Analysis of the Elements Involved in the Enrichment of a Panel of Genomic Regions by Nanopore Sequencing Using Adaptive Sampling

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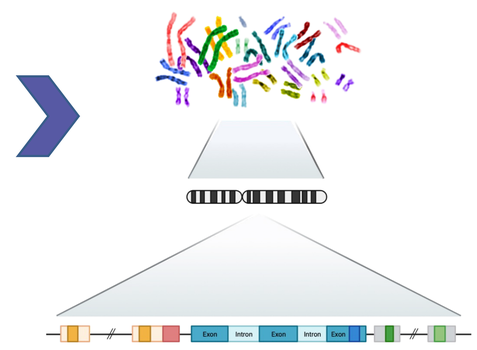
Background

One of the main limitations of nanopore sequencing is the **low coverage** obtained.

Different methods may increase coverage, but **adaptive sampling** is the only one which does not require manipulation and can be used in MinION.



Real-time targeted sequencing

AIM: To dissect the factors involved in the **enrichment** process using adaptive sampling

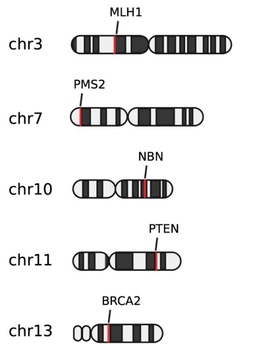
Materials & Methods

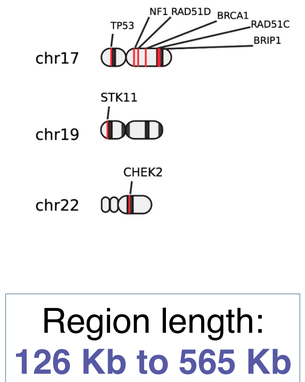
Samples

Sequencing: 18 regions involving 20 genes

Negative for NGS screening

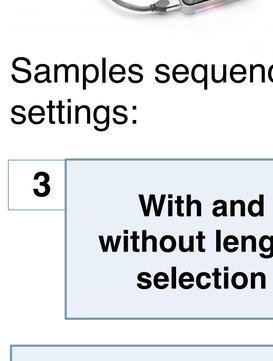
16 cases with familial breast cancer





Region length: **126 Kb to 565 Kb**

Nanopore sequencing



8	**Length selection (>1,000 kb)**
5	**Without length selection**

Samples sequenced **twice** with **two different** settings:

3	**With and without length selection**	1	**DNA fragmentation (g-TUBE Covaris® for 6 Kb fragments)**

Different sequencing times

Results

Coverage reached with this method:	**Region of Interest 5.1x**	Up to **13.0x**	**Rest of the Genome 2.0x**	Up to **3.2x**

We have analyzed the **parameters** and **elements** potentially involved in the enrichment process by using two different approaches:

1) Analysis of samples:

Long Read Selection

$$Ratio = \frac{Coverage\ area}{Coverage\ genome}$$

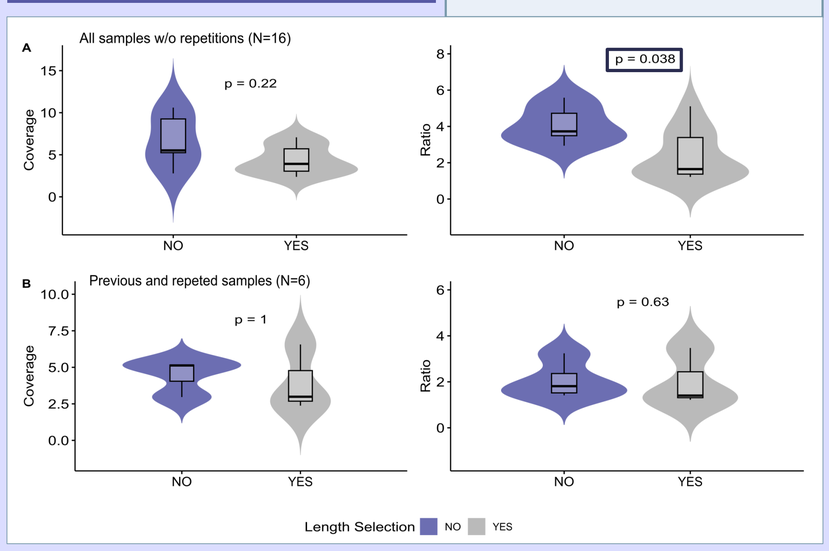


Figure 1. Average coverage and enrichment (ratio) with or without long read selection. **A)** Full cohort of samples **B)** Three Paired samples

Sequencing time

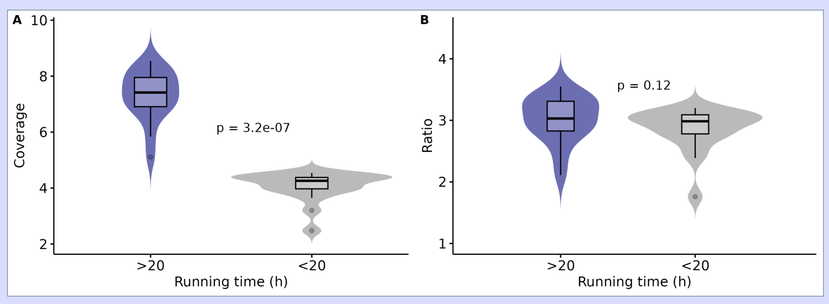


Figure 3. Average coverage **A)** and enrichment **B)** as a function of the running time

Sequencing time (>20 h) is the main element improving the coverage of selected regions. Likewise, it also improves coverage in the rest of the genome.

Selection of long reads (>1,000 Kb) and **DNA fragmentation** did not improve the enrichment yield.

2) Analysis of regions:

One region was **less covered: *PMS2.***

	PMS2	**VS**	**Rest of the regions**
Average	**3.3x**		**4.5x**

Fragmentation

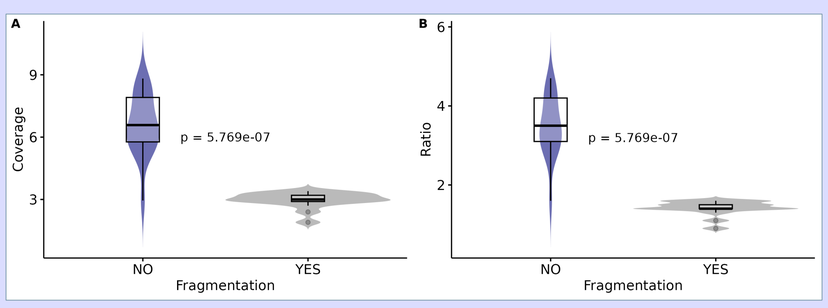


Figure 2. Average coverage **A)** and enrichment **B)** of regions of interest according to DNA fragmentation

Elements evaluated

Repetitive elements | **Coding regions** | **Homopolymers** → Did **not affect** the process

Pseudogenes

NF1	*CHEK2*	***PMS2***
12	7	**14**

Table 1. Regions with a high number of pseudogenes

Coverage of other pseudogenes: Similar or higher than the gene of interest

High homology between genes and pseudogenes

Conclusions

Analysis of factors that could influence the effectiveness of adaptive sampling in a panel of 18 genomic regions using the accessible MinION device revealed these **two factors as the most relevant** for providing **higher coverage** in the areas of interest:

1. **Sequencing time**
2. **Presence of pseudogenes**

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