
Supplementary information

Cryo-EM structure of the transposon-associated TnpB enzyme

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Supplementary Information for

Cryo-EM structure of the transposon-associated TnpB enzyme

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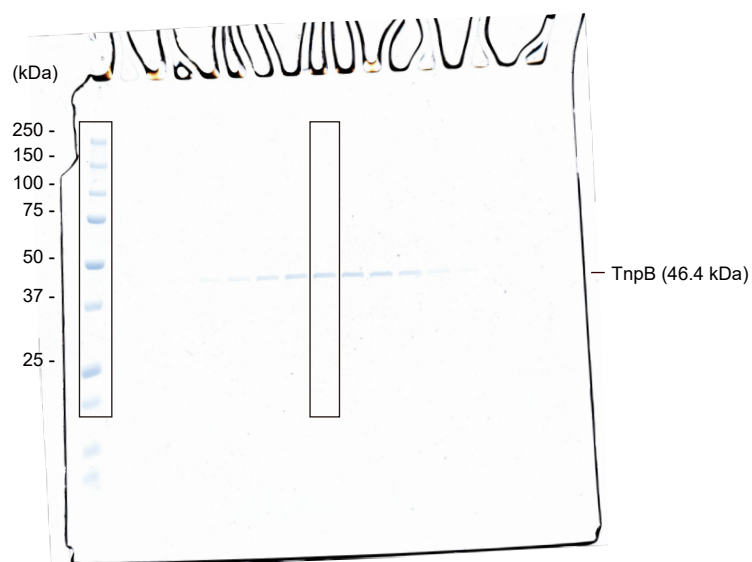
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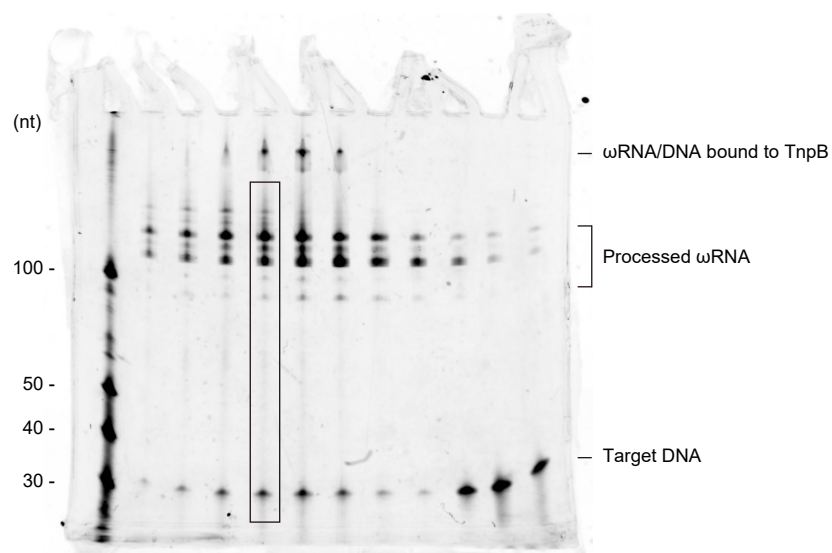
Supplementary Figs. 1, 2

Supplementary Tables 1, 2

a



b

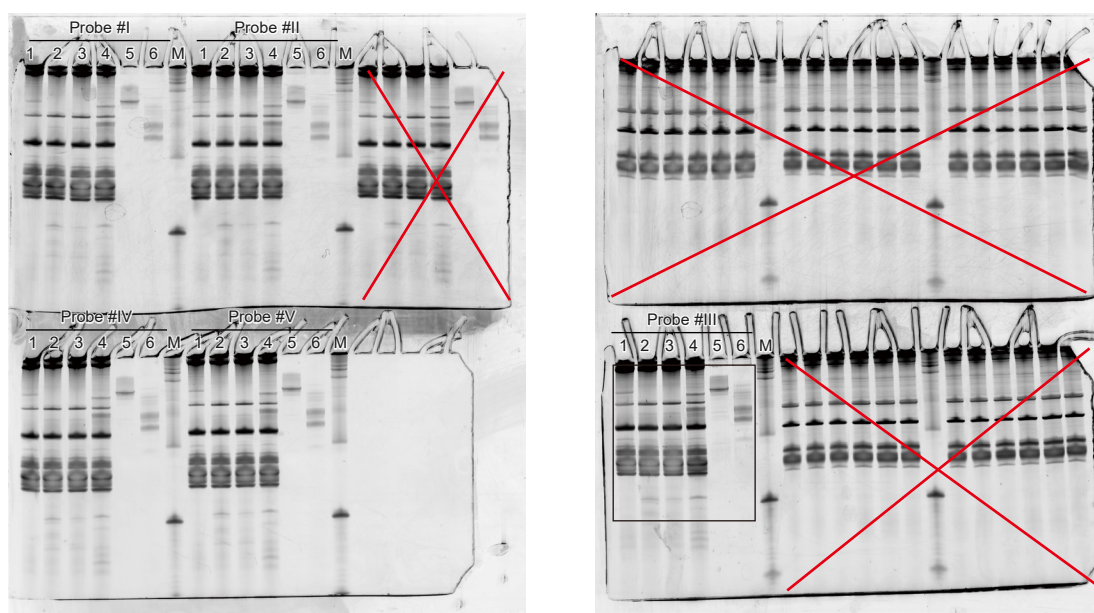


Supplementary Fig. 1. Uncropped images of SDS-PAGE and Urea-PAGE gels.

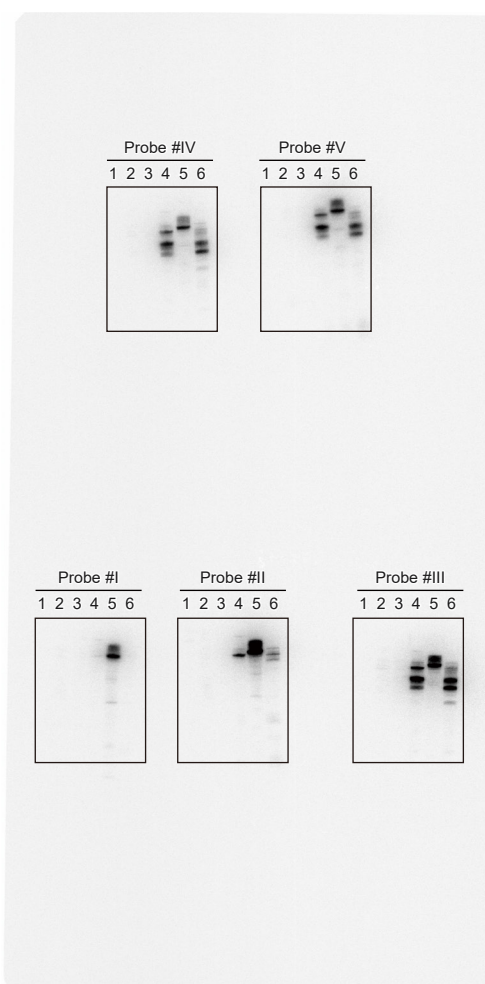
a, SDS-PAGE gel of SEC peak fraction (used for Extended Data Fig. 2a).

b, Urea-PAGE gel of SEC peak fraction (used for Extended Data Fig. 2a).

a



b



Supplementary Fig. 2. Uncropped images of northern blotting analysis.

a, Uncropped 10% gel polyacrylamide gel visualized with GelGreen.

b, Uncropped membrane used for Extended Data Fig. 5c.

Supplementary Table 1. Nucleic-acid sequences used in this study.

cryo-EM analysis	
Name	Sequence
ωRNA	GAUUCAAGAAUCCCGAAGUGAAGAUAUCUUGCCGUCCGUACAUGGACUUGCCCGAACUUGUGGGGAAACCAUGACCGAGACGAGAACGCUUGC GCUGAACAUUUCGGCGUGAAGCGUUGUGUGGUGCGGGAAUCUCAGACACCUUAAACGCUCAUGGAGGCUAUGUCAGACCUGCUUCGGCGGG CAAUGGUCUGCGAAGUGAGAUAUCGCGACUUUAGUCUGUGAGGUUCAAGAGUCCCUUGGCGCCCC
TS	A*T*T*T*G*A*A*T* <u>GGGCGCCAAAGGACT</u> CATCAATAGAAA
NTS	TTTCTA <u>TTGAT</u> <u>GAGTCCCTTGG</u> *C*G*C*C*A*T*T*C*A*A*A*T*

The guide sequence in the wRNA and the target sequence in the TS and NTS are underlined. The TAM sequence (TTGAT) in the NTS is colored purple. * phosphorothioate bond.

In vitro DNA cleavage assays

Name	Sequence
Target DNA	AGCGCCCAATACGCAAAACCGCCTCTCCCCGCGCTTGCCCGATTCTTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAGCGGGCAGTG AGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGGAATTTGTAGC GGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTGCACTCTAGAGGATCCCCGGGTACCGAGC TCGAATTCACCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACTTAATCGCCTTGACGCACATCCCCCTTTTCGCCA GCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCACGCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCC TTACGCATCTGTGCGGTATTTACACCCGCATACGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGT TACGCGCAGCGTGACCGCTACACTTGGCAGCGCCTAGCGCCCGCTCCTTTTCGCTTTTCTCCCTTCCCTTTCTCGCCACGTTGCGCGGCTTTCCC CGTCAAGCTCTAAATCGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTGGGGTATGGTTTCC GTAGTGGGCGCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCAAAACCTGGAACAACA CTCAACCCCTATCTCGGGCTATTCTTTTGATTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAACAAAAATTAACG CGAATTTTAACAAAAATATTACGTTTACAATTTTATGTGCACTCTCAGTACAATCTGCTCTGATGCGCGATAGTTAAGCGACGCCCGACACCCGC CAACACCCGCTGACGCGCCCTGACGGGCTGTCTGCTCCCGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGA GGTTTTACCGCTCATCACGAAACGCGGAGACGAAAGGCCCTCGTGATACGCCTATTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAG ACGTCAAGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>GAGTCCCTTGGCGCCC</u> ATTCAAATATGTATC CGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTT TGCGGCATTTTGCTTCCCTGTTTTTGGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACAGAGTGGTTACATC GAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTGCGCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCGC GGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTACCAAGTCACAGAA AAGCATCTTACGATGGCATGACAGTAAGAGAAATTATGCAGTGCTGCCATAACCATGAGTGATAAACACTGCGGCCAACTTACTCTGACAAACGAT CGGAGGACCGAAGGAGCTAACCCTTTTTTGACAAACATGGGGGATCATGTAACCTGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT ACCAAACGACGAGCGTGACACCAAGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAACTACTTACTAGCTTCCCGG CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGATAAATCTG GAGCGGCTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGCGGGAGT CAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAAGCATTGGTAACGTGCAGACCAAGTTTACTCATATAT ACTTTGATTGATTTAAACTTCAATTTTAAATTTAAAGGATCTAGGTGAAGATCCTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTT CGTTCCTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTCAACAAAAA CCACCGCTACACGCGGTGGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAATA CTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCTGTTACCAAGTGGCT GCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTC GTGCACACAGCCAGCTTGGAGCGAAGACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGA GAAAGGCGGACAGGTATCCGGTAAGCGGCAAGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGT CCTGTGCGGTTTTGCGCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCC TTTTACCGTTCCTGGCCTTTTGTGCGCTTTTGTCTACATGTTCTTCTCGCTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAG TGAGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCACTGAGCGAGGAAGCGGAAG
Target DNA-MM1/2	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM3/4	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTCACCCCTTGGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM5/6	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTGGCTTGGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM7/8	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCGATGGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM9/10	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTACGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM11/12	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCCC</u> ATTCAAATATGTAT~
Target DNA-MM13/14	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGC</u> ATTCAAATATGTAT~
Target DNA-MM15/16	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGG</u> ATTCAAATATGTAT~
Target DNA-1 (TG1)	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGGCGCGCG</u> ATTCGATATGTAT~
Target DNA-2 (TG2)	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGGATCGCG</u> ATTCGATATGTAT~
Target DNA-3 (TG3)	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGGATCG</u> ATTCGATATGTAT~
Target DNA-4 (TG4)	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGGATTCAA</u> ATATGTAT~
Target DNA-5 (TG5)	~GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTTTTTCTA <u>TTGAT</u> <u>CTGTCCCTTGGCGCGGATAT</u> ATATGTAT~

The target sequence are underlined. The TAM sequence (TTGAT) is colored purple. Mismatches, CG, and AT are colored red, blue, and green, respectively.

Target DNA-MM12–Target DNA-ATATAT are shown only around the target sequence.

Genome editing assay

Site #	Gene	Guide sequence
1	AGBL1	AATGAATGGCTACTCTAACC
2	EMX1	GTGATGGGAGCCCTTCTTCT
3	AGBL1	GATTAATAAGTATCTTGTTG
4	DYNC1H1	TTCAAATCAAGCCACTTTTG
5	HPRT1	GTAATCCAGCAGGTGAGCAA
6	VEGFA	GTCAGCTAATTCTGACTCCT
7	EMX1	TTAGATTATGCATATACCAG

Supplementary Table 2. Primers and constructs used in this study.

TnpB mutants			
Mutation	Forward primer	Reverse primer	
ΔCTD	GTTGGTGGCTTAACGGCCACGCGATCGCTG	GTGGCCGTTACGCAGCCACCAACGCTTCAC	
Y52A	CTGACCGCCGGGCAAACGAGTAGCGAACTG	TTGCCCGCGGTCAGTCCCTTCCCGCTTTC	
S56A	GCCAGCGAACTGACCCCTTCTGAAGCAG	CGTTTGCCCGTAGGTCAGTCCC	
K76A	GCCTTTGCTTTGCAGAACTCGTGAAAAAC	ATCTACTTCCGAGAGCCAGGAGGTTTC	
F77A	GCCGCTTTGCAGAACTCGTGAAAAACC	CTTATCTACTTCCGAGAGCCAGGAGG	
Q80A	GTTTGCTTTGGCCAACTCGCTGAAAAACC	TCAGCGAGTTGGCCAAAGCAAACCTTATCTAC	
T123A	GACTCAATTGCGCAACAACAACATCCAAATTG	TGTTGTTGTTGGCGAATTGAGTCCGGTAG	
N124A	TCAATTCACCGCCAACAACATCCAAATTGG	GGATGTTGTTGGCGGTGAATTGAGTCCG	
Target DNA mutation			
Mutation	Forward primer	Reverse primer	
Target DNA-MM1/2	CTGTCCCTTGGCGCCCATTCAAATATG	ATCAATAGAAAAATAACAAATAGGGGTTCCGCG	
Target DNA-MM3/4	CACCCCTGGCGCCCATTCAAATATGTATC	TCATCAATAGAAAAATAACAAATAGGGGTTCCGC	
Target DNA-MM5/6	GGCTTGGCGCCCATTCAAATATGTATCCG	ACTCATCAATAGAAAAATAACAAATAGGGGTTCC	
Target DNA-MM7/8	GATGGCGCCCATTCAAATATGTATCCG	GGACTCATCAATAGAAAAATAACAAATAGGGG	
Target DNA-MM9/10	ACGCGCCCATTCAAATATGTATCCGCTC	AGGGACTCATCAATAGAAAAATAACAAATAGGGG	
Target DNA-MM11/12	CGGCCCATTCAAATATGTATCCGCTCATGAG	CAAGGGACTCATCAATAGAAAAATAACAAATAGG	
Target DNA-MM13/14	CGCCATTCAAATATGTATCCGCTCATGAGAC	GCCAAGGGACTCATCAATAGAAAAATAAAC	
Target DNA-MM15/16	GGATTCAAATATGTATCCGCTCATGAGACAATAAC	GCGCCAAGGGACTCATCAATAGAAAAATAAAC	
Target DNA-1 (TG1)	CGCGCGATATGTATCCGCTCATGAGACAATAACCC	GGGCGCCAAGGGACTCATCAATAG	
Target DNA-2 (TG2)	ATCGCGATATGTATCCGCTCATGAGACAATAACCC	GGGCGCCAAGGGACTCATCAATAG	
Target DNA-3 (TG3)	ATATCGATATGTATCCGCTCATGAGACAATAACCC	GGGCGCCAAGGGACTCATCAATAG	
Target DNA-5 (TG5)	ATATATATATGTATCCGCTCATGAGACAATAACCC	GGGCGCCAAGGGACTCATCAATAG	
DNA probes used in the northern blotting experiment			
Probe number	Oligo sequence	Reverse primer	
Probe #I	TTCTTCACTTCGGGATTCTTGAATC	ATCAATAGAAAAATAACAAATAGGGGTTCCGCG	
Probe #II	CGTCTCGGTCA TGGGTTTCCCCACA	TCATCAATAGAAAAATAACAAATAGGGGTTCCGC	
Probe #III	GTCTGAGATTCCCGCAGCCACCAAC	ACTCATCAATAGAAAAATAACAAATAGGGGTTCC	
Probe #IV	GCAGACCATTGCCCGCCGAAGCAGG	GGACTCATCAATAGAAAAATAACAAATAGGGG	
Probe #V	GGGCGCCAAGGGACTCTTGAACTCT	AGGGACTCATCAATAGAAAAATAACAAATAGGGG	
Genome editing assay			
Site #	Gene	Guide sequence	Reverse primer
1	AGBL1	CAGCATGTTCTCACAAAGAGAGTCTAC	TGCAAAGCCATTATTGCACTTGGAGAG
2	EMX1	CAGCTCAGCCTGAGTGTTGA	CTCGTGGGTTTGTGGTTGC
3	AGBL1	GACTGTCCCATTACTGGGGATGTTAACTTT	TGCCTGAGGCAGAGCAGCA
4	DYNC1H1	GACTAGCCTTGGTCTGGGTGGAATG	AACTAATTTCTGTAAAGGCAGTTCTGTATGC
5	HPRT1	CATCACATTGTAGCCCTCTGTGTGC	CCACACACCTGTAAAAATAAGTATATATCCTCCAAG
6	VEGFA	CCCACCACCTTCTCAGTCTATGT	TAACGTCAACCATACCAACAGCTGTTTAT
7	EMX1	CAGTCATTCCCCTTGCTCTCTGCT	CCCAGGGCTCTTCCCTCATCTC
Construct			
Plasmid			
pETDuet-ISDra2TnpB-ωRNA	https://benchling.com/s/seq-rK3nFj2GszhltgQ1YtfI?m=slm-ojgLECTb9cfZAmxu8knO		
pHS1288	human codon optimized ISDra2 TnpB protein expression		
pHS1201	human U6 promoter ISDra2 scaffold from Karvelis et al with Bpil Golden Gate site		
SN0856	human U6 promoter ISDra2 trim1 scaffold with Bpil Golden Gate site		
SN0857	human U6 promoter ISDra2 trim2 scaffold with Bpil Golden Gate site		
SN1338	human U6 promoter ISDra2 trim3 scaffold with Bpil Golden Gate site		