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Supplementary information

Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing

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Supplementary Information

Programmable deletion, replacement, integration, and inversion of large DNA sequences with twin prime editing

Andrew V. Anzalone, Xin D. Gao, Christopher J. Podracky, Andrew T. Nelson, Luke W. Koblan, Aditya Raguram, Jonathan M. Levy, Jaron A. M. Mercer and David R. Liu

Supplementary Table 1: Sequences of pegRNAs and sgRNAs used in mammalian cell experiments

All sequences are shown in 5' to 3' orientation. pegRNAs are a concatenation of the spacer sequence, the sgRNA scaffold, and the 3' extension (contains PBS and RT template, and a 3' motif in the case of epegRNAs).

sgRNA scaffold sequence (5' to 3')
gttttagagctagaaatagcaagttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgc

Figure 1c

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
HEK3_attB_A_38	ggcccagactgagcacgtga	atgatcctgacgacggagaccgccgtcgtcgacaagcccgtgctcagtctg	indicated on the x-axis
HEK3_attB_A_34	ggcccagactgagcacgtga	tcctgacgacggagaccgccgtcgtcgacaagcccgtgctcagtctg	indicated on the x-axis
HEK3_attB_A_30	ggcccagactgagcacgtga	gacgacggagaccgccgtcgtcgacaagcccgtgctcagtctg	indicated on the x-axis
HEK3_attB_B_38	gtcaaccagtatcccggtgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatccgggatactgg	indicated on the x-axis
HEK3_attB_B_34	gtcaaccagtatcccggtgc	tgtcgacgacggcggtctccgtcgtcaggatcatccgggatactgg	indicated on the x-axis
HEK3_attB_B_30	gtcaaccagtatcccggtgc	gacgacggcggtctccgtcgtcaggatcatccgggatactgg	indicated on the x-axis
HEK3_attP_A_43	ggcccagactgagcacgtga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcgtgctcagtctg	indicated on the x-axis
HEK3_attP_A_39	ggcccagactgagcacgtga	gtacaccactgagaccgcggtggttgaccagacaaacctcgtgctcagtctg	indicated on the x-axis
HEK3_attP_A_35	ggcccagactgagcacgtga	accactgagaccgcggtggttgaccagacaaacctcgtgctcagtctg	indicated on the x-axis
HEK3_attP_B_44	gtcaaccagtatcccggtgc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccgggatactgg	indicated on the x-axis
HEK3_attP_B_40	gtcaaccagtatcccggtgc	ggtcaaccaccgcggtctcagtggtgtacggtacaaacctccgggatactgg	indicated on the x-axis
HEK3_attP_B_36	gtcaaccagtatcccggtgc	aaccaccgcggtctcagtggtgtacggtacaaacctccgggatactgg	indicated on the x-axis

Figure 2b

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
PE3_HEK3_FKBP_ins12bp	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcacacctgcactcccgtgctcagtctg	indicated on the x-axis
PE3_HEK3_FKBP_ins36bp	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcacccgtctcctggggagatggtttccacctgcactcccgtgctcagtctg	indicated on the x-axis
PE3_HEK3_FKBP_ins108bp	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcaaaatttctttccatcttcaagcatcccggtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactcccgtgctcagtctg	indicated on the x-axis
twinPE_HEK3_FKBP_ins108bp_A	ggcccagactgagcacgtga	accacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactcccgtgctcagtctg	indicated on the x-axis
twinPE_HEK3_FKBP_ins108bp_B	gtcaaccagtatcccggtgc	accttccccaagcgcggccagacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaatttccgggatactgg	indicated on the x-axis
sgRNA	**spacer sequence**		
HEK3_3b_+90_nicking	gtcaaccagtatcccggtgc		

Figure 2c

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
PAH_E4.2_45	gcccaagaaccattcaagagc	gttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	exon 4 24-bp overlap (64 bp recoded)
PAH_E4.4_43	gctacgggccatggactcaca	aaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcc	exon 4 24-bp overlap (64 bp recoded)
PAH_E4.2_45_EvoPreQ1	gcccaagaaccattcaagagc	gttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 24-bp overlap epegRNA (64 bp recoded)
PAH_E4.4_43_EvoPreQ1	gctacgggccatggactcaca	aaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 24-bp overlap epegRNA (64 bp recoded)
PAH_E4..2_50	gcccaagaaccattcaagagc	gtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	exon 4 36-bp overlap (64 bp recoded)
PAH_E4..4_50	gctacgggccatggactcaca	gctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcc	exon 4 36-bp overlap (64 bp recoded)
PAH_E4..2_50_EvoPreQ1	gcccaagaaccattcaagagc	gtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 36-bp overlap epegRNA (64 bp recoded)
PAH_E4..4_50_EvoPreQ1	gctacgggccatggactcaca	gctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 36-bp overlap epegRNA (64 bp recoded)
PAH_E4.2_62	gcccaagaaccattcaagagc	aggatgatcagcgtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	exon 4 59-bp overlap (64 bp recoded)
PAH_E4.4_61	gctacgggccatggactcaca	ttgataggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcc	exon 4 59-bp overlap (64 bp recoded)
PAH_E4.2_62_EvoPreQ1	gcccaagaaccattcaagagc	aggatgatcagcgtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 59-bp overlap epegRNA (64 bp recoded)
PAH_E4.4_61_EvoPreQ1	gctacgggccatggactcaca	ttgataggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 4 59-bp overlap epegRNA (64 bp recoded)

PAH_E7.2_34	gtggtttccgcctccgacctg	agaaagtctctactgctcaagagcccggcaacgggtcggaggcg	exon 7 22-bp overlap (46 bp recoded)
PAH_E7.5_34	gagtggaagactcggaaggcc	tcttgagcagtagagactttctgggggtctcgccttccgagtcttc	exon 7 22-bp overlap (46 bp recoded)
PAH_E7.2_34_EvoPreQ1	gtggtttccgcctccgacctg	agaaagtctctactgctcaagagcccggcaacgggtcggaggcgtctctctcttgacg cggttctatctagttacgcgttaaaccaactagaaa	exon 7 22-bp overlap epegRNA (46 bp recoded)
PAH_E7.5_34_EvoPreQ1	gagtggaagactcggaaggcc	tcttgagcagtagagactttctgggggtctcgccttccgagtcttctctctctcttgacg cggttctatctagttacgcgttaaaccaactagaaa	exon 7 22-bp overlap epegRNA (46 bp recoded)
PAH_E7.2_44	gtggtttccgcctccgacctg	gagacccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcg	exon 7 42-bp overlap (46 bp recoded), exon 7 24-bp overlap (64 bp recoded)
PAH_E7.5_44	gagtggaagactcggaaggcc	gttgccgggctcttgagcagtagagactttctgggggtctcgccttccgagtcttc	exon 7 42-bp overlap (46 bp recoded)
PAH_E7.2_44_EvoPreQ1	gtggtttccgcctccgacctg	gagacccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcgtct ctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 7 42-bp overlap epegRNA (46 bp recoded), exon 7 24-bp overlap epegRNA (64 bp recoded)
PAH_E7.5_44_EvoPreQ1	gagtggaagactcggaaggcc	gttgccgggctcttgagcagtagagactttctgggggtctcgccttccgagtcttctct ctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 7 42-bp overlap epegRNA (46 bp recoded)
PAH_E7.6_44	gtctgatgtactgtgtgcag	agtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtaca	exon 7 24-bp overlap (64 bp recoded)
PAH_E7.6_44_EvoPreQ1	gtctgatgtactgtgtgcag	agtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtacatctctc tcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 7 24-bp overlap epegRNA (64 bp recoded)
PAH_E7.2_55	gtggtttccgcctccgacctg	acgcggaatgcgagacccccagaaagtctctactgctcaagagcccggcaacggg tcggaggcg	exon 7 47-bp overlap (64 bp recoded)
PAH_E7.6_56	gtctgatgtactgtgtgcag	gggctcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcaca cagtaca	exon 7 47-bp overlap (64 bp recoded)
PAH_E7.2_55_EvoPreQ1	gtggtttccgcctccgacctg	acgcggaatgcgagacccccagaaagtctctactgctcaagagcccggcaacggg tcggaggcgtctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 7 47-bp overlap epegRNA (64 bp recoded)
PAH_E7.6_56_EvoPreQ1	gtctgatgtactgtgtgcag	gggctcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcaca cagtacatctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	exon 7 47-bp overlap epegRNA (64 bp recoded)

Figure 2e

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
HEK3_DF_A_SA_ del77nt	ggcccagactgagcacgtga	tcctctgccatcacgtgctcagtctg	SA (Δ 77nt)
HEK3_DF_B_SA_ del77nt	gtcaaccagtatcccggtgc	tgatggcagaggaccgggatactgg	SA (Δ 77nt)
HEK3_DF_A_SA_ del56nt	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcacgtgctcagtctg	SA (Δ 56nt)
HEK3_DF_B_SA_ del56nt	gtcaaccagtatcccggtgc	tgatggcagaggaaaggaagccctgcttcctccaccgggatactgg	SA (Δ 56nt)
HEK3_DF_A_HA_ del64nt	ggcccagactgagcacgtga	tgcaggagctgcatcctctgccatcacgtgctcagtctg	HA (Δ 64nt)
HEK3_DF_B_HA_ del64nt	gtcaaccagtatcccggtgc	tgatggcagaggatgcagctcctgcaccgggatactgg	HA (Δ 64nt)
HEK3_DF_A_PD_ del90nt	ggcccagactgagcacgtga	gcccagccaaacttgtcaaccagtatcccggcgtgctcagtctg	PD (Δ 90nt)
HEK3_DF_B_PD_ del90nt	gtcaaccagtatcccggtgc	gggtcaatccttggggcccagactgagcacgccgggatactgg	PD (Δ 90nt)
HEK3_DF_A_SA_ del77nt_EvoPreQ1	ggcccagactgagcacgtga	tcctctgccatcacgtgctcagtctgttaaataacgcggttctatctagttacgcgttaaac caactagaa	SA-EvoPreQ1 (Δ 77nt)
HEK3_DF_B_SA_ del77nt_EvoPreQ1	gtcaaccagtatcccggtgc	tgatggcagaggaccgggatactggaaaatatacgcggttctatctagttacgcgttaa accaactagaa	SA-EvoPreQ1 (Δ 77nt)
HEK3_DF_A_SA_ del56nt_EvoPreQ1	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcacgtgctcagtctgttaataatcgcgg ttctatctagttacgcgttaaaccaactagaa	SA-EvoPreQ1 (Δ 56nt)
HEK3_DF_B_SA_ del56nt_EvoPreQ1	gtcaaccagtatcccggtgc	tgatggcagaggaaaggaagccctgcttcctccaccgggatactggaaaaaaaacgc ggttctatctagttacgcgttaaaccaactagaa	SA-EvoPreQ1 (Δ 56nt)
HEK3_DF_A_HA_ del64nt_EvoPreQ1	ggcccagactgagcacgtga	tgcaggagctgcatcctctgccatcacgtgctcagtctgataaataacgcggttctatct agttacgcgttaaaccaactagaa	SA-EvoPreQ1 (Δ 64nt)
HEK3_DF_B_HA_ del64nt_EvoPreQ1	gtcaaccagtatcccggtgc	tgatggcagaggatgcagctcctgcaccgggatactggaaaaaaggcgcggttctat ctagttacgcgttaaaccaactagaa	SA-EvoPreQ1 (Δ 64nt)
HEK3_DF_A_PD_ del90nt_EvoPreQ1	ggcccagactgagcacgtga	gcccagccaaacttgtcaaccagtatcccggcgtgctcagtctgttaaataccgcggtt ctatctagttacgcgttaaaccaactagaa	PD-EvoPreQ1 (Δ 90nt)
HEK3_DF_B_PD_ del90nt_EvoPreQ1	gtcaaccagtatcccggtgc	gggtcaatccttggggcccagactgagcacgccgggatactggaaaataatcgcggt tctatctagttacgcgttaaaccaactagaa	PD-EvoPreQ1 (Δ 90nt)

Figure 2f

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
DMD-Exon51-A1_a_SA	gattggctttgatttcccta	ttgaataggaagtaaattaatttgaagctggaccctagggaaatcaa	SA-1 (Δ 780nt)
DMD-Exon51-B1_A1_b_SA	gcagttgcctaagaactggt	tagggtccagcttcaaattaatttacttcctattcaaagttcttaggc	SA-1 (Δ 780nt)
DMD-Exon51-a3-b1_a_PD	gtatatgattgttactgaga	tgctgagagagaaacagttgcctaagaactcagtaacaat	PD-1 (Δ 627nt); PD-2 (Δ 627nt)

DMD-Exon51-b1-a3_a-PD	gcagttgcctaagaactggt	accacttccacaatgtatatgattgttactgagttcttagg	PD-1 (Δ 627nt)
DMD-Exon51-b1-a3_b-PD	gcagttgcctaagaactggt	accacttccacaatgtatatgattgttactgagttcttaggcaa	PD-2 (Δ 627nt)
DMD-Exon51-A3_b_attB	gtatatgattgttactgaga	atgatcctgacgacggagaccgccgtcgtcgacaagcccagtaacaatc	tPE-1 attB (Δ 589nt); tPE-2 attB (Δ 589nt); tPE-1 attB (Δ 558nt); tPE-2 attB (Δ 558nt)
DMD-Exon51-A3_c_attB	gtatatgattgttactgaga	atgatcctgacgacggagaccgccgtcgtcgacaagcccagtaacaatcata	tPE-3 attB (Δ 589nt); tPE-4 attB (Δ 589nt); tPE-3 attB (Δ 558nt); tPE-4 attB (Δ 558nt)
DMD-Exon51-B1_b_attB	gcagttgcctaagaactggt	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagttcttaggc	tPE-1 attB (Δ 589nt); tPE-3 attB (Δ 589nt)
DMD-Exon51-B1_c_attB	gcagttgcctaagaactggt	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagttcttaggcaac	tPE-2 attB (Δ 589nt); tPE-4 attB (Δ 589nt)
DMD-Exon51-B2_b_attB	gaggagagtaaagtgattgg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcactttactc	tPE-1 attB (Δ 558nt); tPE-3 attB (Δ 558nt)
DMD-Exon51-B2_c_attB	gaggagagtaaagtgattgg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcactttactctcc	tPE-2 attB (Δ 558nt); tPE-4 attB (Δ 558nt)
sgRNA	**spacer sequence**		**Edits made by the paired sgRNA**
DMD-Exon51-A1	gattggctttgatttcccta		Cas9 (Δ 818nt)
DMD-Exon51-A3	gtatatgattgttactgaga		Cas9 (Δ 627nt)
DMD-Exon51-B1	gcagttgcctaagaactggt		Cas9 (Δ 818nt); Cas9 (Δ 627nt)

Figure 3b

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A223c	gtcatcctgataaactgcaaa	atgatcctgacgacggagaccgccgtcgtcgacaagccgcagtttatcagg	indicated on the x-axis
CCR5_B272a	gaaggaaaaacaggtcagaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgacctg	indicated on the x-axis
CCR5_B291b	gcccagaaggggacagtaaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcattactgtcccct	indicated on the x-axis
CCR5_B305a	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcact	indicated on the x-axis
CCR5_B326b	gatttccaaagtcccactggg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtgggactttg	indicated on the x-axis
CCR5_B330b	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaa	indicated on the x-axis
CCR5_A260c	gtgacatctacctgctcaacc	atgatcctgacgacggagaccgccgtcgtcgacaagcctgagcaggtagat	indicated on the x-axis
CCR5_B305c	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcactatgc	indicated on the x-axis
CCR5_B330c	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaaat	indicated on the x-axis
CCR5_A325b	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagcat	indicated on the x-axis
CCR5_B414a	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
CCR5_A360b	gacaatgtgtcaactcttgac	atgatcctgacgacggagaccgccgtcgtcgacaagccaagagttgacac	indicated on the x-axis
CCR5_A506c	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacactt	indicated on the x-axis
CCR5_B584a	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcatt	indicated on the x-axis
CCR5_A509a	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagcccccaagtga	indicated on the x-axis
CCR5_B535c	gatctggtaaagatgattcc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcatctttaccag	indicated on the x-axis
CCR5_A531c	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaaca	indicated on the x-axis
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	indicated on the x-axis

Figure 3c

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
AAVS1_A1077b	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctgg	indicated on the x-axis
AAVS1_B1154b	gtccttggcaagcccaggag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgcc	indicated on the x-axis
AAVS1_A1098b	gggaaggggcaggagagcca	taccgtacaccactgagaccgcggtggttgaccagacaaacctctctcctgccc	indicated on the x-axis
AAVS1_B1154a	gtccttggcaagcccaggag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttg	indicated on the x-axis
AAVS1_A1246c	gaatatgtcccagatagcac	taccgtacaccactgagaccgcggtggttgaccagacaaacctctatctgggacat	indicated on the x-axis
AAVS1_B1314b	gtgcgtcctaggtgttcacc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaacacctagg	indicated on the x-axis
AAVS1_B1376a	gtcctggcagggctgtggtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacagccct	indicated on the x-axis
AAVS1_A1267c	ggggactctttaaggaaaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctttccttaaagagt	indicated on the x-axis
AAVS1_A1293a	gagaaagagaaagggagtag	taccgtacaccactgagaccgcggtggttgaccagacaaacctctcccttc	indicated on the x-axis
AAVS1_A1307b	gagtagaggcggccacgacc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcgtggccgcct	indicated on the x-axis
AAVS1_B1314a	gtgcgtcctaggtgttcacc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaacaccta	indicated on the x-axis
AAVS1_A1582c	gatcagtgaaacgcaccaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctggtgcgtttcact	indicated on the x-axis
AAVS1_B1640a	gtgacctgcccggttctcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctagaaccggg	indicated on the x-axis
AAVS1_B1676a	gagcttggcaggggtggga	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctg	indicated on the x-axis
AAVS1_B1701a	gagccagagaggatcctggg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctaggatcctc	indicated on the x-axis
AAVS1_B1705b	gatggagccagagaggatcc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttcctctctggc	indicated on the x-axis
AAVS1_A1615b	gcagctcaggttctgggaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcccagaacctg	indicated on the x-axis
AAVS1_B1640a	gtgacctgcccggttctcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctagaaccggg	indicated on the x-axis
AAVS1_A1615a	gcagctcaggttctgggaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcccagaacc	indicated on the x-axis
AAVS1_B1676a	gagcttggcaggggtggga	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctg	indicated on the x-axis
AAVS1_A1647b	gtggccactgagaaccgggc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcggttctcagt	indicated on the x-axis
AAVS1_B1676b	gagcttggcaggggtggga	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctgcc	indicated on the x-axis
AAVS1_B1701a	gagccagagaggatcctggg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctaggatcctc	indicated on the x-axis
AAVS1_B1705a	gatggagccagagaggatcc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttcctctctg	indicated on the x-axis
AAVS1_A1810b	gaatctgcctaacaggaggt	taccgtacaccactgagaccgcggtggttgaccagacaaaccttcctgttaggc	indicated on the x-axis
AAVS1_B1883b	ggggccactagggacaggat	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgtccctagt	indicated on the x-axis

AAVS1_B1902a	gtcccctccaccccacagtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgtggggtg	indicated on the x-axis
AAVS1_A1890b	gtcaccaatcctgtccctag	taccgtacaccactgagaccgcggtggttgaccagacaaacctgggacaggatt	indicated on the x-axis
AAVS1_A1890c	gtcaccaatcctgtccctag	taccgtacaccactgagaccgcggtggttgaccagacaaacctgggacaggattgg	indicated on the x-axis
AAVS1_B1962c	gggaccaccttatattccca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaatataaggtgg	indicated on the x-axis
AAVS1_A3786c	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtgggggggc	indicated on the x-axis
AAVS1_B3839c	gacctgcccagcacaccctg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctggtgtgctgggca	indicated on the x-axis
AAVS1_A3786a	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtggg	indicated on the x-axis
AAVS1_B3903b	gcgactcctggaagtggcca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccagg	indicated on the x-axis
AAVS1_B3930a	ggacttcccagtgtgcatcg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactg	indicated on the x-axis
AAVS1_A3835b	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgccgt	indicated on the x-axis
AAVS1_B3930b	ggacttcccagtgtgcatcg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactggg	indicated on the x-axis
AAVS1_A3856a	ggtgtgctgggcaggtcgcg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgacctgccc	indicated on the x-axis

Figure 3d

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A325a	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagc	325/414
CCR5_B414b	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcgata	325/414
CCR5_A506c	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacactt	506/584
CCR5_A509b	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagccccaagtgatc	509/584
CCR5_A531b	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	531/584
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	506/584, 509/584, 531/584
AAVS1_A1077c	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctggct	1077/1154
AAVS1_B1154c	gtccttggcaagcccaggag	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgccaa	1077/1154
AAVS1_A3786c	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtgggggggc	3786/3903, 3786/3930
AAVS1_B3903c	gcgactcctggaagtggcca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccaggag	3786/3903
AAVS1_B3930c	ggacttcccagtgtgcatcg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactgggaa	3786/3930

Figure 3e

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A531b	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 38
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 38
CCR5_A7_attB_30	gctgtgtttgcgtctctccc	tcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 30
CCR5_B8_attB_30	gtatggaaaatgagagctgc	tgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 30
CCR5_A7_attB_20	gctgtgtttgcgtctctccc	acgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 20
CCR5_B8_attB_20	gtatggaaaatgagagctgc	acgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 20
CCR5_A7_attB_GA_38	gctgtgtttgcgtctctccc	atgatcctgacgacggagtccgccgtcgtcgacaagccagagacgcaaa	attB-GA 38
CCR5_B8_attB_GA_38	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggactccgtcgtcaggatcatgctctcattttc	attB-GA 38
CCR5_A7_attB_GA_30	gctgtgtttgcgtctctccc	tcctgacgacggagtccgccgtcgtcgacaagccagagacgcaaa	attB-GA 30
CCR5_B8_attB_GA_30	gtatggaaaatgagagctgc	tgtcgacgacggcggactccgtcgtcaggatcatgctctcattttc	attB-GA 30
CCR5_A7_attB_GA_20	gctgtgtttgcgtctctccc	acgacggagtccgccgtcgtcgacaagccagagacgcaaa	attB-GA 20
CCR5_B8_attB_GA_20	gtatggaaaatgagagctgc	acgacggcggactccgtcgtcaggatcatgctctcattttc	attB-GA 20
CCR5_A7_attP_50	gctgtgtttgcgtctctccc	gggtttgtaccgtacaccactgagaccgcggtggttgaccagacaaaccaagagacgcaaa	attP 50
CCR5_B8_attP_50	gtatggaaaatgagagctgc	tggtttgtctggtcaaccaccgcggtctcagtggtgtacggtacaaacccgctctcatttttc	attP 50
CCR5_A7_attP_40	gctgtgtttgcgtctctccc	tgtaccgtacaccactgagaccgcggtggttgaccagacaaaccaagagacgcaaa	attP 40
CCR5_B8_attP_40	gtatggaaaatgagagctgc	tgtctggtcaaccaccgcggtctcagtggtgtacggtacaaacccgctctcattttc	attP 40
CCR5_A7_attP_30	gctgtgtttgcgtctctccc	cgtacaccactgagaccgcggtggttgaccagacaaaccaagagacgcaaa	attP 30
CCR5_B8_attP_30	gtatggaaaatgagagctgc	ggtcaaccaccgcggtctcagtggtgtacggtacaaacccgctctcattttc	attP 30
CCR5_A7_attP_GA_50	gctgtgtttgcgtctctccc	gggtttgtaccgtacaccactgagtccgcggtggttgaccagacaaaccaagagacgcaaa	attP-GA 50
CCR5_B8_attP_GA_50	gtatggaaaatgagagctgc	tggtttgtctggtcaaccaccgcggactcagtggtgtacggtacaaacccgctctcatttttc	attP-GA 50
CCR5_A7_attP_GA_40	gctgtgtttgcgtctctccc	tgtaccgtacaccactgagtccgcggtggttgaccagacaaaccaagagacgcaaa	attP-GA 40
CCR5_B8_attP_GA_40	gtatggaaaatgagagctgc	tgtctggtcaaccaccgcggactcagtggtgtacggtacaaacccgctctcattttc	attP-GA 40
CCR5_A7_attP_GA_30	gctgtgtttgcgtctctccc	cgtacaccactgagtccgcggtggttgaccagacaaaccaagagacgcaaa	attP-GA 30
CCR5_B8_attP_GA_30	gtatggaaaatgagagctgc	ggtcaaccaccgcggactcagtggtgtacggtacaaacccgctctcattttc	attP-GA 30

Figure 3f

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A277b	gactgaaacttcacagaata	aacttcacagaggcttgtcgacgacggcggtctccgtcgtcaggatcat	indicated on the x-axis
CCR5_A277c	gactgaaacttcacagaata	gaaacttcacagaggcttgtcgacgacggcggtctccgtcgtcaggatcat	indicated on the x-axis
CCR5_B358b	gatttatgagatcaacagcac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgttgatctc	indicated on the x-axis
CCR5_B358c	gatttatgagatcaacagcac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgttgatctcat	indicated on the x-axis

Figure 3g

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A7_attB_20	gctgtgtttgcgtctctccc	acgacggagaccgccgtcgtcgacaagccagagacgcaaa	CCR5 attB (HEK293T and Huh7)
CCR5_B8_attB_20	gtatggaaaatgagagctgc	acgacggcggtctccgtcgtcaggatcatgctctcattttc	CCR5 attB (HEK293T and Huh7)
CCR5_A277c	gactgaaacttcacagaata	gaaacttcacagaggcttgtcgacgacggcggtctccgtcgtcaggatcat	ALB attB (HEK293T and Huh7)

CCR5_B358c	gatttatgagatcaacagcac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgttgatctcat	ALB attB (HEK293T and Huh7)

Figure 4b

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
IDS2_DF_A1_a_attP_rev	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagtta	A1B2 (attP rev)
IDS2_DF_A1_a_attP_fwd	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagtta	A1B2 (attP fwd)
IDS2_DF_A1_c_attP_rev	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagtttta	A1B3 (attP rev); A1B4 (attP rev)
IDS2_DF_A1_c_attP_fwd	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagtttta	A1B3 (attP fwd); A1B4 (attP fwd)
IDS2_DF_A4_b_attP_rev	gcactcatttcctccaagctc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcttggaggaaa	A4B7 (attP rev)
IDS2_DF_A4_b_attP_fwd	gcactcatttcctccaagctc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcttggaggaaa	A4B7 (attP fwd)
IDS2_DF_B2_a_attP_rev	gtaggtacaggacagggcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccctgtcc	A1B2 (attP rev)
IDS2_DF_B2_a_attP_fwd	gtaggtacaggacagggcag	taccgtacaccactgagaccgcggtggttgaccagacaaacctccctgtcc	A1B2 (attP fwd)
IDS2_DF_B3_a_attP_rev	gagataggtaggtacaggaca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctgtacct	A1B3 (attP rev)
IDS2_DF_B3_a_attP_fwd	gagataggtaggtacaggaca	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctgtacct	A1B3 (attP fwd)
IDS2_DF_B4_b_attP_rev	gtgaaaagataggtaggtac	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctacctatcta	A1B4 (attP rev)
IDS2_DF_B4_b_attP_fwd	gtgaaaagataggtaggtac	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctacctatcta	A1B4 (attP fwd)
IDS2_DF_B7_b_attP_rev	gttatggtttactccatcta	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctatggagtaaacct	A4B7 (attP rev)
IDS2_DF_B7_b_attP_fwd	gttatggtttactccatcta	taccgtacaccactgagaccgcggtggttgaccagacaaacctatggagtaaacc	A4B7 (attP fwd)
IDS_DF_C2_c_attB_rev	gttttggtttaccctatcta	atgatcctgacgacggagaccgccgtcgtcgacaagccatagggtaaacca	C2D2 (attB rev); C2D2 (attB rev)
IDS_DF_C2_c_attB_fwd	gttttggtttaccctatcta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatagggtaaacca	C2D1 (attB fwd); C2D2 (attB fwd)
IDS_DF_D1_b_attB_rev	gctgtggaactgcaacacact	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgtgttgcagt	C2D1 (attB rev)
IDS_DF_D1_b_attB_fwd	gctgtggaactgcaacacact	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgttgcagt	C2D1 (attB fwd)
IDS_DF_D2_c_attB_rev	gtgccacctaacagtgagctg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctcactgttaggt	C2D2 (attB rev)
IDS_DF_D2_c_attB_fwd	gtgccacctaacagtgagctg	atgatcctgacgacggagaccgccgtcgtcgacaagccctcactgttaggt	C2D2 (attB fwd)

Figure 4c

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
IDS2_DF_A4_b_attP_rev	gcactcatttcctccaagctc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcttggaggaaa	pegRNA set1 installing attP_rev in IDS2 and attB_fwd in IDS
IDS2_DF_B7_b_attP_rev	gttatggtttactccatcta	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctatggagtaaacct	pegRNA set1 installing attP_rev in IDS2 and attB_fwd in IDS
IDS_DF_C2_c_attB_fwd	gttttggtttaccctatcta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatagggtaaacca	pegRNA set1 installing attP_rev in IDS2 and attB_fwd in IDS
IDS_DF_D1_b_attB_fwd	gctgtggaactgcaacacact	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgttgcagt	pegRNA set1 installing attP_rev in IDS2 and attB_fwd in IDS
IDS2_DF_A4_b_attP_fwd	gcactcatttcctccaagctc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcttggaggaaa	pegRNA set2 installing attP_fwd in IDS2 and attB_rev in IDS
IDS2_DF_B7_b_attP_fwd	gttatggtttactccatcta	taccgtacaccactgagaccgcggtggttgaccagacaaacctatggagtaaacc	pegRNA set2 installing attP_fwd in IDS2 and attB_rev in IDS
IDS_DF_C2_c_attB_rev	gttttggtttaccctatcta	atgatcctgacgacggagaccgccgtcgtcgacaagccatagggtaaacca	pegRNA set2 installing attP_fwd in IDS2 and attB_rev in IDS
IDS_DF_D1_b_attB_rev	gctgtggaactgcaacacact	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgtgttgcagt	pegRNA set2 installing attP_fwd in IDS2 and attB_rev in IDS

Extended Data Figures

ED Figure 1a - Comparison of twinPE- and PE3-mediated *FKBP* insertion at *CCR5* region 1

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
PE3_CCR5_1_F414a_34_FKBP108	ggtacctatcgattgtcagg	tactgtccccttctgggctcactatgctgccgccaaatttctttccatcttcaagcatcccg gtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggg gagatggtttccacctgcactccgacaatcga	indicated on the x-axis
PE3_CCR5_1_F414a_29_FKBP108	ggtacctatcgattgtcagg	tcccctttctgggctcactatgctgccgccaaatttctttccatcttcaagcatcccggtgta gtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggaga tggtttccacctgcactccgacaatcga	indicated on the x-axis
PE3_CCR5_1_F414a_23_FKBP108	ggtacctatcgattgtcagg	tctgggctcactatgctgccgccaaatttctttccatcttcaagcatcccggtgtagtgca ccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggttt ccacctgcactccgacaatcga	indicated on the x-axis

twinPE_CCR5_1_F414a_FKBP108	ggtacctatcgattgtcagg	accttccccaagcgcggccagacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaatttgacaatcga	indicated on the x-axis
twinPE_CCR5_1_E325b_FKBP108	gctcactatgctgccgcccag	accacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactccggcggcagcat	indicated on the x-axis
sgRNA	**spacer sequence**		
PE3_CCR5_A1_sgRNA	gcatcctgataaactgcaaa		
PE3_CCR5_A2_sgRNA	ggacatctacctgctcaacc		
PE3_CCR5_A4_sgRNA	gcaatgtgtcaactcttgac		

ED Figure 1b - Comparison of twinPE- and PE3-mediated *FKBP* insertion at *CCR5* region 2

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
PE3_CCR5_2_F2_34_FKBP108	gtgaaagacagcctggagtc	agattggagaaacccttgaaaagacatcaagcacaaatttctttccatcttcaagcatccccggtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctgggggagatggtttccacctgcactcctccaggct	indicated on the x-axis
PE3_CCR5_2_F2_28_FKBP108	gtgaaagacagcctggagtc	gagaaacccttgaaaagacatcaagcacaaatttctttccatcttcaagcatcccggtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactcctccaggct	indicated on the x-axis
PE3_CCR5_2_F2_23_FKBP108	gtgaaagacagcctggagtc	acccttgaaaagacatcaagcacaaatttctttccatcttcaagcatcccggtgtagtgccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactcctccaggct	indicated on the x-axis
twinPE_CCR5_2_F2a_FKBP108	gtgaaagacagcctggagtc	accttccccaagcgcggccagacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaattttccaggct	indicated on the x-axis
twinPE_CCR5_2_E2c_FKBP108nt	gaaaagacatcaagcacaga	accacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggttccacctgcactccgtgcttgatgtctt	indicated on the x-axis
sgRNA	**spacer sequence**		
PE3_CCR5_C1_sgRNA	gatgcagagtcagcagaact		
PE3_CCR5_C1.5_sgRNA	ggaagtgagggtcagagagg		
PE3_CCR5_C4_sgRNA	gatggattggtgtaaaagga		

ED Figure 1c - Long twinPE insertions at *CCR5*

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A7_attP_spacer27	gctgtgtttgcgtctctccc	ttcgttatacgcccattcttcgcgaaaggtttgtaccgtacaccactgagaccgcggtggttgaccagacaaaccagagacgcaaa	attB-spacer-attP
CCR5_B8_spacer_attB	gtatggaaaatgagagctgc	tttcgcgaagaatgggcgtataacgaaggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	attB-spacer-attP, attB-spacer-attB
CCR5_A7_attB_spacer27	gctgtgtttgcgtctctccc	ttcgttatacgcccattcttcgcgaaaatgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB-spacer-attB

ED Figure 2 - *PAH* exon recoding via twinPE

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
PAH_2.1.1	gctcaaataagcgcaatactt	tgatcttcagcctgaaggaagaggtgggcgccctggcgaaggtattgcgctt	indicated on the x-axis
PAH_2.1.2	gctcaaataagcgcaatactt	tgatcttcagcctgaaggaagaggtgggcgccctggcgaaggtattgcgcttatt	indicated on the x-axis
PAH_2.1.3	gctcaaataagcgcaatactt	tgatcttcagcctgaaggaagaggtgggcgccctggcgaaggtattgcgcttatttg	indicated on the x-axis
PAH_2.2.1	gaagacaactgcaatcaaaa	tcttccttcaggctgaagatcagagagatagcgccgttctgattgcag	indicated on the x-axis
PAH_2.2.2	gaagacaactgcaatcaaaa	tcttccttcaggctgaagatcagagagatagcgccgttctgattgcagttg	indicated on the x-axis
PAH_2.2.3	gaagacaactgcaatcaaaa	tcttccttcaggctgaagatcagagagatagcgccgttctgattgcagttgtc	indicated on the x-axis
PAH_4.1.1	gttctctgtgtttcagtgccc	aagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaaac	indicated on the x-axis
PAH_4.1.2	gttctctgtgtttcagtgccc	aagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaaacac	indicated on the x-axis
PAH_4.1.3	gttctctgtgtttcagtgccc	aagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaacacag	indicated on the x-axis
PAH_4.1.4	gttctctgtgtttcagtgccc	gacaagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaaac	indicated on the x-axis
PAH_4.1.5	gttctctgtgtttcagtgccc	gacaagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaaacac	indicated on the x-axis
PAH_4.1.6	gttctctgtgtttcagtgccc	gacaagatttgattagcgaacctatcaagttcctgaattgtccgtggaaaccacggcactgaaacacag	indicated on the x-axis
PAH_4.2.1	gcccaagaaccattcaagagc	ggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatgg	indicated on the x-axis
PAH_4.2.2	gcccaagaaccattcaagagc	ggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	indicated on the x-axis
PAH_4.2.3	gcccaagaaccattcaagagc	ggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttcttg	indicated on the x-axis
PAH_4.2.4	gcccaagaaccattcaagagc	gttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatgg	indicated on the x-axis
PAH_4.2.5	gcccaagaaccattcaagagc	gttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	indicated on the x-axis
PAH_4.2.6	gcccaagaaccattcaagagc	gttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttcttg	indicated on the x-axis
PAH_4.2.7	gcccaagaaccattcaagagc	gatgatcagcgtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatgg	indicated on the x-axis
PAH_4.2.8	gcccaagaaccattcaagagc	gatgatcagcgtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttc	indicated on the x-axis
PAH_4.2.9	gcccaagaaccattcaagagc	gatgatcagcgtcaagttcggctccgtaggacaagatttgattagcgaacctatcaagttcttgaatggttcttg	indicated on the x-axis
PAH_4.3.1	gcgggccatggactcacaggg	ataggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtcc	indicated on the x-axis
PAH_4.3.2	gcgggccatggactcacaggg	ataggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccat	indicated on the x-axis

PAH_4.3.3	gcgggccatggactcacaggg	ataggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatgg	indicated on the x-axis
PAH_4.3.4	gcgggccatggactcacaggg	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtcc	indicated on the x-axis
PAH_4.3.5	gcgggccatggactcacaggg	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccat	indicated on the x-axis
PAH_4.3.6	gcgggccatggactcacaggg	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatgg	indicated on the x-axis
PAH_4.4.1	gctacgggccatggactcaca	ggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatg	indicated on the x-axis
PAH_4.4.2	gctacgggccatggactcaca	ggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggc	indicated on the x-axis
PAH_4.4.3	gctacgggccatggactcaca	ggttcgctaatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcc	indicated on the x-axis
PAH_4.4.4	gctacgggccatggactcaca	aaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatg	indicated on the x-axis
PAH_4.4.5	gctacgggccatggactcaca	aaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggc	indicated on the x-axis
PAH_4.4.6	gctacgggccatggactcaca	aaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggcc	indicated on the x-axis
PAH_4.5.1	gaaatctcatcctacgggcca	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggccgtaggat	indicated on the x-axis
PAH_4.5.2	gaaatctcatcctacgggcca	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggccgtaggatga	indicated on the x-axis
PAH_4.5.3	gaaatctcatcctacgggcca	taatcaaatcttgtcctacggagccgaacttgacgctgatcatcctgtgagtccatggccgtaggatgag	indicated on the x-axis
PAH_5.1.1	gcaggtgtctcttttctccta	gaattgcttacggcgggcgcgataaacggggtccttgaagccctaggagaaaagag	indicated on the x-axis
PAH_5.1.2	gcaggtgtctcttttctccta	gaattgcttacggcgggcgcgataaacggggtccttgaagccctaggagaaaagagac	indicated on the x-axis
PAH_5.1.3	gcaggtgtctcttttctccta	gaattgcttacggcgggcgcgataaacggggtccttgaagccctaggagaaaagagacac	indicated on the x-axis
PAH_5.2.1	gaagcaaggcagacttactgg	atcgcgcccgccgtaagcaattcgccgatattgcatataattatcgccagtaagtctg	indicated on the x-axis
PAH_5.2.2	gaagcaaggcagacttactgg	atcgcgcccgccgtaagcaattcgccgatattgcatataattatcgccagtaagtctgc	indicated on the x-axis
PAH_5.2.3	gaagcaaggcagacttactgg	atcgcgcccgccgtaagcaattcgccgatattgcatataattatcgccagtaagtctgcc	indicated on the x-axis
PAH_7.1.1	gcttttcatcccagcttgcac	tactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctggg	indicated on the x-axis
PAH_7.1.2	gcttttcatcccagcttgcac	tactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctgggat	indicated on the x-axis
PAH_7.1.3	gcttttcatcccagcttgcac	tactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctgggatga	indicated on the x-axis
PAH_7.1.4	gcttttcatcccagcttgcac	agaaagtctctactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctggg	indicated on the x-axis
PAH_7.1.5	gcttttcatcccagcttgcac	agaaagtctctactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctgggat	indicated on the x-axis
PAH_7.1.6	gcttttcatcccagcttgcac	agaaagtctctactgctcaagagcccggcaacggggcggagtcgaaagccagtacaagctgggatga	indicated on the x-axis
PAH_7.2.1	gtggtttccgcctccgacctg	agaaagtctctactgctcaagagcccggcaacgggtcggagg	indicated on the x-axis
PAH_7.2.2	gtggtttccgcctccgacctg	agaaagtctctactgctcaagagcccggcaacgggtcggaggcg	indicated on the x-axis
PAH_7.2.3	gtggtttccgcctccgacctg	agaaagtctctactgctcaagagcccggcaacgggtcggaggcgga	indicated on the x-axis
PAH_7.2.4	gtggtttccgcctccgacctg	gagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggagg	indicated on the x-axis
PAH_7.2.5	gtggtttccgcctccgacctg	gagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcg	indicated on the x-axis
PAH_7.2.6	gtggtttccgcctccgacctg	gagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcgga	indicated on the x-axis
PAH_7.2.7	gtggtttccgcctccgacctg	aacacgcggaatgcgagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggagg	indicated on the x-axis
PAH_7.2.8	gtggtttccgcctccgacctg	aacacgcggaatgcgagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcg	indicated on the x-axis
PAH_7.2.9	gtggtttccgcctccgacctg	aacacgcggaatgcgagacccccccagaaagtctctactgctcaagagcccggcaacgggtcggaggcgga	indicated on the x-axis
PAH_7.3.1	ggctggcctgctttcctctc	gcggaatgcgagacccccccagaaagtctctacaggaaagc	indicated on the x-axis
PAH_7.3.2	ggctggcctgctttcctctc	gcggaatgcgagacccccccagaaagtctctacaggaaagcag	indicated on the x-axis
PAH_7.3.3	ggctggcctgctttcctctc	gcggaatgcgagacccccccagaaagtctctacaggaaagcaggc	indicated on the x-axis
PAH_7.3.4	ggctggcctgctttcctctc	tgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagc	indicated on the x-axis
PAH_7.3.5	ggctggcctgctttcctctc	tgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagcag	indicated on the x-axis
PAH_7.3.6	ggctggcctgctttcctctc	tgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagcaggc	indicated on the x-axis
PAH_7.3.7	ggctggcctgctttcctctc	taatatactgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagc	indicated on the x-axis
PAH_7.3.8	ggctggcctgctttcctctc	taatatactgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagcag	indicated on the x-axis
PAH_7.3.9	ggctggcctgctttcctctc	taatatactgggtacaatgaaacacgcggaatgcgagacccccccagaaagtctctacaggaaagcaggc	indicated on the x-axis
PAH_7.5.1	gagtggaagactcggaaggcc	gttgccgggctcttgagcagtagagactttctgggggtctcgccttccgagt	indicated on the x-axis
PAH_7.5.2	gagtggaagactcggaaggcc	gttgccgggctcttgagcagtagagactttctgggggtctcgccttccgagtct	indicated on the x-axis
PAH_7.5.3	gagtggaagactcggaaggcc	gttgccgggctcttgagcagtagagactttctgggggtctcgccttccgagtcttc	indicated on the x-axis
PAH_7.5.4	gagtggaagactcggaaggcc	tcttgagcagtagagactttctgggggtctcgccttccgagt	indicated on the x-axis
PAH_7.5.5	gagtggaagactcggaaggcc	tcttgagcagtagagactttctgggggtctcgccttccgagtct	indicated on the x-axis
PAH_7.5.6	gagtggaagactcggaaggcc	tcttgagcagtagagactttctgggggtctcgccttccgagtcttc	indicated on the x-axis
PAH_7.6.1	gtctgatgtactgtgtgcag	tcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagta	indicated on the x-axis
PAH_7.6.2	gtctgatgtactgtgtgcag	tcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtaca	indicated on the x-axis
PAH_7.6.3	gtctgatgtactgtgtgcag	tcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtacatc	indicated on the x-axis
PAH_7.6.4	gtctgatgtactgtgtgcag	agtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagta	indicated on the x-axis
PAH_7.6.5	gtctgatgtactgtgtgcag	agtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtaca	indicated on the x-axis

PAH_7.6.6	gtctgatgtactgtgtgcag	agtagagactttctgggggtctcgcattccgcgtgtttcattgcacacagtacatc	indicated on the x-axis
PAH_7.6.7	gtctgatgtactgtgtgcag	tgggggtctcgcattccgcgtgtttcattgcacacagta	indicated on the x-axis
PAH_7.6.8	gtctgatgtactgtgtgcag	tgggggtctcgcattccgcgtgtttcattgcacacagtaca	indicated on the x-axis
PAH_7.6.9	gtctgatgtactgtgtgcag	tgggggtctcgcattccgcgtgtttcattgcacacagtacatc	indicated on the x-axis
PAH_7.7.1	gttcgggggtatacatgggct	gggtctcgcattccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatg tatac	indicated on the x-axis
PAH_7.7.2	gttcgggggtatacatgggct	gggtctcgcattccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatg tataccc	indicated on the x-axis
PAH_7.7.3	gttcgggggtatacatgggct	gggtctcgcattccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatg tatacccc	indicated on the x-axis
PAH_7.7.4	gttcgggggtatacatgggct	attccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatgtatac	indicated on the x-axis
PAH_7.7.5	gttcgggggtatacatgggct	attccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatgtataccc	indicated on the x-axis
PAH_7.7.6	gttcgggggtatacatgggct	gtgtttcattgtacccagtatattaggcatggttcaaaacccatgtatacccc	indicated on the x-axis
PAH_7.7.7	gttcgggggtatacatgggct	gtgtttcattgtacccagtatattaggcatggttcaaaacccatgtatac	indicated on the x-axis
PAH_7.7.8	gttcgggggtatacatgggct	gtgtttcattgtacccagtatattaggcatggttcaaaacccatgtataccc	indicated on the x-axis
PAH_7.7.9	gttcgggggtatacatgggct	attccgcgtgtttcattgtacccagtatattaggcatggttcaaaacccatgtatacccc	indicated on the x-axis
PAH_7.8.1	ggacagtactcacggttcgg	gcgtgtttcattgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaac cgtga	indicated on the x-axis
PAH_7.8.2	ggacagtactcacggttcgg	gcgtgtttcattgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaac cgtgagt	indicated on the x-axis
PAH_7.8.3	ggacagtactcacggttcgg	gcgtgtttcattgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaac cgtgagtac	indicated on the x-axis
PAH_7.8.4	ggacagtactcacggttcgg	attgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaaccgtga	indicated on the x-axis
PAH_7.8.5	ggacagtactcacggttcgg	attgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaaccgtgagt	indicated on the x-axis
PAH_7.8.6	ggacagtactcacggttcgg	attgtacccagtatattaggcatggttcaaaaccgatgtacacaccagaaccgtgagta c	indicated on the x-axis
PAH_8.1.1	gtgacatctgccatgagctgt	gaaggacctatcgctgaacagtggcacgtgaccgagaagctcatggc	indicated on the x-axis
PAH_8.1.2	gtgacatctgccatgagctgt	gaaggacctatcgctgaacagtggcacgtgaccgagaagctcatggcag	indicated on the x-axis
PAH_8.1.3	gtgacatctgccatgagctgt	gaaggacctatcgctgaacagtggcacgtgaccgagaagctcatggcagat	indicated on the x-axis
PAH_8.2.1	gtaaaaaatccattccttacc	actgttcagcgataggtccttcgcacagttctctcaggtaaggaatgg	indicated on the x-axis
PAH_8.2.2	gtaaaaaatccattccttacc	actgttcagcgataggtccttcgcacagttctctcaggtaaggaatgga	indicated on the x-axis
PAH_8.2.3	gtaaaaaatccattccttacc	actgttcagcgataggtccttcgcacagttctctcaggtaaggaatggatt	indicated on the x-axis
PAH_9.1.1	gttcccccaattacaggaaat	tatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaatt	indicated on the x-axis
PAH_9.1.2	gttcccccaattacaggaaat	tatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaattgg	indicated on the x-axis
PAH_9.1.3	gttcccccaattacaggaaat	tatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaattggg	indicated on the x-axis
PAH_9.1.4	gttcccccaattacaggaaat	ttctctatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaatt	indicated on the x-axis
PAH_9.1.5	gttcccccaattacaggaaat	ttctctatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaattgg	indicated on the x-axis
PAH_9.1.6	gttcccccaattacaggaaat	ttctctatatactcgtctggtgctcccaggcttgccagaccgatctcctgtaattggg	indicated on the x-axis
PAH_9.2.1	gggagagaagggacttactg	tgggagcaccagacgagtatatagagaaacttgctacagtaagtccctt	indicated on the x-axis
PAH_9.2.2	gggagagaagggacttactg	tgggagcaccagacgagtatatagagaaacttgctacagtaagtcccttc	indicated on the x-axis
PAH_9.2.3	gggagagaagggacttactg	tgggagcaccagacgagtatatagagaaacttgctacagtaagtcccttctc	indicated on the x-axis
PAH_9.3.1	gaccatccacccagggagaga	accagacgagtatatagagaaacttgctacagtaagtcctttctctccctgggt	indicated on the x-axis
PAH_9.3.2	gaccatccacccagggagaga	accagacgagtatatagagaaacttgctacagtaagtcctttctctccctgggtg	indicated on the x-axis
PAH_9.3.3	gaccatccacccagggagaga	accagacgagtatatagagaaacttgctacagtaagtcctttctctccctgggtgga	indicated on the x-axis
PAH_10.2.1_EvoPreQ1	gccagatttactggtttactg	gtaggctttaatgctatcacctgcttacacaatccgaattcgacggtaaaccagtaaat ctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_10.2.2_EvoPreQ1	gccagatttactggtttactg	gtaggctttaatgctatcacctgcttacacaatccgaattcgacggtaaaccagtaaat ctctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_10.2.3_EvoPreQ1	gccagatttactggtttactg	gtaggctttaatgctatcacctgcttacacaatccgaattcgacggtaaaccagtaaat ctgtctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_10.3.1_EvoPreQ1	gtaattcaccaaaggatgac	taagcagggtgatagcattaaagcctacggagcaggtttgctctcatcctttggtgtctct ctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_10.3.2_EvoPreQ1	gtaattcaccaaaggatgac	taagcagggtgatagcattaaagcctacggagcaggtttgctctcatcctttggtgatct ctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_10.3.3_EvoPreQ1	gtaattcaccaaaggatgac	taagcagggtgatagcattaaagcctacggagcaggtttgctctcatcctttggtgaatc tctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.1.1_EvoPreQ1	gaagccaaagcttctccccc	tgtcactgtatagttctgaattgcagtcttttcgagttcgagtgggagaagctctctctcttg acgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.1.2_EvoPreQ1	gaagccaaagcttctccccc	tgtcactgtatagttctgaattgcagtcttttcgagttcgagtgggagaagctttctctctct tgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.1.3_EvoPreQ1	gaagccaaagcttctccccc	tgtcactgtatagttctgaattgcagtcttttcgagttcgagtgggagaagctttggtctct ctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.2.1_EvoPreQ1	gaaagcttctccccctggagc	tgtcactgtatagttctgaattgcagtcttttcgagttccaggggggtctctctcttgacgcg gttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.2.2_EvoPreQ1	gaaagcttctccccctggagc	tgtcactgtatagttctgaattgcagtcttttcgagttccaggggggagtctctctcttgacg cggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.2.3_EvoPreQ1	gaaagcttctccccctggagc	tgtcactgtatagttctgaattgcagtcttttcgagttccaggggggagaatctctctcttga cgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.4.1_EvoPreQ1	gaactctctgccacgtaatag	tgcaattcagaactatacagtgacagaatttcaaccattgtattacgtggctctctctcttg acgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.4.2_EvoPreQ1	gaactctctgccacgtaatag	tgcaattcagaactatacagtgacagaatttcaaccattgtattacgtggcagtctctctct tgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_11.4.3_EvoPreQ1	gaactctctgccacgtaatag	tgcaattcagaactatacagtgacagaatttcaaccattgtattacgtggcagagtctctc tcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.2.1_EvoPreQ1	gactttgctgccacaatacct	ttcgatgcgctgtgtataaggatcatatctcacgctaaatggtcttggtattgtggctctct ctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.2.2_EvoPreQ1	gactttgctgccacaatacct	ttcgatgcgctgtgtataaggatcatatctcacgctaaatggtcttggtattgtggcagtct ctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis

PAH_12.2.3_EvoPreQ1	gactttgctgccacaatacct	ttcgatgcgctgtgtataaggatcatatctcacgctaaatggtcttggtattgtggcagct ctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.3.1_EvoPreQ1	gctacgacccatacacccaa	gatcttcaattgttgagtgttatcgagcacttcgatgcgctgggtgtatggtctctctcttg acgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.3.2_EvoPreQ1	gctacgacccatacacccaa	gatcttcaattgttgagtgttatcgagcacttcgatgcgctgggtgtatgggttctctctctt gacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.3.3_EvoPreQ1	gctacgacccatacacccaa	gatcttcaattgttgagtgttatcgagcacttcgatgcgctgggtgtatgggtctctctctc ttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.4.1_EvoPreQ1	gcagccaaaatcttaagctgc	tatgatccttatacacagcgcatcgaagtgctcgataacactcaacagcttaagatttctc tctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.4.2_EvoPreQ1	gcagccaaaatcttaagctgc	tatgatccttatacacagcgcatcgaagtgctcgataacactcaacagcttaagatttagt ctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.4.3_EvoPreQ1	gcagccaaaatcttaagctgc	tatgatccttatacacagcgcatcgaagtgctcgataacactcaacagcttaagatttag gtctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.5.1_EvoPreQ1	gtgtaaattacttactgttaa	gataacactcaacaattgaagatcctcgcagacagtatcaacagtaagtaatctctctctt gacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.5.2_EvoPreQ1	gtgtaaattacttactgttaa	gataacactcaacaattgaagatcctcgcagacagtatcaacagtaagtaatttctctctc ttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis
PAH_12.5.3_EvoPreQ1	gtgtaaattacttactgttaa	gataacactcaacaattgaagatcctcgcagacagtatcaacagtaagtaatttatctctc tcttgacgcggttctatctagttacgcgttaaaccaactagaaa	indicated on the x-axis

ED Figure 3- pegRNA screen in *CCR5*

3' extension	3' extension	3' extension	3' extension
CCR5_A223a	gtcatcctgataaactgcaaa	atgatcctgacgacggagaccgccgtcgtcgacaagccgcagtttatc	indicated on the x-axis
CCR5_A223b	gtcatcctgataaactgcaaa	atgatcctgacgacggagaccgccgtcgtcgacaagccgcagtttatcag	indicated on the x-axis
CCR5_A223c	gtcatcctgataaactgcaaa	atgatcctgacgacggagaccgccgtcgtcgacaagccgcagtttatcagg	indicated on the x-axis
CCR5_A260a	gtgacatctacctgctcaacc	atgatcctgacgacggagaccgccgtcgtcgacaagcctgagcaggt	indicated on the x-axis
CCR5_A260b	gtgacatctacctgctcaacc	atgatcctgacgacggagaccgccgtcgtcgacaagcctgagcaggtag	indicated on the x-axis
CCR5_A260c	gtgacatctacctgctcaacc	atgatcctgacgacggagaccgccgtcgtcgacaagcctgagcaggtagat	indicated on the x-axis
CCR5_A325a	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagc	indicated on the x-axis
CCR5_A325b	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagcat	indicated on the x-axis
CCR5_A325c	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagcatag	indicated on the x-axis
CCR5_A360a	gacaatgtgtcaactcttgac	atgatcctgacgacggagaccgccgtcgtcgacaagccaagagttgac	indicated on the x-axis
CCR5_A360b	gacaatgtgtcaactcttgac	atgatcctgacgacggagaccgccgtcgtcgacaagccaagagttgacac	indicated on the x-axis
CCR5_A360c	gacaatgtgtcaactcttgac	atgatcctgacgacggagaccgccgtcgtcgacaagccaagagttgacaca	indicated on the x-axis
CCR5_A506a	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcac	indicated on the x-axis
CCR5_A506b	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacac	indicated on the x-axis
CCR5_A506c	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacactt	indicated on the x-axis
CCR5_A509a	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagccccaagtga	indicated on the x-axis
CCR5_A509b	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagccccaagtgatc	indicated on the x-axis
CCR5_A509c	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagccccaagtgatcac	indicated on the x-axis
CCR5_A531a	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgca	indicated on the x-axis
CCR5_A531b	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	indicated on the x-axis
CCR5_A531c	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaaca	indicated on the x-axis
CCR5_B272a	gaaggaaaaacaggtcagaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgacctg	indicated on the x-axis
CCR5_B272b	gaaggaaaaacaggtcagaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgacctgt	indicated on the x-axis
CCR5_B272c	gaaggaaaaacaggtcagaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgacctgttt	indicated on the x-axis
CCR5_B291a	gcccagaaggggacagtaaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcattactgtccc	indicated on the x-axis
CCR5_B291b	gcccagaaggggacagtaaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcattactgtcccct	indicated on the x-axis
CCR5_B291c	gcccagaaggggacagtaaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcattactgtcccctc	indicated on the x-axis
CCR5_B305a	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcact	indicated on the x-axis
CCR5_B305b	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcactat	indicated on the x-axis
CCR5_B305c	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcactatgc	indicated on the x-axis
CCR5_B326a	gatttccaaagtcccactggg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtgggact	indicated on the x-axis
CCR5_B326b	gatttccaaagtcccactggg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtgggactttg	indicated on the x-axis
CCR5_B326c	gatttccaaagtcccactggg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtgggactttgg	indicated on the x-axis
CCR5_B330a	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttgg	indicated on the x-axis
CCR5_B330b	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaa	indicated on the x-axis
CCR5_B330c	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaaat	indicated on the x-axis
CCR5_B414a	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
CCR5_B414b	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcgata	indicated on the x-axis
CCR5_B414c	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcgatagg	indicated on the x-axis
CCR5_B535a	gatctggtaaagatgattcc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcatcttta	indicated on the x-axis
CCR5_B535b	gatctggtaaagatgattcc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcatctttacc	indicated on the x-axis
CCR5_B535c	gatctggtaaagatgattcc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcatctttaccag	indicated on the x-axis
CCR5_B584a	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcatt	indicated on the x-axis
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	indicated on the x-axis
CCR5_B584c	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttcca	indicated on the x-axis
CCR5_B601a	gcagaattgatactgactgta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtcagtat	indicated on the x-axis
CCR5_B601b	gcagaattgatactgactgta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtcagtatca	indicated on the x-axis
CCR5_B601c	gcagaattgatactgactgta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtcagtatcaat	indicated on the x-axis

ED Figure 4 - pegRNA screen at *AAVS1*

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
AAVS1_A1077a	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcct	indicated on the x-axis
AAVS1_A1077b	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctgg	indicated on the x-axis
AAVS1_A1077c	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctggct	indicated on the x-axis

AAVS1_A1098a	gggaaggggcaggagagcca	taccgtacaccactgagaccgcggtggttgaccagacaaacctctctcctgc	indicated on the x-axis
AAVS1_A1098b	gggaaggggcaggagagcca	taccgtacaccactgagaccgcggtggttgaccagacaaacctctctcctgccc	indicated on the x-axis
AAVS1_A1098c	gggaaggggcaggagagcca	taccgtacaccactgagaccgcggtggttgaccagacaaacctctctcctgcccct	indicated on the x-axis
AAVS1_A1246a	gaatatgtcccagatagcac	taccgtacaccactgagaccgcggtggttgaccagacaaacctctatctggg	indicated on the x-axis
AAVS1_A1246b	gaatatgtcccagatagcac	taccgtacaccactgagaccgcggtggttgaccagacaaacctctatctgggac	indicated on the x-axis
AAVS1_A1246c	gaatatgtcccagatagcac	taccgtacaccactgagaccgcggtggttgaccagacaaacctctatctgggacat	indicated on the x-axis
AAVS1_A1267a	ggggactctttaaggaaaga	taccgtacaccactgagaccgcggtggttgaccagacaaaccttttccttaaa	indicated on the x-axis
AAVS1_A1267b	ggggactctttaaggaaaga	taccgtacaccactgagaccgcggtggttgaccagacaaaccttttccttaaaga	indicated on the x-axis
AAVS1_A1267c	ggggactctttaaggaaaga	taccgtacaccactgagaccgcggtggttgaccagacaaaccttttccttaaagagt	indicated on the x-axis
AAVS1_A1293a	gagaaagagaaagggagtag	taccgtacaccactgagaccgcggtggttgaccagacaaacctctccctttc	indicated on the x-axis
AAVS1_A1293b	gagaaagagaaagggagtag	taccgtacaccactgagaccgcggtggttgaccagacaaacctctccctttctc	indicated on the x-axis
AAVS1_A1293c	gagaaagagaaagggagtag	taccgtacaccactgagaccgcggtggttgaccagacaaacctctccctttctctt	indicated on the x-axis
AAVS1_A1307a	gagtagaggcggccacgacc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcgtggccgc	indicated on the x-axis
AAVS1_A1307b	gagtagaggcggccacgacc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcgtggccgcct	indicated on the x-axis
AAVS1_A1307c	gagtagaggcggccacgacc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcgtggccgcctct	indicated on the x-axis
AAVS1_A1582a	gatcagtgaaacgcaccaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctggtgcgttt	indicated on the x-axis
AAVS1_A1582b	gatcagtgaaacgcaccaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctggtgcgtttca	indicated on the x-axis
AAVS1_A1582c	gatcagtgaaacgcaccaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctggtgcgtttcact	indicated on the x-axis
AAVS1_A1615a	gcagctcaggttctgggaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcccagaacc	indicated on the x-axis
AAVS1_A1615b	gcagctcaggttctgggaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcccagaacctg	indicated on the x-axis
AAVS1_A1615c	gcagctcaggttctgggaga	taccgtacaccactgagaccgcggtggttgaccagacaaacctcccagaacctgag	indicated on the x-axis
AAVS1_A1647a	gtggccactgagaaccgggc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcggttctca	indicated on the x-axis
AAVS1_A1647b	gtggccactgagaaccgggc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcggttctcagt	indicated on the x-axis
AAVS1_A1647c	gtggccactgagaaccgggc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcggttctcagtgg	indicated on the x-axis
AAVS1_A1810a	gaatctgcctaacaggaggt	taccgtacaccactgagaccgcggtggttgaccagacaaaccttcctgttag	indicated on the x-axis
AAVS1_A1810b	gaatctgcctaacaggaggt	taccgtacaccactgagaccgcggtggttgaccagacaaaccttcctgttaggc	indicated on the x-axis
AAVS1_A1810c	gaatctgcctaacaggaggt	taccgtacaccactgagaccgcggtggttgaccagacaaaccttcctgttaggcag	indicated on the x-axis
AAVS1_A1890a	gtcaccaatcctgtccctag	taccgtacaccactgagaccgcggtggttgaccagacaaacctgggacagga	indicated on the x-axis
AAVS1_A1890b	gtcaccaatcctgtccctag	taccgtacaccactgagaccgcggtggttgaccagacaaacctgggacaggatt	indicated on the x-axis
AAVS1_A1890c	gtcaccaatcctgtccctag	taccgtacaccactgagaccgcggtggttgaccagacaaacctgggacaggattgg	indicated on the x-axis
AAVS1_A3786a	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtggg	indicated on the x-axis
AAVS1_A3786b	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtggggg	indicated on the x-axis
AAVS1_A3786c	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtgggggggc	indicated on the x-axis
AAVS1_A3835a	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgcc	indicated on the x-axis
AAVS1_A3835b	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgccgt	indicated on the x-axis
AAVS1_A3835c	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgccgtga	indicated on the x-axis
AAVS1_A3856a	ggtgtgctgggcaggtcgcg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgacctgccc	indicated on the x-axis
AAVS1_A3856b	ggtgtgctgggcaggtcgcg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgacctgcccag	indicated on the x-axis
AAVS1_A3856c	ggtgtgctgggcaggtcgcg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgacctgcccagca	indicated on the x-axis
AAVS1_B1154a	gtccttggcaagcccaggag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttg	indicated on the x-axis
AAVS1_B1154b	gtccttggcaagcccaggag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgcc	indicated on the x-axis
AAVS1_B1154c	gtccttggcaagcccaggag	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgccaa	indicated on the x-axis
AAVS1_B1314a	gtgcgtcctaggtgttcacc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaacaccta	indicated on the x-axis
AAVS1_B1314b	gtgcgtcctaggtgttcacc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaacacctagg	indicated on the x-axis
AAVS1_B1314c	gtgcgtcctaggtgttcacc	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaacacctaggac	indicated on the x-axis
AAVS1_B1376a	gtcctggcagggctgtggtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacagccct	indicated on the x-axis
AAVS1_B1376b	gtcctggcagggctgtggtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacagccctgc	indicated on the x-axis
AAVS1_B1376c	gtcctggcagggctgtggtg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacagccctgcca	indicated on the x-axis
AAVS1_B1640a	gtgacctgcccggttctcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctagaaccggg	indicated on the x-axis
AAVS1_B1640b	gtgacctgcccggttctcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctagaaccgggca	indicated on the x-axis
AAVS1_B1640c	gtgacctgcccggttctcag	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctagaaccgggcagg	indicated on the x-axis
AAVS1_B1676a	gagcttggcaggggggtggga	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctg	indicated on the x-axis
AAVS1_B1676b	gagcttggcaggggggtggga	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctgcc	indicated on the x-axis
AAVS1_B1676c	gagcttggcaggggggtggga	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcacccccctgccaa	indicated on the x-axis
AAVS1_B1701a	gagccagagaggatcctggg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctaggatcctc	indicated on the x-axis
AAVS1_B1701b	gagccagagaggatcctggg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctaggatcctctc	indicated on the x-axis
AAVS1_B1701c	gagccagagaggatcctggg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctaggatcctctctg	indicated on the x-axis
AAVS1_B1705a	gatggagccagagaggatcc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttcctctctg	indicated on the x-axis
AAVS1_B1705b	gatggagccagagaggatcc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttcctctctggc	indicated on the x-axis
AAVS1_B1705c	gatggagccagagaggatcc	tctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttcctctctggctc	indicated on the x-axis
AAVS1_B1883a	ggggccactagggacaggat	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgtcccta	indicated on the x-axis
AAVS1_B1883b	ggggccactagggacaggat	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgtccctagt	indicated on the x-axis
AAVS1_B1883c	ggggccactagggacaggat	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgtccctagtgg	indicated on the x-axis
AAVS1_B1902a	gtcccctccaccccacagtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgtggggtg	indicated on the x-axis
AAVS1_B1902b	gtcccctccaccccacagtg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgtggggtgga	indicated on the x-axis
AAVS1_B1902c	gtcccctccaccccacagtg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgtggggtggagg	indicated on the x-axis
AAVS1_B1962a	gggaccaccttatattccca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaatataag	indicated on the x-axis
AAVS1_B1962b	gggaccaccttatattccca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaatataaggt	indicated on the x-axis
AAVS1_B1962c	gggaccaccttatattccca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgaatataaggtgg	indicated on the x-axis
AAVS1_B3839a	gacctgcccagcacaccctg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctggtgtgctg	indicated on the x-axis
AAVS1_B3839b	gacctgcccagcacaccctg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctggtgtgctggg	indicated on the x-axis
AAVS1_B3839c	gacctgcccagcacaccctg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctggtgtgctgggca	indicated on the x-axis
AAVS1_B3903a	gcgactcctggaagtggcca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttcca	indicated on the x-axis
AAVS1_B3903b	gcgactcctggaagtggcca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccagg	indicated on the x-axis
AAVS1_B3903c	gcgactcctggaagtggcca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccaggag	indicated on the x-axis
AAVS1_B3930a	ggacttcccagtgtgcatcg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactg	indicated on the x-axis
AAVS1_B3930b	ggacttcccagtgtgcatcg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactggg	indicated on the x-axis
AAVS1_B3930c	ggacttcccagtgtgcatcg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactgggaa	indicated on the x-axis

ED Figure 5a - Comparison of twinPE- and PE3-mediated *attB* insertion at *CCR5* region 1

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_A325b_attB	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagcat	indicated on the x-axis
CCR5_B414a_attB	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
CCR5_B414_23	ggtacctatcgattgtcagg	tctgggctcactatgctgccgccggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
CCR5_B414_29	ggtacctatcgattgtcagg	tccccttctgggctcactatgctgccgccggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
CCR5_B414_34	ggtacctatcgattgtcagg	tactgtccccttctgggctcactatgctgccgccggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	indicated on the x-axis
sgRNA	**spacer sequence**		
PE3_CCR5_A1_sgRNA	gcatcctgataaactgcaaa		
PE3_CCR5_A2_sgRNA	ggacatctacctgctcaacc		
PE3_CCR5_A4_sgRNA	gcaatgtgtcaactcttgac		

ED Figure 5b - Comparison of twinPE- and PE3-mediated *attB* insertion at *CCR5* region 2

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_C2c_attB	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtctt	indicated on the x-axis
CCR5_D2a_attB	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	indicated on the x-axis
CCR5_D2_23	gtgaaagacagcctggagtc	acccttgaaaagacatcaagcacggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	indicated on the x-axis
CCR5_D2_28	gtgaaagacagcctggagtc	gagaaaacccttgaaaagacatcaagcacggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	indicated on the x-axis
CCR5_D2_34	gtgaaagacagcctggagtc	agattggagaaaacccttgaaaagacatcaagcacggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	indicated on the x-axis
sgRNA	**spacer sequence**		
PE3_CCR5_C1_sgRNA	gatgcagagtcagcagaact		
PE3_CCR5_C1.5_sgRNA	ggaagtgagggtcagagagg		
PE3_CCR5_C4_sgRNA	gatggattggtgtaaaagga		

ED Figure 6b - HTS junction purity

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_A325a	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagc	325/414
CCR5_B414b	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcgata	325/414
CCR5_A506c	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacacctt	506/584
CCR5_A509b	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagcccccaagtgatc	509/584
CCR5_A531b	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	531/584
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	506/584, 509/584, 531/584
AAVS1_A1077c	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctggct	1077/1154
AAVS1_B1154c	gtccttggcaagcccaggag	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgccaa	1077/1154
AAVS1_A3786c	gctggccccccaccgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcggtgggggggc	3786/3903, 3786/3930
AAVS1_B3903c	gcgactcctggaagtggcca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccaggag	3786/3903
AAVS1_B3930c	ggacttcccagtgtgcatcg	tctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactgggaa	3786/3930

ED Figure 6c - Multiplex single transfection knock-in

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
AAVS1_A1077c	gcagagccaggaacccctgt	taccgtacaccactgagaccgcggtggttgaccagacaaacctggggttcctggct	AAVS1 attP, all samples on x-axis
AAVS1_B1154c	gtccttggcaagcccaggag	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctctgggcttgccaa	AAVS1 attP, all samples on x-axis
CCR5_A7_attB_20	gctgtgtttgcgtctctccc	acgacggagaccgccgtcgtcgacaagccagagacgcaaa	CCR5 attB
CCR5_B8_attB_20	gtatggaaaatgagagctgc	acgacggcggtctccgtcgtcaggatcatgctctcattttc	CCR5 attB
CCR5_A7_attB_GA_20	gctgtgtttgcgtctctccc	acgacggagtccgccgtcgtcgacaagccagagacgcaaa	CCR5 attB-GA
CCR5_B8_attB_GA_20	gtatggaaaatgagagctgc	acgacggcggactccgtcgtcaggatcatgctctcattttc	CCR5 attB-GA
CCR5_A7_attP_30	gctgtgtttgcgtctctccc	cgtacaccactgagaccgcggtggttgaccagacaaaccaagagacgcaaa	CCR5 attP
CCR5_B8_attP_30	gtatggaaaatgagagctgc	ggtcaaccaccgcggtctcagtggtgtacggtacaaacccgctctcattttc	CCR5 attP
CCR5_A7_attP_GA_30	gctgtgtttgcgtctctccc	cgtacaccactgagtccgcggtggttgaccagacaaaccaagagacgcaaa	CCR5 attP-GA
CCR5_B8_attP_GA_30	gtatggaaaatgagagctgc	ggtcaaccaccgcggactcagtggtgtacggtacaaacccgctctcattttc	CCR5 attP-GA

ED Figure 6d - Overlap reduction

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_A531b	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 38
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 38
CCR5_A7_attB_30	gctgtgtttgcgtctctccc	tcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 30
CCR5_B8_attB_30	gtatggaaaatgagagctgc	tgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 30
CCR5_A7_attB_20	gctgtgtttgcgtctctccc	acgacggagaccgccgtcgtcgacaagccagagacgcaaa	attB 20
CCR5_B8_attB_20	gtatggaaaatgagagctgc	acgacggcggtctccgtcgtcaggatcatgctctcattttc	attB 20

ED figure 7 - Factor 9 knock-in

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_A7_attB_20	gctgtgtttgcgtctctccc	acgacggagaccgccgtcgtcgacaagccagagacgcaaa	CCR5 attB
CCR5_B8_attB_20	gtatggaaaatgagagctgc	acgacggcggtctccgtcgtcaggatcatgctctcattttc	CCR5 attB
CCR5_A277c	gactgaaacttcacagaata	gaaacttcacagaggcttgtcgacgacggcggtctccgtcgtcaggatcat	ALB attB
CCR5_B358c	gatttatgagatcaacagcac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgttgatctcat	ALB attB

ED figure 8 - TwinPE+Bxb1 mediated inversion in the GFP reporter cells

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
AAVS1_A1077b_ attB_rev	gcagagccaggaacccctgt	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggggttcctgg	twinPE-mediated insertion of attB_rev in AAVS1 upstream of H2B_EGFP sequences
AAVS1_B1154b_ attB_fwd	gtccttggcaagcccaggag	atgatcctgacgacggagaccgccgtcgtcgacaagccctgggcttgcc	twinPE-mediated insertion of attB_rev in AAVS1 upstream of H2B_EGFP sequences
AAVS1_A3835b	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgccgt	twinPE-mediated insertion of attP_fwd in AAVS1 downstream of H2B_EGFP sequences
AAVS1_B3903b	gcgactcctggaagtggcca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccacttccagg	twinPE-mediated insertion of attP_fwd in AAVS1 downstream of H2B_EGFP sequences

ED Figure 9c - *IDS* and *IDS2* pegRNA screen

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
IDS2_A1_a_attP_rev	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagtta	A1B2 (attP rev)
IDS2_A1_a_attP_fwd	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagtta	A1B2 (attP fwd)
IDS2_A1_c_attP_rev	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagtttta	A1B3 (attP rev); A1B4 (attP rev)
IDS2_A1_c_attP_fwd	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagtttta	A1B3 (attP fwd); A1B4 (attP fwd)
IDS2_A4_b_attP_rev	gcactcatttcctccaagctc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcttggaggaaa	A4B7 (attP rev)
IDS2_A4_b_attP_fwd	gcactcatttcctccaagctc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcttggaggaaa	A4B7 (attP fwd)
IDS2_B2_a_attP_rev	gtaggtacaggacagggcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccctgtcc	A1B2 (attP rev)
IDS2_B2_a_attP_fwd	gtaggtacaggacagggcag	taccgtacaccactgagaccgcggtggttgaccagacaaacctccctgtcc	A1B2 (attP fwd)
IDS2_B3_a_attP_rev	gagataggtaggtacaggaca	tctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctgtacct	A1B3 (attP rev)
IDS2_B3_a_attP_fwd	gagataggtaggtacaggaca	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctgtacct	A1B3 (attP fwd)
IDS2_B4_b_attP_rev	gtgaaaagataggtaggtac	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctacctatcta	A1B4 (attP rev)
IDS2_B4_b_attP_fwd	gtgaaaagataggtaggtac	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctacctatcta	A1B4 (attP fwd)
IDS2_B7_b_attP_rev	gttatggtttactccatcta	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctatggagtaaacct	A4B7 (attP rev)
IDS2_B7_b_attP_fwd	gttatggtttactccatcta	taccgtacaccactgagaccgcggtggttgaccagacaaacctatggagtaaacc	A4B7 (attP fwd)
IDS_C2_c_attB_rev	gttttggtttaccctatcta	atgatcctgacgacggagaccgccgtcgtcgacaagccataggtaaacca	C2D2 (attB rev); C2D2 (attB rev)
IDS_C2_c_attB_fwd	gttttggtttaccctatcta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatagggtaaacca	C2D1 (attB fwd); C2D2 (attB fwd)
IDS_D1_b_attB_rev	gctgtggaactgcaacacact	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgtgttgcagt	C2D1 (attB rev)
IDS_D1_b_attB_fwd	gctgtggaactgcaacacact	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgttgcagt	C2D1 (attB fwd)
IDS_D2_c_attB_rev	gtgccacctaacagtgagctg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctcactgttaggt	C2D2 (attB rev)
IDS_D2_c_attB_fwd	gtgccacctaacagtgagctg	atgatcctgacgacggagaccgccgtcgtcgacaagccctcactgttaggt	C2D2 (attB fwd)
IDS2_A1_a_attP_rev_Ev oPreQ1_motif	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagttaaattat tccgcggttctatctagttacgcgttaaaccaactagaa	A1B2 (attP rev)
IDS2_A1_a_attP_fwd_Ev oPreQ1_motif	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagttaaaatt atgcgcggttctatctagttacgcgttaaaccaactagaa	A1B2 (attP fwd)
IDS2_A1_c_attP_rev_Ev oPreQ1_motif	gacaccaaaaactgccacagg	taccgtacaccactgagaccgcggtggttgaccagacaaacctgtggcagttttataca tacacgcggttctatctagttacgcgttaaaccaactagaa	A1B3 (attP rev); A1B4 (attP rev)
IDS2_A1_c_attP_fwd_Ev oPreQ1_motif	gacaccaaaaactgccacagg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctgtggcagttttaaaat atttcgcggttctatctagttacgcgttaaaccaactagaa	A1B3 (attP fwd); A1B4 (attP fwd)
IDS2_A4_b_attP_rev_Ev oPreQ1_motif	gcactcatttcctccaagctc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcttggaggaaacata ataacgcggttctatctagttacgcgttaaaccaactagaa	A4B7 (attP rev)
IDS2_A4_b_attP_fwd_Ev oPreQ1_motif	gcactcatttcctccaagctc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcttggaggaaaaca taatacgcggttctatctagttacgcgttaaaccaactagaa	A4B7 (attP fwd)
IDS2_B2_a_attP_rev_Ev oPreQ1_motif	gtaggtacaggacagggcag	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctccctgtccctcatag tcgcggttctatctagttacgcgttaaaccaactagaa	A1B2 (attP rev)
IDS2_B2_a_attP_fwd_Ev oPreQ1_motif	gtaggtacaggacagggcag	taccgtacaccactgagaccgcggtggttgaccagacaaacctccctgtccataaattc cgcggttctatctagttacgcgttaaaccaactagaa	A1B2 (attP fwd)
IDS2_B3_a_attP_rev_Ev oPreQ1_motif	gagataggtaggtacaggaca	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctgtaccttaaaga atcgcggttctatctagttacgcgttaaaccaactagaa	A1B3 (attP rev)
IDS2_B3_a_attP_fwd_Ev oPreQ1_motif	gagataggtaggtacaggaca	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctgtaccttgaaag aacgcggttctatctagttacgcgttaaaccaactagaa	A1B3 (attP fwd)
IDS2_B4_b_attP_rev_Ev oPreQ1_motif	gtgaaaagataggtaggtac	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctcctacctatctaaata atcccgcggttctatctagttacgcgttaaaccaactagaa	A1B4 (attP rev)

IDS2_B4_b_attP_fwd_EvoPreQ1_motif	gtgaaaagataggtaggtac	taccgtacaccactgagaccgcggtggttgaccagacaaacctcctacctatctaataa atgccgcggttctatctagttacgcgttaaaccaactagaa	A1B4 (attP fwd)
IDS2_B7_b_attP_rev_EvoPreQ1_motif	gttatggtttactccatcta	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctatggagtaaacccc ctttgccgcggttctatctagttacgcgttaaaccaactagaa	A4B7 (attP rev)
IDS2_B7_b_attP_fwd_EvoPreQ1_motif	gttatggtttactccatcta	taccgtacaccactgagaccgcggtggttgaccagacaaacctatggagtaaacccc ctttctcgcggttctatctagttacgcgttaaaccaactagaa	A4B7 (attP fwd)
IDS_C2_c_attB_rev_EvoPreQ1_motif	gttttggtttaccctatcta	atgatcctgacgacggagaccgccgtcgtcgacaagccatagggtaaaccacatttaa ccgcggttctatctagttacgcgttaaaccaactagaa	C2D2 (attB rev); C2D2 (attB rev)
IDS_C2_c_attB_fwd_EvoPreQ1_motif	gttttggtttaccctatcta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatagggtaaaccatttaaaga cgcggttctatctagttacgcgttaaaccaactagaa	C2D1 (attB fwd); C2D2 (attB fwd)
IDS_D1_b_attB_rev_EvoPreQ1_motif	gctgtggaactgcaacacact	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgtgttgcagtagaaatttcgc ggttctatctagttacgcgttaaaccaactagaa	C2D1 (attB rev)
IDS_D1_b_attB_fwd_EvoPreQ1_motif	gctgtggaactgcaacacact	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgttgcagtaattaatccg cggttctatctagttacgcgttaaaccaactagaa	C2D1 (attB fwd)
IDS_D2_c_attB_rev_EvoPreQ1_motif	gtgccacctaacagtgagctg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctcactgttaggtattaaattc gcggttctatctagttacgcgttaaaccaactagaa	C2D2 (attB rev)
IDS_D2_c_attB_fwd_EvoPreQ1_motif	gtgccacctaacagtgagctg	atgatcctgacgacggagaccgccgtcgtcgacaagccctcactgttaggtaataaat ccgcggttctatctagttacgcgttaaaccaactagaa	C2D2 (attB fwd)

ED Figure 10a - twinPE mediated *attB* insertion in *CCR5* region 2

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
CCR5_2_C1c	gatgcagagtcagcagaact	atgatcctgacgacggagaccgccgtcgtcgacaagcctctgctgactctg	indicated on the x-axis
CCR5_2_D1c	ggtgcttgatgtcttttcaa	ggcttgtcgacgacggcggtctccgtcgtcaggatcataaaagacatcaagc	indicated on the x-axis
CCR5_2_C2b	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtc	indicated on the x-axis
CCR5_2_D4c	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactgaggg	indicated on the x-axis
CCR5_2_C2c	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtctt	indicated on the x-axis
CCR5_2_D2a	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	indicated on the x-axis
CCR5_2_C3b	ggtttaggtcaagaagaaga	atgatcctgacgacggagaccgccgtcgtcgacaagcctcttcttgaccta	indicated on the x-axis
CCR5_2_D2b	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggctgt	indicated on the x-axis
CCR5_2_D4a	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactg	indicated on the x-axis
CCR5_2_C5c	cacagtctcacccagactcc	atgatcctgacgacggagaccgccgtcgtcgacaagccgtctgggtgagac	indicated on the x-axis
CCR5_2_D3b	gtatttcagctgggatggga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatcatcccagct	indicated on the x-axis
CCR5_2_D4b	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactgag	indicated on the x-axis
CCR5_2_C6c	gctagatttatgaatacacg	atgatcctgacgacggagaccgccgtcgtcgacaagccgtattcataaatct	indicated on the x-axis
CCR5_2_D5c	gaacccatgaaatgactact	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtcatttcatggg	indicated on the x-axis
CCR5_2_C7c	gcacacatgagatctaggtg	atgatcctgacgacggagaccgccgtcgtcgacaagccctagatctcatgt	indicated on the x-axis
CCR5_2_D6b	gatgtgctaaatgctgcctg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgcagcatttag	indicated on the x-axis

ED Figure 10b - twinPE in multiple human cells

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
IDS2_A4_b_attP_rev	gcactcatttcctccaagctc	taccgtacaccactgagaccgcggtggttgaccagacaaacctcttggaggaaa	IDS2 (attP rev)
IDS2_B7_b_attP_rev	gttatggtttactccatcta	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaacctatggagtaaacct	IDS2 (attP rev)
IDS_C2_c_attB_fwd	gttttggtttaccctatcta	ggcttgtcgacgacggcggtctccgtcgtcaggatcatataggtaaacca	IDS (attB fwd)
IDS_D2_c_attB_fwd	gtgccacctaacagtgagctg	atgatcctgacgacggagaccgccgtcgtcgacaagccctcactgttaggt	IDS (attB fwd)
MYC_pegRNA_F1_22nt-insert	gaggctattctgcccatttg	aaatgatggtgatgatggtgttatgggcaga	MYC (22-nt)
MYC_pegRNA_R1_22nt-insert	gctttaccccgatccagttc	aacaccatcatcaccatcatttctggatcggggt	MYC (22-nt)
TIMM44_pegRNA_F1_22nt-insert	gctggccagcctttctccag	ttcagagaagagatggaagacagagaaaggctgg	TIMM44 (22-nt)
TIMM44_pegRNA_R1_22nt-insert	gtcctgtcctggggccatcg	tgtcttccatctcttctctgaatggccccag	TIMM44 (22-nt)
CCR5_2_C5c	cacagtctcacccagactcc	atgatcctgacgacggagaccgccgtcgtcgacaagccgtctgggtgagac	CCR5_a (attB fwd)
CCR5_2_D3b	gtatttcagctgggatggga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatcatcccagct	CCR5_a (attB fwd)
CCR5_2_C2c	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtctt	CCR5_b (attB fwd)
CCR5_2_D2a	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	CCR5_b (attB fwd)

ED figure 10c – Editing activity of Cas9 nickase, PE2-dead RT variant, and PE2

pegRNA	spacer sequence	3' extension	Edits made by the specified pegRNA
PAH_E7.2_55_EvoPreQ1	gtggtttccgcctccgacctg	acgcggaatgcgagaccccccagaaagtctctactgctcaagagcccggcaacggg tcggaggcgtctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	Also used in Fig. 2c
PAH_E7.6_56_EvoPreQ1	gtctgatgtactgtgtgcag	gggctcttgagcagtagagactttctgggggtctcgcattccgcgtgtttcattgcaca cagtacatctctctcttgacgcggttctatctagttacgcgttaaaccaactagaaa	Also used in Fig. 2c
AAVS1_A3835a	gacgtcacggcgctgcccca	taccgtacaccactgagaccgcggtggttgaccagacaaacctggcagcgcc	Also used in ED Fig. 3
AAVS1_B3930a	ggacttcccagtgtgcatcg	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttgcacactg	Also used in ED Fig. 3
CCR5_2_C2_c_attP fwd_pegRNA1	gaaaagacatcaagcacaga	aggtttgtaccgtacaccactgagaccgcggtggttgaccagacaaacctgtgcttgat gtctt	
CCR5_2_D2_a_attP rev_pegRNA2	gtgaaagacagcctggagtc	aggtttgtctggtcaaccaccgcggtctcagtggtgtacggtacaaaccttccaggct	
PE3_CCR5_1_F414a_34_FKBP108	ggtacctatcgattgtcagg	tactgtccccttctgggctcactatgctgccgccaaatttctttccatcttcaagcatcccg gtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggg gagatggtttccacctgcactccgacaatcga	Also used in ED Fig. 1a

PE3_CCR5_1_F414a_29_FKBP108	ggtacctatcgattgtcagg	tccccttctgggctcactatgctgccgccaaatttctttccatcttcaagcatcccggtgtagtgcaccacgcaggtctggccgcgcttggggaaggtgcgcccgtctcctggggagatggtttccacctgcactccgacaatcga	Also used in ED Fig. 1a
IDS2_DF_A4_b_attP_fwd	gcactcatttcctccaagctc	gtctggtcaaccaccgcggtctcagtggtgtacggtacaaaacctcttggaggaaa	Also used in Fig. 4b
IDS2_DF_B7_b_attP_fwd	gttatggtttactccatcta	taccgtacaccactgagaccgcggtggttgaccagacaaacctatggagtaaacc	Also used in Fig. 4b

Supplementary Note 1 - TwinPE PCR bias assessment

pegRNA	**spacer sequence**	**3' extension**	**Edits made by the specified pegRNA**
CCR5_A223c	gtcatcctgataaactgcaaa	atgatcctgacgacggagaccgccgtcgtcgacaagccgcagtttatcagg	A223c+B272a; A223c+B291b; A223c+B305a; A223c+B326b; A223c+B330b
CCR5_B272a	gaaggaaaaacaggtcagaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatctgacctg	A223c+B272a
CCR5_B291b	gcccagaaggggacagtaaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcattactgtcccct	A223c+B291b
CCR5_B305a	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcact	A223c+B305a
CCR5_B326b	gatttccaaagtcccactggg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatagtgggactttg	A223c+B326b; A260c+B326b
CCR5_B330b	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaa	A223c+B330b
CCR5_A260c	gtgacatctacctgctcaacc	atgatcctgacgacggagaccgccgtcgtcgacaagcctgagcaggtagat	A260c+B305c; A260c+B326b; A260c+B330c
CCR5_B305c	ggcagcatagtgagcccaga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgggctcactatgc	A260c+B305c
CCR5_B330c	gttgtatttccaaagtcccac	ggcttgtcgacgacggcggtctccgtcgtcaggatcatggactttggaaat	A260+B330c
CCR5_A325b	gctcactatgctgccgcccag	atgatcctgacgacggagaccgccgtcgtcgacaagccggcggcagcat	A325b+B414a
CCR5_B414a	ggtacctatcgattgtcagg	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgacaatcga	A325b+B414a; A360b+B414a
CCR5_A360b	gacaatgtgtcaactcttgac	atgatcctgacgacggagaccgccgtcgtcgacaagccaagagttgacac	A360b+B414a
CCR5_A506c	gacaagtgtgatcacttggg	atgatcctgacgacggagaccgccgtcgtcgacaagccaagtgatcacactt	A506c+B584a
CCR5_B584a	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcatt	A506c+B584a
CCR5_A509a	gaagtgtgatcacttgggtgg	atgatcctgacgacggagaccgccgtcgtcgacaagcccccaagtga	A509a+B535c; A509a+B584a
CCR5_B535c	gatctggtaaagatgattcc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatatcatctttaccag	A509_B535c
CCR5_A531c	gctgtgtttgcgtctctccc	atgatcctgacgacggagaccgccgtcgtcgacaagccagagacgcaaaca	A531c+B584b
CCR5_B584b	gtatggaaaatgagagctgc	ggcttgtcgacgacggcggtctccgtcgtcaggatcatgctctcattttc	A531c+B584b
CCR5_2_C2b	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtc	C2b+D4c
CCR5_2_D4c	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactgaggg	C2b+D4c
CCR5_2_C2c	gaaaagacatcaagcacaga	atgatcctgacgacggagaccgccgtcgtcgacaagccgtgcttgatgtctt	C2c+D2a
CCR5_2_D2a	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	C2c+D2a
CCR5_2_C3b	ggtttaggtcaagaagaaga	atgatcctgacgacggagaccgccgtcgtcgacaagcctcttcttgaccta	C3b+D2b; C3b+D4a
CCR5_2_D2b	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggctgt	C3b+D2b
CCR5_2_D4a	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactg	C3b+D4a
CCR5_2_C4b	gatggattggtgtaaaagga	atgatcctgacgacggagaccgccgtcgtcgacaagccttttacaccaatc	C4b+D2a
CCR5_2_D2a	gtgaaagacagcctggagtc	ggcttgtcgacgacggcggtctccgtcgtcaggatcattccaggct	C4b+D2a
CCR5_2_C5c	cacagtctcacccagactcc	atgatcctgacgacggagaccgccgtcgtcgacaagccgtctgggtgagac	C5c+D3b; C5c+D4b
CCR5_2_D3b	gtatttcagctgggatggga	ggcttgtcgacgacggcggtctccgtcgtcaggatcatcatcccagct	C5c+D3b
CCR5_2_D4b	gacccctcagtatttcagct	ggcttgtcgacgacggcggtctccgtcgtcaggatcattgaaatactgag	C5c+D4b
HEK3_DF_A_SA_del77nt	ggcccagactgagcacgtga	tcctctgccatcacgtgctcagtctg	SA (Δ 77nt)
HEK3_DF_B_SA_del77nt	gtcaaccagtatcccggtgc	tgatggcagaggaccgggatactgg	SA (Δ 77nt)
HEK3_DF_A_SA_del56nt	ggcccagactgagcacgtga	tggaggaagcagggcttcctttcctctgccatcacgtgctcagtctg	SA (Δ 56nt)
HEK3_DF_B_SA_del56nt	gtcaaccagtatcccggtgc	tgatggcagaggaaaggaagccctgcttcctccaccgggatactgg	SA (Δ 56nt)
HEK3_DF_A_HA_del64nt	ggcccagactgagcacgtga	tgcaggagctgcatcctctgccatcacgtgctcagtctg	HA (Δ 64nt)
HEK3_DF_B_HA_del64nt	gtcaaccagtatcccggtgc	tgatggcagaggatgcagctcctgcaccgggatactgg	HA (Δ 64nt)
HEK3_DF_A_PD_del90nt	ggcccagactgagcacgtga	gcccagccaaacttgtcaaccagtatcccggcgtgctcagtctg	PD (Δ 90nt)
HEK3_DF_B_PD_del90nt	gtcaaccagtatcccggtgc	gggtcaatccttggggcccagactgagcacgccgggatactgg	PD (Δ 90nt)

Supplementary Table 2. Sequences of primers used for mammalian cell genomic DNA amplification and HTS

Primers (All sequences are shown in 5' to 3' orientation)	
Figure 1	
HEK3_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
HEK3_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCCAAACTTGTCAACC
Figure 2	
PAH_AVA1686	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCATCACCATTGGCTGGGAT
PAH_AVA1687	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGAGGAGAGGCACTGAA
PAH_AVA1696	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACCTAAAGGTCTCCTAGTGCCT
PAH_AVA1697	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCAATGAACCCAAACCTC
HEK3_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
HEK3_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCCAAACTTGTCAACC
DMD_UMI_fwd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNTGCTGGCCAGTTTACTAACAAT
DMD_UMI_fwd2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNCAGAAAGAAGATCTTATCCCATC TTG
DMD_rev0	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTACTTTTGTTATTTGCATT
Figure 3	
AAVS1_AVA1713	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAGCAGGGTCCCGCTTC
AAVS1_AVA1717	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGGGGCTCAGTCTGAAGAG
AAVS1_AVA1651	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCCAAGGACTCAAACCCAGA
AAVS1_AVA1652	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCGTGCGTCAGTTTTACCT
AAVS1_AVA1653	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAACTGCTTCTCCTCTTGGGAA
AAVS1_AVA1715	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTGCCAGAACCTCTAAGGT
AAVS1_AVA1655	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATCCTCTCTGGCTCCATCGTA
AAVS1_AVA1656	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCACTTCAGGACAGCATGTTT
AAVS1_AVA1707	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGCCGGGAACTGCCGCTGGC
AAVS1_AVA1710	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGAGGCCCTCATCTGGCG
CCR5_AVA1678	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATCAATGTGAAGCAAATCGCAGC
CCR5_AVA1679	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCGATTGTCAGGAGGATGATGAA
CCR5_AVA1680	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTTCTTACTGTCCCCTTCTGGGC
CCR5_AVA1681	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACACAGCATGGACGAC
CCR5_AVA1682	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACAATCGATAGGTACCTGGCTGTC
CCR5_AVA1683	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGCCCCAAGATGACTAT
ALB_AVA1760	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGCATTTATTTCTAAAATGGCATA
ALB_AVA1759	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTATCAACAGCAACCAAGAAGACAGACT
Figure 4	
IDS_AVA1763	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGAAAACCTGAGCTTGGAGG
IDS_AVA1764	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTACTCCAGCTTAATGGAAGTGG
IDS_AVA1765	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAAGATGTGGAAATGCCTCAC
IDS_AVA1766	TGGAGTTCAGACGTGTGCTCTTCCGATCTAATCAACATGAAGGGTTGTGTTGT
IDS_AVA1769	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTTCCCACACATGCGTTCCTC
IDS_AVA1770	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATGAAGGGTTGTTTTTAATTGA
IDS_UMI_junc1_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNCCCTGTCCTGTACCTACCTAT
IDS_junc2_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGACTCATGCCCTACGAGG
IDS_universal_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAAATTTACCCGTGGCAGC
ED Figure 1 - Long tPE insertions at CCR5	
CCR5_AVA1682	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACAATCGATAGGTACCTGGCTGTC
CCR5_AVA1683	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGCCCCAAGATGACTAT
ED Figure 2 - pegRNAs screen at PAH	
PAH_AVA1684	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGTCCATGGAGGTTTAACAGGA
PAH_AVA1685	TGGAGTTCAGACGTGTGCTCTTCCGATCTACATGGAAGTTTGCTACGACAT
PAH_AVA1686	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCATCACCATTGGCTGGGAT
PAH_AVA1687	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGAGGAGAGGCACTGAA
PAH_AVA1689	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAAGAGGAAGGGAGGGGA
PAH_AVA1690	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAGCCCCCATTCAAAGCA
PAH_AVA1691	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGGACTCTTCATGCTGGT
PAH_AVA1696	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACCTAAAGGTCTCCTAGTGCCT
PAH_AVA1697	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCAATGAACCCAAACCTC
PAH_AVA1702	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGCCAAGTACTAGGTTGGTTCT
PAH_AVA1703	TGGAGTTCAGACGTGTGCTCTTCCGATCTTAACCTGGCTTCCAGGGGAGT
PAH_exon10_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGACACACCCCAAAATAATGC
PAH_exon10_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTGAAAGCACAATAATGGTTTT
PAH_exon11_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGGGAATACTGATCCTGAT
PAH_exon11_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCAACCACCCACAGATGAGT
PAH_exon12_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGGTGTCCGTGTTCCTAA
PAH_exon12_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCGATGGTAGGGAAAGACAGT
ED Figure 3 - pegRNA screen at CCR5 region 1	
CCR5_AVA1678	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATCAATGTGAAGCAAATCGCAGC

CCR5_AVA1679	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCGATTGTCAGGAGGATGATGAA
CCR5_AVA1680	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTTCTTACTGTCCCCTTCTGGGC
CCR5_AVA1681	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACACAGCATGGACGAC
CCR5_AVA1682	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACAATCGATAGGTACCTGGCTGTC
CCR5_AVA1683	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGCCCCAAGATGACTAT

ED Figure 4 - pegRNA screen at AAVS1

AAVS1_AVA1713	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAGCAGGGTCCCGCTTC
AAVS1_AVA1717	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGGGGCTCAGTCTGAAGAG
AAVS1_AVA1651	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCCAAGGACTCAAACCCAGA
AAVS1_AVA1652	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCGTGCGTCAGTTTTACCT
AAVS1_AVA1653	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAACTGCTTCTCCTCTTGGGAA
AAVS1_AVA1715	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTGCCAGAACCTCTAAGGT
AAVS1_AVA1655	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATCCTCTCTGGCTCCATCGTA
AAVS1_AVA1656	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCACTTCAGGACAGCATGTTT
AAVS1_AVA1707	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGCCGGGAACTGCCGCTGGC
AAVS1_AVA1710	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGAGGCCCTCATCTGGCG

ED Figure 5 - Comparison of twinPE- and PE3-mediated attB insertion at CCR5 region 1

CCR5_AVA1680	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTTCTTACTGTCCCCTTCTGGGC
CCR5_AVA1681	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACACAGCATGGACGAC
CCR5-2_fwd2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGGAGTCAGAGAGAATCCC
CCR5-2_rev2	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTCCTAGACCTCATACCTCGT

ED Figure 6b - HTS Junction Purity

CCR5_AVA1680	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTTCTTACTGTCCCCTTCTGGGC
CCR5_AVA1682	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACAATCGATAGGTACCTGGCTGTC
AAVS1_AVA1717	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGGGGCTCAGTCTGAAGAG
AAVS1_AVA1707	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCGCCGGGAACTGCCGCTGGC
Donor_CJP140	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAACTTCAGGGTCAGCTTGC

ED Figure 6c - HTS Junction Purity (the other donor-genome junction)

CCR5_AVA1681	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACACAGCATGGACGAC
CCR5_AVA1683	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGCCCCAAGATGACTAT
AAVS1_AVA1713	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAGCAGGGTCCCGCTTC
AAVS1_AVA1710	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGAGGCCCTCATCTGGCG
Donor_other_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGCAAGCTTACATCGAGATCC

ED Figure 6e - Overlap reduction

CCR5_AVA1682	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACAATCGATAGGTACCTGGCTGTC
CCR5_AVA1683	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGCCCCAAGATGACTAT

ED Figure 7b

OT1_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGAAATAAGTTATCACAATGGGAAAT
OT1_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCGCGATTCTTAAAAGGAGAGG
OT2_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCATTCATATTTTGAAACAAAAGG
OT2_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCATTGCACTCCTACATACAACA
OT3_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTGTGGTTATTCCCAGCTC
OT3_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGGAACACTGGACAAAATCC
OT4_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGAAAGCTTTGACAAGTGGAA
OT4_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTACTTGCCCTTCTTCCT
OT5_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCATTGCACTCCTACATACAACA
OT5_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTCATATTTTGAAACAAAAGG

ED Figure 7c

Donor_CJP140 (with OT1_fwd or OT2_fwd)	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAACTTCAGGGTCAGCTTGC
Donor_fwd_primer (with OT3_rev, or OT4_rev, or OT5_rev)	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAACTTCAGGGTCAGCTTGC

ED Figure 9

IDS_AVA1763	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGAAAACCTGAGCTTGGAGG
IDS_AVA1764	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTACTCCAGCTTAATGGAAGTGG
IDS_AVA1765	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAAGATGTGGAAATGCCTCAC
IDS_AVA1766	TGGAGTTCAGACGTGTGCTCTTCCGATCTAATCAACATGAAGGGTTGTGTTGT
IDS_AVA1769	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTTCCCACACATGCGTTCCTC
IDS_AVA1770	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATGAAGGGTTGTTTTTAATTGA

ED Figure 10

CCR5-2_Fwd1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTATTCGTGCAGCATATGAG
CCR5-2_Rev1	TGGAGTTCAGACGTGTGCTCTTCCGATCTTATTTCAGCTGGGATGGGAAGG
CCR5-2_fwd2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGGAGTCAGAGAGAATCCC
CCR5-2_rev2	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTCCTAGACCTCATACCTCGT
CCR5-2_Fwd3	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACTGAATGCTTCTGACTTCATAG
CCR5-2_Rev3	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTTGCTCAATGCTTTGCTCAGT
IDS_AVA1765	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAAGATGTGGAAATGCCTCAC
IDS_AVA1766	TGGAGTTCAGACGTGTGCTCTTCCGATCTAATCAACATGAAGGGTTGTGTTGT

IDS_AVA1769	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTTCCCACACATGCGTTCCTC
IDS_AVA1770	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATGAAGGGTTGTTTTTAATTGA
CCR5-2_fwd2	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGGAGTCAGAGAGAATCCC
CCR5-2_rev2	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTCCTAGACCTCATACCTCGT
MYC_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGCACTGGAACTTACAACAC
MYC_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGAAATCTCGAAAGG
TIMM44_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGACCTGTACATTCGGGC
TIMM44_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTAAAAGCCCAGTGCTGCTC

Supplementary Table 5 - TwinPE-mediated off-target genome editing

HEK3_OT1_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCCTGTTGACCTGGAGAA
HEK3_OT1_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTACTTGCCCTGACCA
HEK3_OT2_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGTGTTGACAGGGAGCAA
HEK3_OT2_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGATGTGGGCAGAAGGG
HEK3_OT3_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGAGGGAACAGAAGGGCT
HEK3_OT3_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCAAAGGCCCAAGAACCT
HEK3_OT4_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTAGCACTTTGGAAGGTCG
HEK3_OT4_rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCATCTTAATCTGCTCAGCC

Supplementary Note 1 - Analysis of editing quantification bias

UMI_HEK3_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNATGTGGGCTGCCTAGAAAGG
UMI_CCR5-2_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNAGAGGAGTCAGAGAGAATCCC
UMI_AVA1678_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNAATCAATGTGAAGCAAATCGCAG C
UMI_AVA1680_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNTCCTTCTTACTGTCCCCTTCTGGG C
UMI_AVA1682_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNACAATCGATAGGTACCTGGCTGT C
5p constant primer	ACACTCTTTCCCTACACG

Supplementary Table 3. Sequences of primers and probes used for ddPCR assays

Fig 3d

Assay	fwd	rev	Probe	Samples
CCR5_B6	catctctgacctgtttttcc	tcctcgcccttgctcac	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	325/414
CCR5_B8_1	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	507/584, 510/584, 532/584
AAVS1_1077	ggaacggggctcagtct	tcctcgcccttgctcac	/56-FAM/ACCACCGCG/ZEN/GTCTCCGTCGT/3IABkFQ/	1077/1154
AAVS1_3786	ggcaagcttacatcgagatcc	gaggaggccctcatctggcg	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	3786/3903, 3786/3930
ACTB	acactgtgcccatctac	aatgtcacgcacgatttc	/5HEX/CGGGACCTG/ZEN/ACTGACTACCTCAT/3IABkFQ/	all

Fig 3e

Assay	fwd	rev	Probe	Samples
CCR5_B8_2	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/CTCAGTGGT/ZEN/GTACGGTACAAACCC/3IABkFQ/	all
ACTB	acactgtgcccatctac	aatgtcacgcacgatttc	/5HEX/CGGGACCTG/ZEN/ACTGACTACCTCAT/3IABkFQ/	all

Fig 3g

Assay	fwd	rev	Probe	Samples
CCR5_B8_1	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	CCR5 samples
ALB	gtgactgtaattttcttttgcg	tcctcgcccttgctcac	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	ALB samples
ACTB	acactgtgcccatctac	aatgtcacgcacgatttc	/5HEX/CGGGACCTG/ZEN/ACTGACTACCTCAT/3IABkFQ/	all

ED Fig 6a

Assay	fwd	rev	Probe	Samples
CCR5_B8_2	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/CTCAGTGGT/ZEN/GTACGGTACAAACCC/3IABkFQ/	all
ACTB	acactgtgcccatctac	aatgtcacgcacgatttc	/5HEX/CGGGACCTG/ZEN/ACTGACTACCTCAT/3IABkFQ/	all

ED Fig 6c

Assay	fwd	rev	Probe	Samples
AAVS1_1077	ggaacggggctcagtct	tcctcgcccttgctcac	/56-FAM/ACCACCGCG/ZEN/GTCTCCGTCGT/3IABkFQ/	all
CCR5_B8_1	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/ACGACGGCG/ZEN/GTCTCAGTGGTG/3IABkFQ/	attB
CCR5_B8_2	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/CTCAGTGGT/ZEN/GTACGGTACAAACCC/3IABkFQ/	attB-GA
CCR5_B8_3	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/ACCACCGCG/ZEN/GTCTCCGTCGT/3IABkFQ/	attP
CCR5_B8_4	gccaggacggtcaccttg	tcctcgcccttgctcac	/56-FAM/CTCCGTCGT/ZEN/CAGGATCATCCGT/3IABkFQ/	attP-GA
ACTB	acactgtgcccatctac	aatgtcacgcacgatttc	/5HEX/CGGGACCTG/ZEN/ACTGACTACCTCAT/3IABkFQ/	all

Supplementary Table 4. Sequence of recoded exonic *PAH* sequences

Nucleotides labeled in red indicate positions where silent mutations were introduced.

Spacer 1	Spacer 2	Recoded allele product
2.1	2.2	GAAGACAACTGCAATCAGAACGGCGCTATCTCTCTGATCTTCAGCCTGAAGGAAGAGGTGGGCGCCCTGGC GAAGGTATTGCGCTTATTTGAG
4.1	4.3	TTCTCTGTGTTTCAGTGCCGTGGTTTCCACGGACAATTCAGGAACTTGATAGGTTCGCTAATCAAATCTTGT CCTACGGAGCCGAACTTGACGCTGATCATCCTGTGAGTCCATGGCCCG
4.1	4.4	TTCTCTGTGTTTCAGTGCCGTGGTTTCCACGGACAATTCAGGAACTTGATAGGTTCGCTAATCAAATCTTGT CCTACGGAGCCGAACTTGACGCTGATCATCCTGTGAGTCCATGGCCCGTAG
4.2	4.3	CCCAAGAACCATTCAAGAACTTGATAGGTTCGCTAATCAAATCTTGTCCTACGGAGCCGAACTTGACGCTG ATCATCCTGTGAGTCCATGGCCCG
4.2	4.4	CCCAAGAACCATTCAAGAACTTGATAGGTTCGCTAATCAAATCTTGTCCTACGGAGCCGAACTTGACGCTG ATCATCCTGTGAGTCCATGGCCCGTAG
4.2	4.5	CCCAAGAACCATTCAAGAACTTGATAGGTTCGCTAATCAAATCTTGTCCTACGGAGCCGAACTTGACGCTG ATCATCCTGTGAGTCCATGGCCCGTAGGATGAGATTT
5.1	5.2	CAGGTGTCTCTTTTCTCCTAGGGCTTCAAGGACCCCGTTTATCGCGCCCGCCGTAAGCAATTCGCCGATATT GCATATAATTATCGCCAGTAAGTCTGCCTTGCTT
7.1	7.5	CTTTTCATCCCAGCTTGTACTGGCTTTCGACTCCGCCCCGTTGCCGGGCTCTTGAGCAGTAGAGACTTTCTGG GGGGTCTCGCCTTCCGAGTCTTCCACT
7.1	7.6	CTTTTCATCCCAGCTTGTACTGGCTTTCGACTCCGCCCCGTTGCCGGGCTCTTGAGCAGTAGAGACTTTCTGG GGGGTCTCGCATTCCGCGTGTTTCATTGCACACAGTACATCAGAC
7.2	7.5	TGGTTTCCGCCTCCGACCCGTTGCCGGGCTCTTGAGCAGTAGAGACTTTCTGGGGGGTCTCGCCTTCCGAGT CTTCCACT
7.2	7.6	TGGTTTCCGCCTCCGACCCGTTGCCGGGCTCTTGAGCAGTAGAGACTTTCTGGGGGGTCTCGCATTCCGCGT GTTTCATTGCACACAGTACATCAGAC
7.2	7.7	TGGTTTCCGCCTCCGACCCGTTGCCGGGCTCTTGAGCAGTAGAGACTTTCTGGGGGGTCTCGCATTCCGCGT GTTTCATTGTACCCAGTATATTAGGCATGGTTCAAAACCGATGTACACACCAGAA
7.3	7.6	GGCTGGCCTGCTTTCCTGTAGAGACTTTCTGGGGGGTCTCGCATTCCGCGTGTTTCATTGCACACAGTACAT CAGAC
7.3	7.7	GGCTGGCCTGCTTTCCTGTAGAGACTTTCTGGGGGGTCTCGCATTCCGCGTGTTTCATTGTACCCAGTATATT AGGCATGGTTCAAAACCGATGTACACACCAGAA
7.3	7.8	GGCTGGCCTGCTTTCCTGTAGAGACTTTCTGGGGGGTCTCGCATTCCGCGTGTTTCATTGTACCCAGTATATT AGGCATGGTTCAAAACCGATGTACACACCAGAACCGTGAGTACTGTCC
9.1	9.2	TTCCCCCAATTACAGGAGATCGGTCTGGCAAGCCTGGGAGCACCAGACGAGTATATAGAGAAACTTGCTAC AGTAAGTCCCTTCTCTCCC
9.1	9.3	TTCCCCCAATTACAGGAGATCGGTCTGGCAAGCCTGGGAGCACCAGACGAGTATATAGAGAAACTTGCTAC AGTAAGTCCCTTCTCTCCCTGGGTGGATGGT
10.2	10.3	CCAGATTTACTGGTTTACCGTCGAATTCGGATTGTGTAAGCAGGGTGATAGCATTAAAGCCTACGGAGCAG GTTTGCTCTCATCCTTTGGTGAATTAC
11.1	11.4	GAAGCCAAAGCTTCTCCCACTCGAACTCGAAAAGACTGCAATTCAGAACTATACAGTGACAGAATTTCAAC CATTGTATTACGTGGCAGAGAGTT
11.2	11.4	AAAGCTTCTCCCCCTGGAACTCGAAAAGACTGCAATTCAGAACTATACAGTGACAGAATTTCAACCATTGT ATTACGTGGCAGAGAGTT
12.2	12.4	ACTTTGCTGCCACAATACCAAGACCATTTAGCGTGAGATATGATCCTTATACACAGCGCATCGAAGTGCTC GATAACACTCAACAGCTTAAGATTTTGGCTG
12.3	12.5	GCTACGACCCATACACCCAGCGCATCGAAGTGCTCGATAACACTCAACAATTGAAGATCCTCGCAGACAGT ATCAACAGTAAGTAATTTACAC

Supplementary Table 5. TwinPE-mediated off-target genome editing.

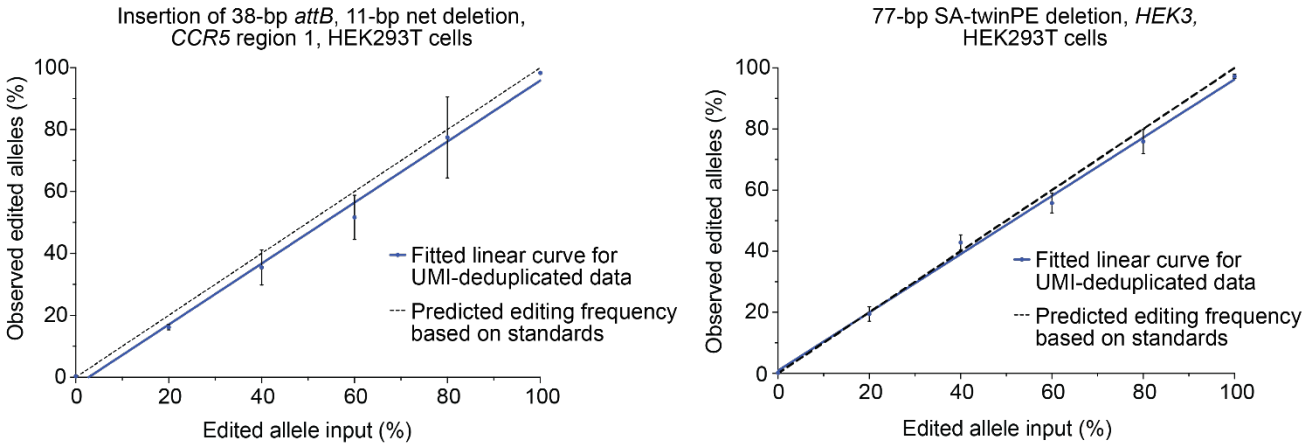
twinPE-mediated % editing at *HEK3* on-target site and off-target sites

HEK3		**standerd pegRNAs**				**enhanced pegRNAs**			
	Untreated	SA (Δ77 nt)	SA (Δ56 nt)	HA (Δ64 nt)	PD (Δ90 nt)	SA (Δ77 nt)	SA (Δ56 nt)	HA (Δ64 nt)	PD (Δ90 nt)
on-target site	<0.1	14.8	18.8	11.7	40.4	29.6	28.5	29.5	59.5
off-target site 1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
off-target site 2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
off-target site 3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
off-target site 4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Supplementary Note 1. Analysis of editing quantification

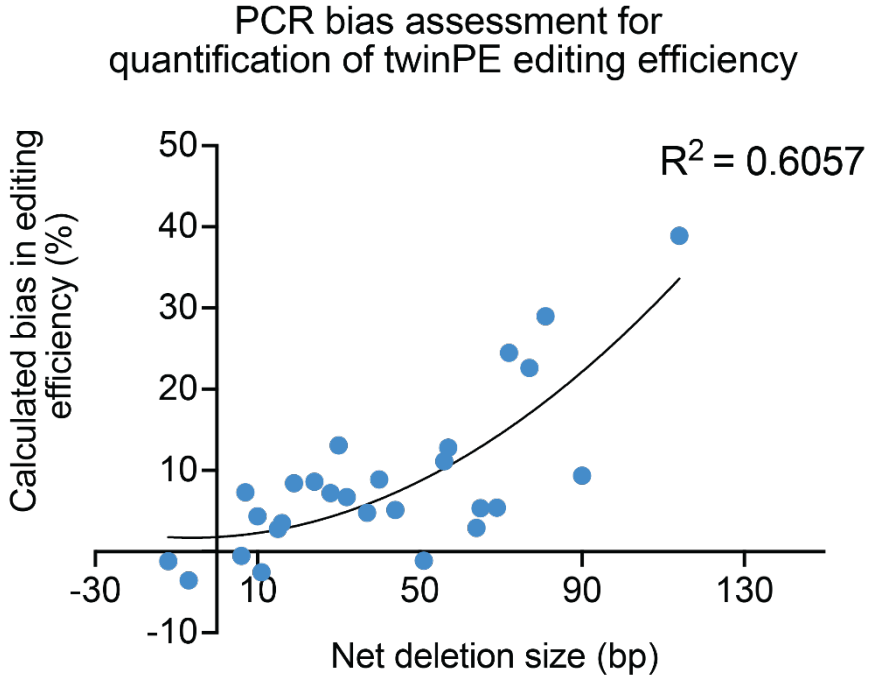
To assess quantification bias, we applied unique molecular identifiers (UMIs) to index individual allele copies using linear amplification followed by bead-based purification, PCR amplification, and Illumina MiSeq amplicon sequencing (see method section for details) similar to the method used by Choi, et al[1] and Bolukbasi, et al[2]. Reads containing identical UMIs are first aligned and then collapsed (deduplicated) to a single consensus sequence. This workflow allows for quantification of allele frequencies based on UMI counts instead of total read counts, which may be subject to PCR amplification bias based on sequence composition or amplicon size differences between different allele products.

To ensure the UMI-sequencing protocol can faithfully quantify the editing efficiency, we mixed chemically synthesized gene fragments (IDT) replicating either an edited or wild-type allele for the given edits (insertion of *attB* at *CCR5* or 77-nt deletion at *HEK3*) to simulate 0%, 20%, 40%, 60%, 80%, and 100% editing efficiencies before diluting the mixed standards to 2000 genetic copies/μL, which is the estimated allele copy number amplified from our cell editing experiments. Diluted standards were then subjected to UMI barcoding, MiSeq analysis, and deduplication. We plotted the percentage of observed edited alleles from three independent biological replicates after sequencing and UMI deduplication against the expected edited allele percentage based on the input allele fraction.



Next, we prepared libraries of twinPE-edited samples from *CCR5* region 1 (15 samples), *CCR5* region 2 (7 samples), *HEK3* (4 samples), and *IDS* (1 sample). By comparing the editing efficiency calculated from total reads and the editing efficiency calculated from reads deduplicated based on UMIs, we estimated the bias in editing efficiency quantification

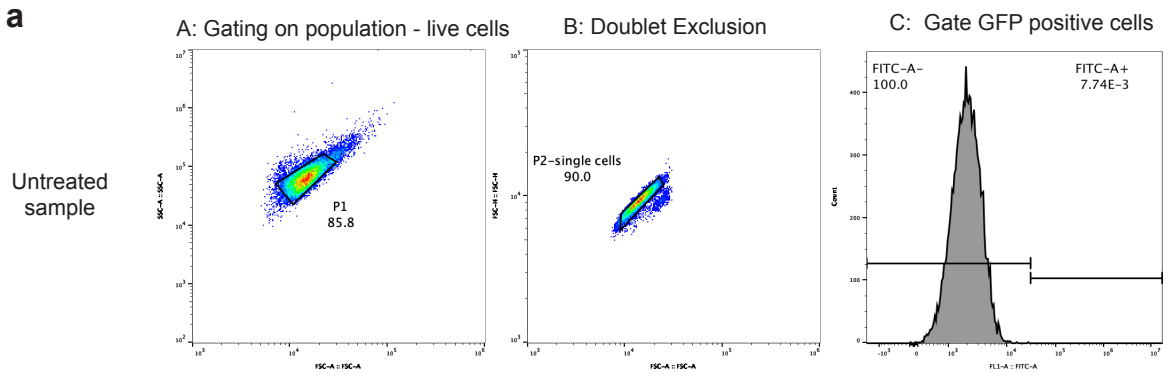
using the linear amplification with UMIs as the “true” editing efficiency. When plotting quantification bias vs. absolute size difference between the starting and edited alleles (net deletion size), we observed a positive correlation between bias and twinPE-induced deletion size. Although bias in this workflow could still exist at the linear amplification stage due to differences in sequence composition at the target DNA locus, this bias does not propagate over the course of repeated PCR cycles and should therefore more accurately represent the true editing efficiency in twinPE editing experiments analyzed by amplicon sequencing.

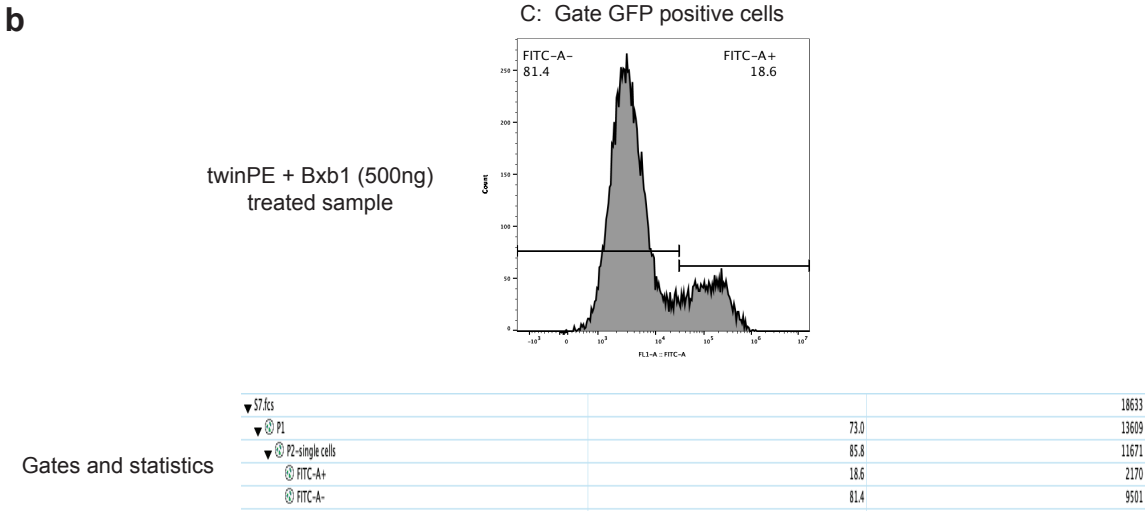


Each point in the above graph represents the mean value of bias calculated from three independent biological replicates. The percentage of bias in editing efficiency was defined by the following equation: 100 x (A – B) ÷ B, where “A” represents the editing efficiency calculated from total read counts without UMI-based deduplication, and “B” represents the editing efficiency calculated after UMI-based deduplication. For example, if the non-deduplicated editing efficiency is 60% and the deduplicated editing efficiency is 46%, the calculated bias is 30%, i.e., the deduplicated editing efficiency is 1.3-fold lower than the non-deduplicated editing efficiency. The bias in editing efficiency is plotted above as a function of the net deletion between the starting and edited alleles, which shows a positive correlation (a second-order polynomial trend line, $R^2 = 0.6057$).

Supplementary Note 2. Representative plot of FACS gating for GFP reporter assay

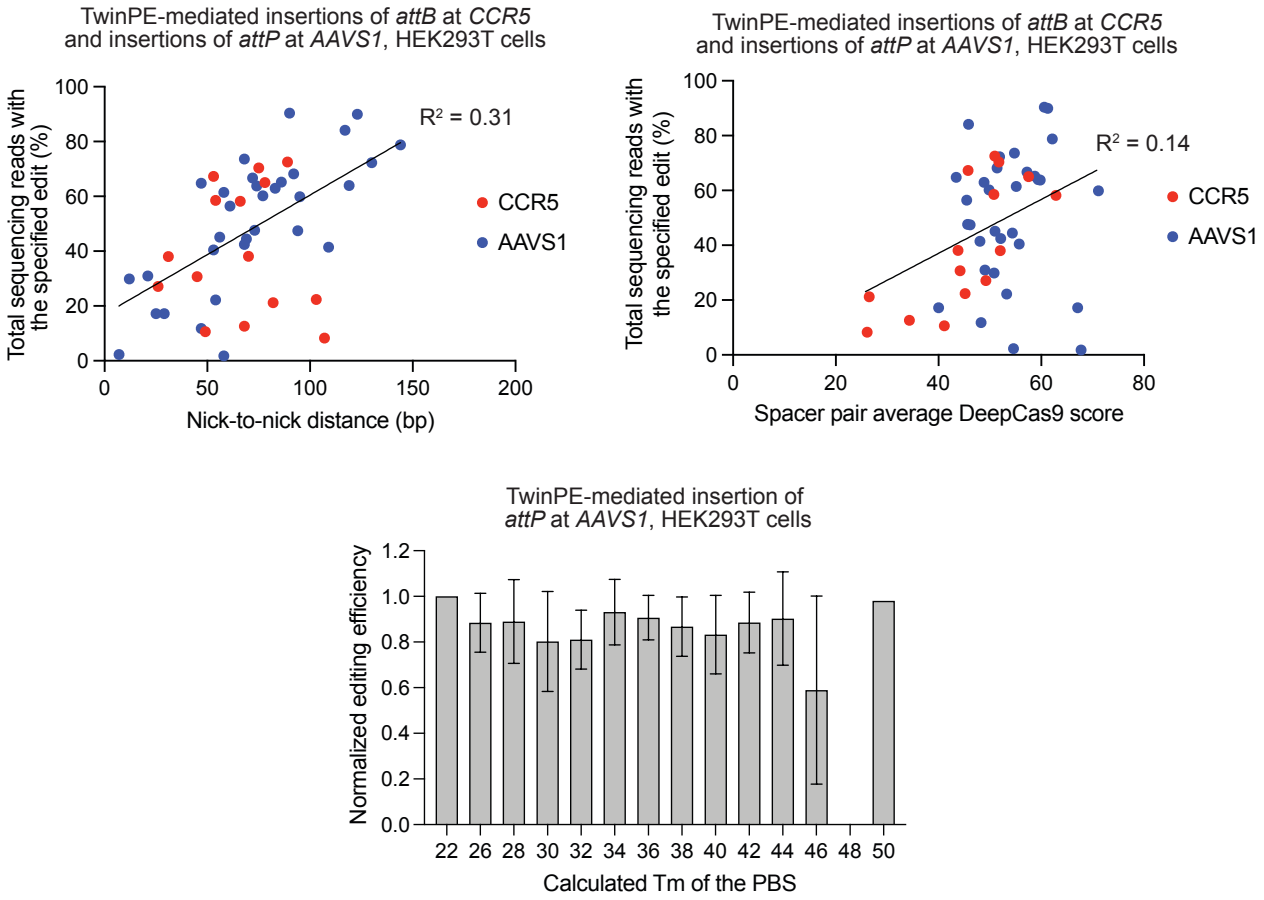
25,000 HEK293T stable GFP reporter cells were seeded into 48-well poly-D-lysine coated plates (Corning). 16-24 h post-seeding, cells were transfected with 1uL of Lipofectamine 2000 (Thermo Fisher Scientific) using the protocol described in the methods section and 750 ng of PE2, 250 ng AAVS1 targeting pegRNAs (62.5 ng each), and Bxb1 plasmid DNA (100 ng, 200 ng, 500 ng, or 1000 ng). The untreated and twinPE+Bxb1 treated cells were cultured for 72 hours, and then collected for flow cytometry analysis. HEK293T stable GFP reporter cells were first gated (Gate A) based on forward (FSC-A) and side scattering (SSC-A) to remove dead cells and other debris. A second gate (Gate B) was used to select singlets based on FSC-H and FSC-A. Finally, GFP positive and GFP negative cells were gated (Gate C) and analyzed via FITC channel.





Supplementary Note 3. Analysis of twinPE design principles

To identify design principles that lead to efficient twinPE editing, we analyzed our dataset of *attP* insertions at *AAVS1* and *attB* insertions at *CCR5*. For each spacer pair, we calculated nick-to-nick distances between predicted pegRNA-induced nick sites, the DeepCas9 score[3] for each spacer, and the predicted T_m of the pegRNA primer binding sequence (PBS) for *AAVS1*. For comparing nick-to-nick distances and DeepCas9 scores, the highest efficiency pegRNA pair for each spacer pair was chosen for analysis (*i.e.*, the optimal individual pegRNAs were used). For analyzing the influence of predicted spacer activities, the average of the individual DeepCas9 scores for each spacer was used. For comparing predicted T_m values for PBS variants, the editing efficiency of each pegRNA was normalized to the maximum editing achieved within the spacer pair group. The editing efficiency for a given PBS variant was then averaged across the three paired pegRNA editing efficiencies (each experiment with the paired spacer). T_m was calculated according to the following formula[4]: $T_m = 4N_{G•C} + 2N_{A•T}$.



Although efficient targeting of both DNA protospacers is likely necessary for achieving efficient twinPE, we observed only weak correlations between *in silico* predictions of Cas9 spacer activity and observed twinPE editing efficiencies (top right panel). This poor correlation could arise from other determinants of twinPE efficiency beyond the protospacer, including RT template and PBS choice. While PBS optimization was important for high editing efficiency at many sites, we did not observe an optimal PBS melting temperature across the pegRNA designs in this study (bottom panel). A correlation between prime editing efficiency and the distance between pegRNA-induced nicks was observed (top left panel), which may suggest an optimal spacing of 50 to 100 bp, although many exceptions exist.

Supplementary Note 4. Discussion of other targeted integration methods

TwinPE and Bxb1-mediated recombination offer advantages over other approaches. The serine integrase phiC31[5] and fusions of zinc fingers, TALEs, or dCas9 to the catalytic domain of Gin recombinase[6-9] have been used to integrate or excise DNA at endogenous pseudo-sites in the human genome, but the efficiency of these sequence manipulations has generally been low and, more importantly, the extensive sequence preferences inherent to these recombinases limit the number of targetable loci to a minute fraction of pseudo-sites in the genome. The programmable integration of attachment sites by twinPE overcomes this challenge by enabling insertion of cognate recombinase recognition sequences at any PE-targetable locus.

Compared to nuclease-based methods, the twinPE and recombinase approach avoids the generation of DSBs, which typically lead to uncontrolled mixtures of by products[10], and can also lead to chromosomal rearrangements[11], chromothripsis[12], large deletions[13-15], and p53 activation[16-18]. Integration orientation using twinPE and Bxb1 recombinase is strictly controlled by the directionality of the *attB* and *attP* sequences[19], in contrast to uncontrolled integration orientation using homology-independent repair[20]. Methods that use HDR also enable control of sequence orientation, and can achieve efficiencies on the order of 5-10% without drug selection or suppression of NHEJ[21-23], but HDR is less efficient than NHEJ in most cell types and typically requires DSBs[24,25]. Methods have also been developed for making targeted gene-sized insertions through paired nicking of the genome and a donor cassette[26,27]. These approaches do not require double strand breaks, but remain reliant on HDR and supportive cell types[27].

Supplementary Sequences 1. Sequences of plasmid donor DNA sequences harboring Bxb1 recombination sites

att site: highlighted in yellow
Promoter-less EGFP: highlighted in green
EF1a promoter: underlined
PuroR: highlighted in red
BFP: highlighted in blue
KanR: highlighted in grey

***attB*-Puro-GA donor DNA:**

gatgccagctcattcctcccactcatgatctatagatcccccgggctgcaggaattctacccactctgtcgatacccccaccgagacc
ccattggggccaatacgcccgcgtttcttccttttccccacccccaccccccaagttcgggtgaaggcccagggctcgcagccaacg
tcggggcggcaagcttacatcgagatcccggcttgtcgacgacggcggactccgtcgtcaggatcatccgtgagcaagggcga
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***attB*-Puro donor DNA:**

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ccattggggccaatacgcccgcgtttcttccttttccccacccccaccccccaagttcgggtgaaggcccagggctcgcagccaacg
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attP Factor IX donor (the vector backbone is pMC.BESPX-MCS1 from System Biosciences)

attP: highlighted in yellow
Factor IX intron 1: highlighted in grey
Factor IX exons 2-8 CDS: highlighted in green
Factor IX exon 8 3'UTR: underlined
bGH poly(A) signal: highlighted in cyan

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Supplementary Sequence 2. Sequence of codon-optimized Bxb1 plasmid

CMV enhancer/promoter: highlighted in grey
SP6 promoter: underlined
Codon-optimized Bxb1: highlighted in green
SV40 poly(A): highlighted in cyan

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Supplementary Sequence 3. Sequence of the lentiviral GFP reporter vector (the vector bone is pLVX-EF1a-IRES-Puro from Takara Bio inc.).

LTR: in bold
EF1α promoter: underlined
AAVS1 target sequence: highlighted in yellow
Inverted H2B-EGFP coding sequence: highlighted in green
IRES: highlighted in grey
Puromycin resistance gene: highlighted in red

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