Facioscapulohumeral muscular dystrophy (FSHD), is one of the most common forms of autosomal dominant, progressive muscular dystrophies affecting approximately 1/10,000-1/25,000 individuals worldwide. The clinical features of this condition vary from a severe infantile form to a less severe adult onset form, including muscular dystrophy involving progressive wasting of the muscles of the face, shoulder blades, upper arms as well as the lower legs. Detection and diagnosis of FSHD is very challenging by Next Generation Sequencing (NGS) methodologies due to the presence of polymorphic microsatellite repeat cluster (D4Z4) at two chromosomal locations in the human genome (4q and 10q). The gold standard for FSHD testing is the accurate detection of the D4Z4 repeat contractions by Southern blot analysis. Due to the time consuming and labor-intensive costs required for detecting the D4Z4 repeat contractions by conventional methodologies, we have evaluated a high-throughput, optical mapping methodology to validate and accurately map the repeats on chromosomes 4q and 10q using the Bionano Saphyr® genome imaging platform.

METHODS

Briefly, whole genome optical mapping involves isolating large molecules of the DNA (150kb to >1Mb), uniformly labeling them at a specific 6-base sequence motifs, loading the labeled DNAs into a cartridge, the molecules are electrophoretically linearized and imaged multiple times using the Bionano Genomics Saphyr® platform. Using the captured images, a de novo genome map indicating the positions of the labels is constructed and compared to a reference genome to detect structural differences in the 2 maps. Molecules aligning to regions of interest in chromosome 4 and chromosome 10 were extracted and assembled. The resulting consensus genomic regions of interest maps were used for the Bionano EnFocus™ FSHD Analysis. The repeat arrays were sized, and the permissive and non-permissive alleles (4qA and 4qB) assigned. A total of 44 DNA samples were utilized for LDT evaluation of this assay including 6 Coriell FSHD positive cell lines (performed in triplicate), 6 normal controls (3 males and 3 females), and 14 clinically diagnosed FSHD patients. Additional structural variants and copy number gains and losses were interrogated in the proximity of the D4Z4 repeat array on chromosome 4. Copy number gains and losses in the proximity of the SMCHD1 gene on chromosome 18 were also interrogated.

RESULTS

We demonstrate 100% analytical accuracy and precision of this assay using the FHS positive Coriell cell lines with an accuracy of ±1 repeat. Normal male and female control samples revealed the D4Z4 repeats to be within normal range (15-48 D4Z4 repeats of either the 4qA or 4qB haplotype, data not shown). In 14 clinically diagnosed FSHD cases, 12 cases were positive for the repeat contractions (ranging from 2-8 repeats) and were reproducible across intra-site, inter-site and inter-instrument and inter method comparison. Two of these positive cases also showed a mosaic pattern of the contracted allele, Chr 10q (not shown) and the normal 4qB alleles were also deemed highly reproducible across different runs performed at 2 sites. The results of our study demonstrate 100% reproducibility and precision of the samples utilized for the LDT evaluation of Bionano’s Saphyr® genome imaging platform as a high resolution, high-throughput and cost-effective next generation cytogenomic tool.

DISCUSSION

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