# Supplementary Information: Linked-read sequencing for detecting short tandem repeat expansions

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*10x sequencing of Coriell samples*

Four and six reference lymphoblastoid cell lines with GAA- and CGG-repeat expansions in the *frataxin* (*FXN*) and *fragile X mental retardation 1* (*FMR1*) genes, respectively, were obtained from the Coriell Cell Repository (Camden, New Jersey, United States; https://www.coriell.org/). The *FXN* and *FMR1* reference samples, their genotypes (normal/NL, premutation/PM, and full-

mutation/FM), and repeat lengths reported by Coriell are shown in Supplemental Table 1. High molecular weight genomic DNA derived from the cell lines was used for library preparation with the Chromium Genome Reagent Kit (v2 Chemistry). Paired-end whole-genome sequence (WGS) reads of 150 base pairs were generated on an Illumina HiSeq X instrument and processed using the Long Ranger™ pipeline (v2.2.2) from 10x Genomics. The generated reads were mapped to the hg19 human reference genome build.

**Table S1.** *FMR1* and *FXN* genotypes and allelic repeats lengths of Coriell samples used for 10x linked-read whole-genome sequencing. The two normal samples, GM06889 and GM06895, were not analyzed using the barcode-based methods presented in the manuscript. On-line information to the cell lines is accessible through the hyperlinks under "Coriell ID".

| Coriell ID | Actual genotype | Gene | Repeat motif | Repeat size (copy number) |
|---|---|---|---|---|
| GM15847 | NL/FM | *FXN* | GAA | NL/760 |
| GM15848 | NL/FM | *FXN* | GAA | NL/830 |
| GM15849 | NL/FM | *FXN* | GAA | NL/920 |
| GM15850 | FM/FM | *FXN* | GAA | 650/1030 |
| GM06889 | NL/NL | *FMR1* | CGG | 23/30 |
| GM06891 | PM | *FMR1* | CGG | 118 |
| GM06894 | NL/PM | *FMR1* | CGG | 30/78 |
| GM06895 | NL | *FMR1* | CGG | 23 |
| GM06896 | NL/PM | *FMR1* | CGG | 23/95-120-140 |
| GM06897 | FM | *FMR1* | CGG | 477 |

**Table S2a.** Information of modified loci in 10x and stLFR simulations used for method comparison.

| Gene | Chromosome | Motif | Size (bp) | Copy number | Zygosity | Sex |
|---|---|---|---|---|---|---|
| *GIPC1* | 19 | CCG | 500 | 167 | het | male |
| *ATXN8OS* | 13 | CTG | 750 | 250 | het | male |
| *FMR2* | X | CCG | 1000 | 334 | hemi | male |
| *C9orf72* | 9 | GGCCCC | 1500 | 250 | het | male |
| *SAMD12* | 8 | TGAAA | 2000 | 400 | het | male |
| *GLS* | 2 | GCA | 2500 | 834 | homo | male |
| *GLS* | 2 | GCA | 3000 | 1000 | homo | male |
| *RFC1* | 4 | AAGGG | 3500 | 700 | homo | male |
| *RFC1* | 4 | AAGGG | 4500 | 900 | homo | male |
| *NOP56* | 20 | GGCCTG | 5000 | 834 | het | male |

**Table S2b.** Information of expanded (relative to reference genome) loci in NA12878 assembly used for method comparison.

| Locus | Motif | Reference Size (bp) | Assembly sizes (allele1/allele2 bp) |
|---|---|---|---|
| chr11:133354203-133354353 | TGG | 151 | 1715/2790 |
| chr11:92705289-92706006 | GTG | 718 | 2437/2411 |
| chr13:112972218-112972863 | TGGA | 646 | 1612/1612 |
| chr13:21725858-21726151 | CACCAC | 294 | 2990/3069 |
| chr18:22231934-22232859 | TCCA | 926 | 2207/2331 |
| chr18:77252855-77253057 | CAC | 203 | 1496/506 |
| chr4:5706294-5706480 | ATAG | 187 | 2456/3136 |
| chr4:7724106-7725052 | GTG | 947 | 1269/1627 |
| chr4:8294583-8295012 | TCCA | 430 | 1640/1640 |
| chr6:3221957-3222326 | CAC | 370 | 1631/1600 |
| chr6:834343-834462 | TCCT | 120 | 1843/1876 |
| chr7:141336723-141337391 | CCA | 669 | 1962/1515 |
| chr7:98810989-98811637 | GTG | 649 | 3389/3385 |

het: heterozygous; homo: homozygous; hemi: hemizygous

**Table S3.** Computing benchmarks of both IRR and JI methods. Computing was performed on an Intel Xeon Gold 6254 3.10 GHz 144-core machine running Centos 7.6.

| Method | Step | Time | Memory (Peak) |
|---|---|---|---|
| IRR | 1. Extract reads based on barcodes mapped to target region (extract_irr.py) | 36m:04s | 550.79 MB |
| | 2. Identify IRR reads from extracted read set (id_irr.py) | 4m:53s | 1.73 GB |
| | 3. Determine repeat size based on IRR counts, read length, and coverage (get_irr_size.py) | 14.35s | 82.71 MB |
| | Benchmarking based on genotyping 13 loci on NA12878 stLRF data (3.34B reads) | | |
| JI | 1. Generate database of JI profiles for varying genomic intervals at random locations (model_span.py) | 14m:11s | 32.25 GB |
| | 2. Match JI profile of target region against database to estimate target's genomic distance (estimate_span.py) | 18.645s | 964.44 MB |
| | Benchmarking based on collecting JI profiles from 50,000 locations at distances from 200 to 10,000 at a step-size of 100 bp (step 1) and generating estimates for 13 loci (step 2) on NA12878 TELL-Seq data (1.11B reads).<br>Step 1 was run with multi-processing using 42 parallel processes. | | |

**Fig. S1.** A comparsion of genotyping performance on simulated STR expansions in BLRS data between ExpansionHunter (EH), barcode-based in-repeat reads (IRR), and Jaccard index (JI) methods. Black vertical bars indicate repeat counts of loci in reference genome. Orange vertical bars indicate repeat counts of simulated alleles. EH_noOTS: EH analysis without off-target sites (OTS) and EH_OTS: EH analysis with OTS.

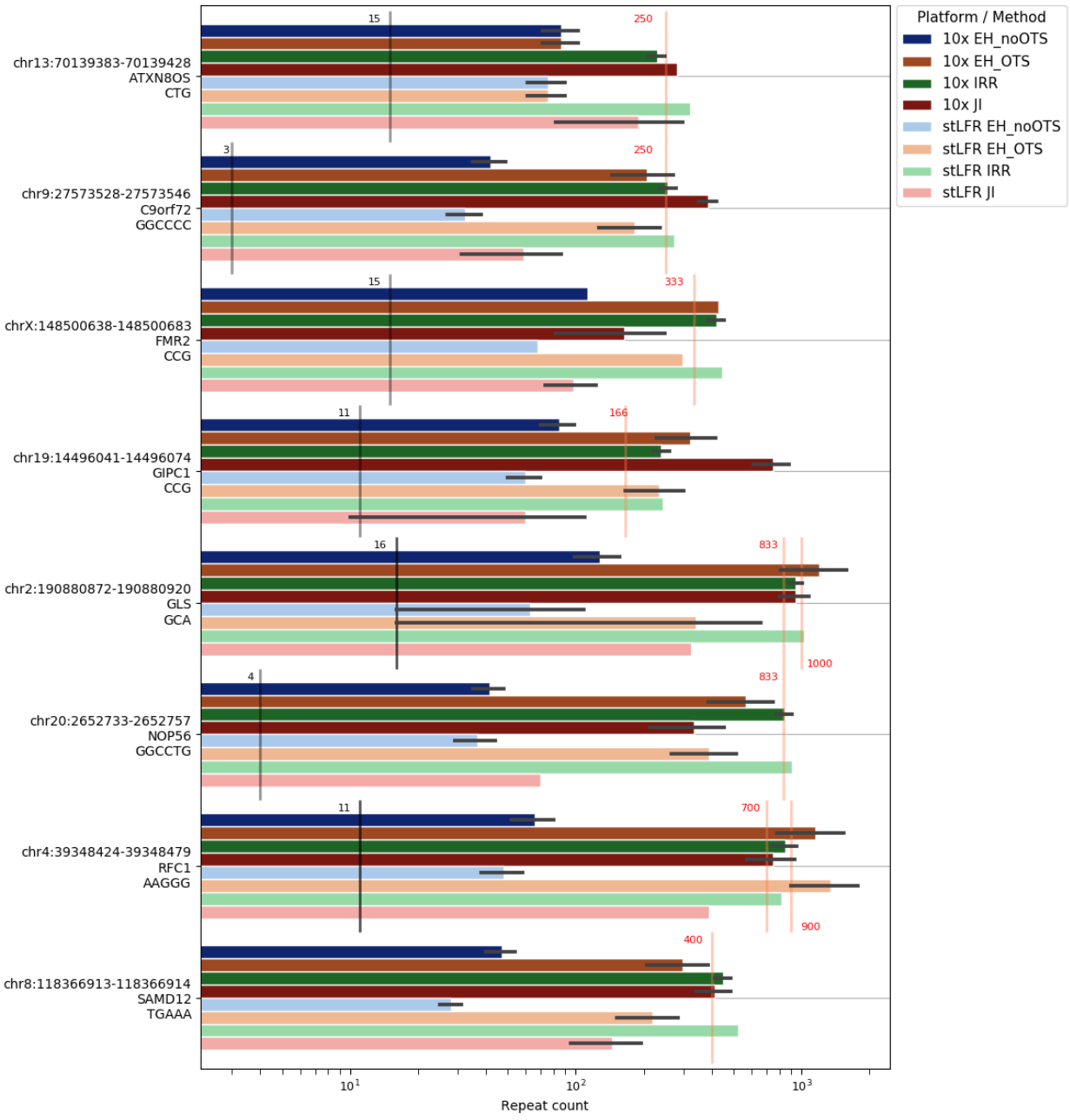
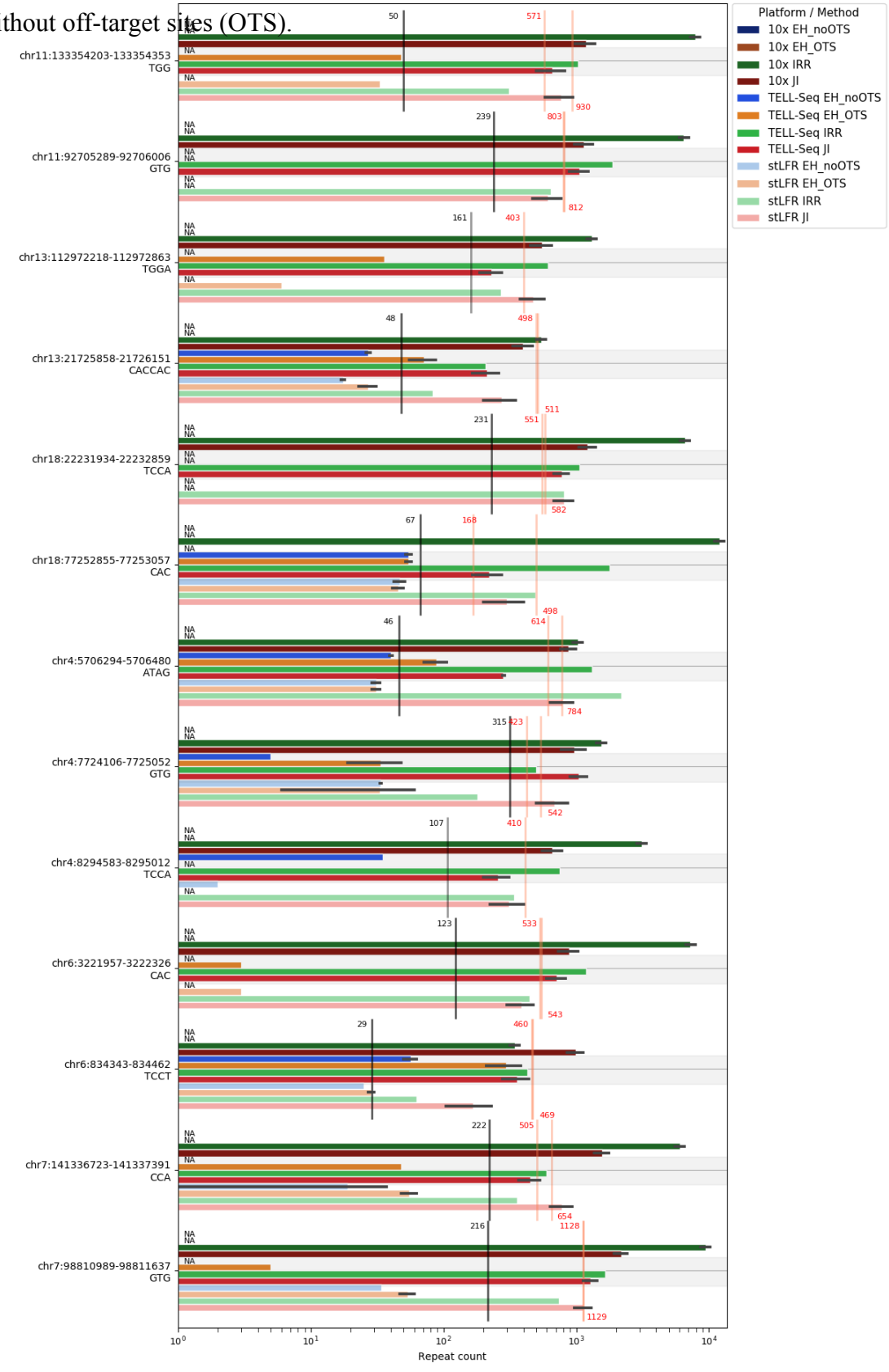


**Fig. S2.** A comparsion of genotyping performance on STR expansions, relative to the human genome and verified by *de novo* assembly, in NA12878 BLRS data between ExpansionHunter (EH), barcode-based in-repeat reads (IRR), and Jaccard index (JI) methods. Black vertical bars indicate repeat counts of loci in reference genome. Orange vertical bars indicate repeat counts of alleles determined from sequence assembly. "NA" indicates results not available because of failed software run or software's failure to genotype specific locus. EH_noOTS: EH analysis without off-target sites (OTS).



*10x and stLFR simulation*

10x and stLFR WGS with repeat expansions were simulated using LRSim[1] (v1.0) and stLFR read simulator[2], respectively. The *ATXN10, ATXN8OS, GIPC1, GLS, NOP56, RFC1, C9ORF72, SAMD12,* and *FMR2* repeat loci in the hg38 human reference genome were modified to contain expanded stretches of repeats (shown in Table S2a) using reform[3]. The modified and unmodified reference FASTA files were processed using LRSim's simulateLinkedReads.pl script in -r (reference) mode:

```
perl simulateLinkedReads.pl -r $modFASTA -p $output_prefix -7 0 -0 0 -z 16

perl simulateLinkedReads.pl -r $unmodFASTA -p $output_prefix -7 0 -0 0 -z 16
```

"clean" FASTA files from the modified and unmodified reference FASTA were used as inputs with the simulateLinkedReads.pl and stLFRSim to generate 10x and stLFR reads as follows:

```
perl simulateLinkedReads.pl -g hg38.hap.0.clean.fasta,hg38.hap.1.clean.fasta -p $output_prefix
-7 0 -0 0 -z 16 -x 350 -i 500 -s 50 -o

stLFRSim --ref hg38.hap.1.clean.fasta
         --o_prefix $output_prefix
         --lr_length_distribution lr_length_dis.txt
         --pe_num_distribution pe_num_dis.txt
         --if_lenth_distribution pe_length_dis.txt
         --readpair_num 225000000

stLFRSim --ref hg38.hap.0.clean.fasta
         --o_prefix $output_prefix
         --lr_length_distribution lr_length_dis.txt
         --pe_num_distribution pe_num_dis.txt
         --if_lenth_distribution pe_length_dis.txt
         --readpair_num 225000000
```

The generated paired-end 150 base pair 10x reads from the modified and unmodified sequences were concatenated and processed using Long Ranger:

```
longranger wgs  --id=sim${i}
                --fastqs=$fq
                --reference=$PATH/reference_genomes/H_sapiens/10X-Genomics/refdata-GRCh38-2.1.0
                --localcores=30
                --localmem=120
                --vcmode=freebayes
                --sex=female
```

The generated paired-end 100 base pair stLFR reads from the modified and unmodified sequences were concatenated and mapped to the hg38 reference genome using BWA-MEM[4], duplicates in the BAM file marked with Picard[5], and indexed using SAMtools[6].

*EH analysis with OTS*

The EH variant catalog file used for *ATXN10* analysis (hg38) is:


```
{
    "OffTargetRegions": [
        "chrX:9791969-9792159"
    ],
    "RepeatId": "ATXN10-withOTS",
    "RepeatUnit": "ATTCT",
    "TargetRegion": "chr22:45795355-45795424"
}
```


The EH variant catalog file used for *FXN* analysis (hg19) is:


```
{
    "OffTargetRegions": [
        "chr1:102123410-102123560",
        "chr13:102813926-102814076",
        "chr2:221410907-221411057",
        "chr5:126583029-126583179",
        "chrX:51364391-51364541"
    ],
    "RepeatId": "FXN-withOTS",
    "RepeatUnit": "GAA",
    "TargetRegion": "chr9:71652203-71652220"
}
```


The EH variant catalog file used for *FMR2* analysis (hg38) is:


```
{
    "OffTargetRegions": [
        "chr17:32142451-32142503",
        "chr19:10871563-10871651",
        "chr2:86914348-86914529",
        "chr7:100694312-100694385",
        "chrX:19990922-19990974",
        "chrX:67546512-67546566",
        "chrX:150983398-150983456"
    ],
    "RepeatId": "FMR2",
    "RepeatUnit": "CCG",
    "TargetRegion": "chrX:148500638-148500683"
}
```


The EH variant catalog file used for *GIPC1* analysis (hg38) is:


```
{
```

```
    “OffTargetRegions”: [
        “chr17:32142451-32142503”,
        “chr19:10871563-10871651”,
        “chr2:86914348-86914529”,
        “chr7:100694312-100694385”,
        “chrX:19990922-19990974”,
        “chrX:67546512-67546566”,
        “chrX:150983398-150983456”
    ],
    “RepeatId”: “GIPC1”,
    “RepeatUnit”: “CCG”,
    “TargetRegion”: “chr19:14496041-14496074”
}
```


The EH variant catalog file used for *GLS* analysis (hg38) is:


```
{
    "OffTargetRegions": [
        "chr16:73546662-73546734",
        "chr17:10995712-10995773",
        "chr17:51831668-51831732",
        "chr18:55586153-55586230",
        "chr19:45770206-45770266",
        "chr3:149766818-149766881",
        "chr7:7673311-7673371",
        "chrX:67545316-67545385"
    ],
    "RepeatId": "GLS",
    "RepeatUnit": "GCA",
    "TargetRegion": "chr2:190880872-190880920"
}
```


The EH variant catalog file used for *NOP56* analysis (hg38) is:


```
{
    "OffTargetRegions": [
        "chr5:146459014-146459174"
    ],
    "RepeatId": "NOP56",
    "RepeatUnit": "GGCCTG",
    "TargetRegion": "chr20:2652733-2652757"
}
```


The EH variant catalog file used for *RFC1* analysis (hg38) is:


```
{
    "OffTargetRegions": [
```

```
            "chr10:25874364-25874549",
            "chr10:100315011-100315116",
            "chr11:63257658-63257763",
            "chr21:39583818-39584123",
            "chr5:79180390-79180505",
            "chr5:79180514-79180629",
            "chrX:48280812-48280912"
        ],
        "RepeatId": "RFC1",
        "RepeatUnit": "AAGGG",
        "TargetRegion": "chr4:39348424-39348479"
}
```


The EH variant catalog file used for *SAMD12* analysis (hg38) is:


```
{
        "OffTargetRegions": [
            "chr21:34718631-34718684",
            "chr3:151368620-151368760",
            "chr4:114319040-114319089",
            "chr4:122718712-122718762",
            "chr6:157534533-157534600",
            "chr8:139313899-139313962",
            "chr9:78008566-78008623"
        ],
        "RepeatId": "SAMD12",
        "RepeatUnit": "TGAAA",
        "TargetRegion": "chr8:118366913-118366914"
}
```


The EH variant catalog file used for *C9ORF72* analysis (hg38) is:


```
{
        "OffTargetRegions": [
            "chrX:154390486-154390564"
        ],
        "RepeatId": "C9ORF72",
        "RepeatUnit": "GGCCCC",
        "TargetRegion": "chr9:27573528-27573546"
}
```


Generation of OTS: To identify the off-target sites, we simulated 100 or 150 bp reads entirely composed of the “motif of interest” and mapped these reads to the reference genome (hg19 or

hg38) using bwa mem. The mapping coordinates of the reads were extracted from the sorted BAM files using bedtools and were provided as off-target sites to EH.

*Illumina Coriell genomes*

The Illumina Coriell genomes[7] analysed in this study can be accessed in the European Genome-phenome Archive repository: https://www.ebi.ac.uk/ega/datasets/EGAD00001003562.

**References**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