



Supplemental Fig. S2. IGV screenshots of long-read adaptive sequencing (LR-AS) and gel images of heterozygous AAGGG *RFC1* expansions miscalled by Straglr.

Individuals called as homozygous pathogenic *RFC1* expansions by Straglr were confirmed by manual inspection and PCR to be heterozygous AAGGG expansion carriers. A. IGV screenshot for P010 shows soft-clipped reads with AAGGG, indicative of a large expansion, and reads aligning to the reference, indicative of a non-expanded AAAAG allele. Gel electrophoresis (right) of flanking PCR products shows a band consistent with a wildtype allele, as seen in the control sample. No band is present for the known homozygous AAGGG sample, as expansions >250 (the pathogenic threshold for AAGGG) are too large to be amplified by standard PCR. B. IGV screenshot for P005 shows soft-clipped reads with the pathogenic AAGGG motif, indicative of a large expansion and soft-clipped reads with the non-pathogenic AAAAG motif (yellow box). Gel electrophoresis (right) of flanking PCR products shows a band consistent with a non-pathogenic allele of ~130 repeats (~1000bp). C. IGV screenshot for P090 shows soft-clipped read of ~600 basepairs of an AAAGG motif (blue box), indicative of an expansion of ~130 repeats, and soft-clipped reads with the pathogenic AAGGG motif (red box), indicative of a large expansion. Gel electrophoresis (right) of long-range PCR products show a band consistent with a non-pathogenic allele of ~130 repeats, similar in size to the bands of the control with known repeat sizes of approximately 14 and 120 repeats.

rpts, repeats; bp, basepairs.