Generating stable knockout zebrafish lines by deleting large chromosomal fragments using multiple gRNAs

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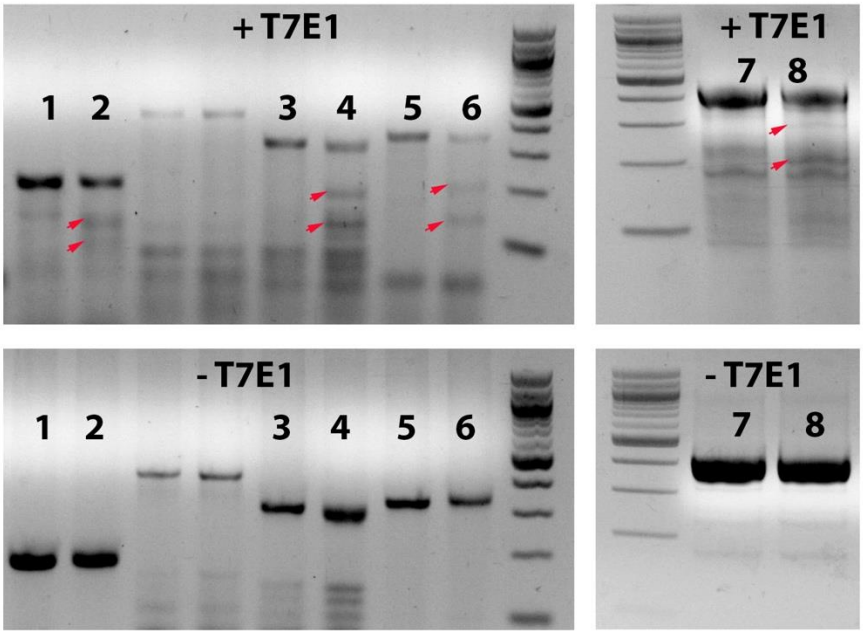
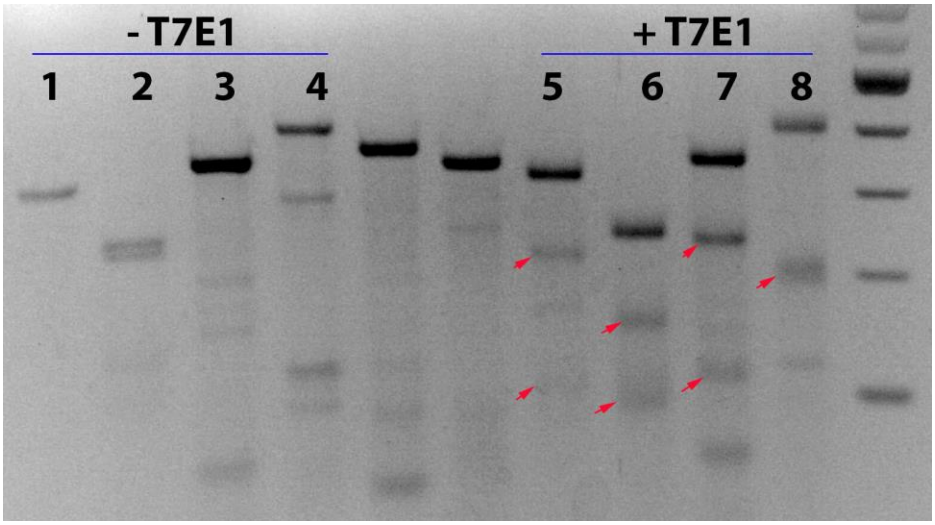
Supplementary Figures

Supplementary Fig.1. T7E1 assay results showed mutations from mixed F_0 zebrafish embryos injected with a cocktail of 7 *smarca2* gRNAs. Lane 1, 3, 5, and 7: un-injected sibling control fish embryos. Lane 2, 4, 6, and 8: mixed gRNA injected fish embryos. Each gDNA sample was extracted from 20-30 fish embryos. Lanes 1-2: CR1 gRNA. Lanes 3- 4: CR15-1 and CR15-2 gRNAs. Lanes 5-6: CR28-1 and CR28-2. gRNAs Lanes 7-8: CR3-1 and CR3-2 gRNAs. Approximate expected sizes (bps) of DNA bands after T7E1 for each gRNA: CR1: 123+87; CR3-1: 305+134; CR3-2: 225+214; CR15-1: 189+134; CR15-2: 211+112; CR28-1: 196+155; CR28-2: 221+130. Positive DNA bands are indicated by red arrows. Some genetic polymorphism among the embryos was noted in some DNA regions such as Lane1 and Lane 7. +T7E1: treated with T7E1. -T7E1: samples underwent the same treatment without T7E1.



Supplementary Fig.2. T7E1 assay results showed mutations from mixed F_0 zebrafish embryos injected with a cocktail of 2 *rnf185* gRNAs and 2 *rnf215* gRNAs. Lanes 1-4: un-injected sibling control fish embryos. Lanes 5-6: mixed gRNA injected fish embryos. Each gDNA sample was extracted from 20-30 fish embryos. Lanes 1 and 5: *rnf185* CR2. The expected sizes of cleaved bands are 214 and 106bps. Lanes 2 and 6: *rnf185* CR4. The expected sizes of cleaved bands are 135 and 94bps. Lanes 3 and 7: *rnf215* CR6. The expected sizes of cleaved bands are 233 and 113bps. Lanes 4 and 8: *rnf185* CR7. The expected sizes of cleaved bands are 204 and 198bps. Positive DNA bands are indicated by red arrows. +T7E1: treated with T7E1. -T7E1: samples underwent the same treatment without T7E1.



Supplementary table 1. DNA sequences of oligonucleotides used in this research.

Oligo Names	DNA Sequences 5'-> 3'
Dr.smarca2-CR1	GGCTCTGTACACAGCATGAT
Dr.smarca2-CR3-1	GCCAATGGATCCGCAGGGGA
Dr.smarca2-CR3-2	GCGTACAAGATTCTGGGACG
Dr.smarca2-CR15-1	CAACCTGAATGGTATTCTGG
Dr.smarca2-CR15-2	AGCGGTACGATGATGAGATA
Dr.smarca2-CR28-1	ATCAGGCCGTCAGCTCAGCG
Dr.smarca2-CR28-2	CGAACTCATAAGGAAACCGG
Dr.rnf185-CR2	GGTCAATCAGCGGGAGAAAG
Dr.rnf185-CR4	GTGATCCCACTGTACGGCAG
Dr.rnf215-CR6	AGCTGCGGTCGTTCAAGAGG
Dr.rnf215-CR7	GACCCCTGTGGAGAGAACAA
Overlap adaptor	GTTTTAGAGCTAGAAATAGCAAG
CRISPR constant oligo	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACG GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
Dr.smarca2-Fw 1	CAATGAGCCACCTGTTGGGATG
Dr.smarca2-Rv 1	TGCATTGGGTGCATTTCTTGTGAGTATTC
Dr.smarca2-Fw 3	CATGTCGCCTCACCCTTCAC
Dr.smarca2-Rv 3	AAGTTCAGAGCCCAGGTGCTAC
Dr.smarca2-Fw 15	CATGAGTCTGCTCGGTTGTCTG
Dr.smarca2-Rv 15	GAAGCCATTAGGCCGCAAATCC
Dr.smarca2-Fw 28	TGATGATAATGTAAACCAGTTGGAG
Dr.smarca2-Rv 28	GGGTAATCCTGTTATCGTCCATCC
Dr.rnf185 Fw 2	AGGGTTGTGTTTAGTGGAGAATGG
Dr.rnf185 Rv 2	TTAAACAATGGCGCAAAGCGTG
Dr.rnf185 Fw 4	GTTTACAGTGGTTAGAGACTCGTCC
Dr.rnf185 Rv 4	GTTGCTTTAGACGCCAGCTCTTAG
Dr.rnf215 Fw 6	AAGCAGCACAGTTTCACTCTCAC
Dr.nf215 Rv 6	ACTGCTACTACTACTGTGCCTTTCC
Dr.rnf215 Fw 7	TCATGGACACATCACCACTTCG
Dr.rnf215 Rv 7	TTGGAAGTTGGAAAGCCACTG