Abstract # 5076

Research Using Rapid Assessment of AML Genes for Mutation Detection

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ABSTRACT

Targeted sequencing using the Ion AmpliSeq™ Library kit combined with the Ion PGM™ sequencing instrument is a fast and effective research method to identify genetic variants in tumor samples. A new targeted primer panel for amplification of genes involved in Acute Myeloid Leukemia (AML) has been developed by Life Technologies. The panel covers 19 genes characterized using 237 specific primer pairs in two highly multiplexed PCRs. To demonstrate the coverage efficiency, we evaluated libraries prepared from isolated genomic DNA. When libraries from 4 samples were run on a single Ion 318™ Chip, the average coverage depth was >3000x, with >97% of the target bases covered >500X. Additionally, >80% of reads were on-target. The panel was tested on control samples and analyzed using the Ion Reporter™ Software, and expected variants were detected with high sensitivity and specificity. In addition, improvements to template preparation and sequencing with the Ion Hi-Q™ Sequencing Chemistry (unreleased product in development) resulted in a higher percentage of end to end reads and uniformity, as well as reduced strand bias.

INTRODUCTION

Acute Myeloid Leukemia (AML) has been shown to be caused by multiple somatically acquired mutations that lead to various outcomes of clinical disease and corresponding treatment[1,2]. Recent advances in next generation sequencing research have lead to the discovery of hundreds of mutations that could lead to a path of informed treatment options in the future[3,4]. The rapid and accurate identification of these mutations may in the future be used to improve a patient's prognosis and potentially prevent relapse of the disease.

The Ion AmpliSeq™ Technology allows single-tube multiplex PCR of up to 24,576 amplicons that together with the Ion PGM™ Sequencing Platform yield fast turn around for results and variant calling[5]. Recently Life Technologies introduced the development of the Ion Hi-Q™ Sequencing Chemistry (unreleased product in development) that reduces consensus insertion and deletion (indel) errors, including homopolymer errors, by 90 percent across 400 base pair read lengths, resulting in higher data quality. Life Technologies has developed an Ion AmpliSeq™ AML Research Panel that can be applied with these tools to further the discovery and analysis of AML mutations in a fast and cost-effective manner.

MATERIALS AND METHODS

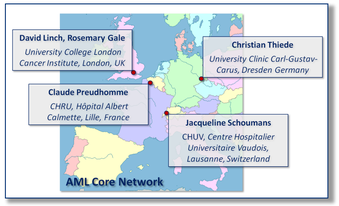
The Ion AmpliSeq™ AML Research Panel was developed with the AML Core Network, a group of leading AML researchers that defined the list of gene targets for design and conduct panel verification testing (Fig. 1). 19 genes were selected for the final design, including genes that are significantly mutated as suggested by the Cancer Genome Atlas Research Network[4] (Fig. 2). The Ion AmpliSeq™ AML Research Panel primer design was generated using the Ion AmpliSeq™ Designer proprietary methodology and customized optimization for challenging regions to create two primer pools of 124 and 113 amplicons (237 total).

The Ion AmpliSeq™ Library Preparation Protocol was used to generate AmpliSeq™ AML Research Libraries with genomic DNA samples from NA12878, NA19240, and NA01201 (Coriell Cell Repositories). Standard Kit Conditions for template preparation and sequencing used the Ion PGM™ Template OT2 200 Kit and Ion PGM™ Sequencing 200 Kit v2, respectively, for evaluation of the generated libraries. Conditions were modified and optimized for the Ion PGM™ OT2 template preparation and the Ion Hi-Q™ Sequencing Chemistry. All libraries were sequenced on Ion 318™ Chips.

All results were analyzed using the Ion Torrent™ Browser, the Coverage Analysis Plugin, and the Ion Reporter™ Software.

RESULTS

Figure 1. AML Core Network

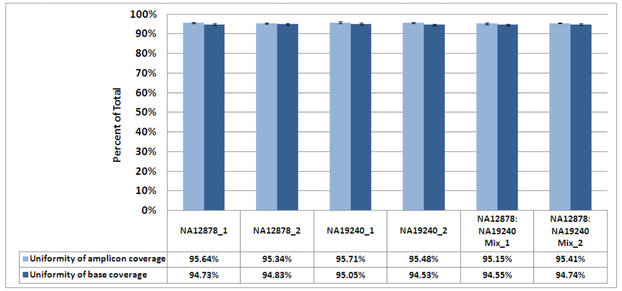


The Life Technologies FALCON team collaborated with leading AML researchers from France, Germany, Switzerland and the UK to define the list of gene targets, regions of interest, and design requirements for the Ion AmpliSeq™ AML Research Panel. The resulting panel is currently being evaluated by the Network researchers, an additional set of researchers across a North American network, and Life Technologies R&D.

Figure 2. Gene targets covered by the Ion AmpliSeq™ AML Research Panel

Gene Target	Chromosome	Design Target	# of Amplicons
ASXL1	20	exon 12	28
BRAF	7	exon 15, codon V600	1
CBL	11	exon 8, 9	5
CEBPA	19	all coding exons	9
DNMT3A	2	all coding exons	42
FLT3	13	codons 676, 830-850	3
GATA2	3	all coding exons	20
IDH1	2	exon 4	3
IDH2	15	exon 4	2
JAK2	9	exon 14	1
KIT	4	exons 8, 10, 11, 17	8
KRAS	12	exons 2, 3, and 4	4
NPM1	5	exon 11	1
NRAS	1	exon 2, 3, and 4	3
PTPN11	12	exons 3, 7, 8, 13	5
RUNX1	21	exons 3-8	15
TET2	4	all coding exons	59
TP53	17	all coding exons	24
WT1	11	exons 7, 9	4

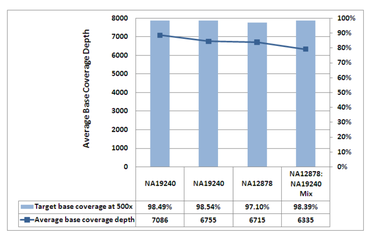
Figure 3. Uniformity of Base Coverage with the Ion AmpliSeq™ AML Research Panel



	NA12878_1	NA12878_2	NA19240_1	NA19240_2	NA12878: NA19240 Mix_1	NA12878: NA19240 Mix_2
Uniformity of amplicon coverage	95.64%	95.34%	95.71%	95.48%	95.15%	95.41%
Uniformity of base coverage	94.73%	94.83%	95.05%	94.53%	94.55%	94.74%

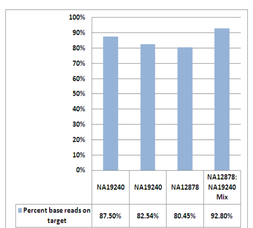
The resulting libraries generated at Life Technologies R&D with the Ion AmpliSeq™ AML Research Panel and two different genomic DNAs show >95% Uniformity of Amplicon Coverage and >94% Uniformity of Base Coverage (percentage of bases covered at >0.2X of the mean coverage).

Figure 4. Ion AmpliSeq™ AML Research Panel coverage depth and on-target results for 4 libraries combined on an Ion 318™ Chip



	NA19240	NA19240	NA12878	NA12878: NA19240 Mix
Target base coverage at 500x	98.49%	98.54%	97.10%	98.39%
Average base coverage depth	7086	6755	6715	6335

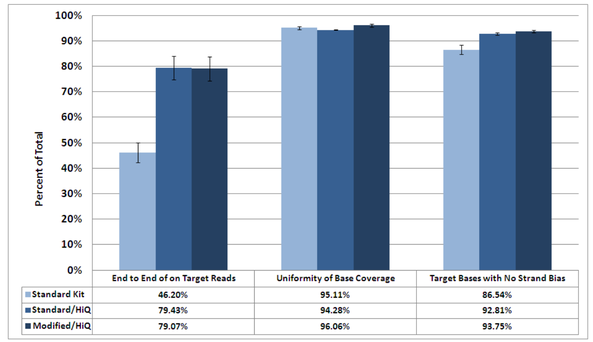
a. Combination of 4 Ion AmpliSeq™ AML Research Libraries on an Ion 318™ Chip results in >3000X average coverage depth with >97% of the target bases covered at >500X.



	NA19240	NA19240	NA12878	NA12878: NA19240 Mix
Percent base reads on target	87.50%	82.54%	80.45%	92.80%

b. The Ion AmpliSeq™ AML Research Libraries show >80% base reads on target.

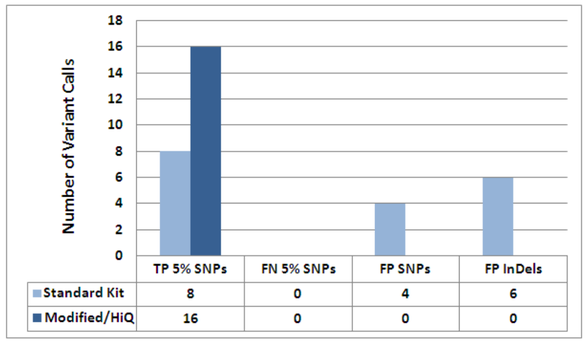
Figure 5. Improvements in AmpliSeq™ AML Research Library metrics with Hi-Q™ Sequencing Chemistry



	End to End of on Target Reads	Uniformity of Base Coverage	Target Bases with No Strand Bias
Standard Kit	46.20%	95.11%	86.54%
Standard/HiQ	79.43%	94.28%	92.81%
Modified/HiQ	79.07%	96.06%	93.75%

Ion Hi-Q™ Sequencing Chemistry together with Standard Template Preparation Conditions show improved percentage of end to end reads, uniformity of base coverage, and reduction in strand bias with the AmpliSeq™ AML libraries generated. Additional improvements can be made with further optimization of the Template Preparation Conditions.

Figure 6. Improvements in variant calling with Hi-Q™ Sequencing Chemistry

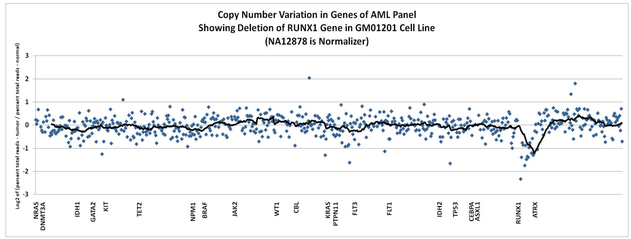


	TP 5% SNPs	FN 5% SNPs	FP SNPs	FP InDels
Standard Kit	8	0	4	6
Modified/HiQ	16	0	0	0

Ion Hi-Q™ Sequencing Chemistry shows improved variant calling over Standard Kit conditions. A DNA sample mix of NA12878 (10%) and NA19240 (90%) was used to evaluate variant calling with the Ion AmpliSeq™ AML Research Panel. Ion Hi-Q™ Sequencing Chemistry identifies a greater number of TP SNPs without generating FN and FP calls.

TP 5% SNPs denotes True Positive Single Nucleotide Polymorphisms detected with 5% variant calling parameters. FN 5% SNPs denotes False Negative Single Nucleotide Polymorphisms identified with 5% variant calling parameters. FP SNPs denotes False Positive Single Nucleotide Polymorphisms identified. FP InDels denotes False Positive Insertion and Deletions identified.

Figure 7. Ion AmpliSeq™ data can be used to detect copy number variation (CNV)



The Ion AmpliSeq™ Comprehensive Cancer Panel was used to detect a CNV deletion in the RUNX1 gene with Standard Kit Conditions. As a normal reference, the data for other genes covered in the Ion AmpliSeq™ AML Research Panel are shown.

CONCLUSIONS

The Ion AmpliSeq™ AML Research Panel combined with the Ion PGM™ sequencing platform is a rapid method to identify variants in archived samples. The Panel described here shows >94% uniformity per base. When 4 barcoded libraries are run on a single Ion 318™ Chip, the average coverage depth was >3000x, with >97% of the target bases covered >500X. Additionally, >80% of reads were on-target. The Ion AmpliSeq™ AML Research Panel together with the latest Ion Hi-Q™ Sequencing Chemistry, results in higher end-to-end reads, uniformity of base coverage, and more accurate variant calling.

REFERENCES

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ACKNOWLEDGEMENTS

Thank you to Hannah Viernes and the Life Technologies Falcon Consortia for their contribution to the Ion Community Panels for the development of clinical research workflows with leading research scientists.

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TRADEMARKS/LICENSING