## Supplemental Material

# Targeted chromosomal deletions in human cells using zinc finger nucleases

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**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1.** ZFN-induced genome deletions at the *CCR2* and *CCR5* loci. PCR products corresponding to the 15-kbp genomic DNA deletions were cloned and sequenced. ZFN target sites are shown in bold letters. Microhomologies are underlined and inserted bases are shown in italics. Dashes indicate deleted bases. Non-conserved bases at the *CCR2* and *CCR5* loci are shown in small letters. In cases where a deletion sequence was detected more than once, the number of occurrences is shown in parentheses. WT, wild-type DNA sequence.

**Supplemental Figure 2.** Large nested deletions. PCR products corresponding to gross genomic DNA deletions were cloned and sequenced. Symbols are as in Supplemental Figure 1.

**Supplemental Figure 3.** Targeted genome deletions using two new ZFNs. PCR products corresponding to gross genomic DNA deletions were cloned and sequenced. Symbols are as in Supplemental Figure 1.

**Supplemental Table 1.** Zinc finger modules.

| ZF No.[1] | ZF name | Amino acid sequence | Target subsite |
|---|---|---|---|
| N.A. | DSAR2 | YSCGICGKSFSDSSAKRRHCILH | GTC |
| ZF108 | DSCR | YTCSDCGKAFRDKSCLNRHRRTH | GCC |
| N.A. | DSNR | YRCKYCDRSFSDSSNLQRHVRNIH | GAC |
| ZF112 | ISNR | YRCKYCDRSFSISSNLQRHVRNIH | GAT |
| ZF113 | KSNR | YGCHLCGKAFSKSSNLRRHEMIH | GAG |
| N.A. | QNTQ | YTCSYCGKSFTQSNTLKQHTRIH | ATA |
| ZF117 | QSHR2 | YKCGQCGKFYSQVSHLTRHQKIH | GGA |
| ZF120 | QSHV | YECDHCGKSFSQSSHLNVHKRTH | YGA |
| ZF123 | QSNR1 | FECKDCGKAFIQKSNLIRHQRTH | GAA |
| ZF126 | QSSR1 | YKCPDCGKSFSQSSSLIRHQRTH | GYA |
| N.A. | RDER2 | YHCDWDGCGWKFARSDELTRHYRKH | GYG |
| ZF132 | RDHT | FQCKTCQRKFSRSDHLKTHTRTH | YGG |
| ZF134 | RSHR | YKCMECGKAFNRRSHLTRHQRIH | GGG |
| N.A. | VDYK | FHCGYCEKSFSVKDYLTKHIRTH | TAT |
| ZF137 | VSNV | YECDHCGKAFSVSSNLNVHRRIH | AAT |
| ZF139 | VSTR | YECNYCGKTFSVSSTLIRHQRIH | GCT |
| ZF140 | WSNR | YRCEECGKAFRWPSNLTRHKRIH | GGT |

[1] ZF No. is based on the numbering scheme of the Zinc Finger Consortium Modular Assembly Kit 1.0 available from Addgene.

**Supplemental Table 2.** List of successful ZFNs.

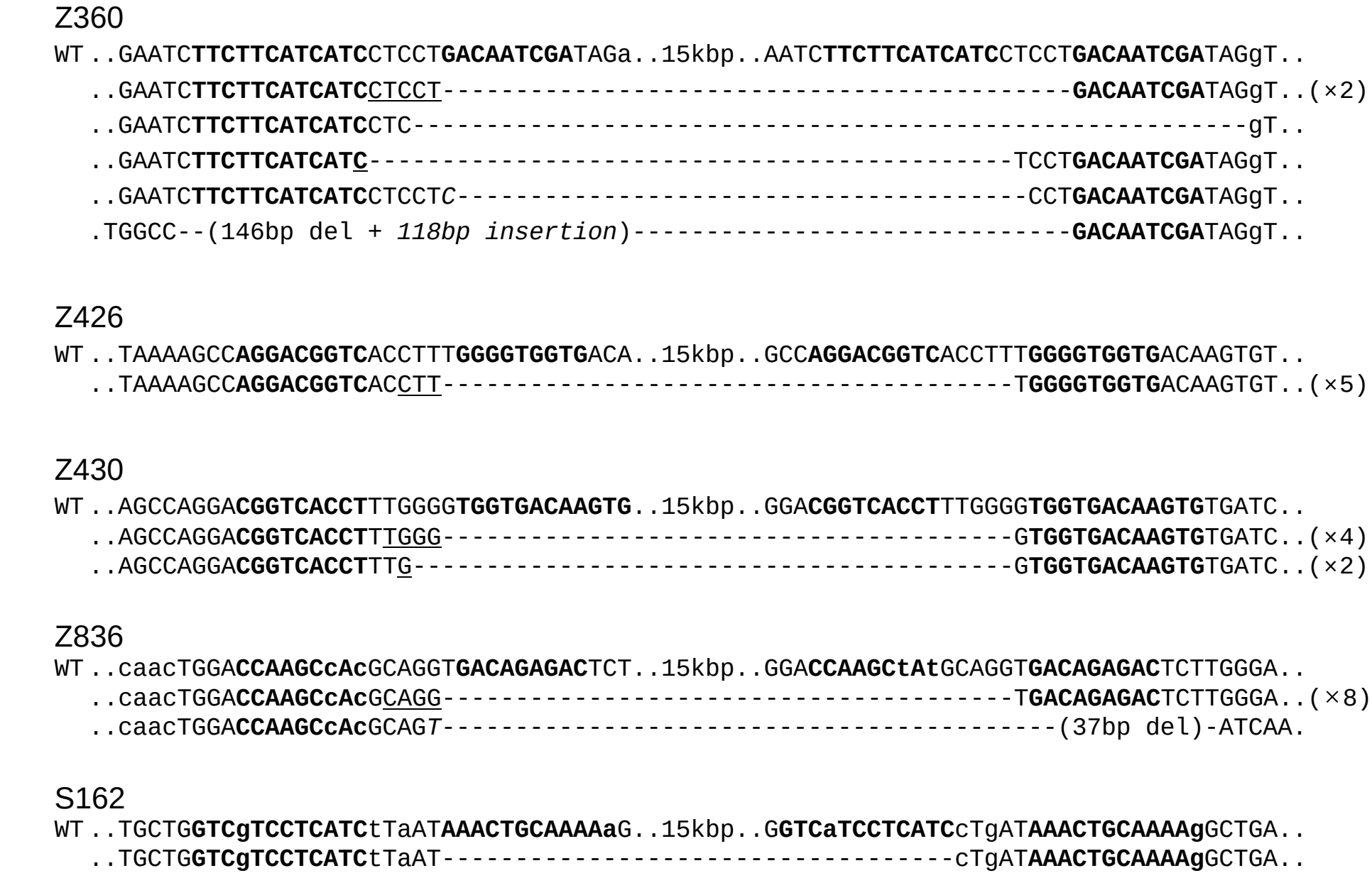
| ZFN name | F1 | F2 | F3 | F4 | Half-site sequence (5' to 3') |
|---|---|---|---|---|---|
| K33R | DSCR | QSHR2 | QSHR2 | QSHR2 | GGA GGA GGA GCC |
| K33F | ISNR | VDYK | ISNR | RSHR | GGG GAT TAT GAT |
| K230R | QSHV | QSHR2 | VSNV | QSSR1 | GCA AAT GGA TGA |
| K230F | DSNR | QSHR2 | VDYK | RSHR | GGG TAT GGA GAC |
| K243R | VDYK | RSHR | WSNR | QSHV | TGA GGT GGG TAT |
| K243F | RDER2 | QSHR2 | VSNV | DSAR2 | GTC AAT GGA GTG |
| K276R | DSNR | RDHT | QSHV | RDHT | TGG TGA TGG GAC |
| K276F | RDHT | DSAR2 | QNTQ | ISNR | GAT ATA GTC TGG |
| K781R | DSNR | QSSR1 | VSTR | RDER2 | GTG GCT GCA GAC |
| K781F | VSTR | QSSR1 | ISNR | RDER2 | GTG GAT GCA GCT |
| K835R | DSAR2 | KSNR | WSNR | WSNR | GGT GGT GAG GTC |
| K835F | KSNR | KSNR | QSNR1 | RDHT | TGG GAA GAG GAG |
| M15R | QSNR1 | QNTQ | DSCR | VSTR | GCT GCC ATA GAA |
| M15F | DSNR | RDHT | QSNR1 | QSHR2 | GGA GAA TGG GAC |

Zinc fingers are named using a four-letter code. F1 is the zinc finger at the N-terminus and F4 is the one at the C-terminus. F2 and F3 are those fingers positioned between F1 and F4. F1 interacts with the 3-bp sub-site at the 3’ terminus, and F4 interacts with the 3-bp sub-site at the 5’ terminus. Zinc fingers are linked to one another using the canonical “TGEKP” sequence to make ZFNs.

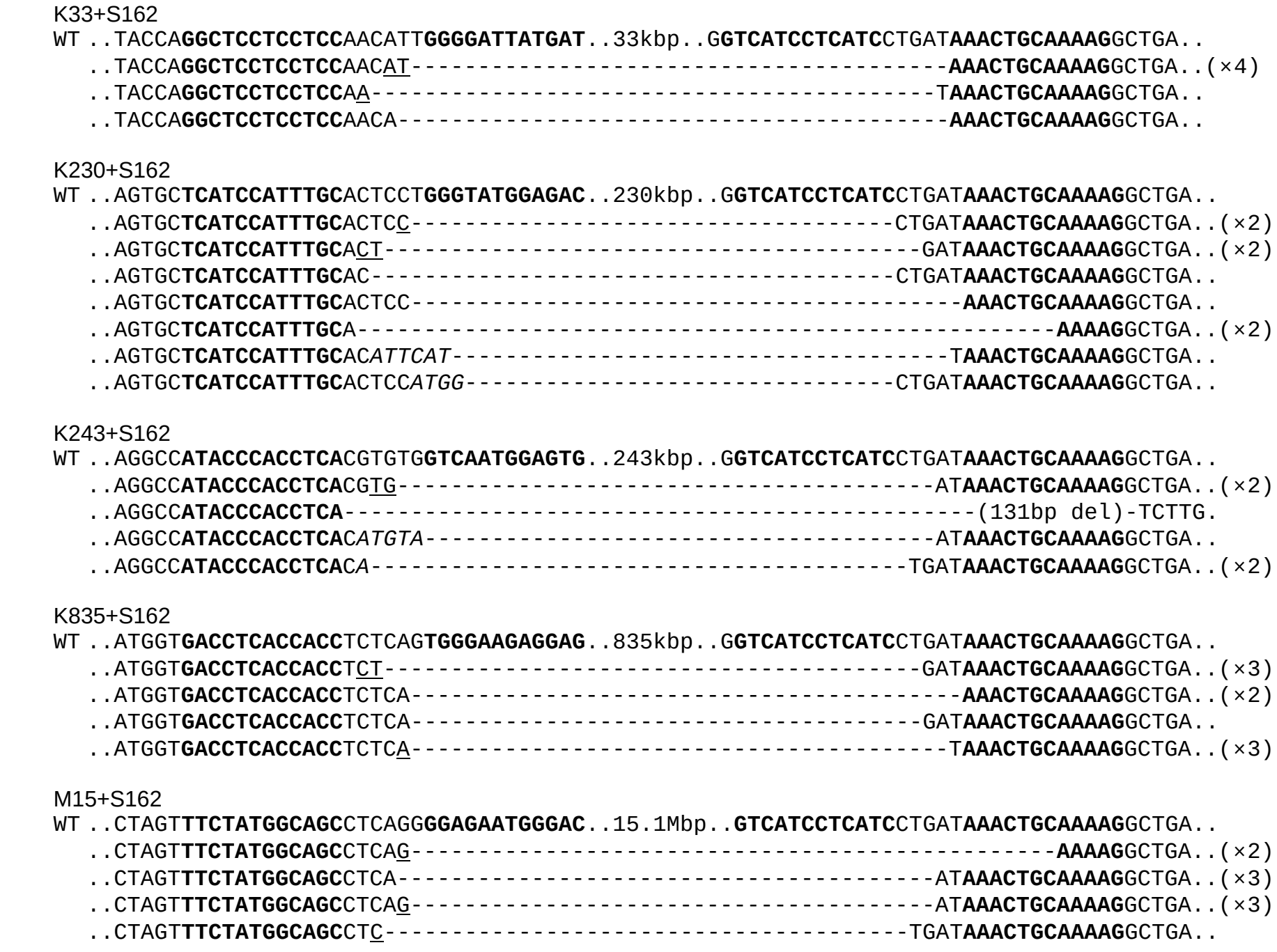
**Supplemental Table 3.** Primer sequences.

| Primer Name | Sequence (5' To 3') |
|---|---|
| F2 | CCACATCTCGTTCTCGGTTT |
| F2f | CCAGCAGACTCCCTTTTCAT |
| R2u | AGAACGAGATGTGGACAGCATGT |
| R2 | GCACCTGCTTTACAGGTTTCT |
| F5 | ATGGATTATCAAGTGTCAAG |
| F5f | GAGCCAAGCTCTCCATCTAGT |
| R5 | TCACAAGCCCACAGATATTT |
| R-S162 | GTATGGAAAATGAGAGCTG |
| R-S162n | CCAGAGAAGAAGCCTATAAAATAG |
| F-K33 | AGCATGGTTCAGAAGGCCAC |
| F-K33n | AGGAAGGCGAAGAGAAAGAA |
| F-K230 | GGGAGCTGAAATACCTTCCTT |
| F-K230n | CATTTGCTTGAAGCAAATCAC |
| R-K230 | ATGTGGCATCACACATGGAG |
| F-K243 | GCCGGGTTTGTACAAGGTAGA |
| F-K243n | GCCAGCTGACGTTAGACATC |
| R-K243 | CCCTGTGTTCCCTTCTAAGC |
| F-K276 | ATCCCTGCCTCACAGCTCAT |
| F-K276n | GAAGCCAGACAGCATTGTGT |
| F-K781 | TGCAGGTACATGCCGAACTG |
| F-K781n | TGTCAGTGCTGCCCTGAGT |
| R-K781 | CCTACCATCCCCTTTCTCAG |
| F-K835 | CCCACTGATGCTCTGATAGTTT |
| F-K835n | ACATGAGGCATAGCAGGGAT |
| F-M15 | CGAGAAGGAAACCTAGCAAGG |
| F-M15n | GGAGGTCTTATGGCCTACAGT |
| R-M15 | CAATTACTCCCCAGGTGTCC |

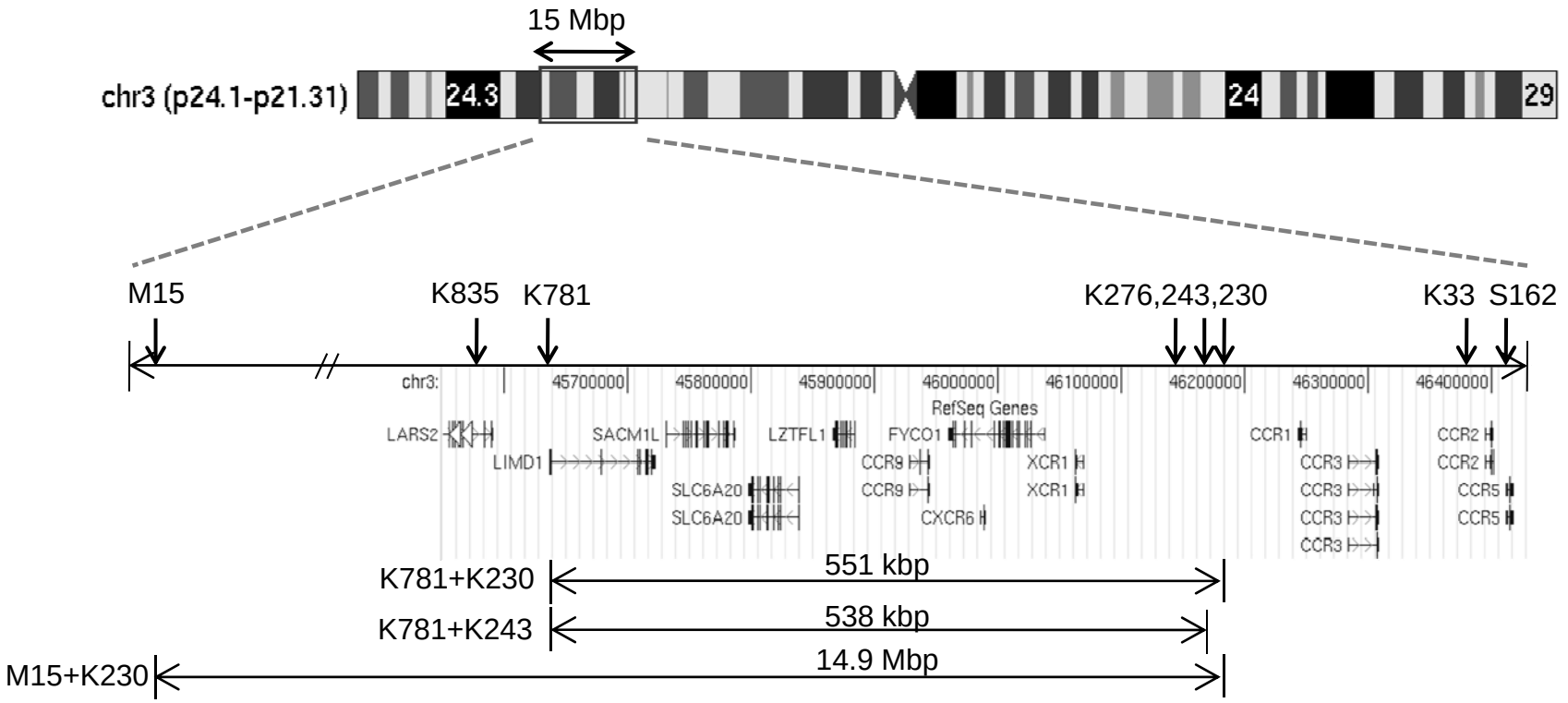
**Supplemental Figure 1**



## Supplemental Figure 2



**Supplemental Figure 3**




K781+K230

```
WT ..CCCAGGTCTGCAGCCACAGCTGGGTGGATGCAGCT..551kbp..TCATCCATTTGCACTCCTGGGTATGGAGACCCACC..
   ..CCCAGGTCTGCAGCCACAGCTG----------------------------------------GGTATGGAGACCCACC..(×2)
   ..CCCAGGTCTGCAGCCACAGCT-------------------------------------CCTGGGTATGGAGACCCACC..
   .ATG-(23bp del)---------------------------------------------CTCCTGGGTATGGAGACCCACC..
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K781+K243

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WT ..CCCAGGTCTGCAGCCACAGCTGGGTGGATGCAGCT..538kbp..ATACCCACCTCACGTGTGGTCAATGGAGTGTACTG..
   ..CCCAGGTCTGCAGCCACAGCTG-----------------------------------TGGTCAATGGAGTGTACTG..(×2)
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M15+K230

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WT ..CTAGTTTCTATGGCAGCCTCAGGGGAGAATGGGAC..14.9Mbp..TCATCCATTTGCACTCCTGGGTATGGAGACCCAC..
   ..CTAGTTTCTATGGCAGCCTC---------------------------------------TCCTGGGTATGGAGACCCAC..
   ..CTAGTTTCTATGGCAGCCTC----------------------------------------CTGGGTATGGAGACCCAC..(×3)
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