

Figure S1 . Homologous donor also promote dsDNA recombination.

a, Schematic diagram of dsDNA rearrangement. Occurrence of NHEJ does not enable the correct ligation between CopGFP fragments while introduction of homologous arms enables the correct ligation of CopGFP to produce active CopGFP.

b, Gel electrophoresis showed that the introduction of homologous arms had a positive effect on the rearrangement of dsDNA.

c, Sanger sequencing shows homology recombination was major repair way.

d, The FACS diagram shows correct ligation of CopGFP fragments only appears when adding homologous donor.

Random rearrangement and pathological rearrangement sites in HEK293T

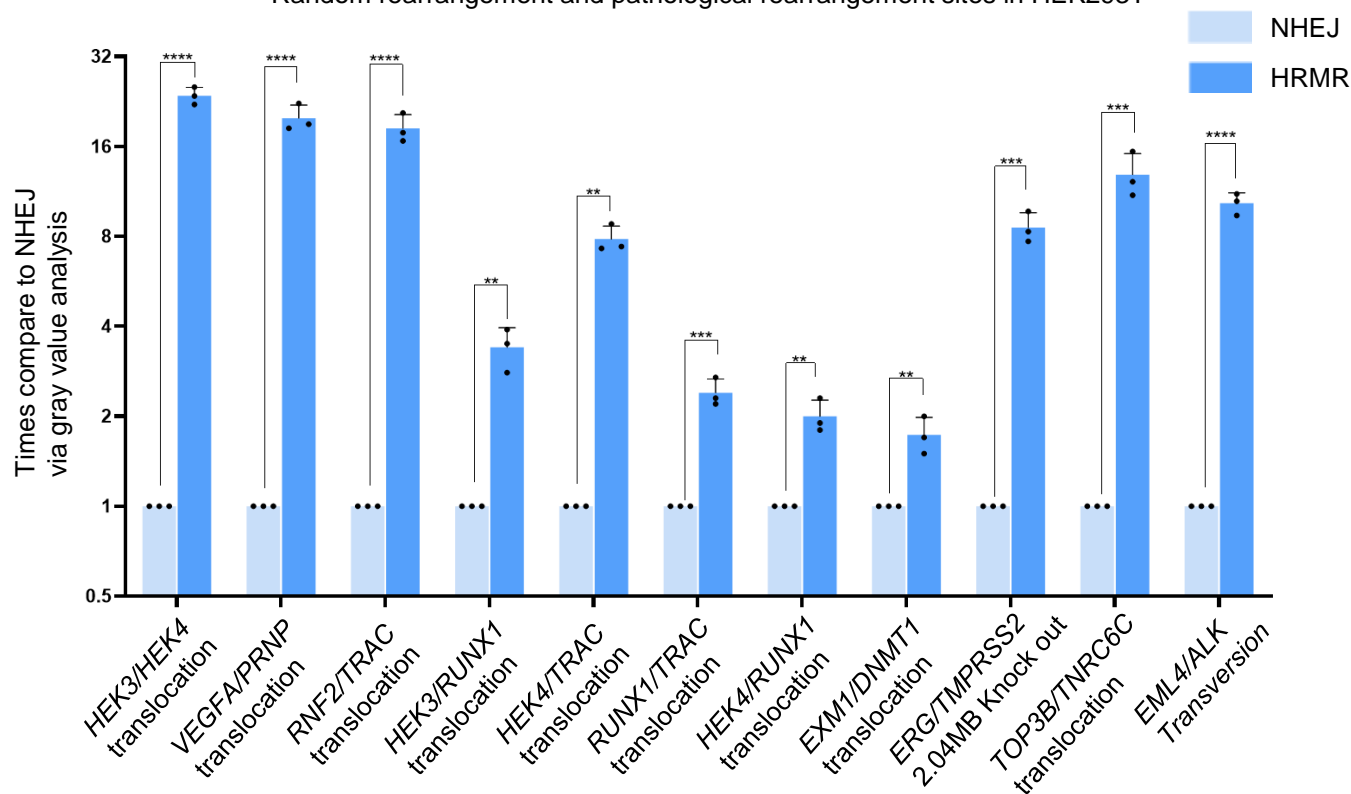


Figure S2 . The versatility of HRMR across various genomic sites encompassing both random and pathological chromosome translocation.

Determination of random rearrangement and pathological rearrangement sites between NHEJ and HRMR in HEK293T cells via quantification of bands gray density. Independent biological replicates were performed ($n = 3$) and error bars show the s.d.

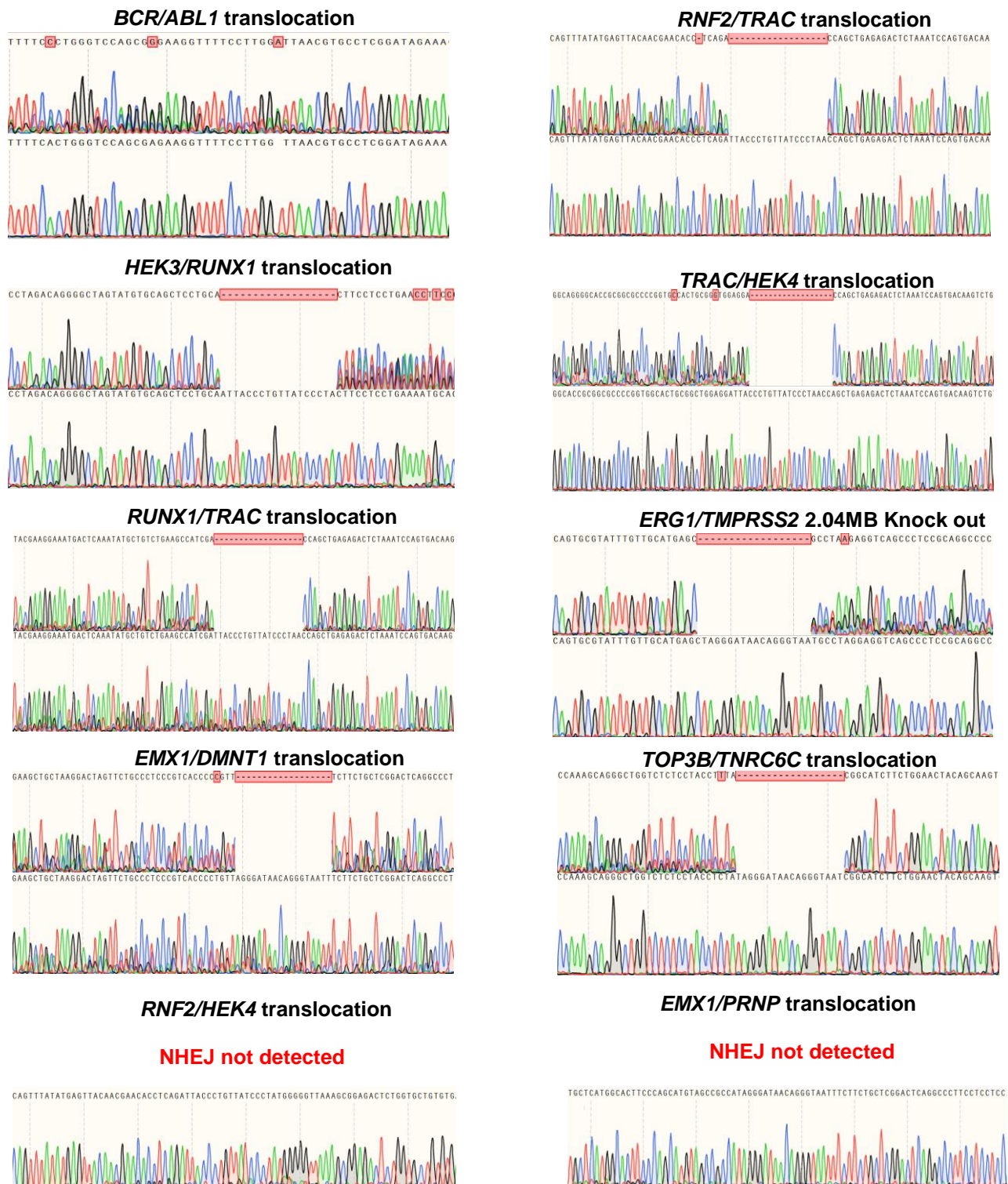


Figure S3. Sanger sequencing shows HRMR is a precise pathway for engineering chromosomal rearrangements.

Sanger sequencing chromatograms showed different repair outcome when using NHEJ (upper panel) and HRMR (lower panel) at different Endogenous loci. Notably, no translocation events of *RNF2/HEK4* translocation and *EMX1/PRNP* were detected because of failure translocation events amplified.

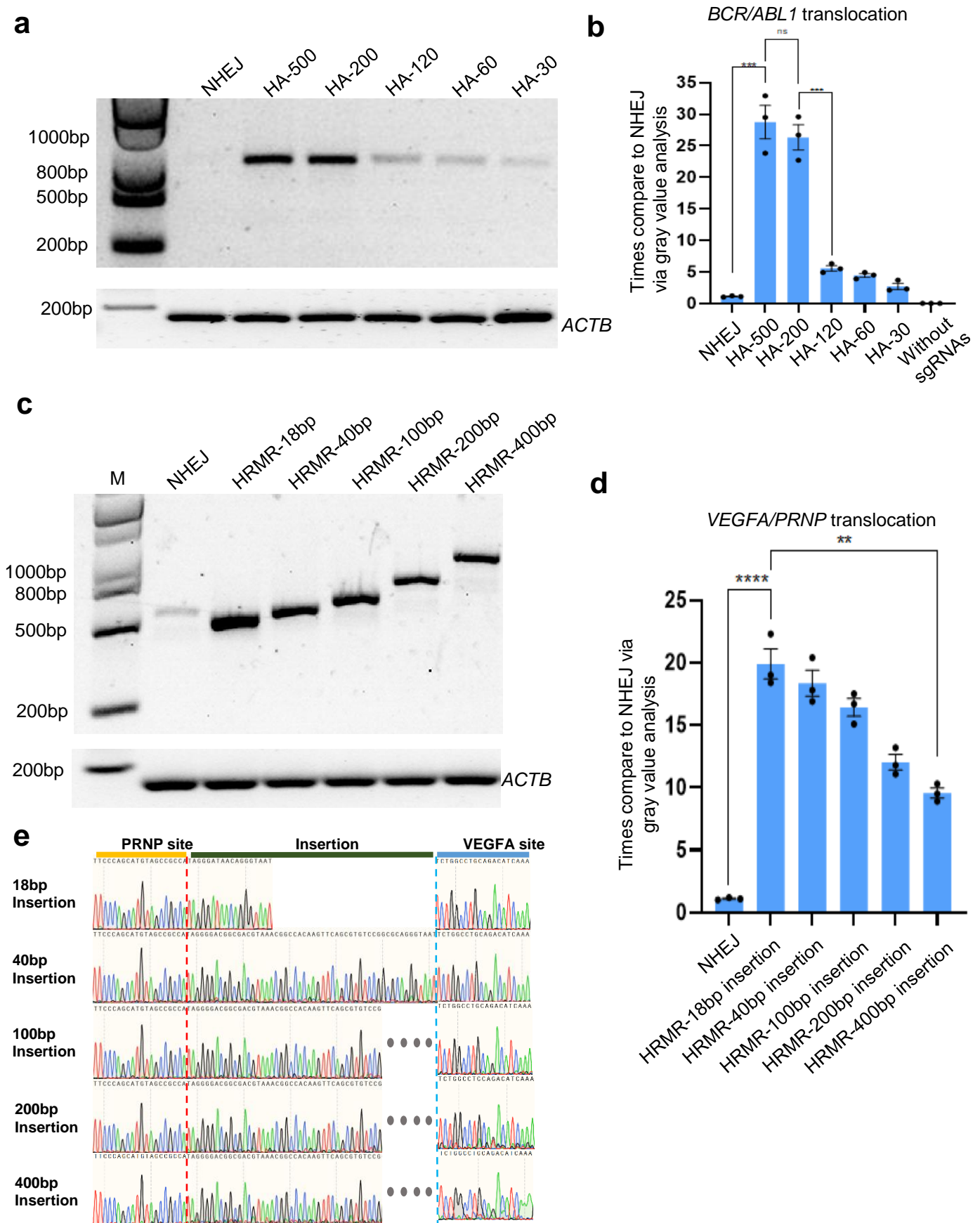


Figure S4 . Optimization of the length of homologous arm and insertion of HRMR.

a,b, Agarose gel analysis of different homologous arm lengths promoting chromosome rearrangement, and quantifying the target rearrangement through image J analysis of band grayscale. Independent biological replicates were performed ($n = 3$) and error bars show the s.e.m.

c,d, Agarose gel analysis of different insertion lengths promoting chromosome rearrangement, and quantifying the target rearrangement through image J analysis of band grayscale. Independent biological replicates were performed ($n = 3$) and error bars show the s.e.m.

e, Sanger sequencing chromatograms showed that different insertion length are integrated into chromosome translocation sites.

The diagram illustrates the CRISPR/Cas9-mediated gene editing strategy for ABL1 and BCR. It shows the initial state with two chromosomes (Chr9 ABL1 and Chr22 BCR) and a donor template. The strategy involves two steps: 1) Homology-Directed Repair (HDR) using a donor template to create a fusion gene (Chr9 ABL1-BCR). 2) Gene conversion using a donor template to create a fusion gene (Chr22 BCR-ABL1). The final products are two fusion genes: Chr9 ABL1-BCR and Chr22 BCR-ABL1. The diagram also shows the location of Primers F1, F2, R1, and R2.

Figure 1 displays RT-PCR analysis of BCR/ABL1 and ABL1/BCR fusion transcripts. The figure is organized into three horizontal panels, each representing a different target. The lanes are labeled at the top: M (Molecular Weight Marker), NHEJ (Negative Control), HRMR-B/A, HRMR-A/B, and HRMR-B/A+A/B.

- Top Panel: BCR/ABL1 (B/A) Junction**
 - Markers: 1000bp, 800bp
 - Results: Bands are visible in lanes M, HRMR-B/A, and HRMR-B/A+A/B, indicating the presence of the BCR/ABL1 fusion transcript. No band is visible in the NHEJ lane.
- Middle Panel: ABL1/BCR (A/B) junction**
 - Markers: 1000bp, 800bp
 - Results: Bands are visible in lanes M, HRMR-B/A, and HRMR-B/A+A/B, indicating the presence of the ABL1/BCR fusion transcript. No band is visible in the NHEJ lane.
- Bottom Panel: ACTB**
 - Marker: 200bp
 - Results: Bands are visible in all lanes (M, NHEJ, HRMR-B/A, HRMR-A/B, HRMR-B/A+A/B), indicating equal loading and serving as a loading control.

Times compare to NHEJ
via gray value analysis

BCR/ABL1(B/A) translocation

ABL1/BCR(A/B) translocation

ns

**

**

ns

NHEJ

B/A junction HA

A/B junction HA

B/A junction HA and A/B junction HA

Category	BCR/ABL1(B/A) translocation	ABL1/BCR(A/B) translocation
NHEJ	1.0	1.0
B/A junction HA	~24	~3
A/B junction HA	~3.5	~24
B/A junction HA and A/B junction HA	~20	~24

a, Schematic diagram for homologous arm mediated translocation, designing a pair of sgRNAs targeting the target gene. Using different donors for different chromosome rearrangement.
b, c, Agarose gel analysis of different homologous donors promoting different forms of chromosome rearrangement, and quantifying the target rearrangement through image J analysis of band grayscale. Independent biological replicates were performed ($n = 3$) and error bars show the s.e.m.

The diagram illustrates the CRISPR-Cas9 genome editing strategy for the RNF2-TRAC fusion. It shows the initial genomic state with Chr1 RNF2 and Chr22 TRAC on separate chromosomes. A donor template with a loxP-flanked HA tag and an I-SceI site is introduced. Cas9 cuts the genomic DNA at the TRAC locus. The donor template is then used for homology-directed repair, resulting in the fusion of Chr1 RNF2 and Chr22 TRAC, with the HA tag and I-SceI site removed.

VEGFA/PRNP translocation

20.8x

NHEJ NEHJ+ I-sceI HRMR HRMR+ I-sceI M

464bp 288bp 176bp 800bp 500bp 200bp

RNF2/TRAC translocation

43.1x

HRMR HRMR+ I-sceI M

376bp 293bp 83bp out of gel 500bp 200bp

***RNF2/TRAC* translocation**

43.1x

HRMR

HRMR⁺

I-SceI

M

500bp

200bp

376bp

293bp

83bp

out of gel

Figure S6 . I-SceI enzyme digestion to determine the relative proportion of HR and NHEJ occurrence.
a, Schematic diagram for homologous arm mediated translocation, designing a pair of sgRNAs targeting the target gene. KU protein mediated NHEJ and homologous recombination mediated by homology donor. A pair of primers was used to detect rearranged chromosomes.
b, c, Agarose gel analysis of I-SceI digestion of targeted translocation amplifiers. The bands with size match sequences with or without enzyme digestion were indicated, and the multiples displayed by grayscale analysis are displayed above the gel plot.

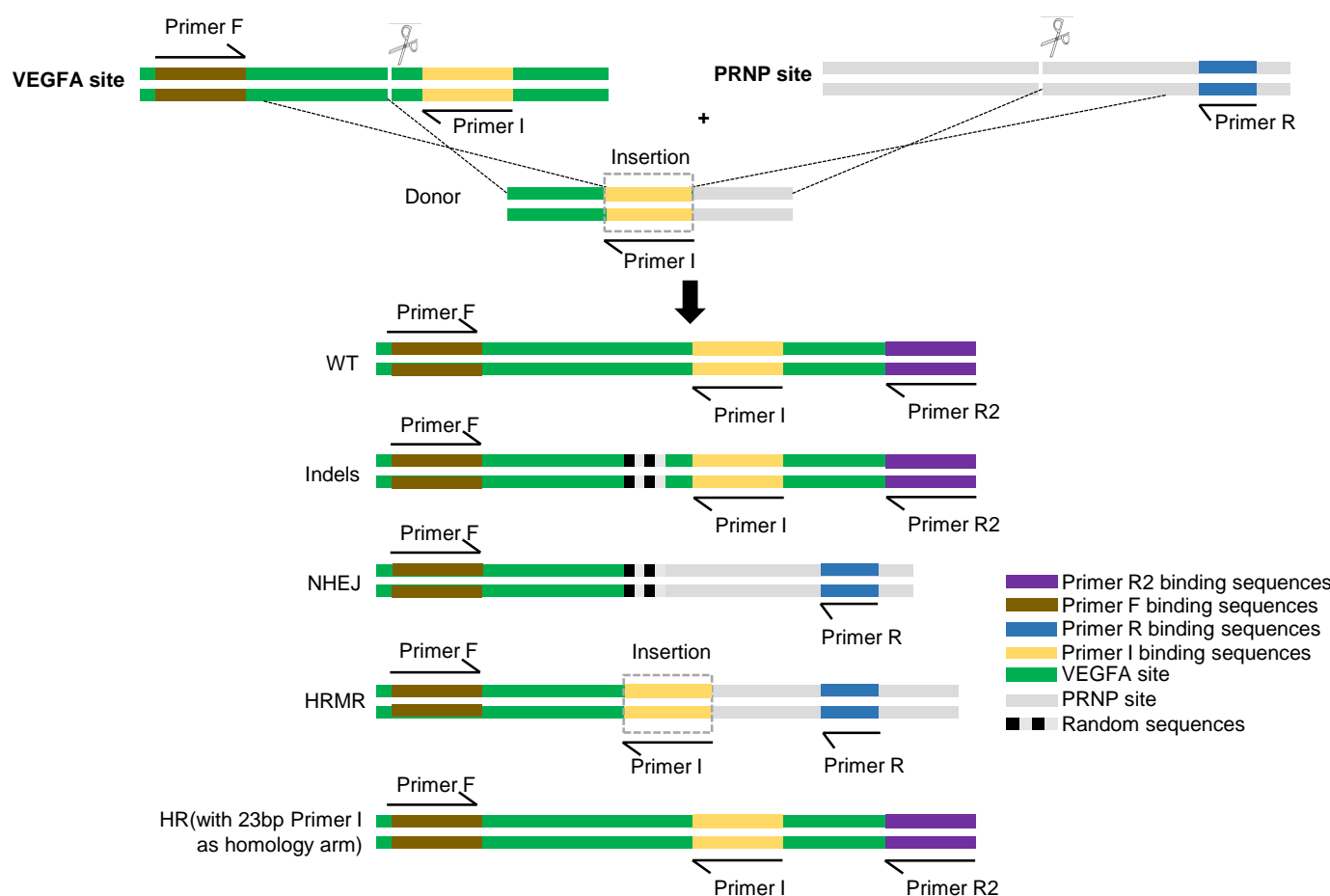


Figure S7. Detailed explanation of the primer insertion method.

Schematic diagram of primer insertion to quantifying the efficiency and accuracy of HRMR-mediated chromosome rearrangement.

1. Wild-Type VEGFA (WT): The VEGFA locus remains unmodified or is repaired without indel formation. Both Primer F and Primer I can amplify this sequence.

2. Indels: Following Cas9-induced cleavage at the VEGFA locus, a double-strand break is repaired through NHEJ pathway, leading to indels (small insertions or deletions). Despite the presence of indels, both Primer F and Primer I can still bind to the genome and amplify the sequence.

3. NHEJ-mediated translocations: Double-strand breaks (DSBs) at VEGFA and PRNP are repaired via the NHEJ pathway, resulting in rearranged chromosomes without the insertion of Primer I (with or without indels). In this case, Primer F cannot amplify the product in combination with Primer I. However, the NHEJ-mediated translocation can still be detected using Primer F and Primer R.

4. HRMR-mediated translocations: Homology-directed repair (HDR) using the homologous donor results in translocations that include the insertion of Primer I. This specific product can be amplified using Primer F and Primer I. Additionally, Primer F and Primer R can be used to detect the products of HRMR-mediated translocations.

5. HR (with 23bp Primer I as homology arm): Primer I exhibits homology to the VEGFA genome, Primer I might function as a homologous arm to mediate HR events. This specific product can be amplified using Primer F and Primer R2.

Primer F and Primer I were used to simultaneously amplify three products: wild-type (WT), indels, HRMR-mediated translocations and rare HR (with 23bp Primer I as homology arm). The proportions of these products were then determined through sequence analysis. Additionally, Primers F and Primer R were used to amplify two products: NHEJ-mediated translocations and HRMR-mediated translocations, allowing further proportion analysis. Primers F and Primer R2 were used to amplify three products: wild-type (WT), indels, and HR (with 23bp Primer I as homology arm) events.

WT	G	C	A	G	G	C	C	A	G	A	T	G	A	G	G	G	C	T	C	C	-	70.92% (2584195 reads)
HRMR	G	C	A	G	G	C	C	A	G	A	-	-	-	-	-	-	C	T	C	C	-	9.92% (361332 reads)
Indel	G	C	A	G	G	C	C	A	G	A	A	T	G	A	G	G	G	C	T	C	C	2.43% (88579 reads)
	G	C	A	G	G	C	C	A	G	A	-	-	-	-	G	G	G	C	T	C	C	1.13% (41299 reads)
	G	-	-	-	-	-	-	-	-	-	-	-	A	G	G	G	C	T	C	C	-	0.88% (32236 reads)
	G	C	A	G	G	C	C	A	G	A	-	G	A	G	G	G	C	T	C	C	-	0.57% (20853 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	G	C	T	C	C	0.48% (17543 reads)
	G	C	A	G	G	A	C	A	G	A	T	T	G	A	G	G	G	C	T	C	C	0.39% (14209 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	G	C	T	A	C	0.38% (13824 reads)
	-	-	-	-	-	-	-	-	-	-	-	-	A	G	G	G	C	T	C	C	-	0.35% (12589 reads)
	G	C	A	G	G	C	C	A	G	A	-	-	A	G	G	G	C	T	C	C	-	0.34% (12455 reads)
	G	C	A	G	G	C	C	A	G	A	C	T	T	G	A	G	G	G	C	T	C	0.32% (11577 reads)
	G	C	A	G	G	C	C	A	G	A	G	T	T	G	A	G	G	G	C	T	C	0.32% (11571 reads)
	G	C	A	-	-	-	-	-	-	-	T	T	G	A	G	G	G	C	T	C	C	0.31% (11477 reads)
	-	-	-	-	-	-	-	-	-	-	-	G	A	G	G	G	C	T	C	C	-	0.31% (11276 reads)
	G	C	-	-	-	-	-	-	-	-	T	T	G	A	G	G	G	C	T	C	C	0.31% (11191 reads)
	G	C	A	G	G	C	C	A	-	-	T	T	G	A	G	G	G	C	T	C	C	0.30% (11057 reads)
	G	C	A	G	G	C	C	A	G	-	T	T	G	A	G	G	G	C	T	C	C	0.30% (10879 reads)
	G	C	A	G	-	-	-	-	-	-	T	T	G	A	G	G	G	C	T	C	C	0.29% (10679 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	G	C	T	T	C	0.29% (10541 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	A	C	T	C	C	0.29% (10507 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	G	C	A	C	C	0.27% (9989 reads)
	G	C	A	G	G	T	C	A	G	A	T	T	G	A	G	G	G	C	T	C	C	0.26% (9355 reads)
	G	C	A	G	G	C	-	-	-	-	-	-	-	-	-	-	-	T	C	C	-	0.25% (9268 reads)
	-	-	-	-	-	-	-	-	-	-	T	T	G	A	G	G	G	C	T	C	C	0.25% (9145 reads)
	G	C	A	G	G	C	C	A	G	A	T	T	G	A	G	G	G	C	C	C	C	0.20% (7396 reads)

Figure S8 . Sequence alignment of *VEGFA* sites edited by HRMR using primer insertion strategy (*VEGFA/PRNP* translocation). Wildtype *VEGFA* sequence was used as a reference and HTS sequencing reads were aligned to the reference sequence and top sequences with a ratio over 0.2% were shown. Vertical dashed line marked the position of Cas9 induced DSBs. Red box marked the inserted bases and the short-term marked the deleted bases. Accurate six base deletion was considered as HRMR.