

Cas9 targeted enrichment of mobile elements using nanopore sequencing

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Abstract

Mobile element insertions (MEIs) are highly repetitive genomic sequences that contribute to inter- and intra-individual genetic variation and can lead to genetic disorders. Targeted and whole-genome approaches using short-read sequencing have been developed to identify reference and non-reference MEIs; however, the read length hampers detection of these elements in complex genomic regions. Here, we pair Cas9 targeted nanopore sequencing with computational methodologies to capture active MEIs in human genomes. We demonstrate parallel enrichment for distinct classes of MEIs, averaging 44% of reads on targeted signals. We show an individual flow cell can recover a remarkable fraction of MEIs (97% L1Hs, 93% *AluYb*, 51% *AluYa*, 99% SVA_F, and 65% SVA_E). We identify twenty-one non-reference MEIs in GM12878 overlooked by modern, long-read analysis pipelines, primarily in repetitive genomic regions. This work introduces the utility of nanopore sequencing for MEI enrichment and lays the foundation for rapid discovery of elusive, repetitive genetic elements.

At least 45% of the human genome is composed of transposable element (TE)-derived sequences¹. TEs can be subdivided into four major categories: (i) DNA transposons; (ii) long terminal repeat (LTR) retrotransposons; (iii) long interspersed elements (LINEs); and (iv) short interspersed elements (SINEs). L1 represents a subclass of LINEs and L1-derived sequences comprise approximately 17% of the human genome^{1,2}. *Alu* elements, a subclass of SINEs, are ancestrally derived from a dimerization of the 7SL RNA gene and make up 11% of the human genome, spread out over 1 million copies³. SVA elements are active elements that have recently evolved and are derived from a SINE-R sequence coupled with a VNTR (variable number of tandem repeats) region and an *Alu*-like sequence⁴. An average human genome contains approximately 80-100 active full-length human-specific L1s (L1Hs)⁵⁻⁷ and a small number of highly active, or “hot,” L1Hs sequences that are responsible for the bulk of human retrotransposition activity^{5,6,8,9}. This includes the mobilization of *Alus* and SVAs which require trans-acting factors from L1s to transpose³. Collectively, the result of such recent mobilization events are referred to as mobile element insertions (MEIs).

Regions harboring these repetitive elements have long been considered part of the ‘dark matter’ of the genome with no expected impact on human phenotypes. However, recent studies indicate that at least some recent insertions indeed play a functional role in various aspects of the cell. L1-mediated retrotransposition events can be mutagenic, and germline retrotransposition events within the exons or introns of genes can result in null or hypomorphic expression alleles, leading to sporadic cases of human disease¹⁰. In addition, Lubelsky and Ulitsky demonstrated that sequences enriched in *Alu* repeats can drive nuclear localization of long RNAs in human cells¹¹. An SVA element insertion was recently reported in an intron of TAF1 that ablated expression through aberrant splicing, and is a driving mutation in X-linked Dystonia-Parkinsonism¹². Another study showed that a recurrent intronic deletion results in the exonization of an *Alu* element that is found in 6% of families with mild hemophilia A in France¹³. Somatic L1 retrotransposition can occur in neuronal progenitor cells¹⁴⁻¹⁸, indicating a possible role for L1s in the etiology of neuropsychiatric diseases¹⁹. In addition, a mutagenic L1 insertion that disrupted the 16th exon of the APC gene has been shown to instigate colorectal tumor development⁹. Beyond a widespread repertoire of disease associations, mobile elements also influence large scale genome structure. Recent work has demonstrated that transposition events are associated with three dimensional genome organization and the evolution of chromatin structure in human and mouse²⁰⁻²².

A tremendous effort has been made to understand the varied functional outcomes of active MEIs. Similar efforts are underway to capture and resolve MEIs to discover additional avenues of genetic pathogenesis^{8,23-28}. While transformative, these studies were confounded by the shortcomings of existing sequencing methodologies and bioinformatics pipelines, and limited in their ability to access a large (~50%), highly repetitive proportion of the genome²⁹. The difficulty in uniquely aligning short-read sequences to repetitive genomic regions likely leads to an under-representation of MEIs that have inserted within these regions. Several tools that have been developed to identify non-reference MEIs from whole genome short-read data, including the Mobile Element Locator Tool (MELT)²⁷, Mobster³⁰, Tangram³¹, TEA³², and others, are further restricted by the short read length and repetitive nature of mobile elements when resolving longer, non-reference insertions, such as L1Hs and SVA^{29,33}. Experimental approaches ranging

from paired-end fosmid sequencing^{8,34} to PCR capture-based approaches^{23,35–37} have also been developed to capture MEIs. However, these methods are intrinsically low throughput and short-read dependent, and they too are hindered by the aforementioned disadvantages.

The advent of long-read sequencing technologies provides a powerful tool for characterizing repeat-rich genomic regions by providing substantially longer sequence reads compared to traditional short-read platforms^{38,39}. We have recently applied these technologies to demonstrate that there are at least 2-fold more polymorphic L1Hs sequences in human populations than previously thought²⁹. Several existing tools and pipelines have the ability to resolve reference and non-reference MEIs in the human genomes; however, most require a whole-genome pipeline for haplotype assembly, local assembly, or cross-platform support^{40–43}. This often necessitates whole genome long-read sequencing, which is currently cost-prohibitive at scale and precludes an in-depth exploration into the impact of MEIs on human biology and disease. One solution to these barriers is the application of Cas9 targeted sequence capture with long read sequencing that allows for alignment to unique flanking genomic regions⁴⁴. This approach significantly lowers costs and enables a focused and efficient computational analysis for MEI discovery. Here, we demonstrate the utility of an *in vitro* Cas9 enrichment of targeted sequence elements combined with Oxford Nanopore long-read sequencing and established computational methodologies to identify a set of MEIs (L1s, *Alu*Ys, and SVAs)⁴⁴ that account for over 80% of currently active mobile elements in the human genome^{45–47}. This technology has been previously utilized to resolve a variety of genomic structural variants, including diseases associated with polynucleotide repeats and oncogenic translocation events^{44,48–50}. By targeting the Cas9 to subfamily-specific sequences within each element, we demonstrate enrichment of mobile elements across the genome that are both annotated and unannotated in the GRCh38 reference (reference and non-reference MEIs, respectively).

Results

Cas9 targeted enrichment strategy for mobile elements using nanopore sequencing

We chose GM12878 (NA12878), a member of the CEPH pedigree number 1463 (GM12878, GM12891, GM12892)⁵¹, as the benchmark genome in this study. GM12878 is one of the most thoroughly investigated human genetic control samples and has been used in many large-scale genomic projects, such as HapMap⁵², 1000 Genomes Project^{34,53,54}, the Human Genome Structural Variation Consortium^{33,40}, Genome In A Bottle^{55,56}, and reference genome improvement projects⁵⁷. To precisely capture MEIs of interest, we applied Cas9 targeted nanopore sequencing^{44,48–50} to enrich for five active subfamilies of MEIs (L1Hs, *Alu*Yb, *Alu*Ya, SVA_F, and SVA_E) in GM12878 (**Fig.1**) as well as L1Hs in the corresponding parental samples.

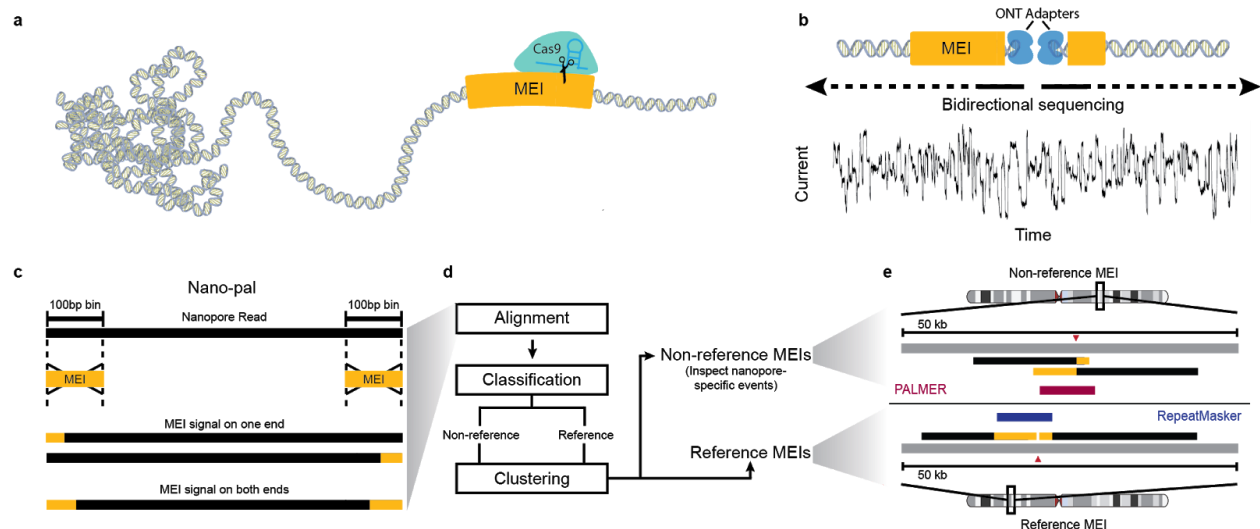


Fig.1: A schematic Cas9 targeted enrichment and Nano-Pal pipeline for mobile elements using nanopore sequencing.

a, Purified genomic DNA (gDNA) is isolated by salting out and then extensively dephosphorylated. Dephosphorylated gDNA is incubated with the Cas9 ribonucleoprotein which is targeted to MEI subfamily-specific sequences near the 3' end of the element. Taq polymerase (not shown), and dATPs (not shown) monoadenylate DNA ends. **b**, Cas9 cleaved sites are ligated with Oxford Nanopore Technologies (ONT) sequencing adapters and sequenced on a flow cell. Sequencing is bi-directional from the cleavage site. **c**, Nano-Pal scans the nanopore sequencing reads (black bars) after Cas9 enrichment for MEI signal on one or both ends. The yellow bar represents MEI consensus sequence or MEI signals in pairwise comparison of Nano-Pal. **d**, All reads with or without annotated MEI signal are imported into the downstream pipeline. Alignment, classification, and clustering processes are sequentially conducted. Nano-Pal identifies reference and non-reference MEIs followed by the inspection of nanopore-specific non-reference MEIs (see **Methods**). **e**, Examples illustrating capture and alignment of reads containing non-reference L1Hs signal (top) and reference L1Hs signal (bottom). Aligned reads display a non-reference insertion (top) with L1Hs signal (yellow bar) and flanking genomic sequence (black bar). MEI components of reads in non-reference insertions are displayed as overlapping (soft clipping) due to lack of reference genome MEI annotation (grey bar). Aligned reads display annotated reference L1Hs (bottom, yellow bar), flanked by surrounding genomic sequence (black bar), separated by the Cas9 cleavage site (red triangle). PALMER and RepeatMasker tracks are illustrated in red and blue, respectively.

We designed guide RNAs using unique subfamily-specific sequences within each element to maximize the specificity of Cas9 targeting (**Fig. 2**, see **Methods**). Using this approach, we generated a list of candidate 20bp guide RNAs for each MEI category (**Fig.2a-c**). We selected an L1Hs candidate guide RNA with the 'ACA' motif at 5929bp of the L1Hs consensus sequence, which distinguishes the L1Hs subfamily from other L1 subfamilies (e.g. L1PA)^{23,37,47}. For *AluY* and SVA, 18 unique guide RNA candidates were obtained (one for *AluYb*, three for *AluYa*, seven for SVA_F, and six for SVA_E) (see **Methods**, **Supplementary Fig.1,2** and **Supplementary Table 1**). Candidates were further prioritized to those with the largest number of subfamily-specific bases and proximity to the 3' end of the MEI sequence, near the polyA tail, which is an obligate component of the TPRT mechanism of retrotransposition^{58,59}. From the pool of candidates, a single guide RNA for each MEI subfamily was selected for downstream

enrichment experiments (**Supplementary Table 1**). After Cas9 enrichment and read processing, we assessed the cleavage sites of all five guide RNAs (**Fig. 2d and Supplementary Fig. 3**). The resulting distribution showed a vast majority of the forward-strand reads start at the third or fourth base-distance from the 'NGG' PAM site, and reverse-strand reads begin at the seventh base. This is consistent with previous characterization studies of Cas9 cleavage activity⁶⁰. Furthermore, we observed strand bias with approximately 4.6-fold more reads on the forward strand compared to the reverse strand, which has been hypothesized to be caused by Cas9 remaining bound after cutting and obstructing adapter ligation and sequencing^{44,61}. We detected directional sequencing biases within different MEI subfamilies and enrichment runs (**Supplementary Table 2, Supplementary Fig.3**).

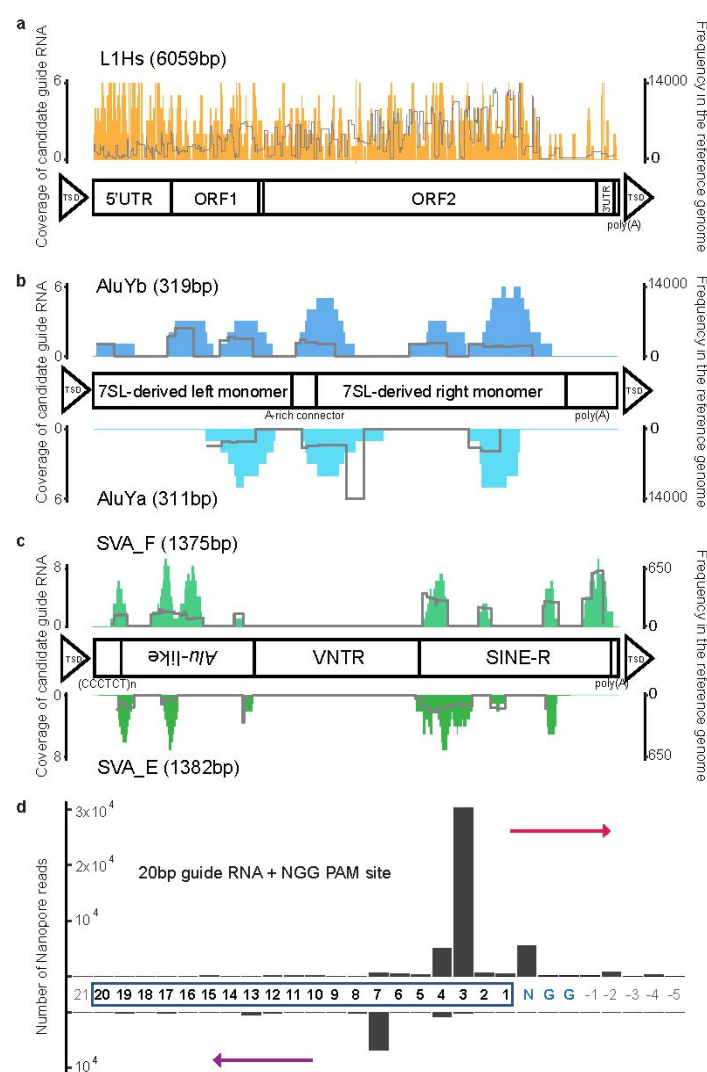


Fig.2: Guide RNA design for MEIs and guide RNA cleavage-site distribution.

a, Distributions of candidate guide RNAs (left Y-axis) in the L1Hs consensus sequence and structure information. The right Y-axis indicates frequency of corresponding candidates in the reference genome sequence. **b**, Upper panel shows the distribution for *AluYb* and the lower panel for *AluYa*. **c**, Upper panel

shows the distribution for SVA_F and the lower panel for SVA_E. **d**, Cleavage-site distribution of all guide RNAs in this project. The x-axis indicates the position where the read ends or begins, with the number depicting the base distance from the PAM site (NGG). The PAM site (NGG) is colored blue and guide RNA bases are highlighted by a rectangle. Bases outside of the guide RNA or the PAM site are colored grey. The y-axis is the number of nanopore reads counted. The upper bar represents reads with forward strand sequencing outward from the 3' end of the guide RNA (red arrow) and the lower bar represents reads with reverse strand sequencing outward from the 5' end of the guide RNA (purple arrow).

We developed a computational pipeline, Nano-Pal, to analyze captured long reads after base-calling and trimming, estimate the on-target rate of Cas9 enrichment from MEI signals on the ends of reads, and identify reference and non-reference MEIs (**Fig.1b,c**). Due to the frequency of targeted MEIs in the genome, an individual nanopore read may harbor a MEI signal on one or both ends. Reads with a single-end MEI signal had similar read lengths within all MEI experiments, yet were significantly larger than reads with two-end MEI signals. This was especially true in L1Hs experiments (L1Hs 1.9-fold, *AluY* 1.1-fold, SVA 1.4-fold, **Supplementary Fig.4**). To better distinguish non-reference MEI signals from those present in the reference, particularly where non-reference MEIs are embedded into reference MEIs²⁹, the pre-masking module from an enhanced version of our long-read non-reference MEI caller, PALMER²⁹, was implemented into Nano-Pal (**Fig.1d**). This enabled identification and masking of reference MEIs in individual long-reads, enhancing detection of non-reference MEI signals within the remaining unmasked portion²⁹. Non-reference and reference MEIs were then summarized by clustering nearby nanopore reads.

Cas9 targeted enrichment efficiently captures mobile element signals in nanopore reads

We performed a total of seven distinct MEI enrichment experiments across two types of nanopore sequencing platforms: Oxford Nanopore Flongles and MinIONs. Five of the experiments, one for each MEI subfamily, were sequenced on Flongle flow cells. One MinION flow cell was used to sequence an L1Hs enrichment, and another MinION flow cell was used to sequence a pooled sample of all five MEIs (see **Methods**) (**Table 1 and Supplementary Table 3**). The N50 ranged from 14.9kb to 32.3kb in all the experiments after base calling and quality controls.

To estimate the enrichment efficiency for different flow cells and MEI subfamilies, all passed reads were classified into three categories: on-target, close-target, and off-target (see **Methods**). The on-target rate for nanopore reads from a single L1Hs experiment on a Flongle flow cell, including both reference and non-reference MEIs, was 56.9%. Relatively lower on-target rates were observed for the other MEIs on the Flongle flow cell: 46.7% for *AluYb*, 23.8% for *AluYa*, 5.8% for SVA_F, and 2.3% for SVA_E (**Table 1**). When an L1Hs enrichment experiment was sequenced on a MinION flow cell, the on-target rate was approximately 35.0% (FAL11389) and 23.3% for a pooled MEI run (FAO84736). Compared to earlier studies (2.09% in Flongle and 4.61% in MinION)⁴⁴, these results show substantially improved enrichment, with a 1- to 25-fold increase relative to the Flongle flow cell, and over 5-fold enrichment relative to the MinION flow cell. These enrichment increases are likely due to the frequency of the targets in the genome. Overall, our approach reaches an average of 44% of nanopore sequencing reads with target MEI signal from these seven flow cell runs.

Table 1 Efficient enrichment of mobile element signals in nanopore reads.

Summary of seven representative flow cells: five individual Flongle flow cells for L1Hs (ABB607), *AluYb* (ACK645), *AluYa* (ACK655), SVA_F (ACK629), and SVA_E (ABO395) each, one individual MinION flow cell for L1Hs (FAL11389), and one pooled MinION flow cell for five MEIs (FAO84736).

MEI	Run	Flow cell	Read number	On-target		Close-target	Off-target
				Reference	Non-reference	Reference	
Individual							
L1Hs	ABB607	Flongle	4,102	49.6%	7.3%	16.8%	26.3%
AluYb	ACK645		2,271	40.2%	6.5%	1.0%	52.2%
AluYa	ACK655		12,513	18.0%	5.8%	10.3%	65.9%
SVA_F	ACK629		14,106	3.7%	2.1%	3.7%	90.6%
SVA_E	ABO395		7,297	1.7%	0.6%	0.2%	97.5%
L1Hs	FAL11389	MinION	110,029	30.7%	4.3%	37.9%	27.1%
Pooled							
Pooled	FAO84736	MinION	105,410	20.1%	3.2%	38.4%	38.3%
L1Hs				8.9%	1.6%	33.9%	
AluYb				7.0%	1.2%	3.2%	
AluYa				2.8%			
SVA_F				1.2%			
SVA_E				0.2%	0.4%	1.3%	

To further assess the improved enrichment of MEI subfamilies, the extent of representation of MEI targets with high sequence identity were examined within the data. A portion of the enrichment data contained ‘close-target’ reads that resemble related subfamilies of the intended targets and can be explained by the base mismatch tolerance between the guide RNA sequence and the targeted MEI sequence^{62,63}. For L1Hs, a rate of 16.8% on the Flongle and 33.9% to 37.9% on the MinION flow cell was observed, with close-target reads mapping to reference L1PA regions. Flongle sequencing of *AluYa* had a rate of 10.3% of close-target reads to other reference *AluY* elements, in contrast to *AluYb* where a dramatically reduced ‘close-target’ rate of 1.0% was observed (**Table 1**). This enhanced specificity may be explained by a specific insertion sequence within *AluYb* (5'-CAGTCCG-3') that was included in the guide RNA and is unique to the youngest *Alu* elements (*AluYb*)⁴⁶ of the genome. For the SVA Flongle sequencing, ‘close-target’ rates of 3.7% and 0.2% to the other reference SVAs were observed in the SVA_F and SVA_E enrichment, respectively.

Cas9 enrichment and nanopore sequencing rapidly saturates reference and non-reference MEIs

Due to the possibility that the guide RNA may bind to off-target sites and direct Cas9 to cut in MEIs that are not perfectly matched to the guide sequence^{62,63}, we established expectation thresholds to evaluate the number of captured reference and non-reference MEIs. The reference MEI sets were obtained from RepeatMasker⁶⁴. A ‘PacBio-MEI’ callset in GM12878 was generated by comprising a mapping-based callset and an assembly-based callset as a comprehensive gold standard set for non-reference MEIs (see **Methods**). The PacBio-MEI includes 215 L1Hs, 362 *AluYb* and 593 *AluYa* in 1404 *Alus*, and 33 SVA_F and 24 SVA_E in 72

SVAs (**Supplementary Fig.5** and **Supplementary Table 4**). Three categories (lower, intermediate, and upper) were defined that contain a number of reference and non-reference MEIs from each subfamily (**Supplementary Table 5**). Each threshold classifies MEIs depending on the extent of allowed mismatches between the guide and the sequence. The lower-bound is the most stringent and requires a perfect match between the guide sequence and the MEI. The intermediate-bound is less stringent and can tolerate three or fewer mismatches. We consider this to be the closest estimation to the number of MEIs that a guide RNA could reasonably capture among these three boundaries. Finally, the upper-bound is the least stringent and most inflated, requiring that at least 60% of the guide sequence matches the MEI (see **Methods**).

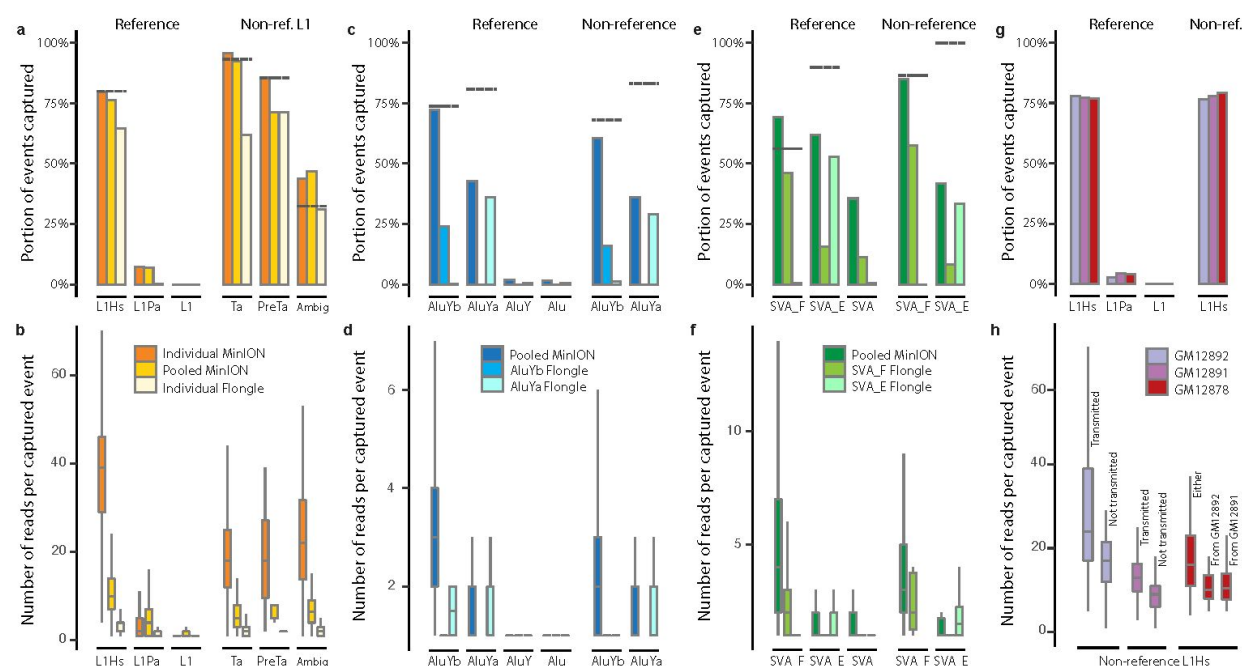


Fig.3: Systematic evaluation of known MEIs captured by nanopore Cas9 enrichment approach in different flow cells.

a, Known L1Hs in GM12878 recovered by Cas9 targeted enrichment from the individual MinION flow cell (FAL11389), pooled-MEI MinION flow cell (FAO84736), and individual Flongle flow cell (ABB607), displayed as a proportion of the upper-bound known reference L1Hs, L1Pa, and other L1 as well as non-reference (non-ref.) L1Hs from the PacBio-MEI set. Non-reference L1Hs were divided into different subfamilies (L1Ta, L1PreTa, and L1Hs with ambiguous subfamilies). Dotted-grey line represents the intermediate values (as proportion) of MEIs that the guide RNA binds when allowing a ≤ 3 bp mismatch or gap. **b**, Number of supporting reads of each captured L1 in the context of **a**. **c**, Known *AluY* elements in GM12878 recovered by Cas9 enrichment on one pooled MinION flow cell (FAO84736), one individual *AluYb* Flongle flow cell (ACK645), and one individual *AluYa* Flongle flow cell (ACK655). **d**, The number of supporting reads of each captured *Alu* element in the context of **c**. **e**, Known SVA elements in GM12878 recovered by Cas9 enrichment on one pooled MinION flow cell (FAO84736), one individual SVA_F Flongle flow cell (ACK629), and one individual SVA_E Flongle flow cell (ACK395). **f**, The number of supporting reads of each captured SVA element in the context of **e**. **g**, Known L1Hs captured in the GM12878 trio by Cas9 enrichment on one pooled MinION flow cell (FAL15177). **h**, The number of supporting reads of each captured non-reference L1Hs based on transmission in the GM12878 trio. The non-reference L1Hs in the parents (GM12892 and GM12891) were categorized into transmitted and

not-transmitted. The non-reference L1Hs in the child (GM12878) were categorized as insertions inherited from GM12892 or GM12891, and from either parents (unknown parental lineage). In **b, d, f, h**, error bars range from Q1-1.5IQR to Q3+1.5IQR (IQR, interquartile range) and outliers are not shown.

Upon comparing our MEI enrichment data to the aforementioned intermediate value estimates (**Fig.3a,b**, **Supplementary Fig.6**, and **Supplementary Table 5**), the individual and the pooled MinION flow cell captured 100% (35.8 mean coverage) and 96.0% (10.5 mean coverage) of known reference L1Hs with on-target reads, respectively. The individual Flongle captured 81.2% (2.7 reads mean coverage) of known reference L1Hs. For the non-reference L1Hs, the individual and pooled MinION flow cell captured 100% and 99.4% for all the L1Hs subfamilies, respectively. The individual Flongle captured 66.4%, 83.3%, and 95.2% for non-reference L1Ta, L1PreTa, and L1Ambig, respectively. Our results showed that only one of the MinION flow cells (FAL11389 or FAO84736) was necessary to capture most of the known reference and estimated non-reference L1Hs subfamilies in the genome when considering intermediate values, indicating a very high sensitivity of guide RNA targeting in the experiments.

Compared to the least stringent upper-bound estimates, 64.5% and 79.7% of known reference L1Hs were captured using the individual Flongle and MinION flow cell, respectively. Non-reference L1Hs capture ranged from 53.0% to 80.0%, compared to 4.1% to 7.3% of close-target L1PA elements, and less than 0.01% of off-targeting to other L1 elements (**Fig.3a,b**, **Supplementary Fig.6**, and **Supplementary Table 5**). The high percentage of elements captured that were on-target versus the other categories, including off-target, indicates the high specificity of the guide RNA to L1Hs in the enrichment. The read depth of the reference and non-reference L1Hs elements observed in these MinION flow cells has an approximate ratio of 2:1 (**Fig.3b**), consistent with the expectation that reference MEIs are homozygous, and a considerable portion of non-reference MEIs are heterozygous⁶⁵.

For *AluY* subfamilies, individual Flongles were utilized for separate runs of *AluYb* (ACK645) and *AluYa* (ACK655), and one pooled MinION flow cell (FAO84736) (**Fig.3c,d**, **Supplementary Fig.6**, and **Supplementary Table 5**). 93.9% (3.5 mean coverage) reference and 88.0% (2.4 mean coverage) non-reference *AluYb*s were captured from the pooled MinION flow cell run, based on intermediate values. Similar to the L1Hs, the MinION flow cell was able to capture a vast majority of *AluYb* elements when considering intermediate values, indicating high sensitivity performance of the *AluYb* enrichment. A relatively lower rate of capture for reference (52.7%, 1.6 mean coverage) and non-reference (42.0%, 1.4 mean coverage) *AluYa* enrichment was observed in the pooled MinION flow cell run based on intermediate values. Cross-capture rate from the two individual Flongle flow cells was less than 0.1%, and close- and off-target reference elements were <0.1% and 2% for the Flongles and MinION, respectively, indicating a high specificity of the guide RNA for each *AluY* subfamily (**Fig.3c** and **Supplementary Table 5**).

Similar enrichment rates were obtained from the two individual Flongle flow cells for SVA_F (ACK629) and SVA_E (ACK395), and in the pooled MinION flow cell (FAO84736) (**Fig.3e,f**, **Supplementary Fig.6**, and **Supplementary Table 5**). 100% (4.6 mean coverage) reference and 96.6% (3.9 mean coverage) non-reference SVA_F were captured from the pooled MinION flow cell run based on intermediate values. A relatively lower rate of capture for reference (68.7%, 1.9 mean coverage) and non-reference (41.7%, 1.5 mean coverage) SVA_E

enrichment was observed in the pooled MinION flow cell run based on intermediate values. The close-target reference SVAs capture rate was relatively high in two of the runs (35.6% in MinION flow cell and 11.4% in ACK629 Flongle flow cell). This could be due to SVAs sharing less base pair substitutions among their subfamilies compared to the other MEI families, as it is the youngest retrotransposon family found in the hominid lineage⁴⁵.

Our results indicate that an individual MinION flow cell (FAL11389) is able to completely (100%) capture reference and non-reference instances of a single MEI subfamily (L1Hs) compared to sequencing on the smaller Flongle flow cells. More importantly, a pooled run of an unbarcoded, five MEI subfamily enrichment experiment can recover the vast majority of known reference and non-reference MEIs (96.5% L1Hs, 93.3% *AluYb*, 51.4% *AluYa*, 99.6% SVA_F, and 64.5% SVA_E) in the genome when considering elements with a ≤ 3 bp mismatch to the guide RNA. Such an approach outperformed individual Flongle flow cells and approached the same capture level as the single MEI subfamily MinION run (**Fig.3, Supplementary Fig.6, and Supplementary Table 3,5**). This suggests that the MinION flow cell has ample sequencing capacity to accommodate each experiment with negligible competition between samples, despite sequencing multiple MEI enrichments on one platform. Finally, a substantial fraction of reference and non-reference MEI events can be captured in a single MinION sequencing experiment with multiple supporting reads. With further optimization of the enrichment and sequencing methodologies, it is plausible to fully saturate reference and non-reference MEIs in a single experimental iteration.

Detectable transmission of non-reference L1Hs within a trio

To trace the transmission of non-reference L1Hs in GM12878 from the parents, another enrichment experiment of L1Hs elements was performed in GM12878, GM12891, and GM12892 (**Fig.3g,h** and **Supplementary Table 3**). The on-target rate for L1Hs ranged from 34.3% to 40.4% in the individual Flongles for GM12891 and GM12892 (parents), which mirrors the on-target rate of GM12878 (child) (**Supplementary Table 6**).

Transmission of non-reference L1Hs to GM12878 from the parental genomes was further examined using available GM12891 and GM12892 sequencing data. As the parental genomes lack sufficient long-read sequencing data, we utilized MELT to resolve non-reference L1Hs callsets from high-coverage Illumina short-read sequencing data. This analysis yielded 123 and 118 high confidence, non-reference L1Hs in GM12892 and GM12891, respectively. The number of MEIs identified in the MELT call sets are relatively lower than the number ($n=205$) detected in the 'PacBio-MEI' set for GM12878, consistent with previous observations that long-reads are more sensitive for MEI discovery^{29,33,40} (**Supplementary Table 4**). Additional evidence of transmission can be derived by the enrichment of reads from non-reference MEIs in GM12878. We expect MEIs that can be transmitted from either parent will have higher read coverage due to a portion of these being homozygous, and single parent transmitted MEIs will be heterozygous in GM12878. As predicted, an enrichment of approximately 1.52-fold (17.6 vs 11.6 mean coverage) was observed for these reads (**Fig.3h**). Similarly, the 'not transmitted to child' non-reference L1Hs in parent samples should be heterozygous and were observed to be depleted by approximately 0.56~0.72-fold (17.4 vs. 31.3 mean coverage in GM12892 and 10.7

vs. 14.8 mean coverage in GM12891) of the nanopore reads that have been transmitted to the child (**Fig.3h**). These observations showed an expected supporting-read distribution of non-reference L1Hs, supporting the efficient nanopore Cas9 targeted enrichment in the pooled trio samples.

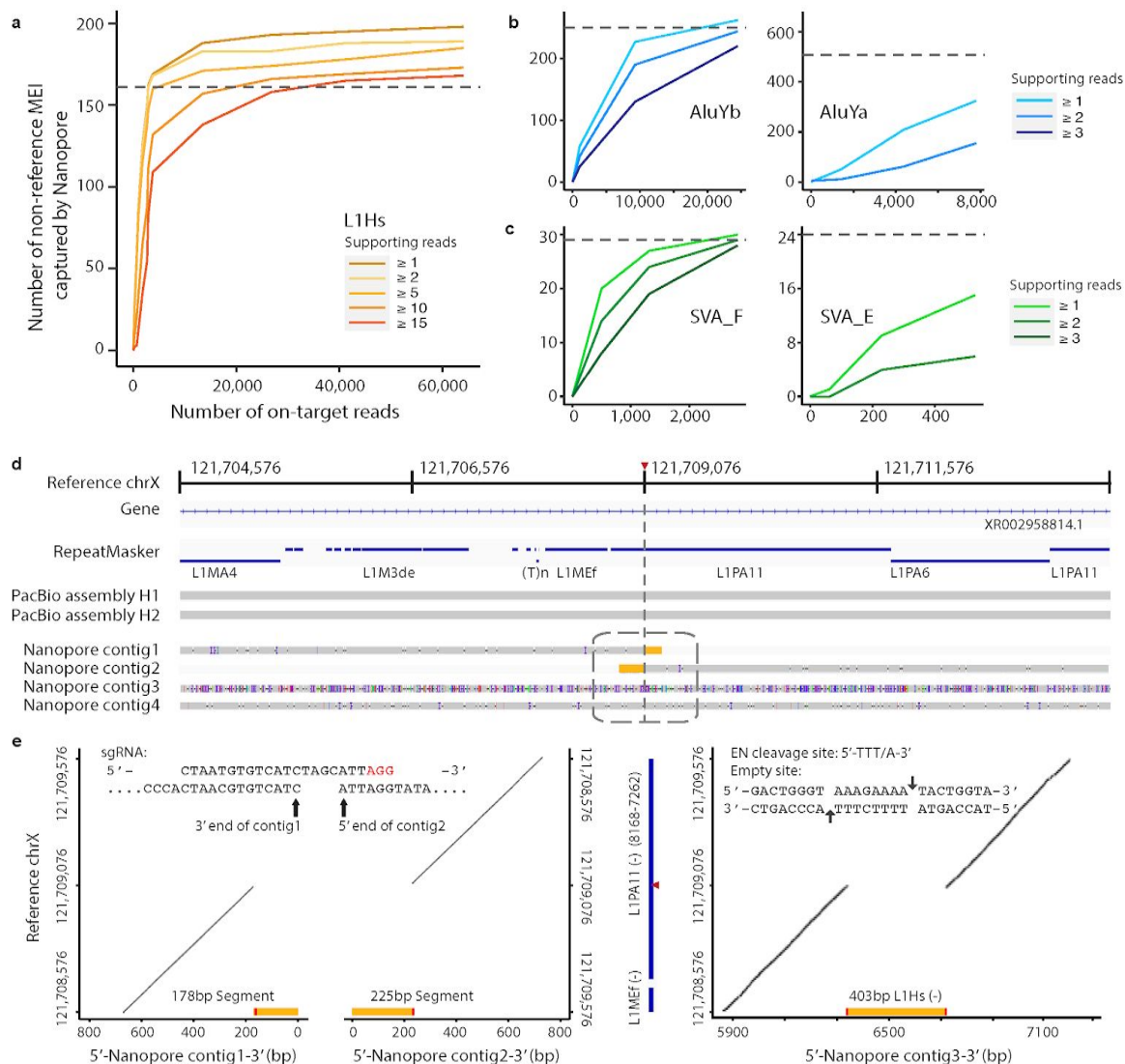


Fig.4. Non-reference MEIs captured by nanopore Cas9 enrichment approach.

a, Number of non-reference L1Hs captured by nanopore Cas9 enrichment at different on-target read coverages for different supporting read cutoffs. The dotted-grey line represents the theoretical number of MEIs that the guide RNA binds when allowing a ≤ 3 bp mismatch or gap in the PacBio-MEI set **b**, **c**, Number of non-reference *AluYb*, *AluYa*, *SVA_F*, and *SVA_E*, respectively, captured by nanopore Cas9 enrichment at different on-target read coverages. Axis labels and theoretic guide number as in **a**. **d**, An example of non-reference L1Hs specifically captured by nanopore sequencing at chrX:121,709,076. The tracks from top to bottom are as follows: reference coordinates with a red triangle represent the insertion site, gene track, RepeatMasker track (blue bars) with reference element annotation, PacBio contigs

assembly for two haplotypes, four nanopore local-assembled contigs by CANU from different classifications of nanopore reads based on insertion signals (contig1, signal on 3' end; contig2, signal on 5' end; contig3, signal in the middle of the read; and contig4, no signal). **e**, Recurrence (dot) plots for nanopore contigs versus the reference region chrX:121,708,576-121,708,576 sequence. Left panel shows the most 3' end of contig1 and the most 5' end of contig2 versus the reference sequence. Yellow bar represents the non-reference L1Hs sequence contained in the contig. The red bar represents one side of the target site duplication motif for the non-reference L1Hs contained in the contig. The upper part of this panel demonstrates sequences at the end of two contigs regarding the cleavage site when aligning to the guide RNA sequence. Blue bars in the middle panel represent the RepeatMasker track with reference L1 information annotated and the red triangle represents the insertion site in the reference L1 region. The right panel shows contig3 versus the reference sequence. Details of this non-reference L1Hs are detailed in the panel, including length, strand, empty site, and endonuclease (EN) cleavage site sequence.

Cas9 enrichment and nanopore sequencing captures non-reference mobile elements in complex genomic regions

To estimate the efficacy of enrichment for non-reference MEIs with different sequencing coverage, we manually inspected each non-reference MEI reported by Nano-Pal and performed subsequent saturation analysis for all flow cells (**Fig.4**, see **Methods**). We find few additional L1Hs insertions by including additional on-target reads beyond approximately 30,000, using a cutoff of 15 supporting reads (**Fig.4a**). This is consistent with the observation that the MinION (individual or pooled, usually with >100k passed reads) has the ability to capture most non-reference L1Hs. In addition, there was no observable enrichment bias of MEI subfamilies from different flow cells (**Supplementary Fig.7**).

We examined the 182 non-reference L1Hs in GM12878 that overlapped with the PacBio-MEI set. Of these 175 (96.2%) could be accounted for by the parental (GM12891 and GM12892) sequencing data (**Supplementary Fig.8**), and three overlapped known polymorphic insertions⁴⁰. The remaining four non-reference L1Hs are located within centromeric regions, which could be missed in the parental samples due to lack of supporting reads. In addition, we observed 601 non-reference *AluY* (including 323 *AluYb* and 263 *AluYa*) and 49 non-reference SVA (including 30 SVA_F and 15 SVA_E) that overlapped with the PacBio-MEI set. We further examined the set of MEIs that were captured exclusively by Cas9 targeted enrichment and nanopore sequencing, but not found in the PacBio-MEI intersection. We identified 16 additional L1Hs insertions as nanopore specific with ≥ 4 supporting reads that had been missed by the PacBio-MEI set. In addition, we detected 5 *AluY* elements that were specifically captured by nanopore reads in the GM12878 genome. After refinement and inspection, we generated a full set of non-reference MEIs (198 L1Hs, 606 *AluY*, and 49 SVA) captured by Cas9 enrichment and nanopore sequencing in the GM12878 genome. Of note, all intermediate-value calls of PacBio-MEI for L1Hs, *AluYb*, and SVA_F were recovered in this study (**Supplementary Table 5,7,8**).

One non-reference L1Hs insertion at chrX:121,709,076 was particularly intriguing. The PacBio genome assembly-based approach overlooked this insertion, as it fell within a 'reference L1 rich' region (**Fig.4d**). Upon further inspection, this event was supported as a 403bp heterozygous

L1Hs insertion by the existence of significant retrotransposition hallmarks, as well as recurrence (dot) plots^{29,66} (**Fig.4e**, see **Methods**). This insertion also shares a high sequence identity with a nearby reference L1PA11 element (**Supplementary Table 7**). The decrease in efficacy of the PacBio assembly-based approach in this region could be explained by the intricate nested ‘L1 in L1’ structure and observed heterozygosity (**Fig.4d**). Likewise, the *AluY* nanopore Cas9 enrichments captured interesting non-reference *AluY* instances: A homozygous *AluYb8* insertion at chr19:52384635, an exonic region within the ZNF880 gene, was reported to alter RNA expression due to the *Alu* element’s effects on the RNA secondary structure⁶⁷. Another heterozygous *AluYa5* insertion at chr16:69157709 was located within a reference *AluJr* region, indicating a potential nested ‘*Alu* in *Alu*’ structure that could hinder non-reference *AluY* discovery. These observations demonstrate the high sensitivity of nanopore Cas9 enrichment, suggesting its feasibility for MEI discovery in complex genomic regions.

Discussion

Here we describe our design and implementation of Cas9 targeted nanopore sequencing to enrich for retrotransposition competent, repetitive mobile elements in the human genome⁴⁴. After carefully designing guide RNAs to each MEI subfamily and coupling the enrichment with an established computational pipeline, our approach reaches an average of 44% nanopore sequencing reads with target MEI signals. We recovered a vast majority of reference and known non-reference mobile elements (96.5% L1Hs, 93.3% *AluYb*, 51.4% *AluYa*, 99.6% SVA_F, and 64.5% SVA_E) in the genome using only a single MinION flow cell. In addition, we discovered 21 non-reference MEIs within the GM12878 genome that were previously missed by other orthogonal long-read pipelines. Our data suggest that a MinION flow cell is ideal for a pooled, multiple-element enrichment experiment, as a prohibitively reduced enrichment or extensive cross-capturing of subfamilies was not observed. The work presented here highlights the potential of targeted enrichment and nanopore sequencing to rapidly discover distinct MEIs, and cements an experimental foundation to probe even the most elusive mobile element insertion events.

Cas9 targeted enrichment paired with nanopore sequencing has the potential for resolving complex structural variation, previously obfuscated by sequencing and computational limitations. We leveraged the nanopore Cas9 targeted sequencing⁴⁴ method to target active retrotransposons in the human genome in a discovery-based approach. To our knowledge, this is the first application of this method for repetitive mobile element detection. Our experiments indicate that by utilizing guides targeted to specific subfamily sequences, both reference and non-reference insertions can be efficiently enriched and mapped with multiple supporting reads on even the smallest of nanopore sequencing flow cells. Moreover, we demonstrate that individual sequencing experiments readily capture a majority of reference and non-reference elements. In both pooled and single element experiments, MEIs of five subfamilies are robustly enriched, suggesting that this method is widely applicable across mobile elements, and most suitable for high copy genomic elements. Guide RNA design process is straightforward, and targeting elements based on subfamily nucleotide differences captures both reference and non-reference elements, with negligible loss of sequencing to related close subfamilies or

off-targeting. Unlike other Cas9 targeted enrichment experiments, on-target (reference and non-reference) rates for our method are comparatively higher, exceeding 50% in some cases. While this is likely a consequence of the number of genomic copies of the targeted element and, to a lesser extent, the fidelity of the guide sequence, it reiterates this method is particularly suitable for MEI discovery.

The preponderance of uncharacterized MEIs, taken together with their potential contribution to genomic variation and disease, emphasizes the critical need for efficient mobile element detection strategies. Our experiments using Cas9 enrichment and nanopore sequencing can quickly map active mobile elements in the human genome, as well as their larger genomic context. Since Cas9 enrichment has been used to target rare rearrangement events⁶⁸, implementation of this approach to detect unique, *de novo*, or somatic mobile element insertions is a feasible endeavor. Identification of particularly rare insertion events, such as mosaicism resulting from retrotransposition in early embryogenesis, is possible. While we surmise that the vast majority of MEIs can be captured using this approach, it is important to recognize that some genomic locations may persistently conceal recently transposed elements. Centromeric regions and long palindromic repeats are examples of complex genomic features that could be recalcitrant to MEI discovery⁶⁹. With N50s of more than 25kb, we observed some MEI signals in centromeric and highly repetitive, palindromic regions from our nanopore sequencing reads. However, these regions still complicate mapping, requiring substantially longer sequencing and comprehensive analysis to confidently pinpoint elusive insertions⁷⁰. Merging the enrichment experiments discussed here with improved commercial kits and extremely high molecular weight genomic DNA, may be critical for preserving the extremely long fragments necessary to map MEIs in complex genomic landscapes. The importance of mobile element activity in shaping the genomes they inhabit cannot be overstated. Even beyond the scope of the human genome, mobile element activity plays an intricate role in evolution across many organisms^{71,72}. Accelerated discovery of active mobile elements and other repetitive genetic elements will expand our understanding of their contributions to phenotypic diversity in genomes from every form of life.

Methods

Cell culture, counting, and genomic DNA isolation

The following cell lines/DNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research: GM12878, GM12891, GM12892. Each cell line was cultured at 37°C, 5% CO₂ in RPMI 1640 media (ThermoFisher, 11875093) with L-glutamine, and supplemented with 15% fetal bovine serum (ThermoFisher, 10437028) and 1x antimycotic-antibiotic (ThermoFisher, 15240112). Cells were regularly passed and the media replenished every 3 days.

High molecular weight genomic DNA was isolated from GM12878, GM12891, and GM12892 using a “salting out” method⁷³ with the following modifications. Lymphoblastoid cells were harvested from culture and centrifuged at 500xg for 10 minutes at 4°C. Each pellet was washed

in 4°C 1X phosphate buffered saline (PBS) and cell number was counted using the Countess II. Washed cell pellets were resuspended in 3 mL of nuclei lysis buffer (10mM Tris-HCl pH 8.2, 400mM NaCl, and 2mM EDTA pH 8.2). 200uL of 10% SDS was added to the resuspension and inverted until evenly mixed. 50uL of RNase A (10mg/mL) was added and the lysate was rotated at 37°C for 30 minutes followed by addition of 50uL of proteinase K (10mg/mL) and rotation at 37°C overnight. 1 mL of saturated NaCl solution was added to the lysate and mixed by handheld shaking until evenly mixed. The sample was then centrifuged at 4000xg for 15 minutes at room temperature. The supernatant was transferred into a new 1.5mL microcentrifuge tube. 2 volumes of 100% ethanol were added to the supernatant and the tube was inverted approximately 20 times, or until the precipitate coalesced. The precipitate was isolated via spooling with a sterile p10 pipette tip and resuspend in a sufficient volume of 1X TE buffer (~250-500uL, depending on starting amount of cell material). Genomic DNA was passed through a 27G needle 3 times and stored at 4°C. The DNA concentration was measured using a Qubit 3 Fluorimeter and the dsDNA Broad Range Assay kit (ThermoFisher, Q32850)

Design of unique guide RNAs for L1Hs, *AluYb*, *AluYb*, SVA_F, and SVA_E

To maximize the enrichment performance for each MEI subfamily, the guide RNA (gRNA) candidates were designed to bind to the unique sequences within each subfamily. A pairwise comparison was conducted for the target MEIs with other, non target subfamilies. The consensus sequences for each target MEI subfamily were obtained from Repbase⁷⁴, namely L1Hs in the L1 family, *AluYa5* and *AluYb8* in the *Alu* element family representing the *AluYa* and *AluYb* subfamily, and SVA_E and SVA_F in the SVA family. These subfamilies account for over 80% of currently active mobile elements in the human genome⁴⁵⁻⁴⁷. The consensus sequences of L1PA2, *AluY*, primate *Alu*, and SVA_D were retrieved from Repbase and included as outgroups in the comparison analysis. Furthermore, *AluYa5* was added as an outgroup in the design of the gRNA for *AluYb*, *AluYb8* for *AluYa*, SVA_E for SVA_F, and SVA_F for SVA_E to avoid enrichment across target MEIs.

Guide RNA target sites (20bp sgRNA + 3bp NGG PAM site) for *S. pyrogenes* Cas9 were identified that are within unique MEI regions to obtain optimal guide candidates. Jellyfish2.0⁷⁵ was utilized to create a *k*-mer (*k* = 23) index for the sequences of these unique regions, and 23mers with a 5' 'CC' or 3' 'GG' were selected as gRNA candidates. The frequency of gRNA candidates and the three base substitution options in the 'NGG' PAM site for each candidate in the reference genome was calculated to confirm that the number of unique guide sequences is similar to the genomic reference MEI sequence frequency (**Supplementary Table 1 and Supplementary Fig.1,2**). The guide RNA candidates of *AluY* and SVA with unique sequences into were categorized into different tiers: Tier0, sequence has subfamily-specific bases in GG/CC of the PAM site; Tier1, the frequency of 23mer falls into a reasonable range (<2-fold of target MEI frequency) in reference genome; Tier2, the sequence has only subfamily-specific bases at the N site of the PAM or the frequency of the 23mer falls out of a reasonable range. A gRNA sequence falling in Tier0 was considered an ideal candidate.

On-target boundary calculations for MEIs

Using the final gRNA selection as a reference, an upper-bound, lower-bound, and intermediate value of the theoretical numbers of target MEIs could be estimated. The lower-bound for target MEIs was defined as a MEI sequence that contains the sequence the gRNA binds to with 100% (or 23bp) matched sequence, the intermediate bound allows for ≤ 3 bp mismatch or gap between the gRNA sequence and the matched MEI sequence, and the upper bound is a gRNA that aligns with more than 60% matched sequence (or ≥ 14 bp) to the MEI.

In vitro transcription of guide RNA and Cas9 ribonucleoprotein formation

Single stranded DNA oligos were designed using the EnGen sgRNA Designer tool (<https://sgRNA.neb.com/#!/sgRNA>, New England Biolabs) and purchased from IDT (Integrated DNA Technologies) to be used in the EnGen sgRNA Synthesis Kit (New England Biolabs, E3322S). Lyophilized oligos were resuspended in molecular biology grade water to a concentration of 100 μ M, and 1:10 dilutions were made for working stocks. Each reaction was set up containing 10 μ L of EnGen 2X sgRNA Reaction Mix (*S. pyrogenes*), 0.5 μ L of 100mM DTT, 2.5 μ L of 10 μ M oligonucleotide, 2 μ L of EnGen sgRNA Enzyme Mix, and brought to 20 μ L total with PCR grade water. The reactions were incubated at 37°C for 30 minutes to 1 hour. To degrade leftover DNA oligonucleotides, the reaction volume was adjusted to 50 μ L using PCR grade water, and 2 μ L of DNase (New England Biolabs, E3322S) was added to the sample and incubated for 15 minutes at 37°C. The sgRNA was purified by adding 200 μ L of Trizol and 50 μ L of chloroform to the sample, vortexed to mix and centrifuged at 20,000xg at room temperature. The aqueous layer was removed and placed into a new 1.5mL microcentrifuge tube and extracted again using 50 μ L of chloroform. The aqueous layer was removed and placed into a new 1.5mL microcentrifuge tube and ethanol precipitated in 2 volumes of 100% ethanol, and sodium acetate was added to a final concentration of 0.3M. The sample was centrifuged at max speed at 4°C for 30 minutes. The RNA pellet was washed with 70% ethanol, air dried, and resuspended in 10 μ L of PCR grade water. RNA concentration was measured using the Qubit RNA BR Assay Kit (ThermoFisher, Q10211). Fresh guide RNA was transcribed for every experiment, and prepared no more than a day in advance.

The Cas9 ribonucleoprotein (RNP) was formed by combining 850ng of *in vitro* transcribed guide RNA, 1 μ L of a 1:5 dilution of Alt-R *S.p.*Cas9 Nuclease V3 (Integrated DNA Technologies, 1081058), and 1X Cutsmart buffer (New England Biolabs, B7204S) in a total of 30 μ L. To allow for sufficient RNP formation, the reaction was incubated at room temperature for 20 minutes.

Cas9 enrichment for L1Hs on a MinION flow cell

To perform a Cas9 sequencing enrichment for L1Hs, a modified Cas9 enrichment experiment was performed⁴⁴. Three identical aliquots of 10 μ g of GM12878 genomic DNA were exhaustively dephosphorylated in a total volume of 40 μ L, with 1X Cutsmart buffer, 6 μ L of Quick CIP (New England Biolabs, M0525S), 10 μ g of gDNA, and H₂O for 30 minutes at 37°C. The Quick CIP was heat inactivated at 80°C for 20 minutes. 20 μ L of RNP (Cas9 + gRNA) was added to the

reaction along with 2uL of Taq polymerase (New England Biolabs, M0273L) and 1.5uL of 10mM dATP. The reaction was mixed by tapping and incubated at 37°C for 30 minutes for Cas9 cleavage, and 72°C for 10 minutes for monoadenylation. Following monoadenylation, each reaction was combined with 50uL of ligation mix: 25uL Ligation Buffer (LNB; Oxford Nanopore Technologies, SQK-LSK109), 5uL of Adapter Mix X (AMX; Oxford Nanopore Technologies, SQK-LSK109), 12.5uL of T4 DNA ligase (New England Biolabs, M0202M), and 5uL of nuclease-free water. The nanopore adapters were ligated to the genomic DNA at room temperature for 30 minutes on a tube rotator. Once completed, the ligations were diluted with 1 volume of 1X TE buffer (100uL). 60uL of SPRI beads (Beckman Coulter, B23317) were added to the adapter ligated samples and incubated at room temperature for 10 minutes with rotation and for another 5 minutes without rotation. Beads were immobilized using a magnet and the supernatant was removed. Immobilized beads were resuspended with 200uL of room temperature L fragment buffer (LFB; Oxford Nanopore Technologies, SQK-LSK109). At this step, the resuspended beads from the three samples were pooled into one Eppendorf tube. The magnet was applied again to immobilize the beads and remove the supernatant and the wash was repeated. Washed samples were pulse spun on a tabletop centrifuge for 1 second to collect beads at the bottom. Residual LFB was aspirated with a pipette. Beads were resuspended in 16.8uL of Elution Buffer (EB; Oxford Nanopore Technologies, SQK-LSK109) and incubated at room temperature for 10 minutes. Following the elution, the magnet was applied and the supernatant was collected and placed into a sterile Eppendorf tube. In some sample preparations, the adapter ligated library eluted in the last step may be viscous and the beads will resist immobilization on the magnet. A maximum speed centrifugation step prior to applying the magnet will help to immobilize the beads. Once the supernatant was separated from the beads into a sterile Eppendorf tube, 26uL of Sequencing Buffer (SQB; Oxford Nanopore Technologies, SQK-LSK109) was added and placed on ice until the sequencing flow cell was prepared. Immediately prior to loading of the sample, 0.5uL of Sequencing Tether (SQT, Oxford Nanopore Technologies, SQK-LSK109) was added along with 9.5 uL of Loading Beads (LB; Oxford Nanopore Technologies, SQK-LSK109). The sample was mixed evenly by pipetting with a p20 and loaded onto the sequencing platform.

Pooled Cas9 enrichment for L1Hs, *AluYb*, *AluYa*, SVA_F, and SVA_E in GM12878 (MinION)

Five parallel Cas9 enrichment experiments were performed for the five MEI subfamilies in GM12878 for a pooled sequencing run. Five separate aliquots of 10ug of genomic DNA were dephosphorylated in 40uL total (30uL of gDNA, 4uL of 10X CutSmart, 6uL of Quick CIP) for 25 minutes at 37°C then heat inactivated at 80°C for 5 minutes. Following the heat inactivation, each dephosphorylated genomic DNA sample was combined with 20uL of Cas9 RNP, 1uL of Taq polymerase, and 1uL of 10mM dATP. After briefly mixing by tapping, the reaction was incubated at 37°C for 30 minutes to enable Cas9 cleavage, then incubated to 75°C for monoadenylation by Taq polymerase. The Cas9 digested and monoadenylated samples were pooled into the ligation reaction (164uL of Custom LNB, 10uL of AMX, 20uL of T4 DNA ligase, and 164uL of nuclease-free water) and rotated at room temperature for 30 minutes. One volume of 1X TE buffer was added to the ligation and mixed by inversion approximately 10 times, or

until evenly mixed. 0.3X sample volume of SPRI beads (394.8uL) was added and incubated at room temperature with rotation for 5 minutes. The beads were immobilized using a magnet and washed twice with 100uL of room temperature LFB. After the final wash, the beads were pulse spun for 1 second in a table top centrifuge, immobilized on a magnet, and residual LFB was removed. The washed beads were eluted in 13uL of EB for 10 minutes at room temperature and removed using a magnet. The supernatant was collected and combined with 26uL of SQB. The library was incubated on ice until the flow cell was prepared. 0.5uL of SQT and 9.5uL of LB were added to the library before the loading onto the flow cell.

Cas9 enrichment for single MEI subfamily on a Flongle flow cell

10ug of purified genomic DNA was dephosphorylated using 4uL of Quick CIP in 1X Cutsmart buffer and brought to a total reaction volume of 40uL, then incubated for 30 minutes at 37°C. The sample was then incubated at 80°C for 5 minutes to inactivate the Quick CIP. 1uL of Taq polymerase, 1uL of 10mM dATP, and 20uL of the corresponding Cas9 RNP (targeting L1Hs, AluYb, AluYb, SVA_F, or SVA_E), was added to the dephosphorylated genomic DNA, gently mixed, and incubated at 37°C for 30 minutes, followed by a 10 minute incubation at 75°C. The sample was added to the ligation solution (25uL of custom LNB, 6uL of T4 DNA ligase, 5uL of AMX, and nuclease-free water to 100uL total), and incubated at room temperature for 20 minutes with rotation. The ligation was mixed with 1 volume (100uL) of 1X TE buffer and mixed by inversion approximately 10 times, or until evenly mixed. SPRI beads were added to a final 0.3X (60uL) to the sample volume (200uL) and the sample was rotated at room temperature for 5 minutes. The SPRI beads were immobilized on a magnet and washed twice with 100uL of room temperature LFB. After the final wash, the beads were pulse spun for 1 second on tabletop centrifuge and residual LFB was removed. The beads were resuspended in 9uL of EB and incubated at room temperature for 10 minutes. After the elution, the beads were immobilized on a magnet and the supernatant was transferred to a new 1.5mL microcentrifuge tube. 13uL of SQB was added to the supernatant and this library was placed on ice until the flow cell was prepared. Before loading the sample onto the flow cell, 0.5uL of SQT and 9.5uL of LB were added and mixed by gentle tapping.

Cas9 enrichment for L1Hs in trio (MinION)

To detect L1Hs in the lymphoblastoid trio cells (GM12878/91/92), a modified Cas9 enrichment assay, originally described by Gilpatrick et al. 2020, was performed. 10ug of genomic DNA for each genome (30ug total) was exhaustively dephosphorylated using 6uL Quick CIP for 45 minutes at 37°C, and heat inactivated at 80°C for 20 minutes. 20uL of the RNP (Cas9 and sgRNA) was added to the dephosphorylated genomic DNA along with 2uL of Taq DNA polymerase and 1.5uL of 10mM dATP. The reaction was incubated at 37°C for 30 minutes, then 72°C for 10 minutes. Each genomic DNA reaction was combined with an equal volume (50uL) of ligation mix for nanopore adapter ligation: 25uL Ligation Buffer (Oxford Nanopore Technologies, EXP-NBD104), 5uL of AMII (Adapter Mix II; Oxford Nanopore Technologies, EXP-NBD104), 12.5uL of T4 DNA ligase, 2.5uL of the barcode (NB01/02/03; Oxford Nanopore

Technologies, EXP-NBD104), and 2.5uL of nuclease-free water, and incubated at room temperature for 30 minutes on a tube rotator. After adapter ligation, an equal volume of 1X TE buffer was added to the reaction, and SPRI beads were added to a final 0.3X (~60uL). The library was incubated at RT for 10 minutes with rotation, and 10 minutes without rotation for a total of 20 minutes to allow for DNA binding to the SPRI beads. The beads were washed twice with 200uL of L Fragment Buffer (LFB; Oxford Nanopore Technologies, EXP-NBD104). The uniquely barcoded samples were pooled by combining the resuspended beads in the first wash, then washed again. The washed beads were resuspended in 16.8uL of the Elution Buffer (EB; Oxford Nanopore Technologies, EXP-NBD104). Resuspended beads were incubated at room temperature for 10 minutes. Following the incubation, the beads were collected with a magnet and the supernatant collected into a separate 1.5mL microcentrifuge tube and placed on ice. The sample was prepared for sequencing by adding 26uL of Sequencing Buffer (SQB; Oxford Nanopore Technologies, EXP-NBD104) and kept on ice while the flow cell was primed. 0.5uL of Sequencing Tether (SQT; Oxford Nanopore Technologies, EXP-NBD104) and 9.5uL of Loading Beads (LB; Oxford Nanopore Technologies, EXP-NBD104) were added after flow cell priming, before the sample was loaded onto the flow cell.

Nanopore flow cell preparation, sequencing, base-calling, and cleavage-site analysis

A MinION flow cell was purchased from Oxford Nanopore Technologies and stored at 4°C per manufacturer's instructions. The Ligation Sequencing Kit (SQK-LSK109) and Native Barcoding Kit (EXP-NBD104) were used to prepare the pooled libraries. Upon arrival and prior to usage, MinION flow cell QC was performed using the MinKNOW software. No appreciable loss of active pores was noted during storage. Prior to loading the library, the MinION was flushed with 800uL of FLB in the priming port, followed by priming of the flow cell with 200uL of 0.5x SQB diluted with water..

Flongle flow cells were purchased from Oxford Nanopore Technologies in batches and stored at 4°C. Flow cells were QC'd upon arrival and the number of active pores was noted. Flongles to be used in sequencing were QC'd immediately before use to assess pore loss during the storage period.

Base-calling was processed by Guppy 4.0.15 (Oxford Nanopore Technologies) using the high accuracy, modified base model (dna_r9.4.1_450bps_modbases_dam-dcm-cpg_hac.cfg). Porechop⁷⁶ was used to trim nanopore adapters and barcodes from the reads with Q-score > 7, as well as demultiplex the reads in the pooled sample MinION run.

To determine cut site preferences, reads were aligned to the consensus sequence of each mobile element class that were investigated; L1Hs, *AluYa5*, *AluYb8*, SVA_E and SVA_F. Only the first 80 base pairs of each read were used for alignment to focus on the cut site region and to ensure that the beginning of the reads aligned correctly. Pairwise alignments were performed using the Biopython Bio.Align package⁷⁷ with FASTA files as input. Each read was aligned to the mobile element consensus sequence as well as the reverse complement to determine sequence orientation. To obtain high-confidence alignments, strict gap penalties were enforced (open gap:-10, extend gap:-5). In order for an alignment to be considered for cut-site analysis, it

had to meet two criteria; the alignment had to start at the very first base of the read, and needed to have an alignment score of at least 100. The 5' ends of reads meeting this criteria were then used to estimate cleavage site location.

Nano-Pal for detection and refinement of MEIs from nanopore Cas9 enrichment

To resolve both the reference and non-reference MEI signals from nanopore Cas9 enrichment, we developed a computational pipeline, Nano-Pal, to analyze the nanopore reads and customized it for different MEI subfamilies (**Fig.1c,d**). Information of the potential targeting MEI signals was obtained by Nano-Pal scanning through both sides (100bp bin size) of all quality-passed nanopore raw reads using BLASTn^{78,79}. Next, it aligned the reads to the reference genome (GRCh38) using minimap2⁸⁰ and discarded reads with low mapping quality (MAPQ < 10). The aligned reads were screened by RepeatMasker⁶⁴ and the pre-masking module in PALMER²⁹ to bin them into different categories: reads with reference MEI signals, reads with non-reference MEI signals, and off-target reads. All reads that were reported by the PALMER pre-masking module fell into the on-target non-reference MEI category. If reads were not reported by PALMER, but were annotated by RepeatMasker, they fell into the reference MEI category. They will be further classified as on-target, close-target, and off-target reads depending on where they mapped to the reference regions. For L1Hs experiments, the reads mapped to the reference L1PA are considered as close-target and the ones mapped to other reference L1 are considered as off-target. For *AluY* experiments, the close-target reads are those that mapped to other reference *AluY* besides *AluYb* and *AluYa* and the off-target reads are defined when mapped to other reference *Alu* elements besides the reference *AluY*. For SVA experiments, the close-target reads are the ones mapped to other reference SVAs besides SVA_F and SVA_E and no off-target reads were defined when they have the MEI signals. Any remaining reads with no MEI signals were classified as off-target reads as well. The reads in the first and second category were then clustered into non-reference MEIs and reference MEIs, respectively. Nano-Pal was performed for each Flongle and MinION flow cell separately for GM12878, GM12891, and GM12892.

GM12878 trio data, reference genome, and reference MEI information

We obtained Pacific Bioscience (PacBio) long-read CLR sequencing data from Audano et al. 2019⁵⁷ for the GM12878 genome (50x coverage). The 30x Illumina NovaSeq sequencing data for GM12878 and the related samples (GM12891 and GM12892) were obtained from the 1000 Genomes project phase 3 sample set, which were generated at the New York Genome Center (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/1000G_2504_high_coverage/)^{54,81}. All analyses in this project were carried out using the GRCh38 (GRCh38+decoy) reference genome obtained from the 1000 Genomes Project (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/GRCh38_reference_genome/). Information of reference MEIs, including the five target subfamilies, were obtained from RepeatMasker⁶⁴.

Enhanced PALMER for resolving non-reference MEIs from whole-genome long-read sequencing

We developed an enhanced version of PALMER (Pre-mASKing Long reads for Mobile Element inSeRTion)²⁹ in this study to detect non-reference MEIs across the long-read sequenced genomes (<https://github.com/mills-lab/PALMER>). Reference-aligned BAM files from long-read technology were used as input. Known reference repetitive sequences (L1s, *Alu*s or SVAs) were used to pre-mask portions of individual reads that aligned to these repeats and also utilized in the Nano-Pal pipeline. After the pre-masking process, PALMER searched subreads against a library of consensus mobile element sequences within the remaining unmasked sequences and identified reads with a putative insertion signal (including 5' inverted L1 sequences, if available) as supporting read candidates. PALMER opens bins 5' upstream and 3' downstream of the putative insertion sequence for each read and identifies hallmarks of mobile elements, such as target site duplication (TSD) motifs, transductions, and poly(A) tract sequences. All supporting reads are clustered at each locus and those with a minimum number of supporting events are reported as putative insertions.

To improve the accuracy of non-reference MEI sequences derived from individual subreads, which tend to have lower per-read base-pair accuracy, local sequence alignments and error correction strategies were performed. Error correction was conducted by CANU⁸² (ver2.2) using default parameters on the subreads with MEI signals reported by PALMER, allowing the generation of error-corrected reads that served as inputs for local realignment using minimap2. A second-pass of the PALMER pipeline then was executed using these locally aligned error-corrected reads to generate a high-confidence call set of germline non-reference MEIs. CAP3⁸³ was used with default parameters to assemble all MEI sequences reported by the second-pass of the PALMER pipeline to generate a high-confidence consensus contig for each non-reference MEI event.

MEI callsets in orthogonal short-read and long-read data

As there is no public long-read data available for GM12891 and GM12892, we used the Mobile Element Locator Tool (MELT) to identify non-reference MEIs in the short-read Illumina sequencing data for the GM12878 trio^{27,65} as a benchmark set in the trio analysis. We applied the enhanced version of PALMER and carried out the non-reference MEI calling in GM12878. To generate a more comprehensive callset of non-reference MEIs in GM12878, the Phased Assembly Variant (PAV) caller was included (<https://github.com/EichlerLab/pav>), which can discover genetic variants based on a direct comparison between two sequence-assembled haplotypes and the human reference genome⁴⁰. The callset by PAV for GM12878 was generated from the PacBio HiFi sequencing data after haplotype-assembly. A 'PacBio-MEI' callset in GM12878 was generated by applying the union set of the mapping-based PALMER callset and the assembly-based PAV callset, both of which resolved the MEIs from PacBio long-read sequencing data. The details of MEI merging and subfamily defining strategy for the two approaches are described in a prior study⁴⁰.

Inspection and validation of nanopore-specific non-reference MEIs

All non-reference MEI calls were further intersected with the PacBio-MEI set and classified as known non-reference calls and potential nanopore-specific non-reference calls. A filtering module, an empirical curation of read-depth (<2-fold difference) within the 500bp bin of the insertion site from public data⁵⁷, and manual inspection were applied to exclude false-positive (FP) signals in the nanopore-specific non-reference MEIs. All potential nanopore-specific non-reference MEIs were classified into three categories: a) true positive (TP) non-reference event, b) FP non-reference event, and c) an ambiguous event (**Supplementary Table 7**). The category was further defined as TP missed by the PacBio sequencing data, TP missed by the PacBio mapped-based and assembly-based pipelines but with PacBio read signals, or TP redundant with the called non-reference one. Category (b) was broken down into three subcategories; FP redundant with the called reference event, FP targeting on the other off-target reference repeat, or ambiguous. All subcategories in (a), and the first (b) subcategory, were on-target reads with or without correct annotations. For example, one FP nanopore-specific non-reference call originated from reads targeted to reference MEIs, yet was categorized as non-reference due to a mapping error introduced by flanking structural variations (deletions, duplications, or inversions) (**Supplementary Table 7**). A recurrence plot analysis was further employed for further in-depth validation. For the recurrence plot analysis, a region of one sequence (X-axis) is compared to another sequence (Y-axis) and small (i.e. 10 bp) segments that are identical between the two sequences are denoted with a plotted point. Thus, a continuous diagonal line comprising multiple points indicates portions of the compared sequences that are identical. By comparison, gaps and shifts from the diagonal denote an insertion or deletion in one sequence relative to the other.

Non-reference MEIs captured by nanopore Cas9 enrichment sequencing in GM12878

The flow cell runs for each MEI subfamily were merged to investigate the read coverage enrichment performance and generate the final non-reference MEIs in GM12878. For the saturation analysis, the flow cells were ranked by the number of on-target reads. Reads from the flow cells were then added and merged, one flow cell at a time, based on the above ranking. Non-reference calls for L1Hs, *AluYb*, *AluYa*, SVA_F, and SVA_E were resolved by Nano-Pal and our validation process after every merging instance. By merging all batches for each subfamily, a final non-reference MEI callset in GM12878 captured by nanopore Cas9 enrichment approach was produced.

Data availability

The nanopore sequencing data for the Cas9 targeted enrichment of MEIs in this study are available in the SRA repository under BioProject accession PRJNA699027.

Code availability

All script and pipelines in this publication, including Nano-Pal, are available on GitHub: <https://github.com/Boyle-Lab/NanoPal-and-Cas9-targeted-enrichment-pipelines>.

The enhanced version of PALMER is available at <https://github.com/mills-lab/PALMER>.

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Author Contributions

A.P.B., R.E.M., W.Z., and T.M. conceived the project. J.S. established and cultured cell lines and isolated gDNA. T.M. performed the Cas9 targeted enrichment and nanopore sequencing. W.Z. developed the NanoPal pipeline and PALMER software. W.Z., C.C., and C.M. performed computational analysis. All authors guided the data analysis strategy. A.P.B., R.E.M., W.Z., and T.M. wrote the manuscript. All authors edited the manuscript. All authors read and approved the final manuscript.

Competing Interests statement

The authors declare no competing interests.

Reference

1. Smit, A. F. Interspersed repeats and other mementos of transposable elements in mammalian genomes. *Curr. Opin. Genet. Dev.* **9**, 657–663 (1999).
2. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
3. Deininger, P. Alu elements: know the SINEs. *Genome Biol.* **12**, 236 (2011).
4. Ostertag, E. M., Goodier, J. L., Zhang, Y. & Kazazian, H. H., Jr. SVA elements are nonautonomous retrotransposons that cause disease in humans. *Am. J. Hum. Genet.* **73**, 1444–1451 (2003).

5. Kazazian, H. H., Jr & Moran, J. V. Mobile DNA in Health and Disease. *N. Engl. J. Med.* **377**, 361–370 (2017).
6. Brouha, B. *et al.* Hot L1s account for the bulk of retrotransposition in the human population. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 5280–5285 (2003).
7. Sassaman, D. M. *et al.* Many human L1 elements are capable of retrotransposition. *Nat. Genet.* **16**, 37–43 (1997).
8. Beck, C. R. *et al.* LINE-1 retrotransposition activity in human genomes. *Cell* **141**, 1159–1170 (2010).
9. Scott, E. C. *et al.* A hot L1 retrotransposon evades somatic repression and initiates human colorectal cancer. *Genome Res.* **26**, 745–755 (2016).
10. Kazazian, H. H., Jr *et al.* Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man. *Nature* **332**, 164–166 (1988).
11. Lubelsky, Y. & Ulitsky, I. Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells. *Nature* **555**, 107–111 (2018).
12. Aneichyk, T. *et al.* Dissecting the Causal Mechanism of X-Linked Dystonia-Parkinsonism by Integrating Genome and Transcriptome Assembly. *Cell* **172**, 897–909.e21 (2018).
13. Jourdy, Y. *et al.* Recurrent F8 Intronic Deletion Found in Mild Hemophilia A Causes Alu Exonization. *Am. J. Hum. Genet.* **102**, 199–206 (2018).
14. Evrony, G. D., Lee, E., Park, P. J. & Walsh, C. A. Resolving rates of mutation in the brain using single-neuron genomics. *Elife* **5**, (2016).
15. Upton, K. R. *et al.* Ubiquitous L1 mosaicism in hippocampal neurons. *Cell* **161**, 228–239 (2015).
16. Coufal, N. G. *et al.* L1 retrotransposition in human neural progenitor cells. *Nature* **460**, 1127–1131 (2009).
17. Muotri, A. R. *et al.* Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. *Nature* **435**, 903–910 (2005).

18. Zhu, X. *et al.* Machine learning reveals bilateral distribution of somatic L1 insertions in human neurons and glia. doi:10.1101/660779.
19. McConnell, M. J., Moran, J. V., Abyzov, A. & Akbarian, S. Intersection of diverse neuronal genomes and neuropsychiatric disease: The Brain Somatic Mosaicism Network. (2017).
20. Diehl, A. G., Ouyang, N. & Boyle, A. P. Transposable elements contribute to cell and species-specific chromatin looping and gene regulation in mammalian genomes. *Nat. Commun.* **11**, 1796 (2020).
21. Zhang, Y. *et al.* Transcriptionally active HERV-H retrotransposons demarcate topologically associating domains in human pluripotent stem cells. *Nat. Genet.* **51**, 1380–1388 (2019).
22. Choudhary, M. N. K. *et al.* Co-opted transposons help perpetuate conserved higher-order chromosomal structures. doi:10.1101/485342.
23. Iskow, R. C. *et al.* Natural mutagenesis of human genomes by endogenous retrotransposons. *Cell* **141**, 1253–1261 (2010).
24. Steranka, J. P. *et al.* Transposon insertion profiling by sequencing (TIPseq) for mapping LINE-1 insertions in the human genome. *Mob. DNA* **10**, 8 (2019).
25. Huang, C. R. L. *et al.* Mobile interspersed repeats are major structural variants in the human genome. *Cell* **141**, 1171–1182 (2010).
26. Erwin, J. A. *et al.* L1-associated genomic regions are deleted in somatic cells of the healthy human brain. *Nature Neuroscience* vol. 19 1583–1591 (2016).
27. Gardner, E. J. *et al.* The Mobile Element Locator Tool (MELT): population-scale mobile element discovery and biology. *Genome Res.* **27**, 1916–1929 (2017).
28. Kvikstad, E. M., Piazza, P., Taylor, J. C. & Lunter, G. A high throughput screen for active human transposable elements. *BMC Genomics* **19**, 115 (2018).
29. Zhou, W. *et al.* Identification and characterization of occult human-specific LINE-1 insertions using long-read sequencing technology. *Nucleic Acids Res.* **48**, 1146–1163 (2020).

30. Thung, D. T. *et al.* Mobster: accurate detection of mobile element insertions in next generation sequencing data. *Genome Biol.* **15**, 488 (2014).
31. Wu, J. *et al.* Tangram: a comprehensive toolbox for mobile element insertion detection. *BMC Genomics* **15**, 795 (2014).
32. Lee, E. *et al.* Landscape of somatic retrotransposition in human cancers. *Science* **337**, 967–971 (2012).
33. Chaisson, M. J. P. *et al.* Multi-platform discovery of haplotype-resolved structural variation in human genomes. *Nat Commun* **10**: 1784. (2019).
34. Kidd, J. M. *et al.* A human genome structural variation sequencing resource reveals insights into mutational mechanisms. *Cell* **143**, 837–847 (2010).
35. Beck, C. R., Garcia-Perez, J. L., Badge, R. M. & Moran, J. V. LINE-1 elements in structural variation and disease. *Annu. Rev. Genomics Hum. Genet.* **12**, 187–215 (2011).
36. Ha, H., Loh, J. W. & Xing, J. Identification of polymorphic SVA retrotransposons using a mobile element scanning method for SVA (ME-Scan-SVA). *Mob. DNA* **7**, 15 (2016).
37. Flasch, D. A. *et al.* Genome-wide de novo L1 Retrotransposition Connects Endonuclease Activity with Replication. *Cell* **177**, 837–851.e28 (2019).
38. Chin, C.-S. *et al.* Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* **10**, 563–569 (2013).
39. Jain, M. *et al.* Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nat. Biotechnol.* **36**, 338–345 (2018).
40. Ebert, P. *et al.* De novo assembly of 64 haplotype-resolved human genomes of diverse ancestry and integrated analysis of structural variation. *bioRxiv* (2020).
41. Zook, J. M. *et al.* A robust benchmark for detection of germline large deletions and insertions. *Nat. Biotechnol.* **38**, 1347–1355 (2020).
42. Chu, C., Zhao, B., Park, P. J. & Lee, E. A. Identification and Genotyping of Transposable Element Insertions From Genome Sequencing Data. *Curr. Protoc. Hum. Genet.* **107**, e102

- (2020).
43. Ewing, A. D. *et al.* Nanopore sequencing enables comprehensive transposable element epigenomic profiling. doi:10.1101/2020.05.24.113068.
 44. Gilpatrick, T. *et al.* Targeted nanopore sequencing with Cas9-guided adapter ligation. *Nat. Biotechnol.* **38**, 433–438 (2020).
 45. Wang, H. *et al.* SVA elements: a hominid-specific retroposon family. *J. Mol. Biol.* **354**, 994–1007 (2005).
 46. Bennett, E. A. *et al.* Active Alu retrotransposons in the human genome. *Genome Res.* **18**, 1875–1883 (2008).
 47. Boissinot, S., Chevret, P. & Furano, A. V. L1 (LINE-1) retrotransposon evolution and amplification in recent human history. *Mol. Biol. Evol.* **17**, 915–928 (2000).
 48. Karamitros, T. & Magiorkinis, G. Multiplexed Targeted Sequencing for Oxford Nanopore MinION: A Detailed Library Preparation Procedure. *Methods Mol. Biol.* **1712**, 43–51 (2018).
 49. Gabrieli, T. *et al.* Selective nanopore sequencing of human BRCA1 by Cas9-assisted targeting of chromosome segments (CATCH). *Nucleic Acids Res.* **46**, e87 (2018).
 50. Giesselmann, P. *et al.* Analysis of short tandem repeat expansions and their methylation state with nanopore sequencing. *Nat. Biotechnol.* **37**, 1478–1481 (2019).
 51. Dausset, J. *et al.* Centre d'étude du polymorphisme humain (CEPH): collaborative genetic mapping of the human genome. *Genomics* **6**, 575–577 (1990).
 52. International HapMap Consortium. The International HapMap Project. *Nature* **426**, 789–796 (2003).
 53. Mills, R. E. *et al.* Mapping copy number variation by population-scale genome sequencing. *Nature* **470**, 59–65 (2011).
 54. Consortium, T. 1000 G. P. & The 1000 Genomes Project Consortium. A global reference for human genetic variation. *Nature* vol. 526 68–74 (2015).
 55. Zook, J. M. *et al.* Extensive sequencing of seven human genomes to characterize

- benchmark reference materials. *Scientific data* vol. 3 160025 (2016).
56. Zook, J. M. *et al.* Integrating human sequence data sets provides a resource of benchmark SNP and indel genotype calls. *Nat. Biotechnol.* **32**, 246–251 (2014).
 57. Audano, P. A. *et al.* Characterizing the Major Structural Variant Alleles of the Human Genome. *Cell* **176**, 663–675.e19 (2019).
 58. Mills, R. E., Bennett, E. A., Iskow, R. C. & Devine, S. E. Which transposable elements are active in the human genome? *Trends Genet.* **23**, 183–191 (2007).
 59. Moran, J. V. *et al.* High frequency retrotransposition in cultured mammalian cells. *Cell* **87**, 917–927 (1996).
 60. Ran, F. A. *et al.* Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* **8**, 2281–2308 (2013).
 61. Sternberg, S. H., Redding, S., Jinek, M., Greene, E. C. & Doudna, J. A. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature* **507**, 62–67 (2014).
 62. Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
 63. Hsu, P. D. *et al.* DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat. Biotechnol.* **31**, 827–832 (2013).
 64. Smit, A. F. A., Hubley, R. & Green, P. 2015 RepeatMasker Open-4.0. (2013).
 65. Sudmant, P. H. *et al.* An integrated map of structural variation in 2,504 human genomes. *Nature* **526**, 75–81 (2015).
 66. Zhao, X., Weber, A. M. & Mills, R. E. A recurrence-based approach for validating structural variation using long-read sequencing technology. *Gigascience* **6**, 1–9 (2017).
 67. Hehir-Kwa, J. Y. *et al.* A high-quality human reference panel reveals the complexity and distribution of genomic structural variants. *Nat. Commun.* **7**, 12989 (2016).
 68. Stangl, C. *et al.* Partner independent fusion gene detection by multiplexed CRISPR-Cas9 enrichment and long read nanopore sequencing. *Nat. Commun.* **11**, 2861 (2020).

69. Niu, Y. *et al.* Genome-wide analysis of mobile element insertions in human genomes. *bioRxiv* (2021).
70. Miga, K. H. *et al.* Telomere-to-telomere assembly of a complete human X chromosome. *Nature* **585**, 79–84 (2020).
71. Bennetzen, J. L. Transposable element contributions to plant gene and genome evolution. *Plant Mol. Biol.* **42**, 251–269 (2000).
72. Yu, T. *et al.* A benchmark and an algorithm for detecting germline transposon insertions and measuring de novo transposon insertion frequencies. *Nucleic Acids Res.* (2021) doi:10.1093/nar/gkab010.
73. Miller, S. A., Dykes, D. D. & Polesky, H. F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215 (1988).
74. Jurka, J. Repeats in genomic DNA: mining and meaning. *Curr. Opin. Struct. Biol.* **8**, 333–337 (1998).
75. Marçais, G. & Kingsford, C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics* **27**, 764–770 (2011).
76. Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb Genom* **3**, e000132 (2017).
77. Cock, P. J. A. *et al.* Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* **25**, 1422–1423 (2009).
78. Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **7**, 203–214 (2000).
79. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
80. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018).
81. Byrska-Bishop, M. *et al.* High coverage whole genome sequencing of the expanded 1000

Genomes Project cohort including 602 trios. doi:10.1101/2021.02.06.430068.

82. Koren, S. *et al.* Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res.* **27**, 722–736 (2017).
83. Huang, X. & Madan, A. CAP3: A DNA sequence assembly program. *Genome Res.* **9**, 868–877 (1999).