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How single-molecule real-time sequencing can improve the prognosis and genetic counselling in Myotonic dystrophy type 1

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Abstract

Myotonic dystrophy type 1 (DM1) results from the expansion of an unstable CTG repeat that usually increases across generations and over time in somatic tissues. CTG repeat instability and DM1 clinical manifestations depend on the length of the repeat itself and the purity of the repeated sequence. The genetic counseling in DM1 is very complex, due to the highly variable clinical presentation and technical difficulties in determining the size and purity of the CTG expansion. We used PacBio Single-Molecule Real-Time sequencing (SMRT) to precisely measure large CTG repeat size and identify sequence interruptions of expanded allele to understand clinical and genetic variability in DM1 patients. We sequenced several DM1 patients with CTG repeat expansions ranging from 130 to > 1000 CTG repeats on the Sequel I and II systems from purified amplicons. We obtained more than 77% full DM1 reads per sample, with >70% of the reads from expanded alleles. The data includes long reads in the expected size range for all samples, including DM1 patients with more than 1000 CTG repeats (Table1and Ref1). SMRT sequencing is very promising to sequence large triplet repeat expansions, to identify CTG repeat interruptions and to estimate somatic mosaicism in DM1 patients. This method can significantly improve the prognosis and counseling offered to patients.

Table 1. Sequel II results in family E and L2 and L3 patients carrying more than 1000 CTG repeats.

Sample	Total CCS Reads	% CCS Reads on-Target	% on-Target Reads Full-Length	Full DM1 Reads	Total Reads Analyzed	Reads < 50 CTG	Reads ≥ 50 CTG	% Expanded Allele	Estimated Repeat Size (Mode)	CCG Interruptions
E1	94,411	99.37	84	79,224	10,000	724	9276	93	5, ~447 (1099 max rpt)	No obvious interruption
E2.1	110,578	99.54	87	95,756	9999	627	9372	94	13, ~383 (831 max rpt)	2–3x CCG
E3	67,182	99.61	89	59,342	10,000	375	9625	96	31, ~173 and ~215 (478 max rpt)	6x CCG
L2	48,310	99.10	79	37,727	10,000	1291	8709	87	5, ~957 (2138 max rpt)	No obvious interruption
L3	29,512	99.02	77	22,625	9999	1670	8329	83	5, ~1156 (2081 max rpt)	No obvious interruption
A4.1	50,300	99.62	89	44,745	10,000	721	9279	93	5, ~109 (245 max rpt)	1x CAG
1201	37,407	99.44	85	31,683	9999	1813	8186	82	5, ~118 (619 max rpt)	No obvious interruption
B2	57,255	99.3	84	47,745	9995	1075	8920	89	5, ~292 (802 max rpt)	3x CCG
5289	42,349	99.31	82	34,690	9994	1595	8399	84	5, ~185 (896 max rpt)	No obvious interruption

Biography

Tomé has been working for more than 15 years on trinucleotide repeats instability observed in several neurological and neuromuscular disorders such as Huntington's disease and myotonic dystrophies. Dr Tomé is focusing her research on myotonic dystrophy type 1 (DM1) and the mechanisms of CTG repeat instability, in particular CTG contractions in the research group led by Dr. Gourdon (Paris, FRANCE). Dr. Tomé has acquired a solid expertise in Single-molecule real-time (SMRT) sequencing developed by Pacific Biosciences. Recently, she applied this method to precisely measure and characterize the sequence of large CTG repeats in DM1 patients to better understand the high clinical and genetic variability observed in the DM1 population. Dr Tomé and her colleagues wish to introduce SMRT sequencing as an alternative molecular diagnostic method that could improve the prognosis and counseling offered to DM1 patients, but also use it routinely in research.