

QIAseq[®] xHYB Long Read Hereditary Cancer Panel targeted-capture accurately detects large structural variants through a streamlined workflow and straightforward analysis pipeline



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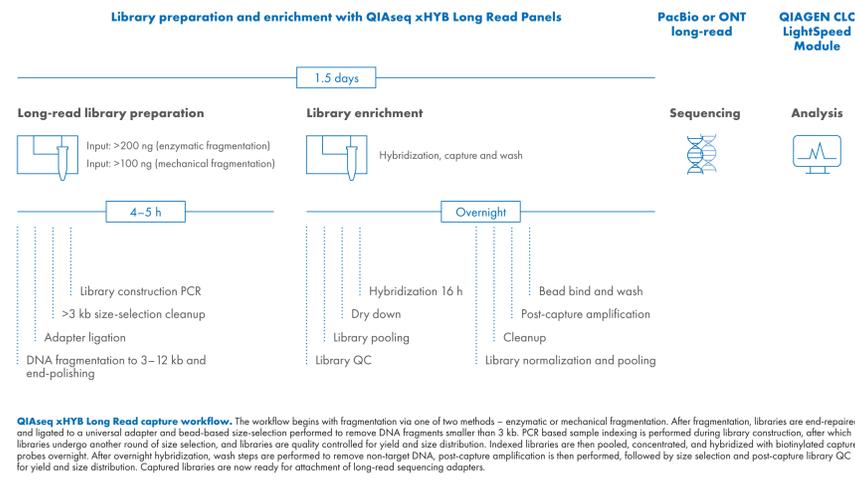
Large structural variant detection with the QIAseq xHYB Long Read Hereditary Cancer Panel

Introduction: Targeted long-read sequencing enables characterization of novel, complex mutations since long reads can span and be phased across large genomic regions. Long-read hybrid capture offers probe design flexibility for capturing targeted regions with unresolved structural variants. Here, we describe the development of QIAseq xHYB Long Read Hereditary Cancer Panel and chemistry for detection of large structural variants in 95 known cancer driver genes.

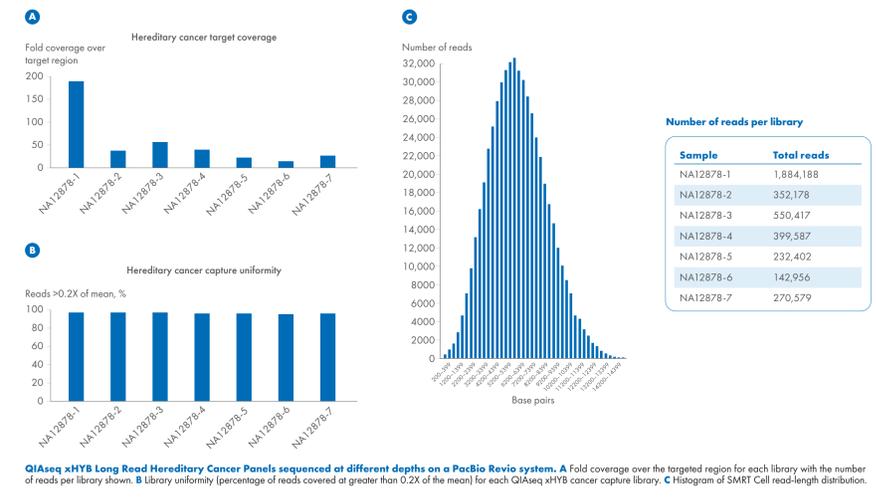
Methods: The QIAseq xHYB Long Read Hereditary Cancer Panel and analysis pipelines were used to identify large structural variants in genes involved in cancer progression that human reference DNA is known to harbor. Libraries were prepared using either enzymatic or mechanical fragmentation modules, after which targeted regions were captured with probes optimally designed for long DNA fragments. Captured DNA was amplified with chemistry developed for long, fast PCR and sequenced on both Pacific Biosciences[®] (PacBio[®]) and Oxford Nanopore Technologies (ONT) platforms. The resulting long-read sequencing data was mapped and large structural variants detected with QIAGEN[®] CLC LightSpeed Module on the CLC Genomics Workbench.

Results: Enzymatic and mechanical fragmentation produced libraries with average read lengths of 4.5 kb and 5.5 kb, respectively. Both fragmentation methods produce libraries with similar coverage and uniformity. Sequencing eight QIAseq xHYB Hereditary Cancer Panel libraries from one capture pool on a PacBio Revio[®] SMRT[®] Cell yields 30X average coverage, and uniformity greater than 95% of reads >0.2X of the mean. Sequencing the same libraries on the ONT MinION[®] platform for 24 hours produced 1 million reads, which resulted in 12X average coverage, and uniformity greater than 90% of reads >0.2X of the mean. Downstream analysis with the CLC LightSpeed Module clearly identified the expected large structural variants in different samples.

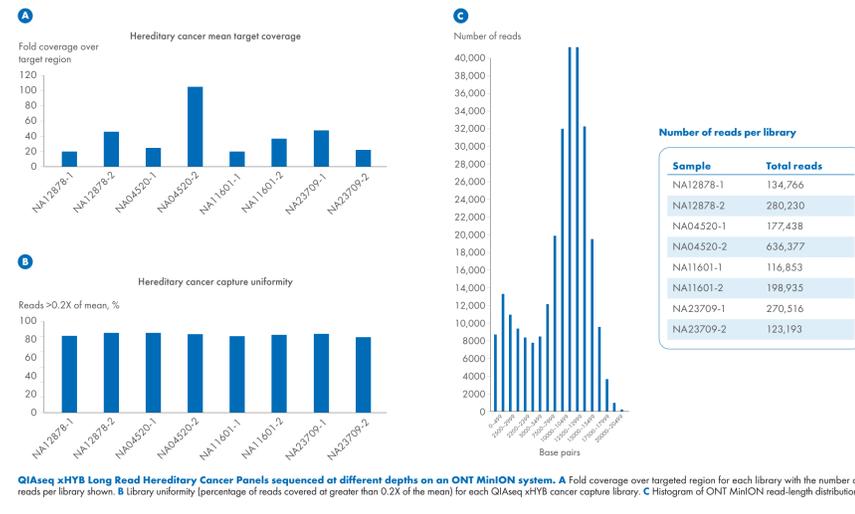
Flexible sample to sequencing workflow with two fragmentation methods



QIAseq xHYB Long Read Hereditary Cancer Panels sequenced on the PacBio Revio System show excellent uniformity



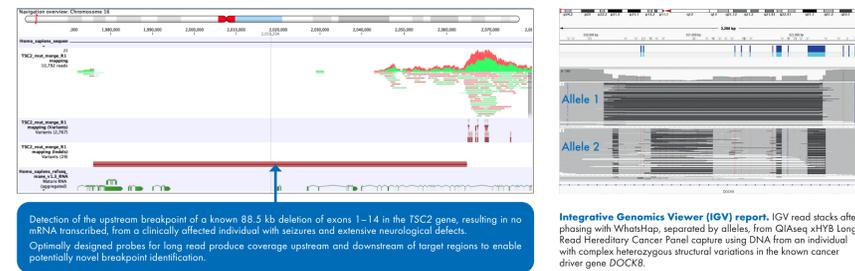
QIAseq xHYB Long Read Hereditary Cancer Panels sequenced on ONT MinION provide guidelines for coverage depth and uniformity



Detection of large structural and complex heterozygous variants

The QIAseq xHYB Long Read Hereditary Cancer Panel enables detection of large structural and complex heterozygous variants with QIAGEN CLC LightSpeed Module. Phasing with haplotagging using WhatsHap shows clear allelic separation. Two examples are shown below.

- *TSC2* which regulates mTOR signaling to control cell growth and division and plays an important role in neuronal homeostasis
- *DOCK8*, which regulates signal transduction in immune cells. *DOCK8* deficiency is associated with increased risk for lymphoma and squamous cell carcinoma



QIAGEN CLC LightSpeed Module analysis of the *TSC2* gene in a clinically affected individual. A large deletion upstream of *TSC2* is shown. The 88.5 kb deletion was detected using QIAseq xHYB Long Read Hereditary Cancer Panel capture and CLC LightSpeed Module structural variant caller for long reads.

Conclusions

- The QIAseq xHYB Long Read Hereditary Cancer Panel produces even coverage through a robust workflow. It enables accurate detection of large structural variants in known cancer-driver genes with a user-friendly, long-read large structural variant calling pipeline using QIAGEN CLC LightSpeed Module on the CLC Genomics Workbench.
- QIAseq xHYB Long Read enzymatic and mechanical fragmentation options both produce long-read libraries that result in 4–6 kb average read lengths with robust uniformity and coverage that are compatible for sequencing on PacBio and Oxford Nanopore platforms.
 - After long-read sequencing, the known large structural variants were accurately identified. The probe design targets entire genes, including introns and UTR's, capturing extended regions of genes in an unbiased manner.
 - This allows for the detection of novel structural variants and breakpoints without prior knowledge.

QIAseq xHYB Long Read Panels are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

PacBio products are for research use only. Not for use in diagnostic procedures.

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