

Figure S1. Previously reported genome-wide STR mutation rates. Mutation rates as a function of STR motif size.

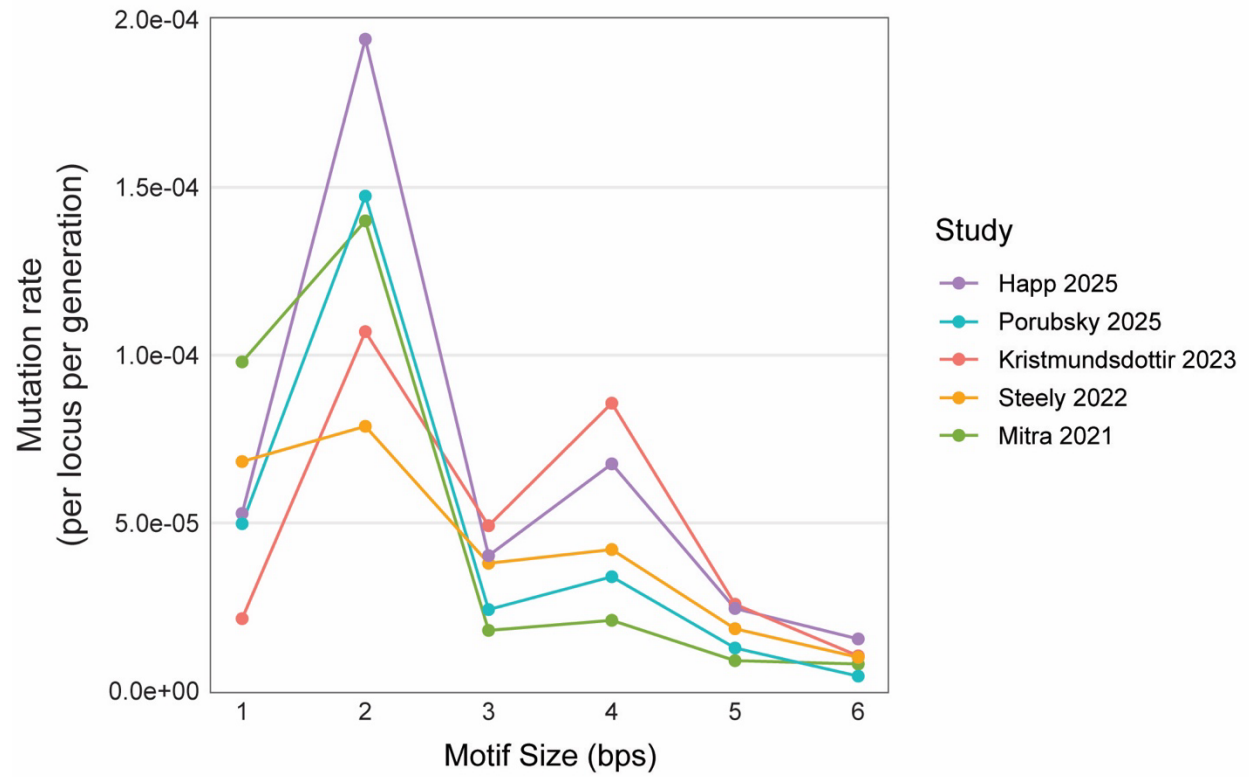


Figure S2. Correlation of observed parental allele lengths at homopolymer loci and the allele length in the reference genome (GRCh38).

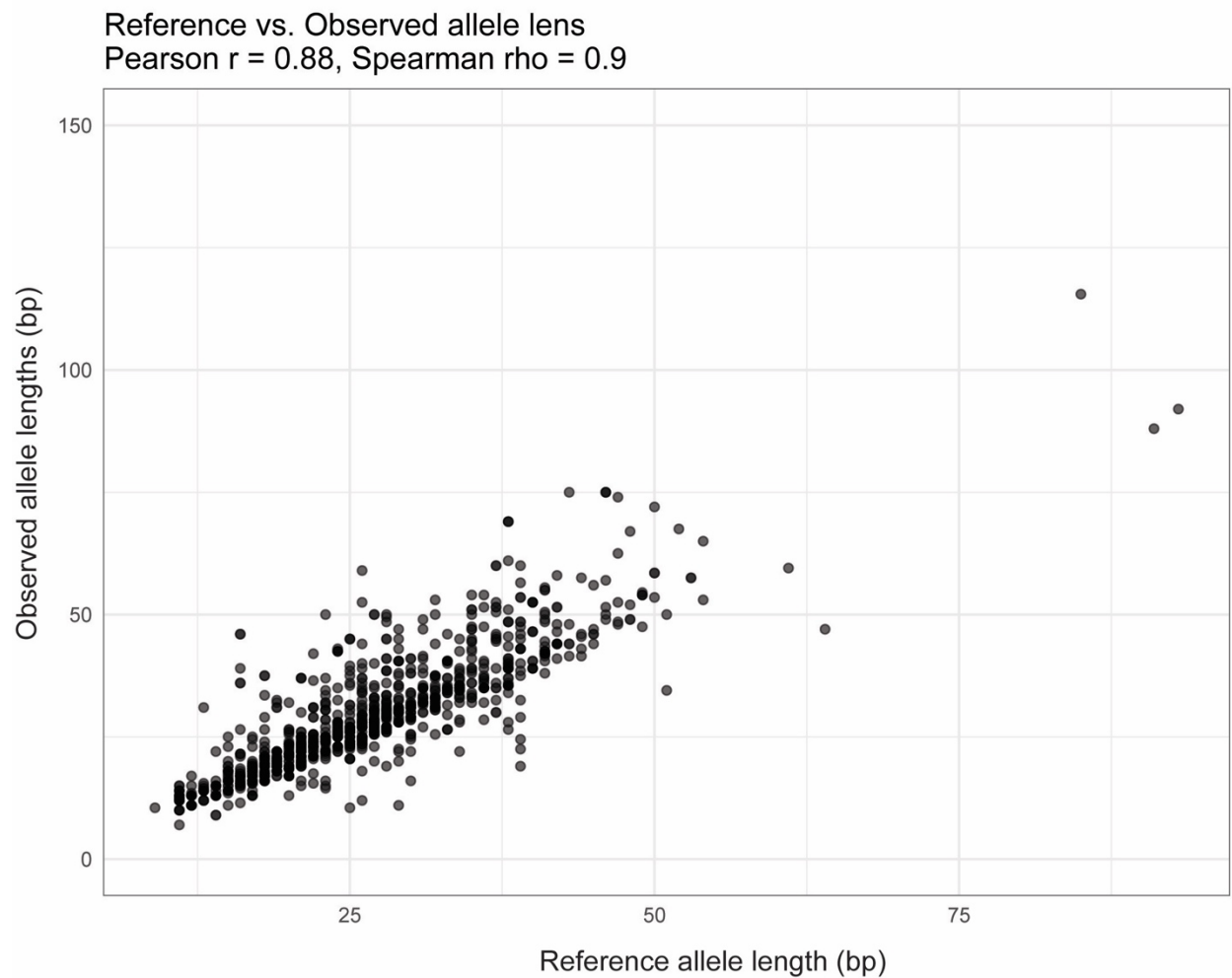


Figure S3. Mutation rates across homopolymer, dinucleotide, and trinucleotide loci as a function of motif nucleotide content. Homopolymers were grouped by base content (A/T, G/C), dinucleotide motifs were collapsed by reverse complement (e.g. AG = CT) and trinucleotide motifs were collapsed by shifting and reverse complementation (e.g. AAC/GTT = ACA/TGT = CAA/TTG).

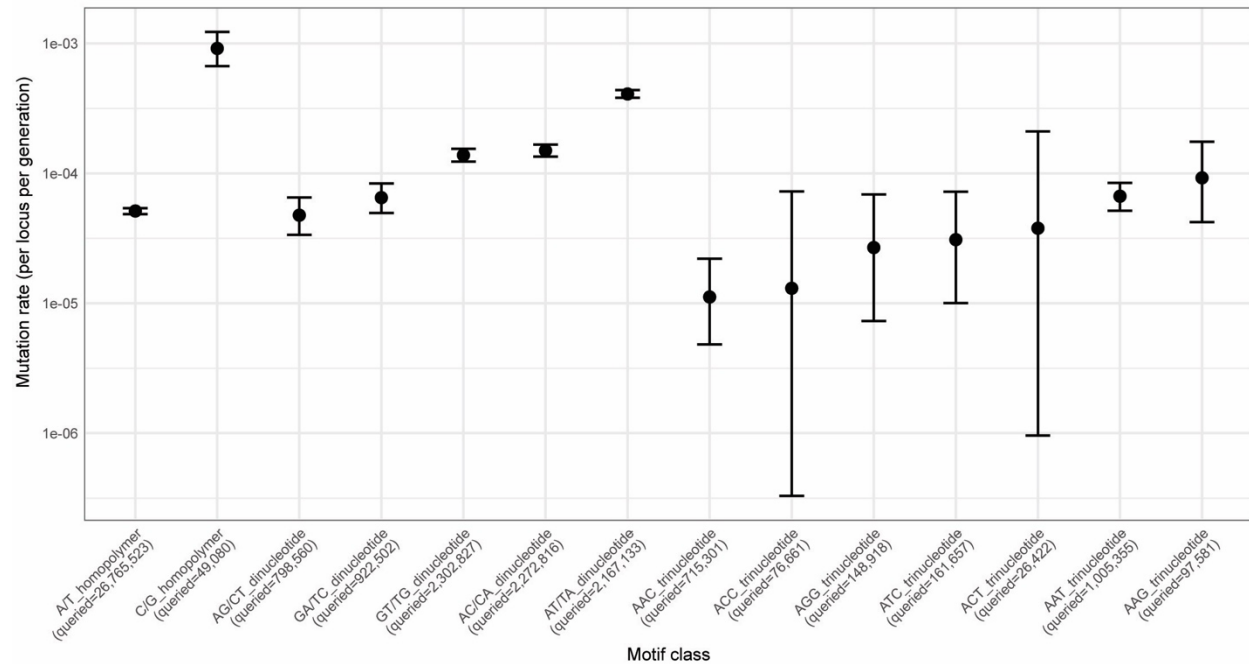


Figure S4. Distributions of DNM-supporting reads in the child with respect to A) the minimum number of supporting reads required in the child and B) the number of STR-spanning reads in the child. A) For our analyses, we required a minimum of 2 (for Q50 sequencing) or 3 (for Q40 sequencing) supporting reads. **B)** The number of DNM-supporting reads scales with the number of STR-spanning reads at a locus (Spearman correlation = 0.74).

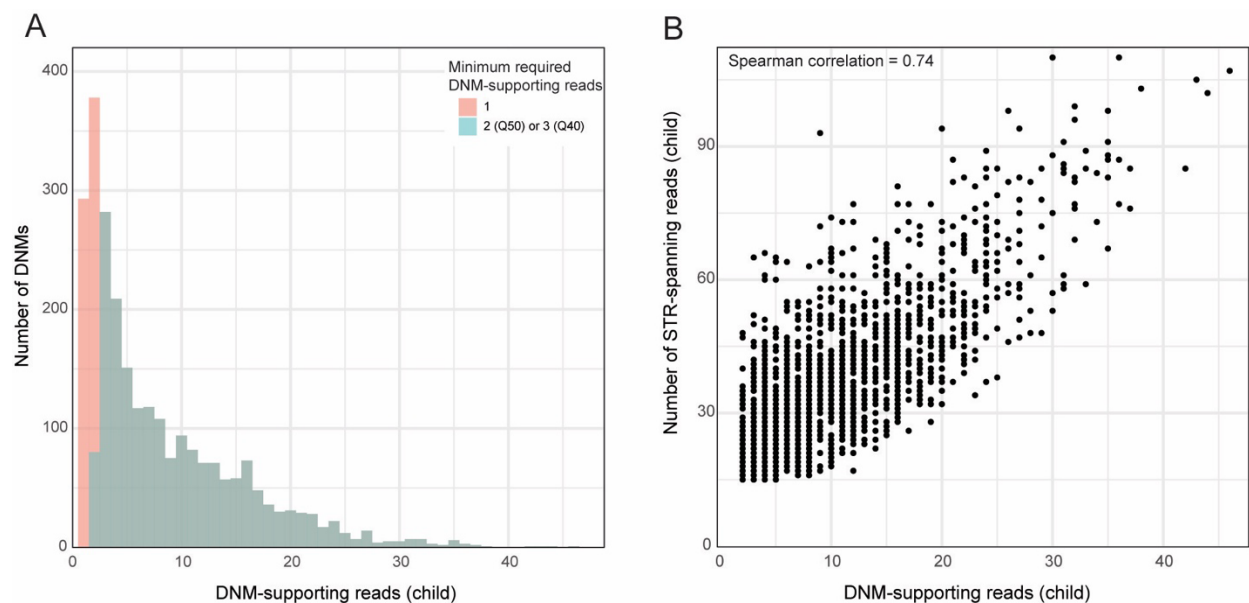


Figure S5. Number of candidate DNMs that pass BAM-level inspection versus the maximum allowable parental reads matching the DNM allele. During BAM-level inspection, we count the number of reads in both parents that match the DNM allele. Each line in the figure represents a trio as the threshold of parental evidence changes. The analyses in the manuscript use a threshold of 1, meaning that if a DNM allele was present in more than a single read across both parents combined, we excluded that DNM call.

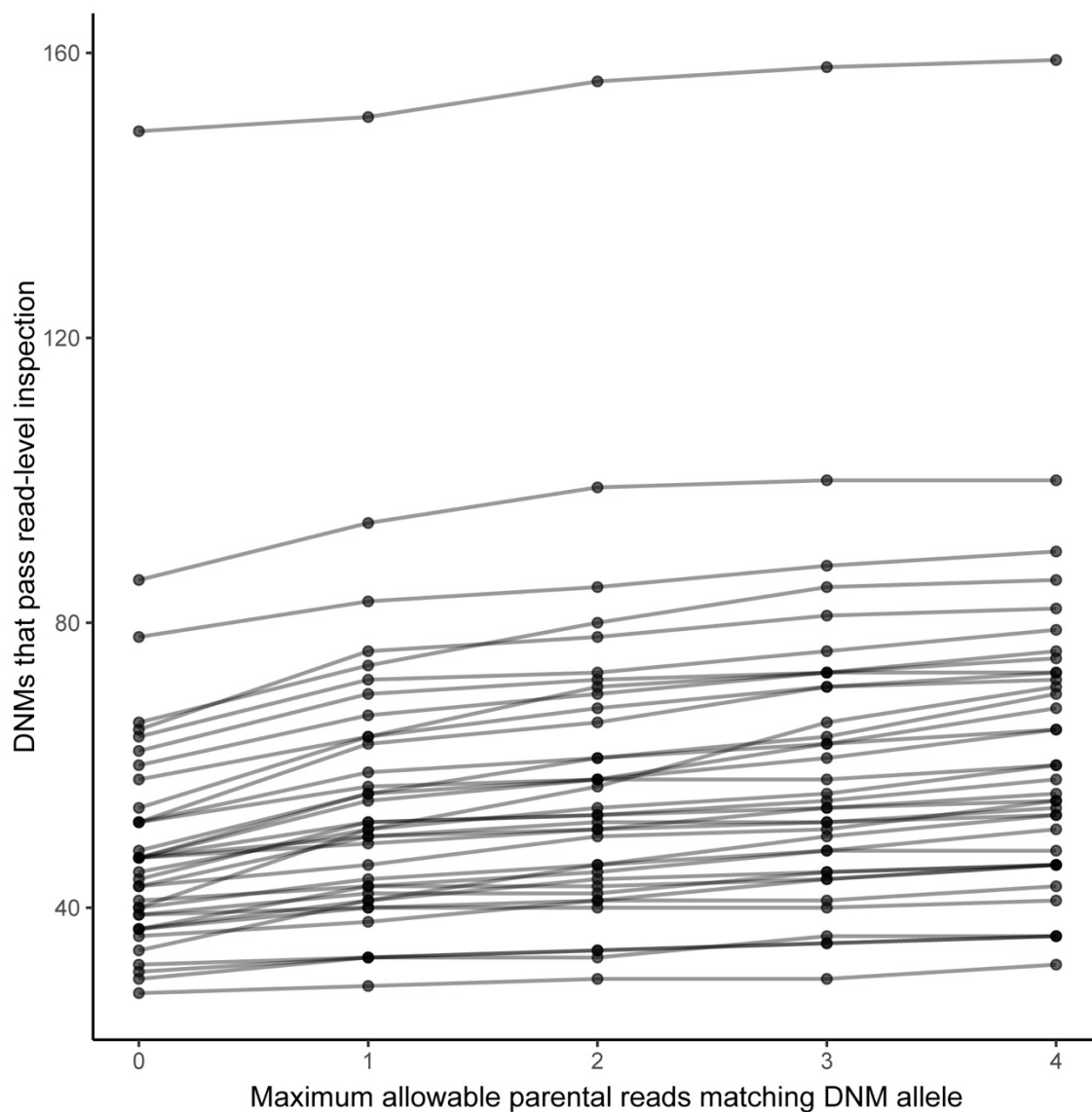


Table S2. Homopolymer *de novo* mutation rates stratified by length bin.

Length bin (bp)	DNMs	Queried sites	Mutation rate (per locus per generation)	95% CI
10-24	727	23280405	3.12×10^{-5}	$2.90 \times 10^{-5} - 3.36 \times 10^{-5}$
25-39	594	3735034	1.59×10^{-4}	$1.47 \times 10^{-4} - 1.72 \times 10^{-4}$
40-54	90	379175	2.37×10^{-4}	$1.91 \times 10^{-4} - 2.91 \times 10^{-4}$
55-49	2	39669	5.04×10^{-5}	$6.11 \times 10^{-6} - 1.82 \times 10^{-4}$
70-84	0	6043	0	$0 - 6.1 \times 10^{-4}$
85-99	3	822	3.65×10^{-3}	$7.53 \times 10^{-4} - 1.07 \times 10^{-2}$