Utilization of Dual-Label Optical Genome Mapping for Genetic/Epigenetic Diagnosis

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Introduction

Short-read exome/genome sequencing **(SRS)** and chromosomal microarrays **(CMA)** have helped increase diagnostic rates across many genetic disorders. However, despite this success, about half of the cases remain undiagnosed.

Technological limitations of SRS and CMA

a. Genetic:

SRS struggles to sensitively identify structural variants **(SVs)** due to utilization of reads (~150bp) that do not span a region. CMA is unable to identify balanced rearrangements such as inversions or translocations

b. Epigenetic:

Both SRS and CMA do not provide long-range haplotype specific methylation states, rather the detected signals are averaged for individual genomic positions

These limitations are alleviated with utilization of a novel dual-label optical genome mapping **(DL-OGM)** technology for detection of **both genetic and epigenetic changes in one assay over long stretches of single DNA molecules and phased haplotypes[1].**

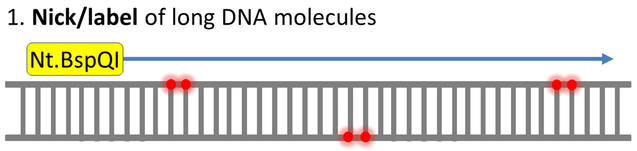
- The method relies on differential labeling of high molecular weight DNA (methods and **Poster # 305**).
  - Sharim H., et al., Genome Res 2019 (PMID:30846530)

Methods

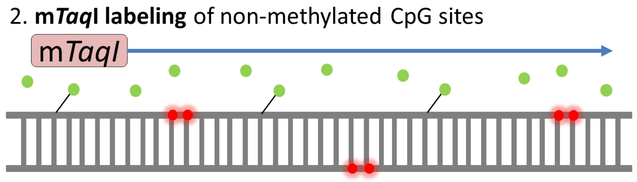
Step 1: long DNA molecules are nicked with either BspQI or BssSI endonucleases and labeled with red fluorescent nucleotides.

Step 2: the same DNA molecules undergo treatment with M.TaqI methyltransferase that attaches green fluorescent cofactor to TCGA sequences throughout the genome given that Cytosine is TCGA is on-methylated.

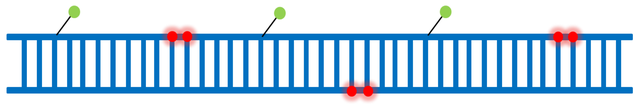
1. **Nick/label** of long DNA molecules



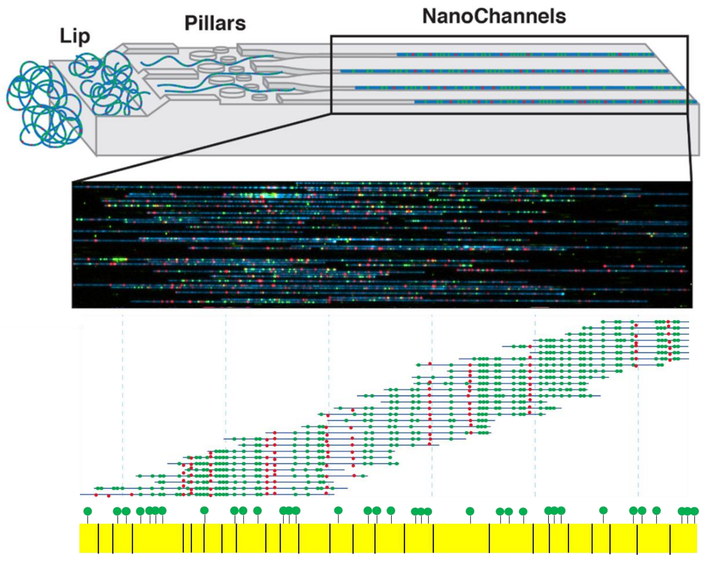
2. **m*TaqI* labeling** of non-methylated CpG sites



3. **Staining** of the DNA backbone

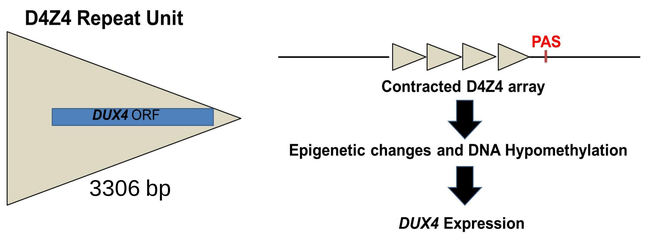


Step 3: the pattern of fluorescent labels is captured in nanochannel arrays for de novo genome assembly, variant calling and quantification of epigenetic marks.



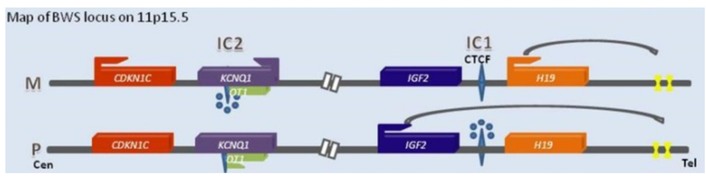
Results

Facioscapulohumeral Muscular Dystrophy (FSHD)



- Incidence 1:8,000 to 1:20,000
- Repression of myogenesis and progressive muscle atrophy
- Clinical diagnosis: restriction enzyme digestion, pulsed-field gel electrophoresis and Southern Blotting

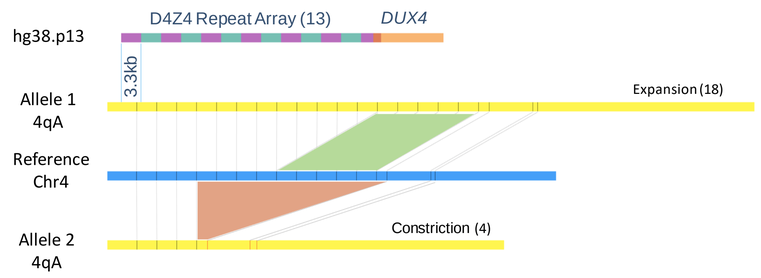
Beckwith-Wiedemann Syndrome (BWS)



- Incidence 1:10,500 to 13,700
- Abnormal gene regulation resulting in overgrowth
- Clinical diagnosis: methylation-sensitive multiplex ligation-dependent probe amplification

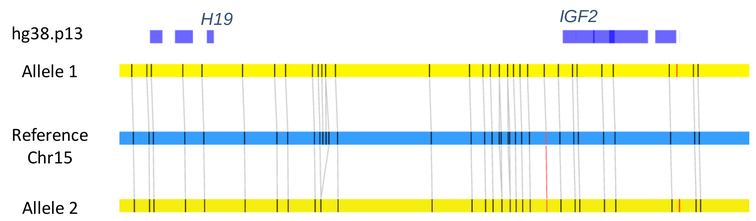
Dual-Label Optical Genome Mapping

De Novo Assembly of 4q35



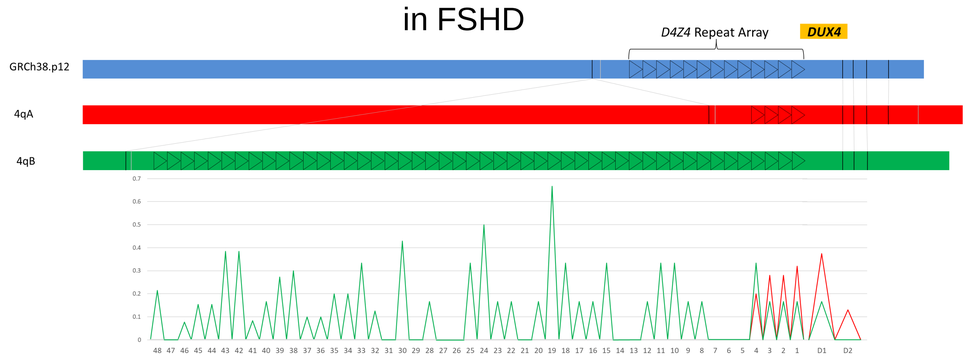
- Affected and Unaffected Haplotypes
  - Allele 1 shows expansion of D4Z4 array from 13 to 18 units
  - Allele 2 shows constriction of D4Z4 array from 13 to 4 units

De Novo Assembly of 11p15



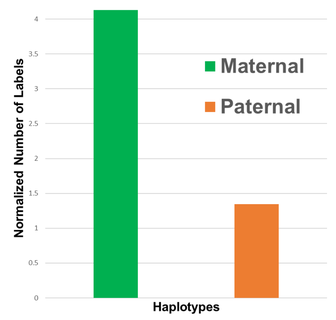
- Maternal and Paternal Haplotypes
  - Allele 1 – identified as maternal based on trio
  - Allele 2 – identified as paternal based on trio

Genetic Structure and Epigenetic Profile in FSHD



- Red and Green lines represent de novo assemblies of consensus optical maps of two alleles, 4qA and 4qB. Each peak in the plot corresponds to the average non-methylation level of one repeat unit (higher peaks correspond to lower methylation).

Epigenetic Profiles of Maternal and Paternal Alleles

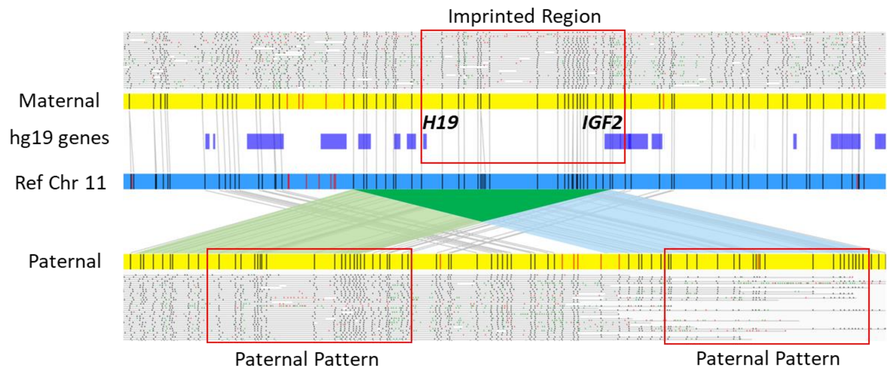


- Methylation level differences between maternal (green) and paternal (orange) alleles in BWS locus. Lower bar indicates less labels corresponding to hypermethylation.

Epigenetic Profiles of D4Z4 Region

- Methylation level differences between alleles carrying less (red) or more (green) than 10 D4Z4 repeat units. Lower bar indicates less labels corresponding to hypermethylation.

DL-OGM Achieves Diagnosis for BWS



- Duplication in BWS carrying paternal imprinting. Top and bottom yellow maps – de novo assembled haplotypes of the case. Highlighted green region in the middle – duplicated region identified in the proband.

Conclusions

- We successfully identified the molecular diagnosis (constriction of D4Z4 array and associated hypomethylation) in FSHD case/control samples in the sub-telomeric region of chromosome 4q35
- Additionally, we tested the method for a case diagnosed with BWS, where DL-OGM identified a duplication in the paternally inherited allele carrying epigenetic states resulting in the syndrome
- DL-OGM technology offers substantial advantages over the current clinical diagnostic practices for specific disorders tested here (FSHD, BWS) and can be applied to other types of disorders such as CHARGE syndrome