One-Day Construction Of Multiplex Arrays to Harness Natural CRISPR-Cas Systems

Robert M. Cooper, Jeff Hasty

Supporting Information

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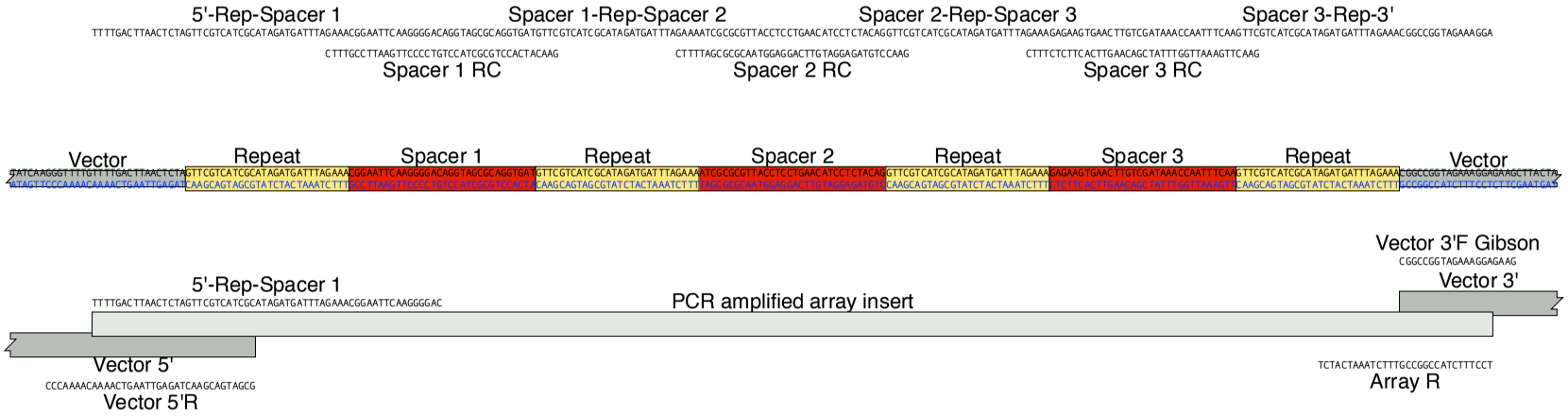
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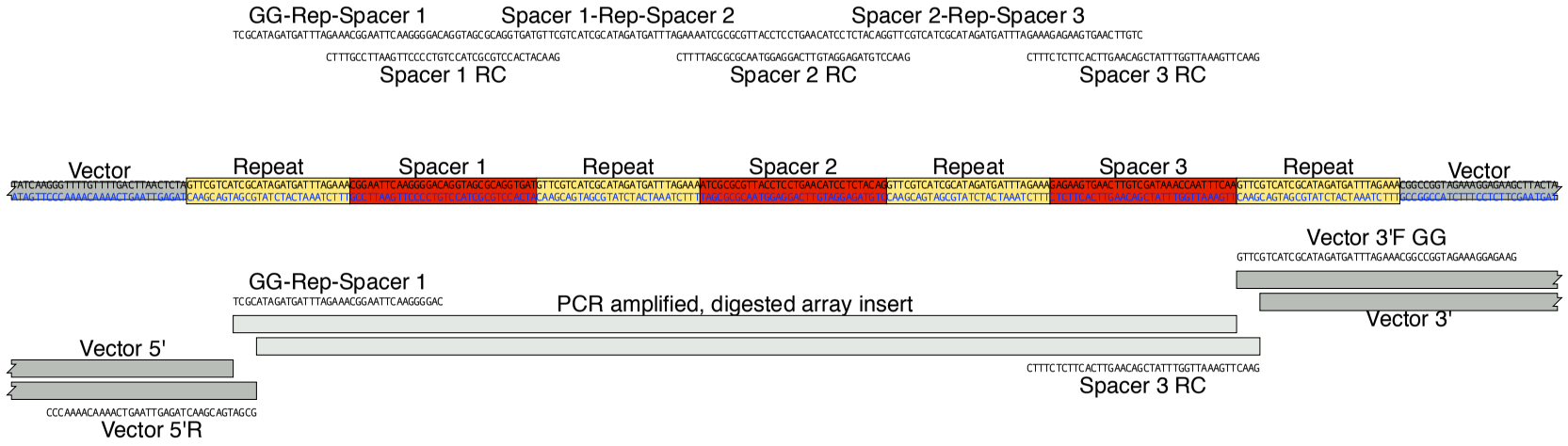
Vector 5'
Array R
Vector 5'R

B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Repeat
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Repeat
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Repeat
Vector
Vector 3'F GG
Vector 3'
GG-Rep-Spacer 1
PCR amplified, digested array insert
Vector 5'
Spacer 3 RC
Vector 5'R

Figure S1: Detailed multiplex natural CRISPR array assembly. A more detailed version of Figure 2 showing DNA sequences for the 3xBAP CRISPR array. The two array assembly strategies are for insertion into a vector using Gibson assembly or fusion PCR (A) or Golden Gate assembly (B). Note that primers used for Golden Gate assembly (denoted "GG" in B) have an additional BsaI site-containing tail appended to their 5′ ends that is not shown, specifically, TTTGGTCTCA.

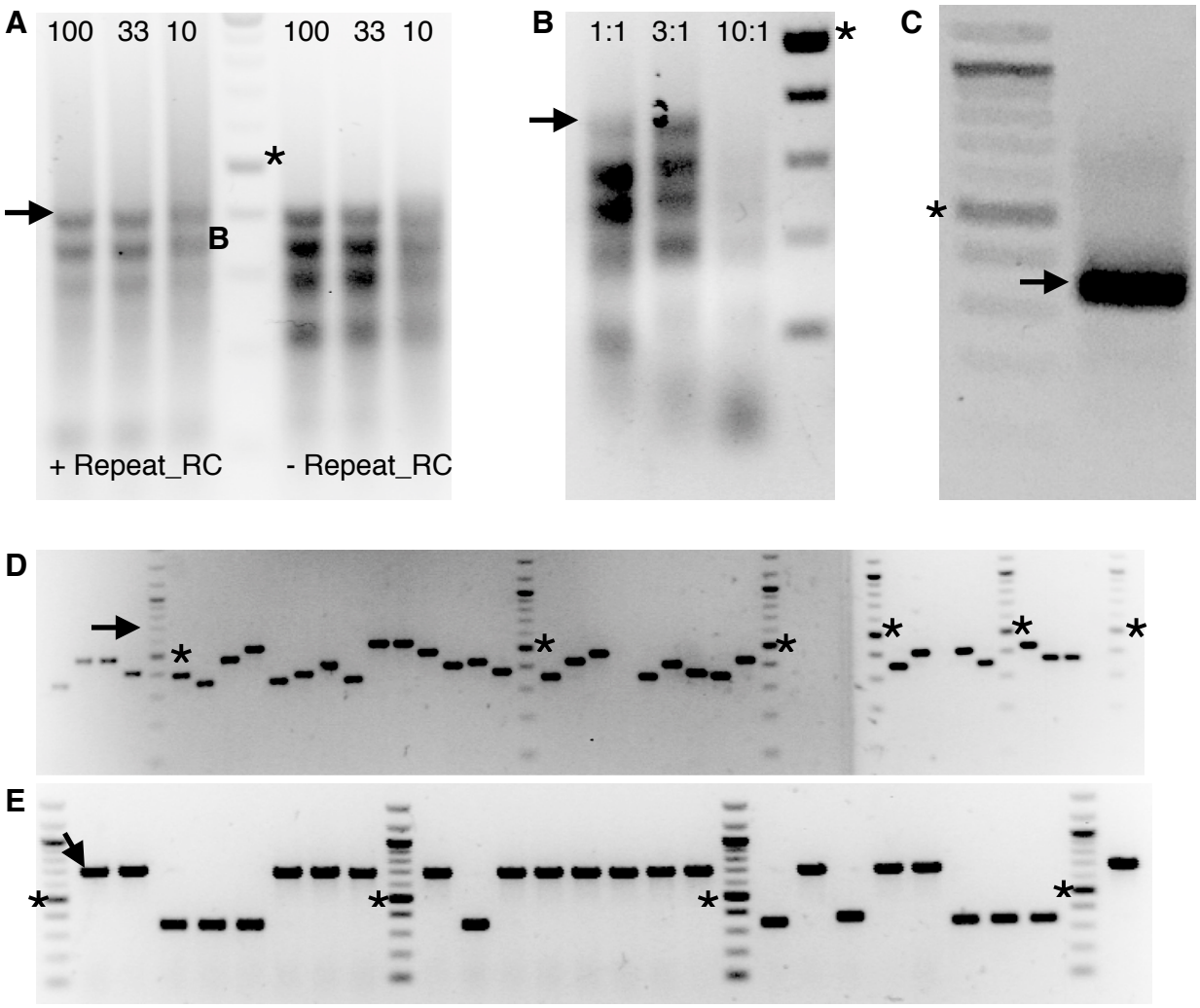

Figure S2: Multiplex array assembly optimization. Protocol optimizations were performed using a 6xIS-CRA array and inserted into pBAV using Golden Gate assembly. A,B: raw ligations, C: PCR amplification, D,E: Colony PCR screening of clones. Asterisks on all gels indicate the 500 bp band of the ladder, and arrows indicate the correctly sized assembly. A) Including the Repeat_RC oligo increases incorrect, higher-molecular-weight smearing (left 3 vs right 3 lanes), and 100 uM stock oligos (lanes 1 and 5) work better than 33 uM (lanes 2 and 6) or 10 uM (lanes 3 and 7) stock solutions. The center (lane 4) is a 100 bp ladder. B) Annealing and ligation is most efficient using 3 parts bottom oligos to 1 part top oligos. The lanes from left to right are ligations using 1:1, 3:1, and 10:1 ratios of bottom oligos to top oligos, followed by a 100 bp ladder. C) PCR amplification of the resulting ligation improves yield of the correct-sized product. D) Golden Gate assembly directly from ligation products yielded no correct-sized arrays out of 36 tested clones. All of 6 sequenced clones were correct at the 3′ end, but truncated at the 5′ end of the array. E) As for D, but the ligation product was PCR amplified and gel extracted before inserting into the vector. 16 of 25 colonies were the correct size, and all incorrect clones had 0x arrays (a single repeat only).

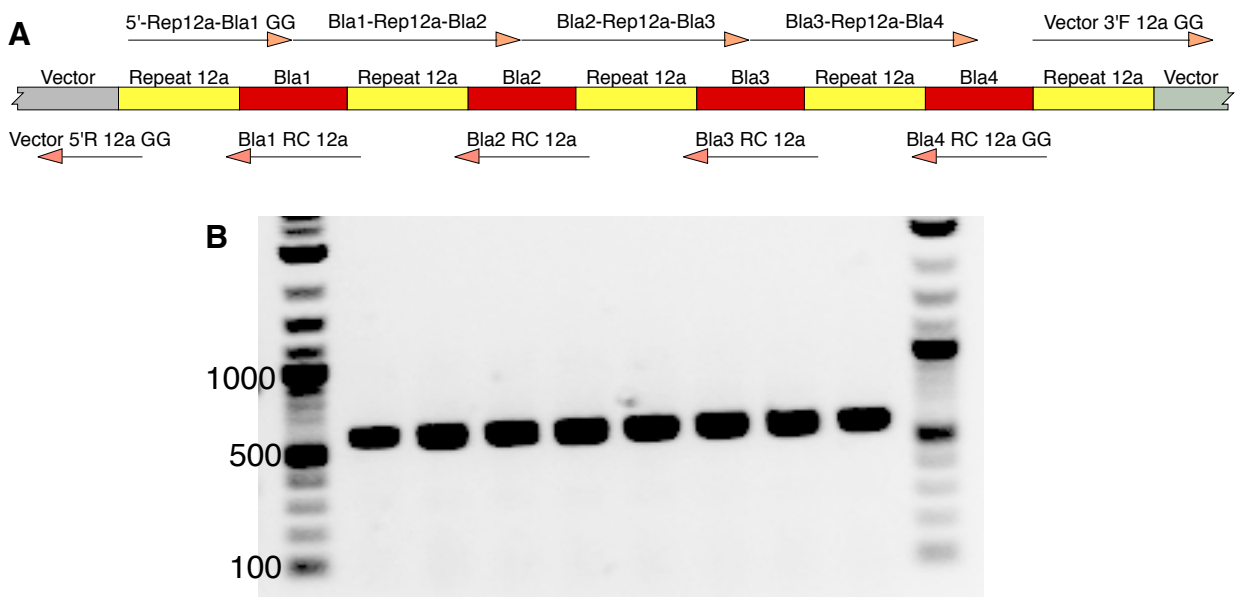

Figure S3: Assembly of a 4-spacer Cas12a array. A) Design and oligonucleotides for a 4-spacer FnCas12a CRISPR array, to be inserted into the vector using Golden Gate assembly. This is analogous to Figure 2B for *A. baylyi* arrays. All oligos denoted by GG also contain a 5′ Golden Gate tail (see Methods and Table S1). B) Screening of 8 clones for the 4-spacer array, of which all had the desired 603 bp product. The primer pair hybridized to the backbone of the vector, outside the inserted CRISPR array. The ladder on the end lanes contains 100 bp increments up to 1 kb.

Table S1: Oligos/Primers

Purpose	Name	Sequence
$CRISPR_{4xKan1}$ Gibson	kan1 5'-R-T1	TTTTGACTTAACTCTAGTTCGTCATCGCATAGATGATTTAGAA AGGTCGATCAGGGAGGA
	kan1 T1-R-B1	TATCGGGGAAGAACAGGTTCGTCATCGCATAGATGATTTAGA AATTGCATTCTAAAACCT
	kan1 B1-R-T2	TAAATACAGAAAACAGGTTCGTCATCGCATAGATGATTTAGAA AGTCGATACTATGTTAT
	kan1 T2-R-B2	ACGCCAACTTTGAAAAGTTCGTCATCGCATAGATGATTTAGA AAAAGCGAGCTCGGTACT
	kan1 B2-R-3'	AAAACAATTCATCCAGGTTCGTCATCGCATAGATGATTTAGAA ACGGCCGGTAGAAAGGA
	Kan1 T1 RC	GAACCTGTTCTTCCCCGATATCCTCCCTGATCGACCTTTC
	Kan1 B1 RC	GAACCTGTTTTCTGTATTTAAGGTTTTAGAATGCAATTTC
	Kan1 T2 RC	GAACTTTTCAAAGTTGGCGTATAACATAGTATCGACTTTC
	Kan1 B2 RC	GAACCTGGATGAATTGTTTTAGTACCGAGCTCGCTTTTTC
$CRISPR_{4xKan2}$ Gibson	kan2 5'-R-T1	TTTTGACTTAACTCTAGTTCGTCATCGCATAGATGATTTAGAA ATCGCCGTCGGGCATGC
	kan2 T1-R-B1	GCGCCTTGAGCCTGGCGTTCGTCATCGCATAGATGATTTAGA AAGGCTACCTGCCCATTC
	kan2 B1-R-T2	GACCACCAAGCGAAACGTTCGTCATCGCATAGATGATTTAGA AACAACCTTACCAGAGGG
	kan2 T2-R-B2	CGCCCCAGCTGGCAATGTTCGTCATCGCATAGATGATTTAGA AAGGCCGCTTGGGTGGAG
	kan2 B2-R-3'	AGGCTATTCGGCTATGGTTCGTCATCGCATAGATGATTTAGAA ACGGCCGGTAGAAAGGA
	Kan2 T1 RC	GAACGCCAGGCTCAAGGCGCGCATGCCCGACGGCGATTTC
	Kan2 B1 RC	GAACGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCTTTC
	Kan2 T2 RC	GAACATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGTTTC
	Kan2 B2 RC	GAACCATAGCCGAATAGCCTCTCCACCCAAGCGGCCTTTC
Array PCR	Array R	TCCTTTCTACCGGCCGTTTCTAAATCATCT
	Array R GG	TTTGGTCTCATCCTTTCTACCGGCCGTTTCTAAATCATCT
$CRISPR_{8xKan}$ Gibson	Kan1 B2-R-Kan2 T1	AAAACAATTCATCCAGGTTCGTCATCGCATAGATGATTTAGAA ATCGCCGTCGGGCATGC
Vector Gibson	Vector 5'R	TAGAGTTAAGTCAAAACAAAACCC

	Vector 3'F	GAAACGGCCGGTAGAAAGGA
Vector Golden Gate (GG)	Vector 5'R GG	TTTGGTCTCAGCGATGACGAACTAGAGTTAAGTCAAAACAAAACCC
	Vector 3'F GG	TTTGGTCTCAGTTCGTCATCGCATAGATGATTTAGAAACGGCCGGTAGAAAGGAGAAG
Genomic integrating CRISPR vector	pp 5'F	TGAGCCGACATTTTATTACCCTCT
	pp 3'R	TTACCTGAAAGCCAATCGCTG
$CRISPR_{3xBAP}$ GG	GG-R-BAP1	TTTGGTCTCATCGCATAGATGATTTAGAAACGGAATTCAAGGGGAC
	BAP1-R-BAP2	AGGTAGCGCAGGTGATGTTCGTCATCGCATAGATGATTTAGAAAATCGCGCGTTACCTCC
	BAP2-R-BAP3	TGAACATCCTCTACAGGTTCGTCATCGCATAGATGATTTAGAAAGAGAAGTGAACTTGTC
	BAP1 RC	GAACATCACCTGCGCTACCTGTCCCCTTGAATTCCGTTTC
	BAP2 RC	GAACCTGTAGAGGATGTTCAGGAGGTAACGCGCGATTTTC
	BAP3 RC GG	TTTGGTCTCAGAACTTGAAATTGGTTTATCGACAAGTTCACTTCTCTTTC
$CRISPR_{3xCRA-3xBAP}$ GG	GG-R-CRA1	TTTGGTCTCATCGCATAGATGATTTAGAAATCTCCGCGCTTGCTTC
	CRA1-R-CRA2	GCATAATGCAGATTGAGTTCGTCATCGCATAGATGATTTAGAAAGTCACTATGACCATGT
	CRA2-R-CRA3	TGCTTTGTATTGTGAAGTTCGTCATCGCATAGATGATTTAGAAACCCGGATTTTGACTGG
	CRA3-R-BAP1	CGAAATGTAGAAGATAGTTCGTCATCGCATAGATGATTTAGAAACGGAATTCAAGGGGAC
	CRA1 RC	GAACTCAATCTGCATTATGCGAAGCAAGCGCGGAGATTTC
	CRA2 RC	GAACTTCACAATACAAAGCAACATGGTCATAGTGACTTTC
	CRA3 RC	GAACTATCTTCTACATTTCGCCAGTCAAAATCCGGGTTTC
PCR screening of arrays	Array screen F	GGAGTTCTGAGGTCATTACTGGATCTA
	Array screen R	CAAATGTACGGCCAGCAACG
bap deletion donor DNA	BAP 5'F	AGCAGCTGAGAGCCTGAATG
	BAP 5'R	ACATGCCAGCACTTAATCTGA

	BAP 3’F	TCAGATTAAGTGCTGGCATGTGCACCCAATCCCTAACATTAAACA
	BAP 3'R	GGTTCGGGCACCTCATCATT
CRAΦ deletion donor DNA	CRA 5’F	ACAGGGCAGCCATTAACTGA
	CRA 5'R	TCTGAGACTGTAGCCTACGCA
	CRA 3’F	TGCGTAGGCTACAGTCTCAGAACGAAGTTATGTGCCACAAGAAA
	CRA 3'R	TCAGACGCAAGCGTGAAGAT
bap deletion screening	BAP checkF	GCCTCCTAAAATTGGGGGCT
	BAP checkR	CTTGGTTCTGCATTGGGTGC
CRAΦ deletion screening	CRA checkF	GACTTGCGTAGGCTTGGACT
	CRA checkR	GCATGTCATGGTTTGGTGGG
	CRA circular F	ATGAACGCGATCATTGCAGC
	CRA circular R	TACGGCCAATTGATCACCCA
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	Bla1-R12a-Bla2	TAATACCGCGCCACATGTCTAAGAACTTTAAATAATTTCTACTGTTGTAGATGGAGCTGAATGAAGCC
	Bla2-R12a-Bla3	ATACCAAACGACGAGCGTCTAAGAACTTTAAATAATTTCTACTGTTGTAGATCTCCCGTATCGTAGTT
	Bla3-R12a-Bla4	ATCTACACGACGGGGAGTCTAAGAACTTTAAATAATTTCTACTGTTGTAGATAGCCGGAAGGGCCGAG
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	Vector 5'R 12a GG	TTTGGTCTCACTTAGACTAGAGTTAAGTCAAAACAAAACCC
	Bla1 RC 12a	AGACATGTGGCGCGGTATTATCCCGTATTGACGCCGATCT
	Bla2 RC 12a	AGACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCATCT
	Bla3 RC 12a	AGACTCCCCGTCGTGTAGATAACTACGATACGGGAGATCT
	Bla4 RC 12a	TTTGGTCTCAAGACCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTATCT

Table S2: CRISPR spacers

Name	Sequence
Kan1_T1	GGTCGATCAGGGAGGATATCGGGGAAGAACAG
Kan1_T2	GTCGATACTATGTTATACGCCAACTTTGAAAA
Kan1_B1	TTGCATTCTAAAACCTTAAATACAGAAAACAG
Kan1_B2	AAGCGAGCTCGGTACTAAAACAATTCATCCAG
Kan2_T1	TCGCCGTCGGGCATGCGCGCCTTGAGCCTGGC
Kan2_T2	CAACCTTACCAGAGGGCGCCCCAGCTGGCAAT
Kan2_B1	GGCTACCTGCCCATTCGACCACCAAGCGAAAC
Kan2_B2	GGCCGCTTGGGTGGAGAGGCTATTCGGCTATG
CRA1	TCTCCGCGCTTGCTTCGCATAATGCAGATTGA
CRA2	GTCACTATGACCATGTTGCTTTGTATTGTGAA
CRA3	CCCGGATTTTGACTGGCGAAATGTAGAAGATA
BAP1	CGGAATTCAAGGGGACAGGTAGCGCAGGTGAT
BAP2	ATCGCGCGTTACCTCCTGAACATCCTCTACAG
BAP3	GAGAAGTGAACTTGTCGATAAACCAATTTCAA
Random	TAGGGGAAAGCCTACTAGCCGGAGTGTTGCGA

DNA sequence of sample genomically integrating vector, pp2.1-CRISPR$_{8xKan}$-Spec-pp2.2

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DNA sequence of sample replicating vector, pBAV1spec-CRISPR$_{3xCRA}$-Spec

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