CRISPR-Assisted Electronic Genome Mapping for Robertsonian Translocations and FSHD

Erika Headrick, Wentao Huang, John F. Thompson, Lindsay Schneider Opsahl, and Syndi Koltz
Nabsys 2.0 LLC, Providence, RI

Nabsys

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Abstract

Assays for detecting and characterizing Robertsonian translocations (ROB) and FSHD are challenging for all genomic technologies due to the repetitive nature of the affected DNA. ROBs are particularly problematic due to their proximity to centromeres, the extreme repetitiveness of the satellite repeats, and the balanced nature of the changes. No genetic material is lost, so copy number methods cannot be used. Long-read sequencing methods are challenged by the highly repetitive nature of these regions. FSHD detection faces similar problems due to the near identity of sequences near the telomeres of chr4/chr10.

Electronic genome mapping (EGM) is increasingly being used to detect large structural variants. Ultra-long DNA is passed through nanochannels, where the presence of DNA and DNA tags can be readily distinguished through voltage changes. The time between tags can then be converted to distances to generate single-molecule maps. To insert tags, sample preparation currently uses nickases with 6-7 nucleotide recognition sequences, but in situations like ROB and FSHD, the tags are not suitably positioned to identify differences between normal and disease-state DNA. To circumvent this issue, we have used CRISPR D10A nickase to tag DNA at virtually any desired location.

Efforts are underway to optimize gRNAs for use with both ROB and FSHD assays. Because there are so few sequence differences, the range of gRNAs available is limited. Furthermore, many changes between the sequences examined differ by only one or two base pairs. Thus, it is necessary to assess how mismatches may impact nicking to ensure proper tag assignment during mapping. By choosing a gRNA that nicks at a desired frequency and spacing in the repetitive acrocentric region, a single gRNA can be used to create a characteristic pattern for all human acrocentric centromeres. The entire centromere cannot be analyzed with a single read, but molecules that flank the centromeric/non-centromeric boundary can be analyzed to determine whether recombination has occurred. Similarly, there are multiple changes in both the repeat region and flanking regions of chr4/chr10 responsible for FSHD that gRNAs can be designed and tested for specificity.

Combining CRISPR nicking with EGM offers the possibility of analyzing ROB without the need for cell culture or karyotyping as well as distinguishing pathogenic from non-pathogenic FSHD alleles. Progress towards creating such assays will be described.

Background

Electronic genome mapping (EGM) reveals large-scale DNA structure by electronically detecting sequence-specific tags on long, single DNA molecules. Distances between tags allow molecules to be aligned and structural differences to be identified.

Robertsonian translocations involve fusion of the long arms of two acrocentric chromosomes. Balanced carriers may be unaffected but have an increased risk of pregnancy loss or offspring with an unbalanced chromosome complement.

- **Challenging to characterize:** Highly repetitive centromeric sequences complicate molecular mapping, while balanced rearrangements can escape detection by copy-number methods.
- **Limited standard tagging:** Many centromeric regions contain few or no sites for standard EGM nicking enzymes, limiting mapping across these regions.
- **An opportunity for CRISPR:** Guide-directed nicking introduces additional tags, using sequence differences between centromeric repeats to develop chromosome-specific patterns.

Our objective is to develop CRISPR-assisted EGM tagging patterns that support mapping of acrocentric regions and detection of Robertsonian translocations.

Methods

Guide RNAs were designed to target repetitive sequences in human acrocentric chromosomes and complexed with Cas9 D10A nickase to introduce additional nick sites. DNA was prepared using CRISPR-directed nicking alone or in combination with standard nicking enzymes, then tagged and analyzed on the OhmX™ Platform. Experimental maps were compared with predicted tagging patterns to assess mapping across centromeric regions.

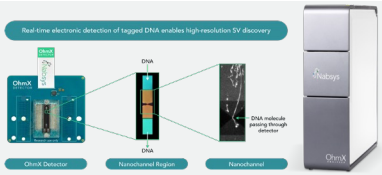


Figure 1. EGM detector and analyzer. EGM is enabled by the novel, solid-state nanodetectors developed by Nabsys. The OhmX Detector houses 256 parallel nanochannels, each with its own electronic sensor. Nabsys has developed DNA tagging chemistry that provides a high signal-to-noise. The high signal and spatial resolution allows tags to be closely spaced on the high molecular weight DNA molecules. This yields optimal tag density such that structural variations as small as 300 bp can be routinely identified and mapped genome-wide.



Figure 2. Overview of EGM and OhmX Genome Preparation workflow plus CRISPR. Cas9 ribonucleoprotein (RNP) complexes are made using sgRNA and dCas9 to block nick sites, or Cas9 D10A nickase to add nick sites to target DNA. RNPs are mixed with DNA prior to the OhmX Genome Preparation workflow. Then, the DNA is loaded onto the OhmX Detector on the OhmX Analyzer for sample analysis.

Mapping the Centromeric Challenge

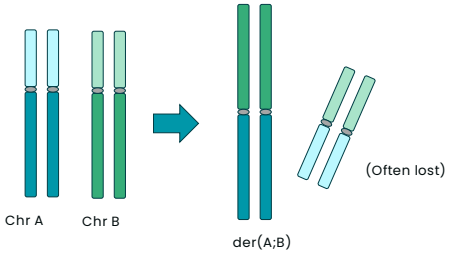


Figure 3. Formation of a Robertsonian translocation. The long arms of two acrocentric chromosomes fuse near the centromeres to form a single derivative chromosome. The short arms are typically lost. Balanced carriers retain the major long-arm genetic content but may produce gametes with an unbalanced chromosome complement.

Few standard tags: Centromeric regions contain few or no recognition sites for standard EGM nickases, limiting assembly across these sequences.

Potential sequence specificity: Small differences among centromeric satellite repeats provide possible targets for chromosome-specific guide RNAs.

Centromere length: Centromeres can stretch for more than a megabase, longer than EGM reads. As a result, DNA from junction regions where the translocated chromosomes meet must be detected and analyzed.

Central question: Can CRISPR-directed nicking create tagging patterns specific enough to distinguish acrocentric chromosomes?

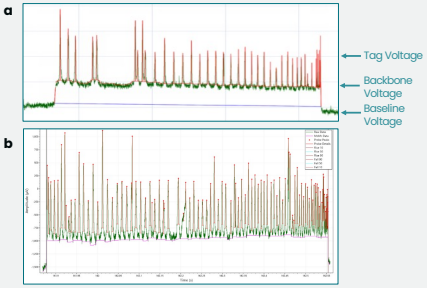


Figure 4. Electronic signals from tagged DNA molecules. (a) A typical genomic molecule produces distinct, well-spaced tag signals that can be converted into genomic distances. **(b)** Centromeric DNA produces a dense, repetitive signal pattern that is difficult to resolve using standard tagging.

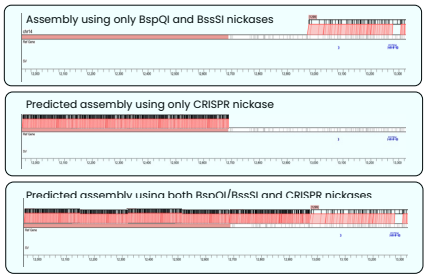


Figure 5. CRISPR-directed nicking increases predicted tag coverage across the chromosome 14 centromere. The assembly generated with standard Nt.BspQI and Nb.BssSI nickases contains few tags within the centromeric region. Predicted CRISPR-directed nicking introduces tags throughout the repetitive sequence, while combining CRISPR and standard nickases links the added centromeric tags with the surrounding genomic pattern. This combined strategy is designed to support mapping across centromeric boundaries and distinguish chromosome-specific patterns.

Initial Mapping with CRISPR-Directed Tags

DNA prepared with CRISPR-directed nicking produced mapped molecules containing the expected added tags. Combining CRISPR-directed and standard nicking generated a more uniform nicking/tagging pattern that retained genomic context around the targeted region.

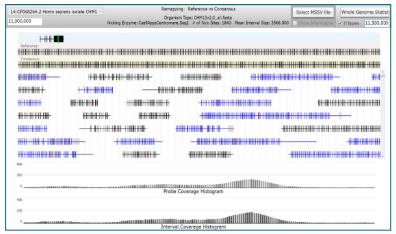


Figure 6. Mapping of DNA tagged using CRISPR-directed nicking. Molecules containing CRISPR-introduced tags aligned to the predicted tagging pattern, demonstrating targeted nicking and detection by EGM.

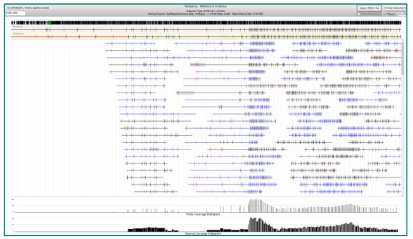


Figure 7. Mapping with standard and CRISPR-directed nicking. Combining Nt.BspQI and CRISPR-introduced tags produced a complementary tagging pattern. Standard tags provide genomic context, while CRISPR-directed tags increase coverage within the targeted repetitive region.

What These Results Show

- CRISPR D10A nickase can introduce custom detectable tags throughout the human genome.
- CRISPR and standard nickases can be used in the same EGM workflow with few changes.
- The combined pattern provides a foundation for mapping poorly tagged centromeric regions and allowing discrimination of highly similar regions like the telomeric regions of chr4 and chr10 in FSHD.. gRNAs for FSHD have been designed and are being tested.

Conclusions

- Targeted centromeric tagging: CRISPR D10A nickase introduced detectable tags into repetitive centromeric DNA, where standard EGM nickases provide few or no recognition sites.
- Compatible workflows: CRISPR-directed nicking was compatible with standard EGM sample preparation and nicking chemistry.
- Complementary information: CRISPR tags increase coverage within centromeric repeats, while standard tags retain the surrounding genomic context needed for molecule alignment.
- Chromosome-specific potential: Sequence differences among centromeric satellite repeats may enable guide RNAs to generate distinct tagging patterns for individual acrocentric chromosomes.
- Path toward Robertsonian detection: These results support continued development of CRISPR-assisted EGM for mapping acrocentric chromosomes and identifying Robertsonian derivatives.

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