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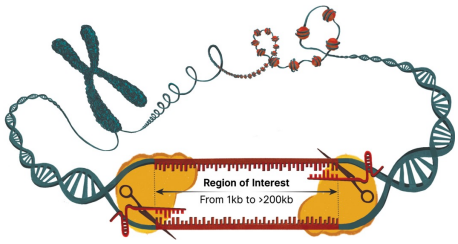
Targeted long-read sequencing and epigenetic profiling

We are evaluating technologies to interrogate specific genomic regions using long-read sequencing. These tools can resolve complex genomic regions, while also providing information about DNA methylation, and there are important applications both in clinical diagnostics and biomedical research.

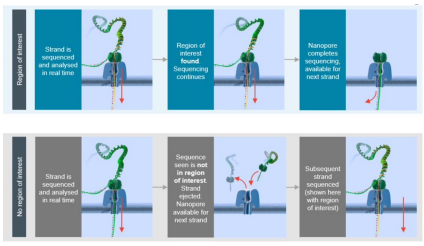
Methods

Two methods for amplification-free long-read sequencing are currently being tested. They allow for rapid sequencing on the portable Nanopore MinION instrument.

Cas9 capture sequencing



Adaptive sampling

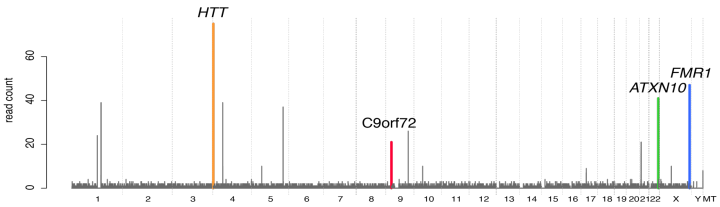


Use case 1: Repeat expansion disorders

Long-read sequencing enables to study regions that are challenging to amplify by PCR. This opens new possibilities for diagnostics of diseases caused by repeat expansions. Together with Clinical Genomics Uppsala, we are designing assays that allow to interrogate several repeat expansion genes simultaneously. The methods are tested on DNA from patient samples.

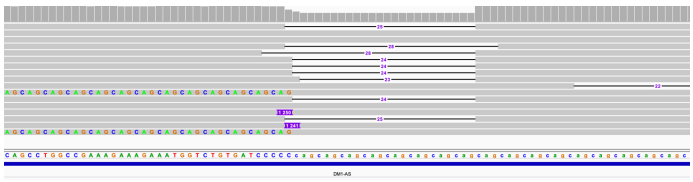
Coverage for selected repeat expansion genes

Cas9 enrichment on DNA from a Huntington's Disease patient



DM1 gene (Myotonic Dystrophy)

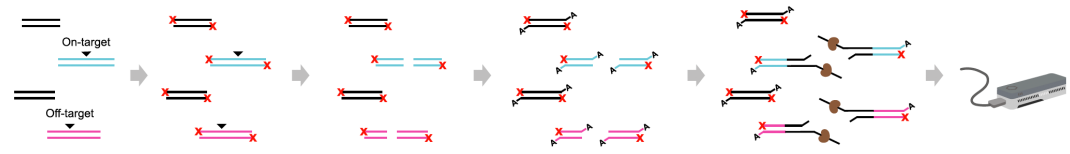
A ~1250bp heterozygous repeat resolved by adaptive sampling

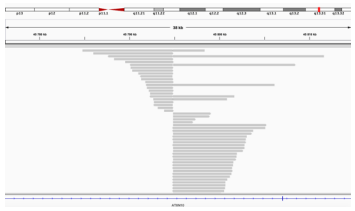


Use case 2: Genome editing outcomes

We also develop long-read sequencing assays to characterize of outcomes of CRISPR-Cas9 genome editing, both at the intended (on-target) site as well as at unintended (off-target) sites.

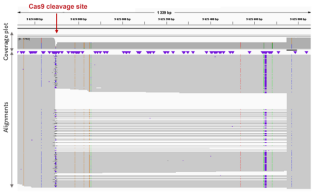
Nano-OTS: Identification of off-target Cas9-cleavage sites in vitro



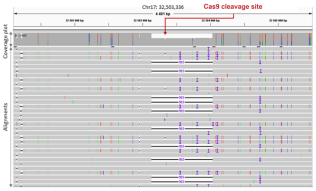


After CRISPR-Cas9 editing, the on-target site and all identified off-target sites can be analyzed using long-read sequencing. This enables identification of *off-target mutations*, *mosaicism* and *structural variation* in the edited cells. Two examples of large deletions in edited zebrafish are shown to the right.

On-target deletion, *ywhaqa* (1054 bp)



Off-target deletion, *sh2b3* (903 bp)



SciLifeLab has been created by the coordinated effort of four universities in Stockholm and Uppsala: Stockholm University, the Karolinska Institutet, KTH Royal Institute of Technology and Uppsala University.