

# **Clinical Application of Optical Genome Mapping for Molecular Diagnosis of Facioscapulohumeral Muscular Dystrophy**

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**Supplemental Data Table S1. OGM data quality**

Sample ID	Total DNA (Gbp)	Average N50 ( $\geq 150$ Kbp)	Average label density	Average mapping rate (%)	Coverage ( $\times$ )	PLV (%)	NLV (%)	Assessment of molecular quality*	Assessment of stable regions <sup>†</sup>
P1	1,336.618	296	14.37	88.0	363.96	0.031	0.113	Pass	Pass
P2	1,306.0158	248	14.85	92.0	371.36	0.033	0.086	Pass	Pass
P3	1,302.6004	248	13.79	81.1	324.98	0.031	0.121	Pass	Pass
P4	1,054.177	243	7.74	<b>22.6<sup>‡</sup></b>	<b>58.49</b>	0.029	0.263	Fail	Pass
P5	1,302.586	229	13.04	77.4	308.78	0.02	0.193	Pass	Pass
P6	1,251.508	233	14.41	89.8	345.56	0.028	0.105	Pass	Pass
P7	906.0857	<b>187</b>	14.67	85.2	238.42	0.016	0.103	Fail	Pass
P8	1,315.772	314	13.47	77.4	313.25	0.033	0.135	Pass	Pass
P9	1,327.779	283	10.76	<b>55.1</b>	211.14	0.033	0.221	Fail	Pass
P10	458.4844	200	14.48	<b>52.5</b>	75.48	0.033	0.121	Fail	Pass
P11	450.508	215	14.85	74.4	104.91	0.046	0.107	Pass	Pass

P12	273.001	202	14.05	85.4	<b>71.22</b>	0.03	0.107	Fail	Pass
<b>Family 1</b>									
P13	834.5647	218	14.64	<b>47.1</b>	124.34	2.1	12.3	Fail	Pass
P14	1,316.357	263	14.34	87.0	370.73	3.0	10.8	Pass	Pass
P15	1,317.544	315	13.49	55.7	228.04	0.019	0.193	Pass	Pass
<b>Family 2</b>									
P16	1,306.891	236	14.05	73.4	296.48	4.6	13.3	Pass	Pass
P17	1,042.404	<b>188</b>	14.46	81.3	260.36	3.0	10.7	Fail	Pass
P18	1,336.028	227	15.03	80.5	335.11	0.05	0.111	Pass	Pass
<b>Family 3</b>									
P19	1,053.411	196	14.49	77.1	262.43	3.3	9.7	Pass	Pass
P20	977.873	206	15.4	<b>53.1</b>	168.12	2.9	11.5	Fail	Pass
P21	505.605	212	15.34	65.4	102.91	0.033	0.104	Pass	Pass
<b>Family 4</b>									
P22	1,307.931	296	14.99	85.8	348.42	2.8	13.1	Pass	Pass

P23	405.408	232	13.92	75.8	94.90	3.4	15.9	Pass	Pass
P24	1,307.974	263	12.9	76.2	307.65	2.7	17.3	Pass	Pass
P25	1,301.117	222	13.76	80.9	324.48	0.03	0.132	Pass	Pass

\*Sufficient molecular quality for the FSHD pipeline was defined as a mapping rate of  $\geq 70\%$ , an effective coverage of  $\geq 75\times$ , and a molecular N50 of  $\geq 200$  Kbp.

<sup>†</sup>To assess consensus map-level quality, we employed a pipeline that analyzes 22 stable regions selected based on the analysis of 59 Bionano human control samples. The absolute percent difference between the map and reference should not exceed 1.2%.

<sup>‡</sup>Values outside the optimal range recommended in the manufacturer's guidelines are indicated in bold. Average N50 ( $\geq 150$  Kbp): the average molecular N50 length for all molecules that are  $\geq 150$  Kbp in length. Average label density (per 100 Kbp): the average number of labels detected using the image-detection algorithm per 100 Kbp of DNA for molecules  $\geq 150$  Kbp long. Average mapping rate: the average percentage of molecules that map to the reference for molecules  $\geq 150$  Kbp.

Abbreviations: OGM, optical genome mapping; PLV %, percentage of molecule labels absent in reference labels. NLV %, percentage of reference labels absent in molecular labels; FSHD, facioscapulohumeral muscular dystrophy.

**Supplemental Data Table S2.** Comparison of the workflow and test time between Southern blot analysis and OGM

<b>Technique</b>	<b>Southern blot analysis</b>	<b>OGM</b>
Total test time	Seven days	Four days
Workflow	<p>Day 1: DNA extraction, quality-control analysis, and enzymatic reaction (overnight)</p> <p>Day 2: Purification of enzyme-treated DNA (overnight)</p> <p>Day 3: Gel electrophoresis (overnight; 1× Tris-borate-EDTA, 30 V)</p> <p>Day 4: Denaturation (30 mins), renaturation (40 mins), and transfer (overnight)</p> <p>Day 5: Pre-hybridization (3 hrs) and hybridization (overnight)</p> <p>Day 6: Washing (overnight)</p> <p>Day 7: Detection and analysis</p>	<p>Day 1: DNA extraction (2 hrs, 15 mins)</p> <p>Day 2: DNA quality control, labeling, quality-control analysis, and run start (5 hrs)</p> <p>Day 3: Pipeline run (analysis)</p> <p>Day 4: Check report</p>

Abbreviation: OGM, optical genome mapping.