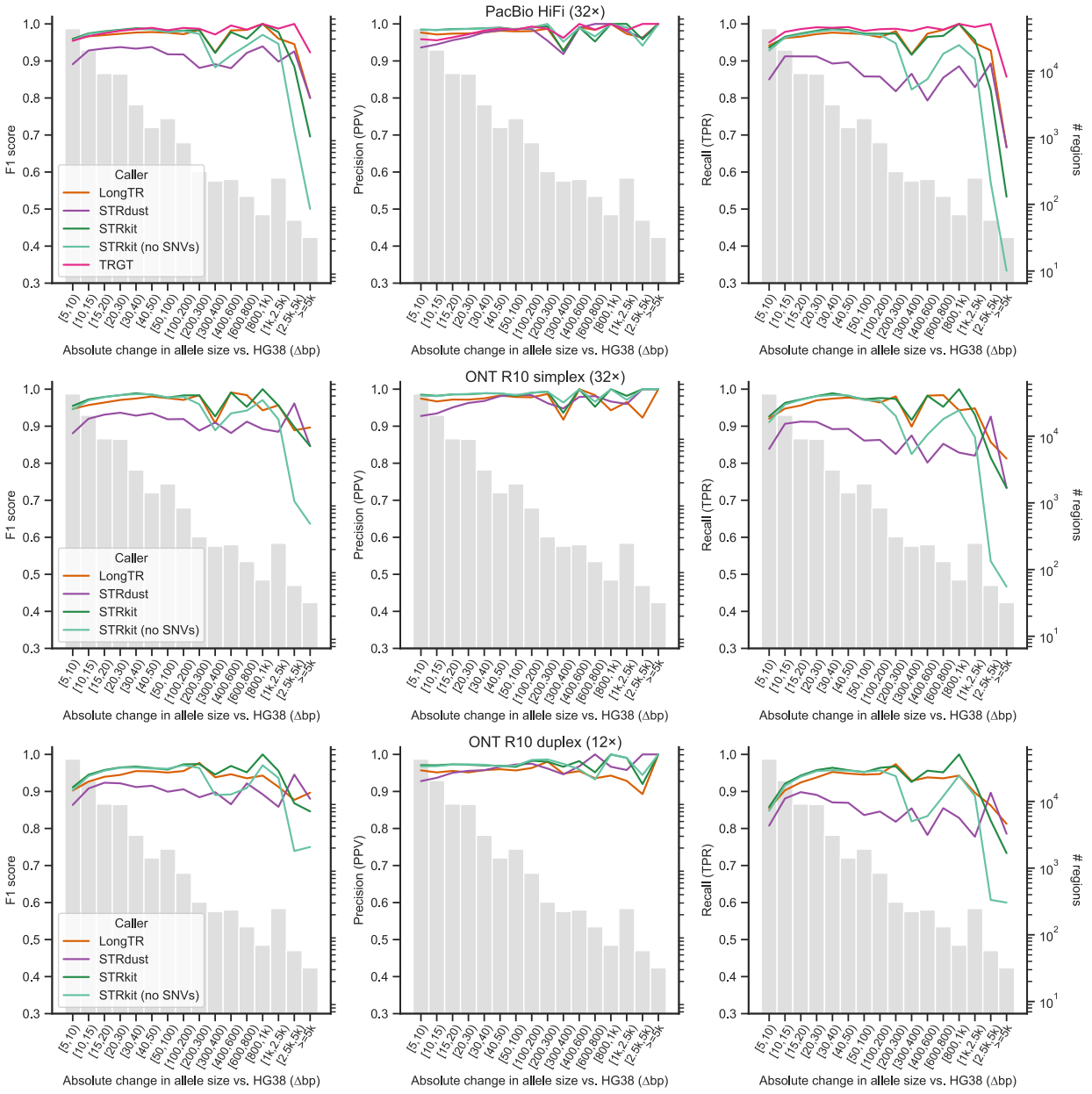
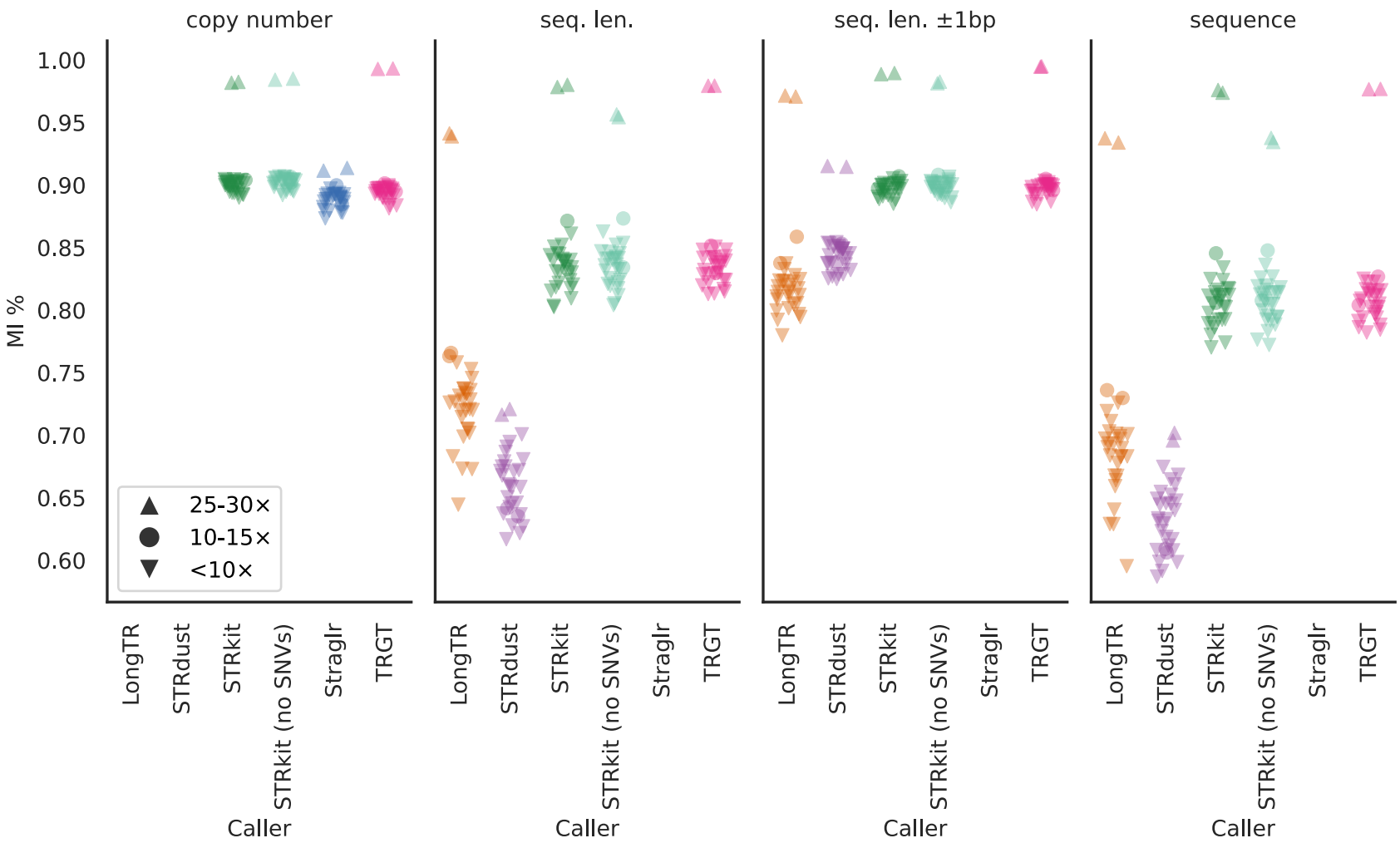
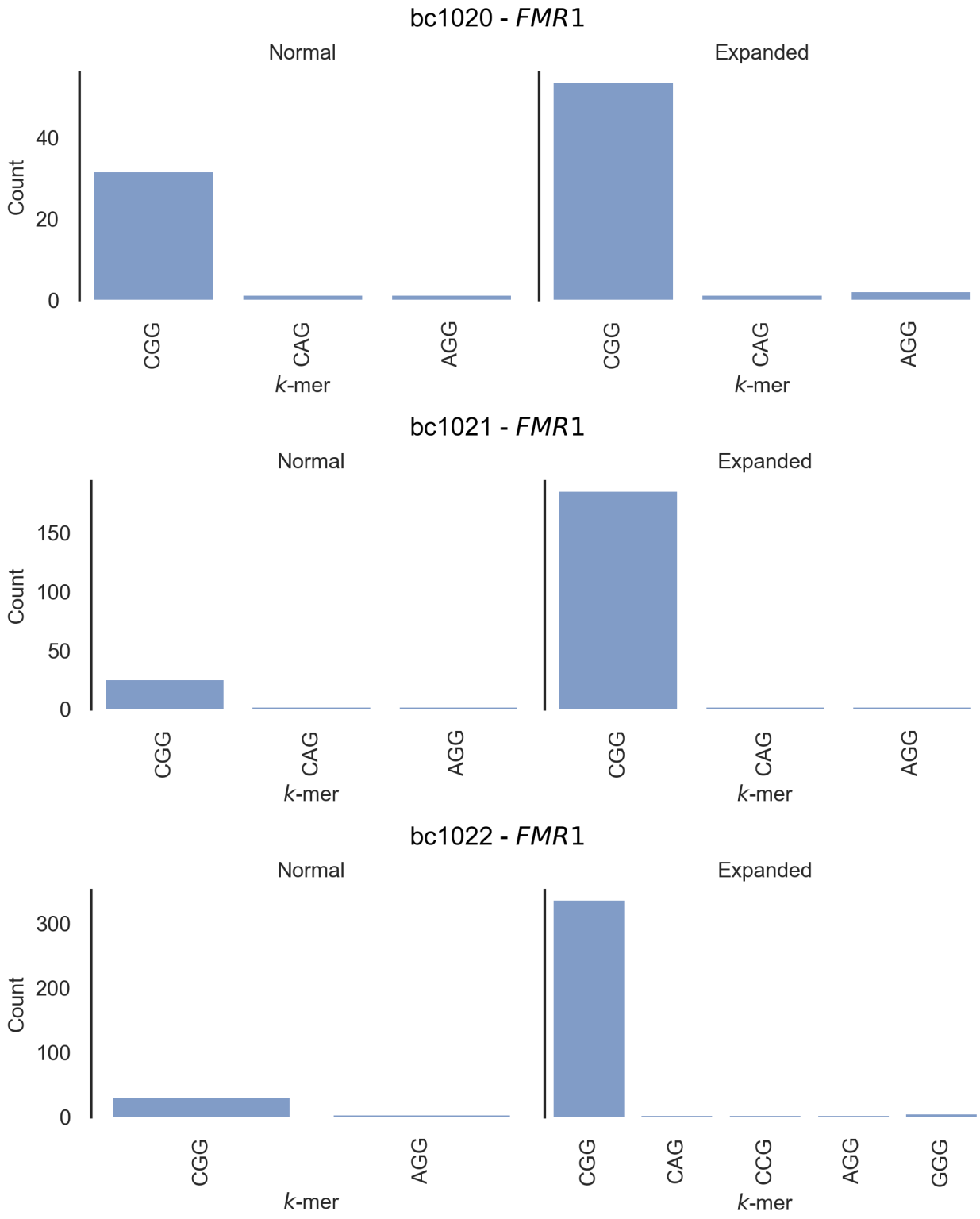


Supplemental Figure S1: F1 score, precision, and recall of STR genotyping tools by locus-maximum allele size delta, i.e., the maximum change in allele size versus the HG38 reference genome of the maternal and paternal alleles for each locus, as output by the Truvari benchmarking utility and Laytr reporting tool.



Supplemental Figure S2: Rates of Mendelian inheritance metrics by caller on a dataset of 30 trios from the Genomic Answers for Kids (GA4K) cohort, sequenced with PacBio HiFi data. Most of the trios used here have very low proband and parental coverage, limiting genotype success.



Supplemental Figure S3: *k*-mer breakdowns for *FMR1* normal and expanded alleles in three samples with *FMR1* expansions, using read-level output from STRkit.

Supplemental Table S1: STRkit STR allele peak-calling method for Genome-in-a-Bottle Ashkenazi trio samples

Tech.	Sample	SNV	SNV+Dist	Dist	Single	% SNV use
HiFi	HG002	36.71%	34.58%	25.55%	3.17%	71.28%
	HG003	32.39%	36.01%	28.48%	3.17%	68.40%
	HG004	35.18%	36.87%	27.94%	*N/A**	72.06%
ONT Simplex	HG002	31.01%	43.77%	22.05%	3.17%	74.78%
	HG003	29.38%	44.40%	23.06%	3.17%	73.77%
	HG004	35.78%	42.01%	22.21%	*N/A**	77.79%
ONT Duplex	HG002	51.85%	20.17%	24.87%	3.11%	72.02%

STRkit STR allele peak-calling method for Genome-in-a-Bottle Ashkenazi trio samples. “SNV” means 2+ SNVs were used to peak-call alleles. “SNV+Dist” means a combined one-SNV and copy number distance metric was used. “Dist” means only copy-number distance was used. “Single” means the locus was called as haploid (i.e., the X/Y-chromosomes for standard chromosomal males).

*HG004 is the maternal parent in the trio, with a standard XX karyotype and thus no haploid chromosomes (i.e., single-peak genotypes).

Supplemental Table S2: STRkit SNV call and phase set error rates

	SNVs					Phase sets		
Technology	**False Het.**	**Other Incorr.**	**Total Incorr.**	**Total Corr.**	**Total SNVs**	**Invalid phase sets**	**Valid phase sets**	**Total phase sets**
HiFi (~32×)	50 (0.0043%)	37 (0.0032%)	87 (0.0075%)	1 159 704 (99.9925%)	1 159 791	370 (0.3122%)	118 154 (99.6878%)	118 524
ONT simplex (~32×)	107 (0.0094%)	178 (0.0157%)	285 (0.0251%)	1 133 202 (99.9749%)	1 133 487	355 (0.3427%)	103 221 (99.6573%)	103 576
ONT duplex (~12×)	1126 (0.1059%)	211 (0.0199%)	1337 (0.1258%)	1 061 633 (99.8742%)	1 062 970	379 (0.5084%)	74 173 (99.4916%)	74 552

Comparison of SNV calls from STRkit to a phased version of the HG002 Genome-in-a-Bottle NIST v4.2.1 small variant benchmark, in terms of SNV calls (falsely heterozygous SNV calls, other incorrect SNV calls, correct SNV calls, and overal total SNV calls) and phase sets (invalid phase sets, with “flipped” SNV genotypes, valid phase sets, and total phase sets).

Supplemental Table S3: Rates of Mendelian inheritance (MI) for STR genotypers on the Ashkenazi trio

Panel A: PacBio HiFi

Caller	MI % (copy num.)	MI % (seq.)	MI % (seq. len.)	MI% (seq. len. ±1bp)	# trio calls
STRkit	98.54%	97.56%	97.98%	99.04%	883 983
STRkit (-SNV)	98.67%	93.64%	95.58%	98.42%	883 860
LongTR	*N/A*	94.52%	94.85%	97.54%	882 515
Straglr	86.98%	*N/A*	*N/A*	*N/A*	854 887
STRdust	*N/A*	69.48%	71.51%	93.09%	882 322
TRGT	97.68%	94.77%	97.19%	98.98%	883 777

Panel B: ONT R10 simplex

Caller	MI % (copy num.)	MI % (seq.)	MI % (seq. len.)	MI% (seq. len. ±1bp)	# trio calls
STRkit	96.99%	94.44%	94.99%	97.96%	884 262
STRkit (-SNV)	96.65%	90.23%	92.25%	96.42%	884 135
LongTR	*N/A*	90.60%	91.06%	95.49%	883 259
Straglr	85.51%	*N/A*	*N/A*	*N/A*	856 934
STRdust	*N/A*	46.84%	49.76%	73.55%	883 333

Rates of Mendelian inheritance (MI) for STR genotypers on the Ashkenazi trio with HiFi and ONT R10 simplex sequencing data, using regions from the Genome-in-a-Bottle tandem repeats v1.0 benchmark, as measured by STRkit's MI calculator. Up to four different versions of the MI metric are measured, depending on the caller: copy number MI (exact MI in terms of [approximate or reported] copy number), sequence MI (exact MI in terms of allele sequence), sequence length MI (exact MI in terms of allele length, in base pairs [bp]), and sequence length MI ±1 bp. The "# trio calls" figure indicates the number of loci with successful calls in all trio individuals, out of 914 676. Underlines indicate the best value for the Mendelian inheritance metric within the sequencing technology.

Supplemental Table S4: Runtime performance and maximum memory usage of STR genotyping software

Panel A: Runtime (core-minutes)

	PacBio HiFi (Coverage: 32.3×, 31.6×, 32.6×)				Ont R10 Simplex (Coverage: 32.2×, 31.7×, 30.2×)				ONT R10 Duplex (~12×)
Caller	**HG002**	**HG003**	**HG004**	**Avg.**	**HG002**	**HG003**	**HG004**	**Avg.**	**HG002**
STRkit	490	489	491	490	495	494	493	494 (*best m.c.)*	438 (*best m.c.*)
STRkit (-SNV)	490	490	492	491	981	979	978	979	442
LongTR	123	123	183	143	245	305	305	285 *(best)*	123 *(best)*
Straglr	2447	2447	3417	2770	6351	5862	7814	6676	2934
STRdust	6840	6346	9766	7651	12207	15623	14156	13995	6350
TRGT	106	99	97	101 *(best)*	*N/A*				*N/A*

Panel B: Maximum memory usage (gigabytes [GB])

Caller	**HG002**	**HG003**	**HG004**	**Avg.**	**HG002**	**HG003**	**HG004**	**Avg.**	**HG002**
STRkit	10.6 GB	8.6 GB	7.2 GB	8.8 GB	8.4 GB	8.6 GB	15.5 GB	10.8 GB	11.2 GB
STRkit (-SNV)	5.9 GB	6.2 GB	5.9 GB	6.0 GB	5.6 GB	6.8 GB	14.8 GB	9.1 GB	5.5 GB
LongTR	3.5 GB	2.9 GB	2.4 GB	2.9 GB	11.2 GB	5.5 GB	8.0 GB	8.2 GB	5.0 GB
Straglr	79.9 GB	78.4 GB	80.2 GB	79.5 GB	65.4 GB	64.1 GB	55.3 GB	61.6 GB	33.7 GB
STRdust	4.4 GB	4.3 GB	4.4 GB	4.4 GB	6.9 GB	7.2 GB	6.7 GB	6.9 GB	5.9 GB
TRGT	1.9 GB	3.0 GB	3.4 GB	2.8 GB	*N/A*				*N/A*

Runtime performance and maximum memory usage of STR genotyping software on our GIAB tandem repeats benchmark subset for the Ashkenazi trio. Underlines indicate the best value for the performance metric, within the sequencing technology. *N/A* is not available because of licensing restrictions. *m.c.* = multi-core.

Supplemental Table S5: *HTT* and *FMR1* expansion genotypes from long-read STR calling tools

Panel A: *HTT* genotyping results

Sample	Documented genotype*	STRkit	LongTR[1]	Straglr	STRdust[1]	TRGT[1]
NA13505	22/**50**	22/**51**	22/**51**	22/**51**	23/**52**	23/**53**
NA13509	15/**70**	16/**75**	15/**74**	15/**75**	15/**75**	20/**76**
NA20253	22/**96-103**[a]	22/**114**	22/**113**	22/**111**	23/**121**	23/**111**
NA14044	19/**250**[b]	19/**804**	19/**723**	20/*20*	20/**1210**	22/**646**
HEK293	Control (no expansion)	17/18	17/18	17/17	17/18	18/19

* The Tandem Repeats Finder (TRF) catalogue includes a tailing CAACAG as part of the *HTT* repeat, so we subtracted 2 from reported repeat counts for comparison against the documented genotypes.

1 These tools occasionally included additional non-CAA/CAG repeats in the VCF output for the locus. We identified the CAG stretch in the reported expanded allele and calculated the copy number.

a Mean PCR genotype across 10 volunteer laboratories from Kalman *et al.* 2007. Multiple peaks are visible in this sample, which De Luca *et al.* (2021) also found via repeat-primed PCR.

b In this sample, there is a wide range of copy numbers in the expanded allele (i.e., mosaicism or somatic instability) visible in the read-level data.

Panel B: *FMR1* genotyping results

Sample	Documented genotype†	STRkit	LongTR[1]	Straglr	STRdust[1]	TRGT
NA13664	28±3/49±3	31/**54**	31/**53**	32/**54**	32/**53**	32/**54**
NA06896	23/**95-140**[c]	24/**187**	24/**196**	24/**196**	24/**150**	24/**192**
NA07537	28-29/**>200**	30/**343**	29/**348**	30/**341**	29/*29*	30/**339**
HEK293	Control (no expansion)	30/31	30/32	32/32	30/30	31/32

† The TRF catalogue includes an interrupting section (4 amino acids) in the *FMR1* repeat which we subtracted from reported repeat counts in order to match the Coriell genotype.

c Pre-mutation expansion range (linked to FXPOI and FXTAS).

HTT and *FMR1* genotypes from long-read STR calling tools using high-depth targeted CCS in seven samples with expansions plus a control sample. Pathogenic-length expansions are shown in bold text; expansions at the upper limit of “normal” are underlined; expansion alleles which were missed are italicized. Documented sample genotypes are from the Coriell institute, except where noted; accessed from https://www.coriell.org/ Oct 4, 2022.