Candidate gene screening using long-read sequencing

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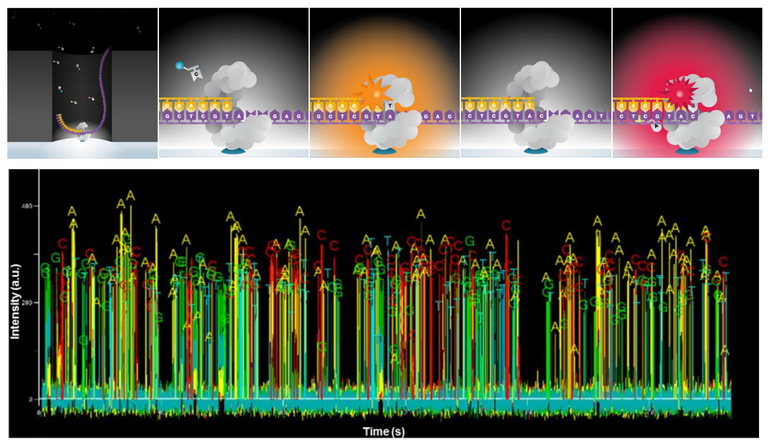
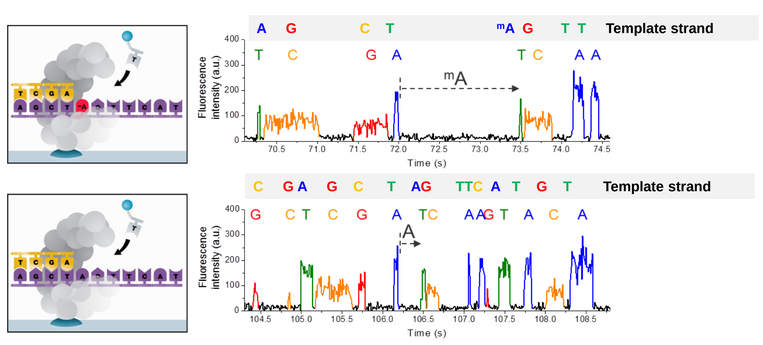
SMRT Sequencing overview

We have developed several candidate gene screening applications for both Neuromuscular and Neurological disorders. The power behind these applications comes from the use of long-read sequencing. It allows us to access previously unresolvable and even unsequencable genomic regions. SMRT Sequencing offers uniform coverage, a lack of sequence context bias, and very high accuracy. In addition, it is also possible to directly detect epigenetic signatures and characterize full-length gene transcripts through assembly-free isoform sequencing.



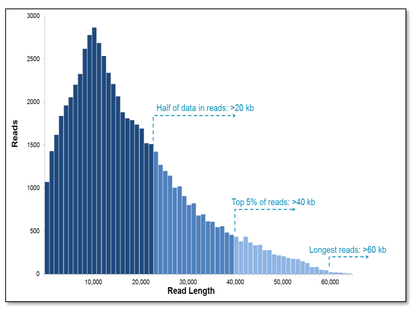
In addition to calling the bases, SMRT Sequencing uses the kinetic information from each nucleotide to distinguish between modified and native bases.



Example: N^6-methyladenine

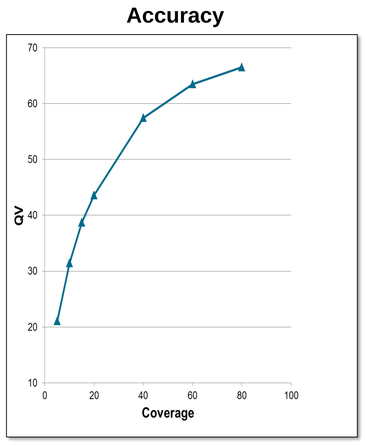
Long-read sequencing data properties

Read Length



Data generated with 20 kb size-selected human library using 6 hour movies with P6-C4 chemistry using PacBio RS II, analyzed with SMRT® Analysis v 2.3. Each SMRT Cell generates ~55,000.

Accuracy



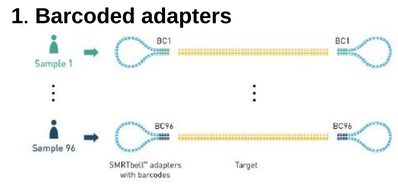
E. coli 20 kb-insert library, SMRT Analysis v2.3

Targeted sequencing and multiplexing

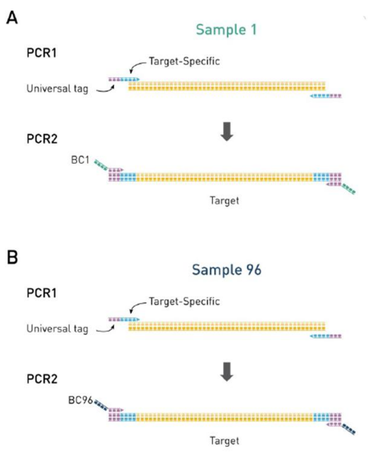
Targeted sequencing workflow

Barcoding options

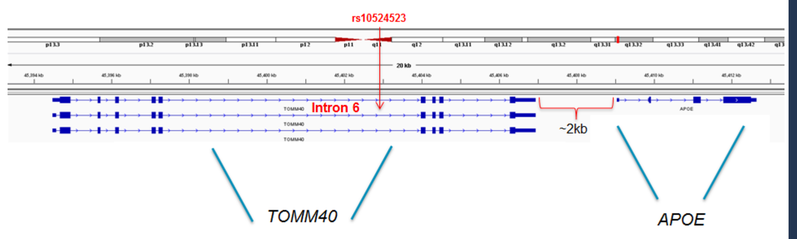
1. Barcoded adapters



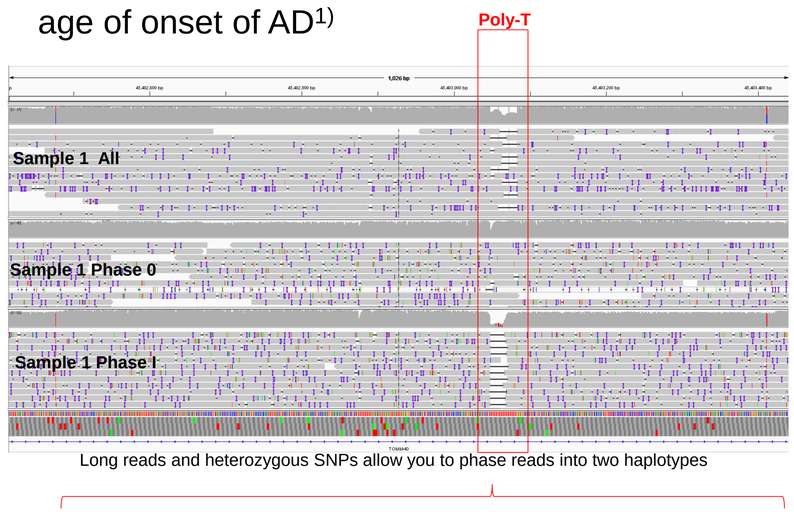
2. Barcoded Universal Primer



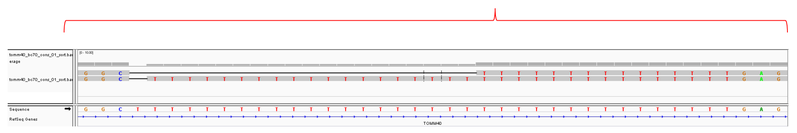
Alzheimer's disease (AD): *TOMM40* gene



A variable poly-T repeat at the rs10524523 SNP within intron 6 of the *TOMM40* gene that in combination with *APOE3* allele will affect the age of onset of AD[1)]

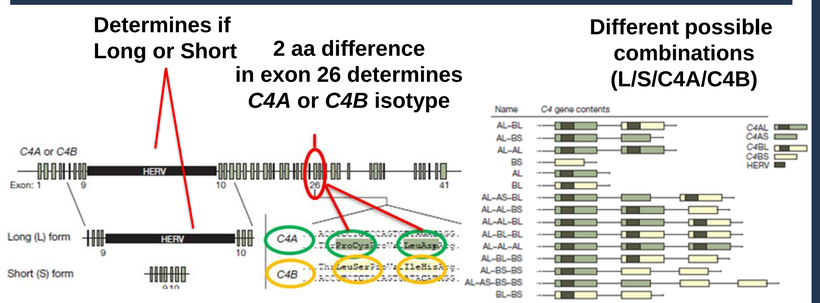


Long reads and heterozygous SNPs allow you to phase reads into two haplotypes



We successfully captured and sequenced the associated poly-T repeat in the *TOMM40* gene and were able to determine that the sample had one short (15) allele and one Very Long (34) allele.

Schizophrenia: *C4* gene



<u>The *C4* structural variant is highly complex:[2)]</u>

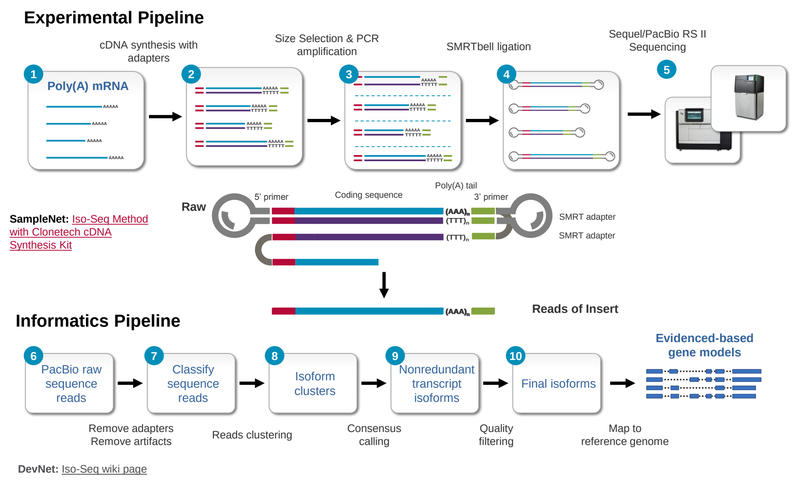
- Two functionally distinct genes (isotypes); *C4A* and *C4B*
- Both isotypes can have 1 - 3 functional copies
- A human endogenous retroviral (HERV) insertion in intron 9 changes the length of the gene

C4 plays a role in signaling which connections between neurons should be “pruned” or removed, as the brain develops after childhood. And the more *C4* was present, the higher the risk of developing schizophrenia. Certain versions of the *C4* gene seem to increase people’s risk for developing schizophrenia by 27 to 50 percent.

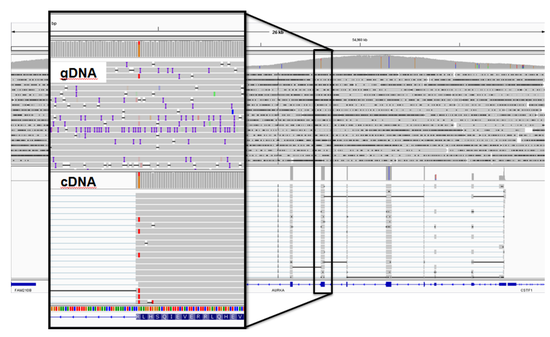
We successfully captured and sequenced the *C4* gene as part of a MHC capture panel. We were able to see the two different *C4* isotypes (*C4A* and *C4B*) as well as seeing the 7 kb HERV insertion in intron 9.

Example: Isotype C4A/L was successfully called for a sample using long-read sequencing

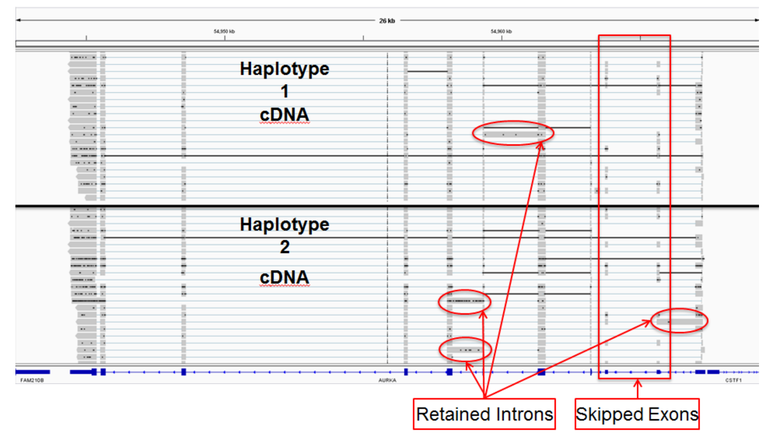
Gene isoform characterization



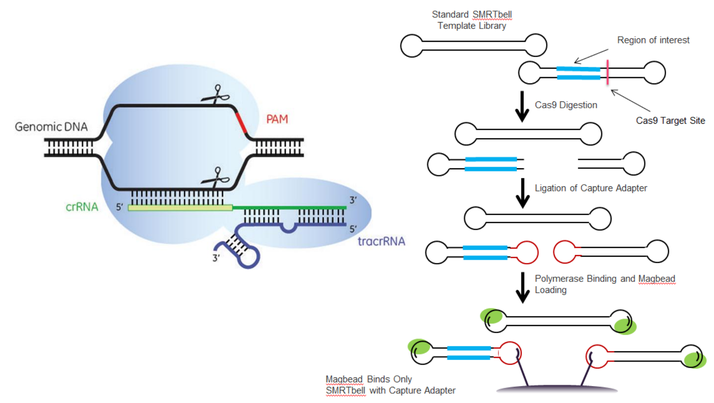
gDNA & Transcripts from SK-BR-3 Cell Line Captured with NimbleGen Oncology Panel - example *AURKA* gene



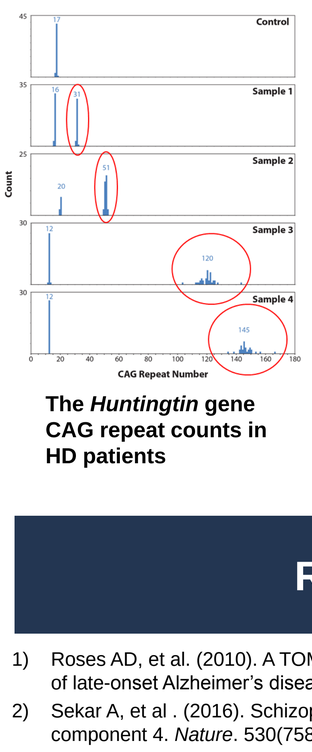
Phased Transcripts reveal retained introns and skipped exons



Amplification-free targeted sequencing using CRISPR/Cas9

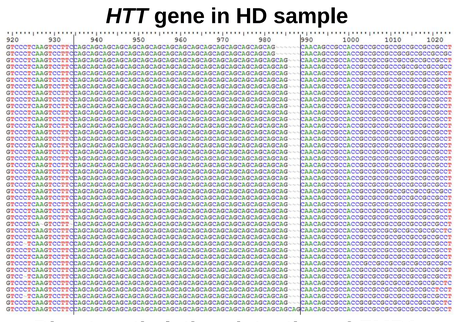


Repeat expansion disorders are challenging to interrogate due to the long repetitive regions. Using CRISRP/Cas9 we are able to access the repeat counts, interruption sequences as well as epigenetic information without introducing PCR bias.

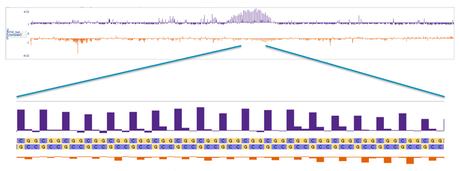


The *Huntingtin* gene CAG repeat counts in HD patients

***HTT* gene in HD sample**



Direct methylation detection of *FMR1* pre-mutation sample



CGG repeat region appears to be heavily methylated (5mC)

References

1) Roses AD, et al. (2010). A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer’s disease. Pharmacogenomics J. 10(5): 375-84
2) Sekar A, et al . (2016). Schizophrenia risk from complex variation of complement component 4. *Nature*. 530(7589):177-83

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