A Consensus Model of Homology-Directed Repair Initiated by CRISPR/Cas Activity

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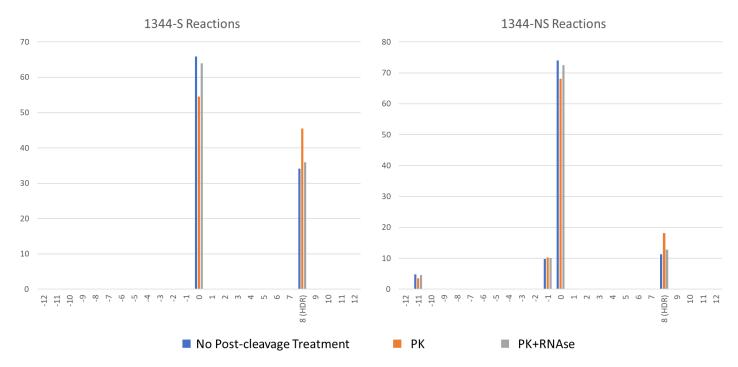
SUPPLEMENTAL MATERIAL

Experiment Goal: Asymmetric Insertion

Ref.	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCC ATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGGCAGCAAAATGTTGCAGCACTGACCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCGTGAGGGGG GACCGGCGGCGGCG 1364 10/50HDR-NS			
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	CCGTCG T	TT TTACAACGTCGTGACTGGGAAAACCCTGGCG	
Output Sequences	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сстссссссссто	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сстссссссссто	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сстссссссссто	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сстсссссссст	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TGTAAAAGCGGCCGCCGACGGCCGCTTTTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	ссадса т	T <mark>GTAAAAGCGGCCGCCGACAGCCGCTT</mark> TTACAAC <mark>R</mark> TCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	CGTCGCCGCCGCT	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	T <mark>GTAAAAGCGGCCGCCGACGGCCGCTT</mark> TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	CGTCG <mark>GCGGCCGC</mark> T	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сстссссссст	TTACAACGTCGTGACTGGGAAAACCCTGGCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGC	сбтсб т	TGTAAAAGCGGCCGCCGACGGCCGCTT]TACAACGTCGTGACTGGGAAAACCCTGGCG	
	-TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT- ATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGGCAGCAA <u>A</u> -	Cas12a 1364 CG Cleavage	10/50 HDR-NS STITTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC =ATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCG	
Plasmid Oligo Mismatcl De novo	-GCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGT	11111 11 1111		
	h 🗸			
			-ATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACGTCG	
	TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGT		ciation of bound, corporated oligo	
	5'>3' Resection of unmatched bases -GCGATTAAGTTGGGTAACGCCAGGGTTTCCCAGTCACGACGTTGT -ATCTCAGCTGGACGGTCCGTACGTTCGAACCGTGACCGGCAGCAA	FAAAAgeggeegeCGACGGC -	CCCCTTTTACAACGTCGTGACTGGGAAAACCCCTGGCGTTACCCAACTTAATCGCCTTGCAGC	
	5'>3' Resection of unmatched bases			
	-TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACCGGCCGCGCGTGTTGTAAAAgeggecgeCGACGGCCGCTTTTACAACGTCGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC-+ ATCTCAGCTGGACGTCCGTACGTTCGAACCGTGGACCGGCAGCAA + Fill-in and ligation -TAGAGTCGACCTGCAGCGACGGCAGCAATGGGATGAATTAGCGGAACGTCG -TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACCGGCGTGGTGGTAGAAAgeggeccgCCGACGGCCGCTTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC-+ -TAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTGGTGGTAGAAAgeggeccgCCGACGGCCGCTTTTACAACGTCGTGACTGGGAAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC-+			
	#ATCTCAGCTGGACGTCCGTACGTTCGAACCGTGACCGGCAGCAACATTTTcgccggcgGCTGCCGGCGAAAATGTTGCAGCACTGACCCTTTTGGGACCGCAATGGGTTGAATTAGCGGAACG			

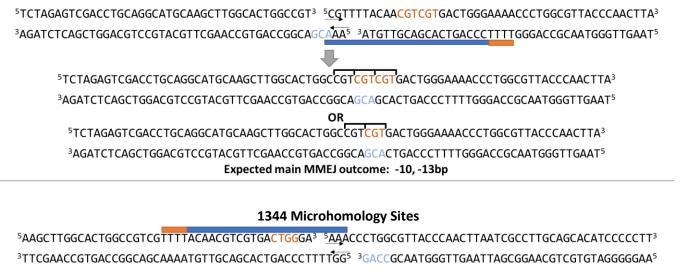
Supplemental Figure 1: Analysis and pathway of atypical repair resulting in ssODN dimer-driven repair outcomes.

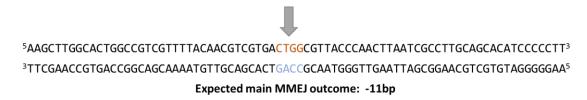
Sanger 1344-S/NS Reaction Parameters Comparison



Supplemental Figure 2: Persistence of Strand Bias within *In Vitro* gene editing reactions in response to post-cleavage Cas12a degradation

1364 Microhomology Sites





1228 Microhomology Sites

Supplementary Figure 3: Suggested patches of microhomology responsible or non-HDR indel events exhibited in Figures 3 and 4.