



Supplementary Figure S1. Purification of MBP-Hsmar1 transposase and dCas9-transposase. Purified protein was analysed by SDS-PAGE to gauge protein purity. **A)** MBP-Hsmar1 transposase accounts for ~95% total protein. **B)** dCas9-transposase accounts for ~85% total protein.

## Supplemental Table 1 Plasmid Sequences

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CCTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTA  
CCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG  
ACGATAGTTACCGGATAAGGCGCAGC

Spacer 1a	AAAC AGGCACCCCAGGCTTTACACTTTATGCTTCG
Spacer 1b	AAAACGAAGCATAAAGTGTAAGCCTGGGGTGCCT
Spacer 2a	AAAC TACGAGCCGGAAGCATAAAGTGTAAGCCTG
Spacer 2b	AAAACAGGCTTTACACTTTATGCTTCCGGCTCGTA
Spacer 3a	AAAC TCCGGCTCGTATGTTGTGTGAAATTGTGAGG
Spacer 3b	AAAACCTCACAATTTACACAACATACGAGCCGGA
Spacer 4a	AAAC TCCGCTCACAATTTACACAACATACGAGCG
Spacer 4b	AAAACGCTCGTATGTTGTGTGAAATTGTGAGCGGA
Spacer 5a	AAAC TGAAATTGTGAGCGGATAACAATTTACACAG
Spacer 5b	AAAACGTGTGAAATTGTTATCCGCTCACAATTTCA
Spacer 6a	AAAC AAACAGCTATGACCATGATTACGGATTACAG
Spacer 6b	AAAACGTGAATCCGTAATCATGGTCATAGCTGTTT
Spacer 7a	AAAC GGTTTTCCAGTCACGACGTTGTAAAACGAG
Spacer 7b	AAAACTCGTTTTACAACGTCGTGACTGGGAAAACC
Spacer 8a	AAAC TGAATGGCGAATGGCGCTTTGCCTGGTTTTCG
Spacer 8b	AAAACGAAACCAGGCAAAGCGCCATTCGCCATTCA
Spacer 9a	AAAC ACCCGTCGGATTCTCCGTGGGAACAAACGGG
Spacer 9b	AAAACCCGTTTGTTCACGAGGAGAAATCCGACGGGT
Spacer 10a	AAAC TCACATTTAATGTTGATGAAAGCTGGCTACG
Spacer 10b	AAAACGTAGCCAGCTTTCATCAACATTAATGTGA

### Supplemental Table 2 CRISPR spacer sequences

Sequences of the oligonucleotides that were annealed together to make the oligoduplexes encoding the spacer sequences encoding the ten candidate gRNAs used in Figure 1.

RNC target top	<u>CGGATTCACTGGCCGT</u> <u>CGTTTTACAACGTCGT</u> <u>GACTGGGAAAACCCTG</u> GC	Substrate used in EMSAs to demonstrate reconstituted sgRNA-dCas9-transposase binding to target DNA. Anneals with RNC target bottom. sgRNA hybridizes with underlined sequence.
RNC target bottom	[Cy5]- GCCAGGGTTTTCCAG TCACGACGTTGTAAAA CGACGGCCAGTGAATC CG	Substrate used in EMSAs to demonstrate reconstituted sgRNA-dCas9-transposase binding to target DNA. Anneals with RNA target top
Hsmar1 substrate top	ACTAGTGGATCCCCCG GGCTGCAGGAATTCTA <u>TTAGGTTGGTGCAAAA</u> <u>GTAATTGCGGTTTTGG</u> ATCCCTGT-[Cy5]	Substrate used in EMSAs to demonstrate transposase binding to inverted terminal repeat (ITR). Anneals with Hsmar1 substrate bottom. ITR is underlined.
Hsmar1 substrate bottom	ACAGGGATCCAAAACC <u>GCAATTACTTTTGCACC</u> <u>AACCTAATAGAATTCCT</u> GCAGCCCGGGGGATC CACTAGT-[Cy5]	Substrate used in EMSAs to demonstrate transposase binding to ITR. Anneals with Hsmar1 substrate top. ITR is underlined.
sgRNA	<u>GUCACGACGUUGUAAA</u> <u>ACG</u> <i>aguuuagagcua</i> <b>GA</b> <b>AA</b> <i>UAGCAAGUAAAAU</i> <i>AAGGCUAGUCCGUUA</i> <i>UCAACUUGAAAAAGUG</i> <i>GCACCGAGUCGGUGC</i> <i>UUUU</i>	sgRNA used for reconstitution with purified dCas9-transposase. Green sequence is the spacer (crRNA targeting sequence), Italicized sequence is the scaffold composed of: i) lowercase = crRNA repeat, ii) bold = tetraloop and iii) blue = tracrRNA sequence.

Supplemental Table 3 Oligonucleotide sequences