Nestekah PatchPCR™ for Highly Multiplexed Targeted Sequencing in Cancer Samples

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Abstract

The ability to deeply sequence hundreds to thousands of targeted genomic regions across a large number of patient samples is crucial for adopting next-generation sequencing in clinical research and clinical practice setting. Various targeted capture methods have been developed for next-generation sequencing, but there is limited consensus on which approach is best when applied to large-scale clinical research applications. Many next-generation sequencing bias of targeted exome sequencing. High cost of reagents, low sample throughput, specialized equipment, and limited content design. To address these limitations, we have developed Nestekah PatchPCR™ to perform targeted sequencing across hundreds of regions in a 96-well format.

We have used Nestekah PatchPCR™ to simultaneously amplify 760 genomic regions, covering the exons of 62 genes that are frequently mutated in cancer. We used Nextera library construction and barcoding to sequence 96 samples on a single Illumina GAIIx platform. Nestekah PatchPCR™ was highly compatible with standard library construction/barcoding for sequencing on various platforms.

We have used Nestekah library construction and barcoding to sequence 86 samples on a single Illumina GAIIx platform. Nestekah PatchPCR™ was highly specific and sensitive, allowing us to achieve high coverage across the loci for sensitive detection of cancer mutations.

We have also created a more focused cancer panel (177 regions covering exons from 10 genes) that is sequenced down to take advantage of the throughput of the Archer Dx platform. The CRX panel is designed to maximize the number of samples that can be sequenced simultaneously using the Nestekah PatchPCR™ reaction so that all samples can be sequenced directly without any consuming library construction.

Nestekah PatchPCR Workflow

Targeting PCR
- 1 hr 20 min

Primer Removal
- 1 hr 30 min

Patch Ligation
- 2 hr 40 min

Off-Target Amplicon Degradation
- 2 hr 40 min

Universal PCR
- 2 hr 40 min

TargetRich™ Kits

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<th>Cancer Survey (CSP)</th>
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Results

CRX Kit Performance

% with >200X Read Depth

Target | Mean of Read Depth | SD | SD/Target

Target | Mean of Read Depth | SD | SD/Target

CRX-Rep1 92.04% 81 0.06 0.01 0.01
CRX-Rep2 91.00% 79 0.03 0.01 0.01
CRX-Rep3 91.46% 80 0.03 0.01 0.01
CRX-Rep4 93.39% 81 0.02 0.01 0.01
CSP-Rep1 92.53% 82 0.03 0.01 0.01
CSP-Rep2 92.45% 81 0.03 0.01 0.01
CSP-Rep3 93.07% 82 0.03 0.01 0.01
CSP-Rep4 93.07% 81 0.03 0.01 0.01

CSP Kit Performance

% with >200X Read Depth

Target | Mean of Read Depth | SD | SD/Target

Target | Mean of Read Depth | SD | SD/Target

CRX-Rep1 90.32% 81 0.03 0.01 0.01
CRX-Rep2 92.24% 81 0.03 0.01 0.01
CRX-Rep3 91.46% 80 0.02 0.01 0.01
CRX-Rep4 93.39% 81 0.03 0.01 0.01
CSP-Rep1 93.45% 81 0.02 0.01 0.01
CSP-Rep2 92.24% 81 0.03 0.01 0.01
CSP-Rep3 93.07% 82 0.03 0.01 0.01
CSP-Rep4 93.07% 81 0.03 0.01 0.01

Spec Comparison

Kailos CRX versus Ion AmpliSeq Cancer Panel

Target | Mean of Read Depth | SD | SD/Target

Target | Mean of Read Depth | SD | SD/Target

Kailos CRX 94.72% 81 0.03 0.01 0.01
Ion AmpliSeq 86.11% 81 0.03 0.01 0.01

Conclusions

Nestekah PatchPCR provides highly specific, multiplexed PCR of a large number of targeted loci for next-generation sequencing. New analysis is Kailos Genetics TargetRich™ Kits:

- SPECIFIC - high on-target alignment rate across co-targeted sequence
- SENSITIVE - improved uniformity provides high coverage across targets for sensitive mutation detection
- LOW STARTING MATERIAL - requires just 20 ng DNA
- SCALABLE - samples can be prepared in parallel in 96-well plates
- EASY - addition only reactions on standard thermocyclers
- FAST - single day workflow

PARTICIPATE IN THE EARLY ACCESS PROGRAM
For additional information, call 1-844-625-8300 or visit www.kailosgenetics.com
kailosgenetics.com/targetrich