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Optical Genome Mapping Analysis of *FMR1* Expansions in Fragile X Syndrome and Multi-site Validation

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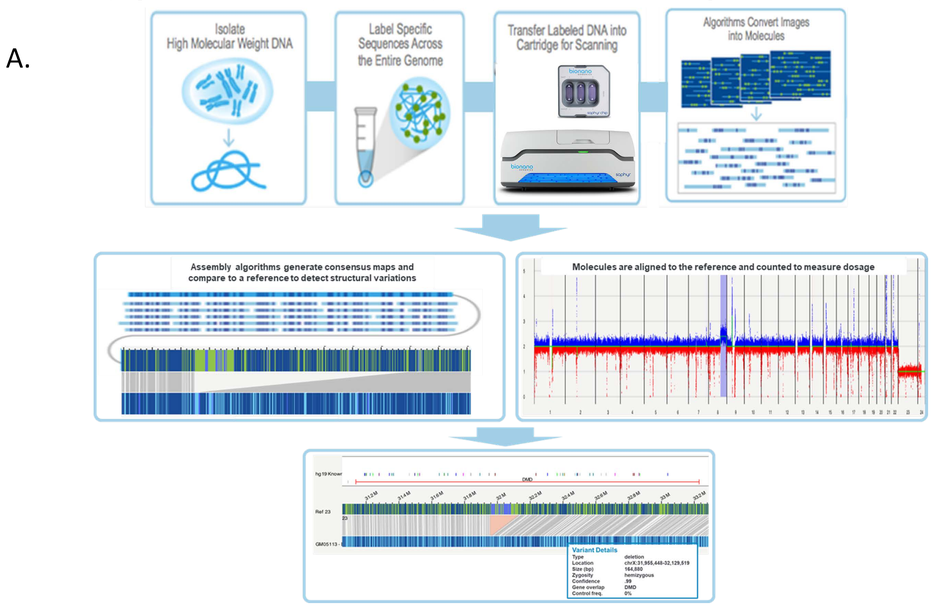
Background/Objectives

Fragile X syndrome (FXS) is associated with intellectual disability, and is usually due to CGG expansion in *FMR1* [1]. Phenotype severity being correlated with expansion size, accurate sizing is crucial. The repetitive nature of these regions presents difficulties: 1. PCR is unable to traverse through long repeats; 2. Sequencing is limited short-read lengths; 3. Southern-blot is inaccurate, time-consuming, and expensive. Optical genome mapping (OGM) has the potential to address some of these shortcomings [2].

Methods

OGM images ultra-long DNA molecules, labeled at specific motifs linearized in nanochannel arrays, and can be used for SV and CNV calling. We developed a targeted analysis workflow for *FMR1* CGG repeat analysis. To evaluate the capability of measuring repeat arrays in ranges consistent with normal, premutation, and full expansions, we analyzed 75 FXS samples and 20 control subjects.



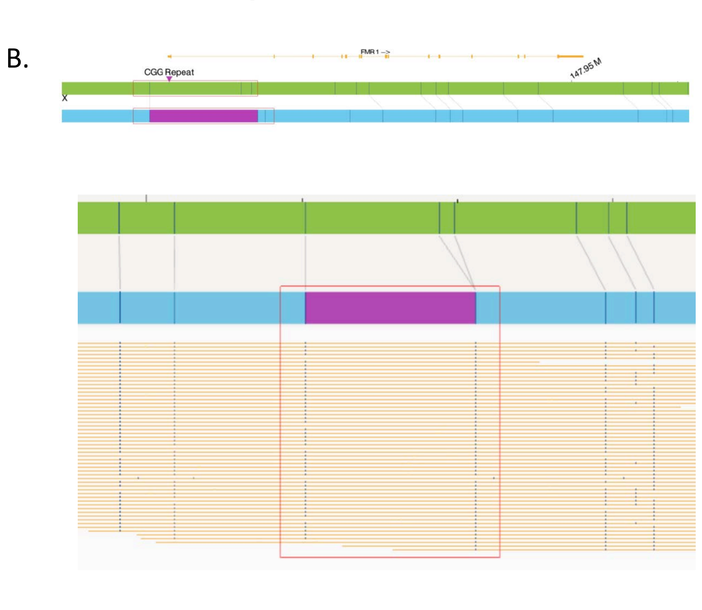
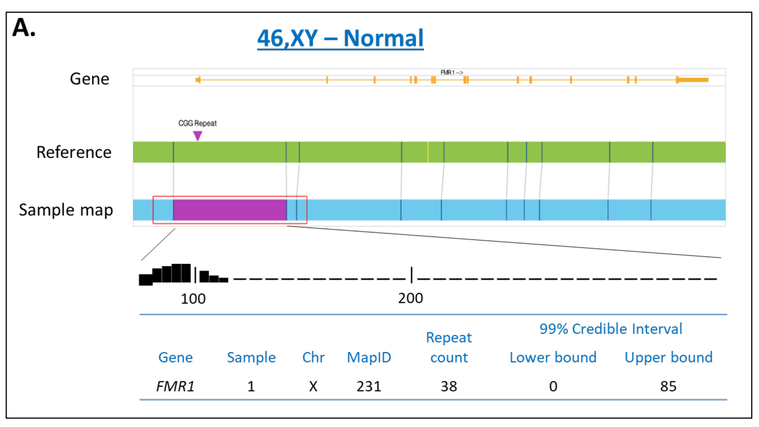


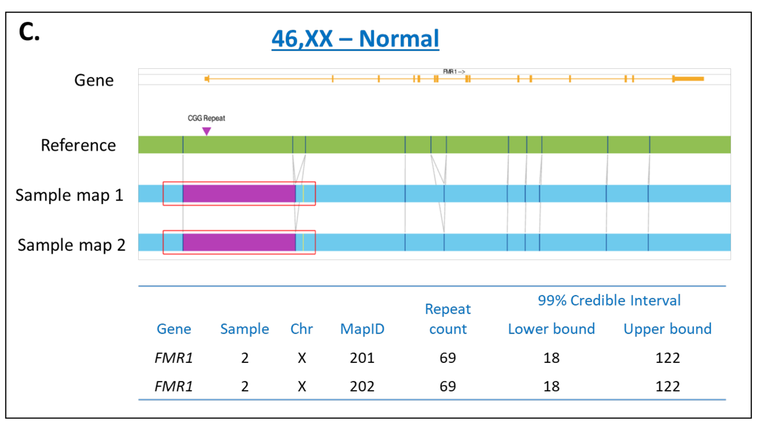
Figure 1. A) OGM pre-analytical and analytical workflow. High molecular-weight DNA is isolated, labeled and counterstained, then imaged sequentially in nanochannels on the Saphyr instrument. Analytical steps assemble molecules into consensus maps and construct an orthogonal copy number profile, which are aligned to GRCh37 or GRCh38 and annotated. B) Bionano optical map with *FMR1* CGG expansion (645 units).

Results

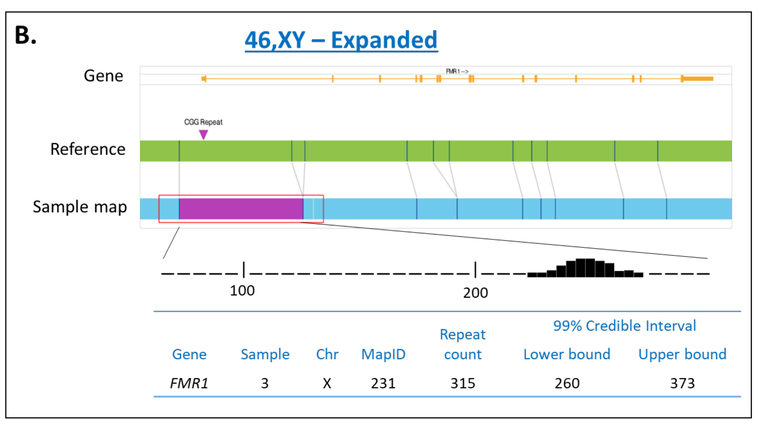
In annotated samples, we observed alleles consistent with annotation across the entire range of repeat counts. Sensitivity was measured at 97% with 100% PPV for expansions >200 repeats [3]. The largest expansion detected was ~1000 repeats. In controls, we measured CN below the full mutation cutoff in all cases. Repeatability studies were carried out to show analytical consistency. EnFocus™ analysis report provides pass/fail for QC metrics as well as analytic measurement quality using internal control regions on each autosome chromosome.



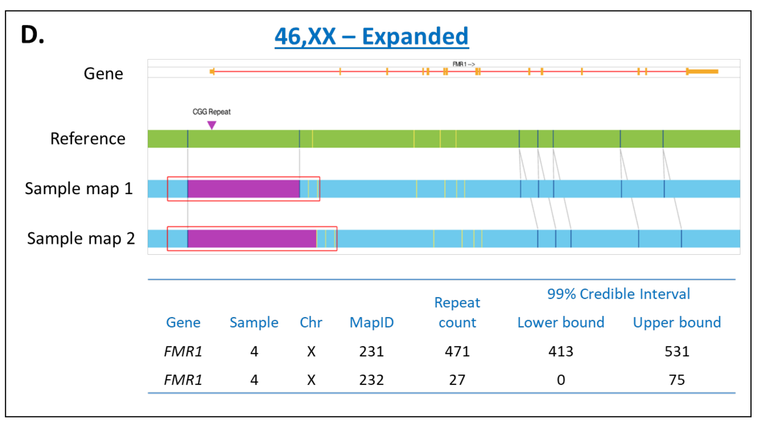
Gene	Sample	Chr	MapID	Repeat count	99% Credible Interval Lower bound	99% Credible Interval Upper bound
FMR1	1	X	231	38	0	85



Gene	Sample	Chr	MapID	Repeat count	99% Credible Interval Lower bound	99% Credible Interval Upper bound
FMR1	2	X	201	69	18	122
FMR1	2	X	202	69	18	122



Gene	Sample	Chr	MapID	Repeat count	99% Credible Interval Lower bound	99% Credible Interval Upper bound
FMR1	3	X	231	315	260	373



Gene	Sample	Chr	MapID	Repeat count	99% Credible Interval Lower bound	99% Credible Interval Upper bound
FMR1	4	X	231	471	413	531
FMR1	4	X	232	27	0	75

Figure 2. Visualization of A) Normal male with allele 1 of 38 repeat units; B) Male with allele of 315 repeat units; C) Female with allele 1 of 69 repeats and allele 2 of 69 repeats; and D) Female with allele 1 of 27 repeat units and allele 2 of 471 repeat units.

Conclusions

OGM performance for the *FMR1* promoter CGG repeat lengths show a much higher dynamic range compared to PCR, NGS, and higher precision compared to Southern-blot.

References

1. Paulson, Handb. Clin. Neurol. 2018.
2. Sahajpal, Genes 2021.
3.https://bionanogenomics.com/wp-content/uploads/2021/11/30457-Bionano-Solve-Theory-of-Operation-Bionano-EnFocus-Fragile-X-Analysis.pdf

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