GNAQ*	2	*KDR*	9	*NOTCH1*	3	*RET*	6	*VHL*	3

Two Liquid Biopsy Assays: Genome-Wide Hypomethylation and Mutation Detection



The circos plot depicts methylation density of 1 Mb bins across chromosomes 1-22 for the metastatic colorectal adenocarcinoma sample, where red is hypomethylated (>3 SD lower than normal mean MD) and green is comparable to normal.

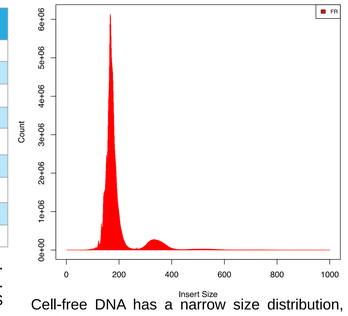
mL Plasma	ng/mL cfDNA	Pathology	cfDNA Hypomethylation	56G Mutation	Normal Adjacent	FFPE Tumor	cfDNA
2.5	6.3	Fallopian tube high-grade papillary serous carcinoma	0.4 %	TP53 E285K	0%	48%	0%
5.0	4.3	5 cm ovarian 'borderline' serous content	1.1 %	BRAF V600E	0%	14%	0%
3.8	4.4	Recurrent pT2, pN0 mammary carcinoma	2.4 %	PIK3CA H1047R	0%	17%	0%
4.0	10.5	pT1/pN1 pancreatic adenocarcinoma	3.6 %	-	-	-	-
3.0	6.7	Metastatic colon cancer to the liver	4.4 %	-	-	-	-
4.5	7.1	14 cm ovarian 'borderline' serous content	18.0 %	BRAF V600E	0%	23%	1%
4.5	2.6	Colon-cancer, non-resectable Adenocarcinoma	18.0 %	TP53 frameshift exon 8	0%	15%	2%
4.5	2.9	Metastatic colorectal adenocarcinoma	43.4 %	PIK3CA E545K APC Q1429* TP53 Q38* KRAS G13D	0% 0% 0% 0%	23% 20% 21% 22%	11% 5% 14% 5%

A correlation between cfDNA hypomethylation and detection of tumor mutations in cfDNA may exist. Significant hypomethylation was detected in 6 of the 8 samples, and the 56 gene amplicon panel identified point mutations in the cfDNA of the three samples with the highest observed hypomethylation. Concordance was observed between corresponding cfDNA and FFPE tumors, when mutations were detected.

Uniform Coverage from PCR-Free cfDNA WGS

15 ng cfDNA	Sample 1	Sample 2
Total Reads	238,230,712	254,136,768
Aligned	99%	99%
Genome Coverage	14.6x	15.6x
Genome Missing	1.9%	2.0%
Genome ≥ 5X	99.6%	99.6%
Genome ≥ 10X	94.9%	95.7%
Genome ≥ 14x	92.6%	93.2%
Duplication	0.04%	0.08%
Median Insert Size	172 bp	168 bp

Cell-free DNA was extracted by PerkinElmer using a Chemagic™ 360 system. Libraries were prepared with the Accel-NGS 2S PCR-free library prep kit (Swift Bio). Paired-end sequencing was performed on a HiSeq 2500 by Perkin Elmer NGS services. Analysis was performed using BWA, GATK and Picard tools.



Cell-free DNA has a narrow size distribution, centering around 165 bp.

Conclusions

Accel-NGS Methyl-Seq library prep kit:
- Provides uniform, comprehensive methylome coverage.
- Enables liquid biopsy for genome-wide hypomethylation from 5 ng cfDNA.

Accel-Amplicon 56G targeted sequencing panel:
- Provides quality performance with > 95% on target and > 95% coverage uniformity.
- Enables a limit of mutation detection of 1% for liquid biopsy from 10 ng cfDNA.

Using both kits for liquid biopsy, a correlation was observed between percent hypomethylation and mutation detection for the tumor bearing cfDNA sample set presented.

The Accel-NGS 2S library prep kit:
- Provides uniform, comprehensive genome coverage.
- Enables PCR-free cfDNA sequencing from 10-15 ng input .

Thanks to Brian Gerwe, Alex Lopez and Mike Benway (Perkin Elmer) for cfDNA extraction and sequencing

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