

Supplementary Material

Pairwise selection assembly for sequence-independent construction of long-length DNA

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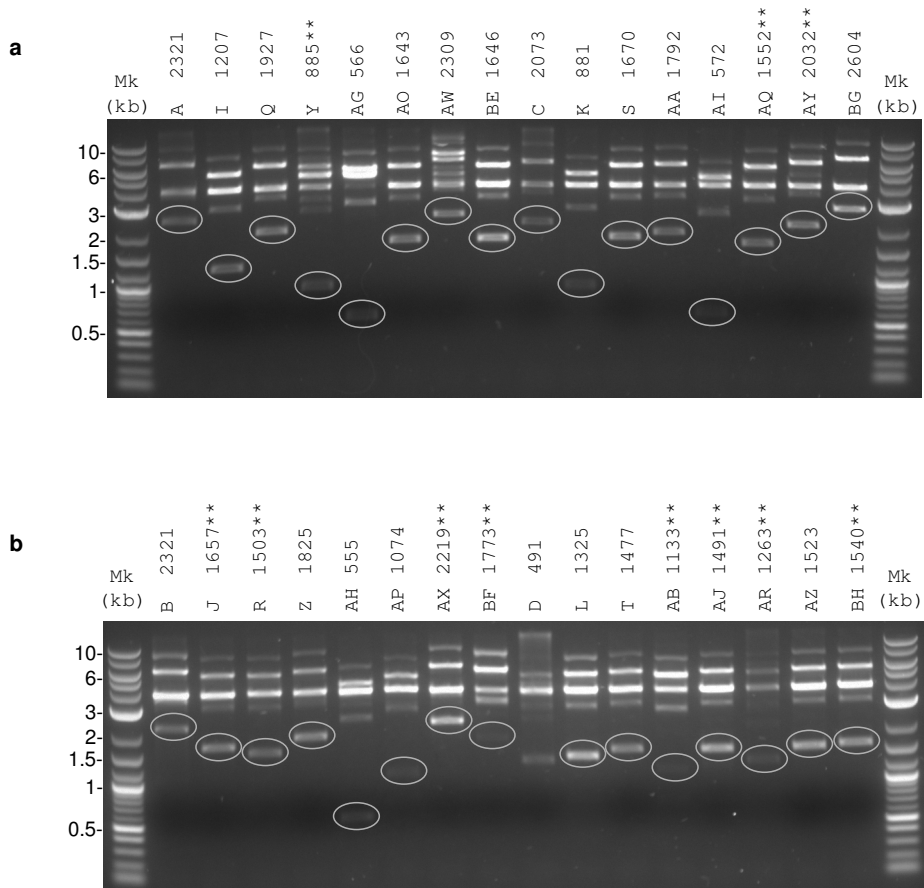
Table S1: PSA cassette sequences. Intergenic regions in CK and TS cassettes containing abutting BsaI, NotI, and BsmBI sites are underlined.

<p>CK cassette</p>	<p>TTACGCCCCGCCCTGCCACTCATCGCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGC CATCACAAACGGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATAT TTGCCCATGGTGAAAACGGGGCGAAGAAGTTGTCCATATTGGCCACGTTTAAATCAAACTGGTGAA ACTCACCCAGGGATTGGCTGATACGAAAAACATATTCTCAATAAACCCCTTTAGGGAAATAGGCCAGGT TTTCACCGTAACACGCCACATCTTGCGAATATATGTGTAGAACTGCCGAAATCGTCGTGGTATTCA CTCCAGAGCGATGAAAACGTTTCAGTTTGCTCATGAAAAACGGTGTAAACAAGGGTGAACACTATCCCA TATCACCAGTACCCTTTTTCATTGGCATAACGGATCCGGATGAGCATTTCATCAGGCGGGCAAGAA TGTGAATAAAGGCCGGATAAAAACCTTGCTTATTTTTCTTTACGGTCTTTAAAAAAGGCCGTAATATCC AGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATG CCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTTGTTCAGAGACC GCGGCCGCTCTC TTGAGCCATATTC AACGGGAAACGTCCTTGCTCGAGGCCGCGATTAAATCCAACATGGATGCTGATTT ATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGA AGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAG ATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCC TGATGATGCATGGTTACTCACCCTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATC CTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTTGCATTTCGATTCCTGTT TGTAATTGTCCTTTT AACAGCGATCGCGTATTTTCGCCTCGCTCAGGCGCAATCACGAATGAATAACGG TTTGTTGATGCGAGTGATTTTGTGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAA TGCATAAATGTTGCCATTTCTACCGGATTCAGTGTCACTCATGGTGATTTCTCACTTGATAACCTT ATTTTTGACGAGGGGAAATTAATAGGTTGATTTGATGTTGGACGAGTCGGAATCGCAGACCGATAACA GGATCTTGCCATCCTATGGAACGCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTTCAA AATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAA</p>
<p>TS cassette</p>	<p>TTAGGTCGAGGTGGCCCGGCTCCATGCACCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGG CGCCTACAATCCATGCCAACCCTGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGC GGTCCAGTGATCGAAGTTAGGCTGGTAAGAGCCCGGAGCGATCCTTGAAGCTGCCTGATGGTCTGTC ATCTACCTGGCGGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCCGGGAAGCGAGAAGAATCA TAATGGGGAAGGCCATCCAGCCTCGCGTCCGGAACGCCAGCAAGACGTAGCCCAGCGCGTCCGGCCG ATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGACCAGTGACGAAGGCTTGAGC GAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCGATCATCGTCGCGCTCCAGCGAAAGCGGT CCTCGCCGAAAATGACCCAGAGCGCTGCCGGCACCCTGTCCTACGAGTTGCATGATAAAGAATACAGTC ATAAGTTCCGGGACGATAGTCATGCCCGCCACCCTGGGAAGGAGCTGACTGGGTTGAAGCTTCTCAA GGCATCGGTGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTGGTTGAGGC CGTTGAGCACCGCCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACG GGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCAGTGGCGAGCCCAGTCTTCCCC ATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTATGCCGGCCACGATGC GTCCGGCGTAGAGGATCCACAGGACGGGTGTGGTCCCATGATCGCGTAGTCGATAGTGGCTCCAAGT AGCGAAGCGAGCAGGACTGGGCGGGCCAAAGCGGTCCGACAGTGTCCGAGAACGGGTGCGCATAG AAATTGCATCAACGCATATAGCGCTAGCAGCACGCCATAGTACTGGCGATGCTGTGCGAATGGACGA TATCCCGCAAGAGGCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGACGGTG CCGAGGATGACGATGAGCGCATTGTTAGATTTCAAGAGACC GCGGCCGCTCTCTT GAGGGAAGCGG TGATCGCCGAAGTATCGACTCAACTATCAGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACG TTGCTGGCCGTACATTTGTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGATTT GCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACCGCGGAGCTTTGATCAACGACCTTTTGAAA CTTCCGGCTTCCCCTGGAGAGAGCGAGATTTCCCGCTGTAGAAGTCACCATTGTTGTGACAGCAGC ATCATTCCGTGGCGTTATCCAGCTAAGCGCAACTGCAATTTGGAGAATGGCAGCGCAATGACATTCT TGCTGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAAC ATAGCGTTGCCTTGGTAGGTCCAGCGCGGAGGAACCTTTGATCCGGTTCCTGAACAGGATCTATTT GAGGCGCTAAATGAAACCTTAACGCTATGAAACTCGCCGCCGACTGGGCTGGCGATGAGCGAAATGT AGTGCTTACGTTGTCCGCATTTGGTACAGCGCAGTAAACCGGAAAATCGCGCAAGGATGTCGCTG CCGACTGGGCAATGGAGCGCCTGCCGGCCAGTATCAGCCCGTCATACTTGAAGTCAGCAGGCTTAT CTTGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAA AGGCGAGATACCAAGGTAGTCGGCAATAA</p>

Table S2: PSA tags and blocking oligo sequences. BsmBI and BtgZI restriction sites are underlined, and marker start codons are shown in bold. Overhangs generated within the sequence being constructed are indicated by NNNN.

upstream tag	TT CAT <u>GAGACGATCTCCTTCTCTT</u> GATGGCTGTAATAATAGCTCTAGGG <u>GCGAT</u> GTTAAGACAACNNNN
downstream tag	NNNNGAGACGTTGACAACATGAAGTAAACAGCGTAAGATGTACCACATGAAATT <u>GCGATGAGGAAATCTATGAG</u>
L block upstream	CATGAGACGATCTCCTTCTCTT <u>GATGGCTGTAATAATAGCTCTAGGGGCGATGTTAAGAC</u>
L block downstream	CGAGACGTTGACAACATGAAGTAAACAGCGTAAGATGTACCACATGAAATT <u>GCGATGAGG</u>
R block upstream	GTCTTAACATCGCCCTAGAGCTATTATTACAGCCATCAAGAGGAAGGAGATCGTCTCATG
R block downstream	CATAGATTTCTCATCGCAATTTCATGTGGTACATCTTACGCTGTTTACTTCATGTTGTC

Figure S1: PSA level 0 sub-fragment block/digest. 64 sub-fragments from PSA level 0 in pCK were blocked and digested, and 15 μ l (225 ng) of the heat-inactivated digest was run on a 0.7% agarose gel for analysis. Sub-fragments with internal sites that require blocking are indicated with asterisks (**), and all sub-fragment sizes are indicated. L sub-fragments (**a** and **c**) were digested with 1 unit of BsmBI, and R sub-fragments (**b** and **d**) were digested with 2 units of BtgZI. DNA marker (Mk) is indicated and expected insert bands are circled.



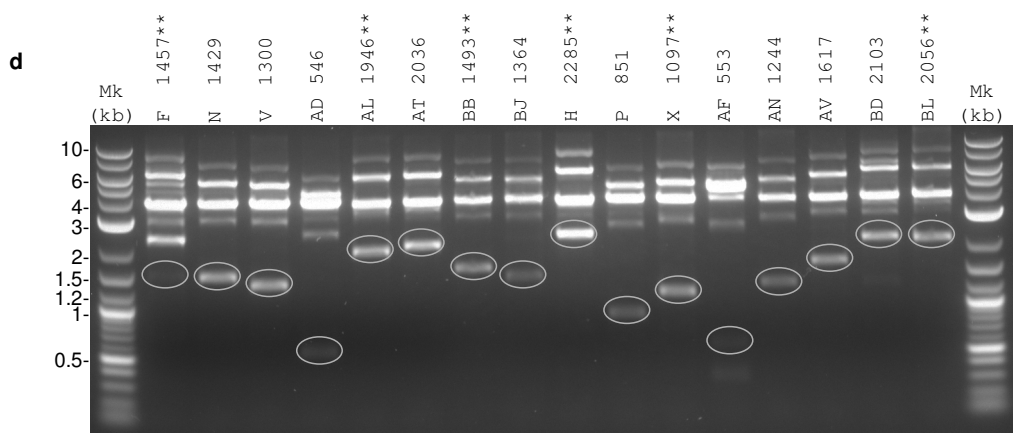
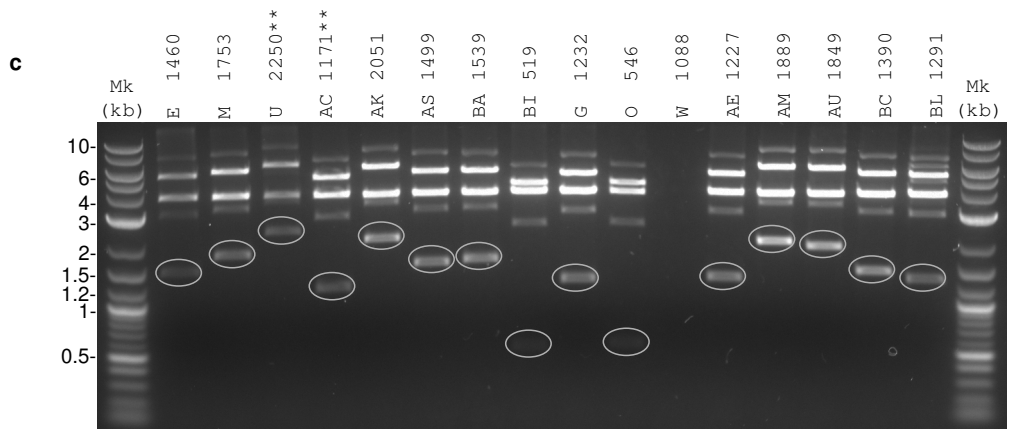
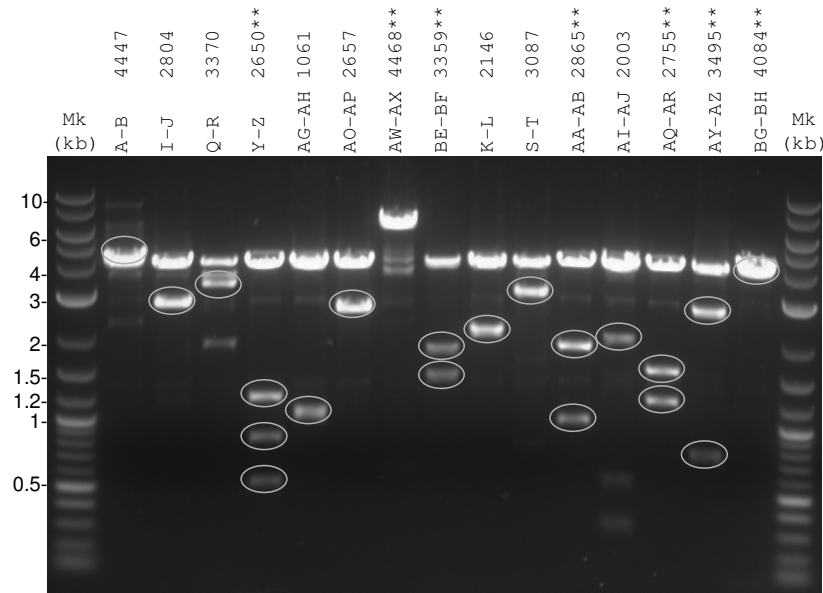


Figure S2: PSA level 1 product screening for selection in culture. Approximately 400 ng of prepped DNA was digested with BsmBI and run on a 0.7% agarose gel for analysis (**a,c**). Products containing internal BsmBI sites are indicated with asterisks (**), and expected fragment sizes are given in (**b,d**). DNA marker (Mk) is indicated and expected insert bands are circled.

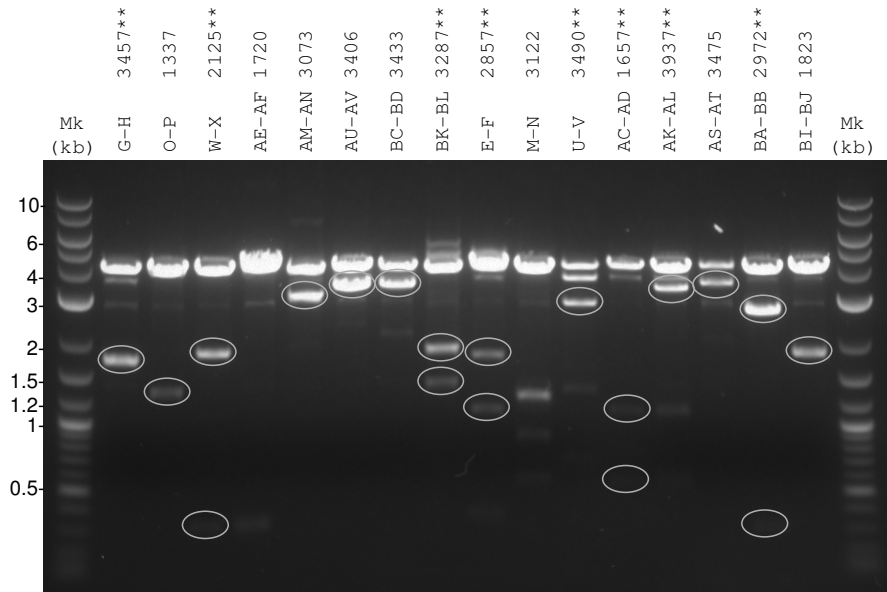
a



b

sub-fragment	expected sizes for internal cuts (**)	sub-fragment	expected sizes for internal cuts (**)
A-B		C-D	not pictured
I-J		K-L	
Q-R		S-T	
Y-Z	563, 862, 1230	AA-AB	1008, 1862
AG-AH		AI-AJ	
AO-AP		AQ-AR	1199, 1561
AW-AX	664, 3809	AY-AZ	789, 2711
BE-BF	1499, 1865	BG-BH	87, 4002

c



d

sub-fragment	expected sizes for internal cuts (**)
E-F	1095, 1763
M-N	
U-V	696, 2799
AC-AD	10, 548, 1104
AK-AL	681, 3347
AS-AT	
BA-BB	308, 2669
BI-BJ	

sub-fragment	expected sizes for internal cuts (**)
G-H	1713, 1734
O-P	
W-X	312, 1818
AE-AF	
AM-AN	
AU-AV	
BC-BD	
BK-BL	1402, 1890

Figure S3: PSA level 1 sub-fragment block/digest. 32 sub-fragments from PSA level 1 in pTS were blocked and digested, and 15 μ l (225 ng) of the heat-inactivated digest was run on a 0.7% agarose gel for analysis. Sub-fragments with internal sites that require blocking are indicated with asterisks (**), and all sub-fragment sizes are indicated. L sub-fragments (a) were digested with 1 unit of BsmBI, and R sub-fragments (b) were digested with 2 units of BtgZI. DNA marker (Mk) is indicated and expected insert bands are circled.

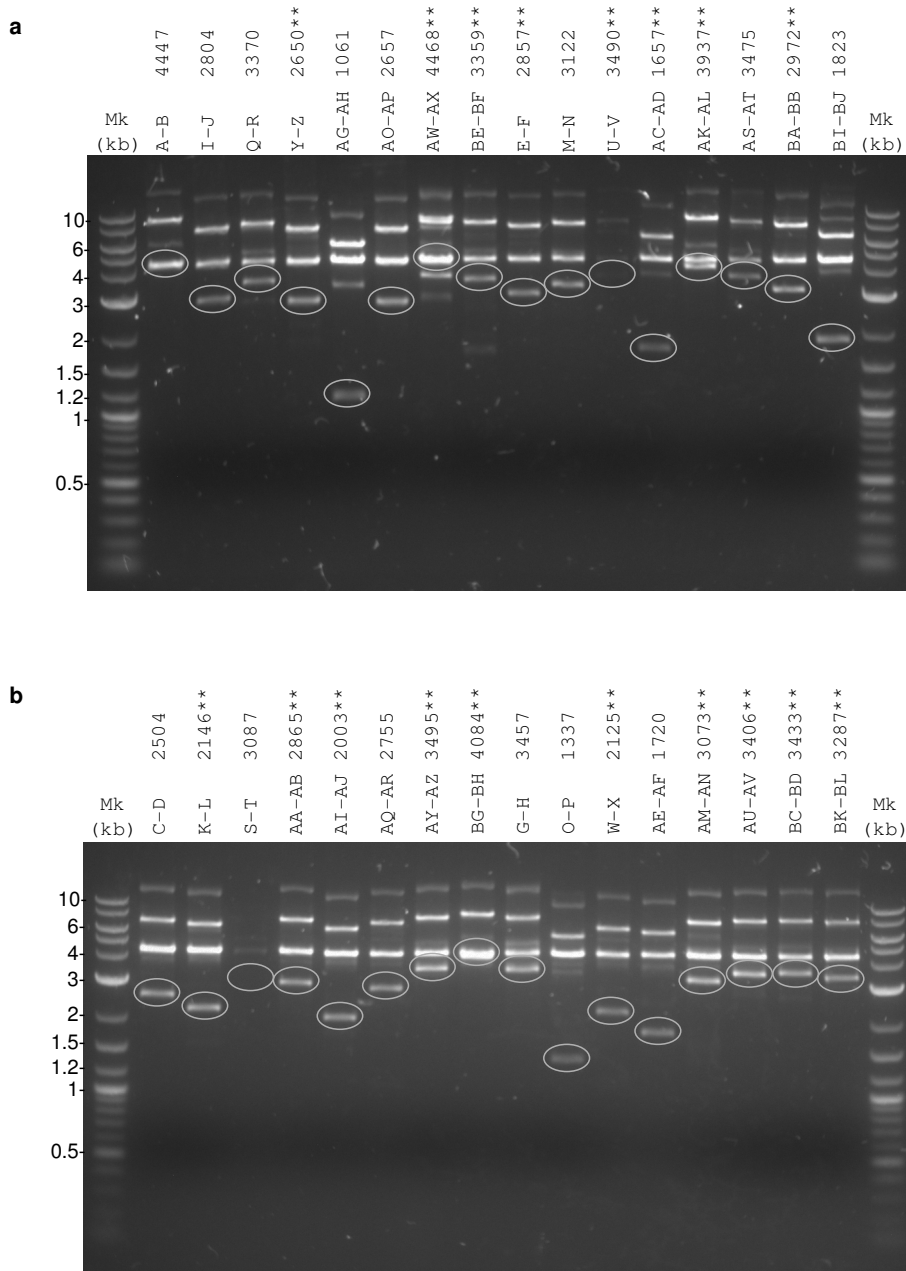
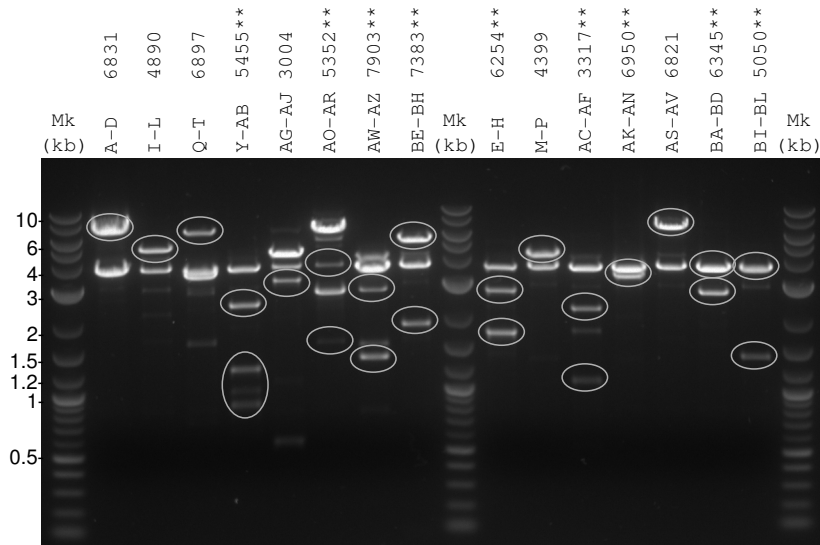


Figure S4: PSA level 2 product screening for selection in culture. **(a)** Approximately 400 ng of prepped DNA was digested with BsmBI and run on a 0.7% agarose gel for analysis. Products containing internal BsmBI sites are indicated with asterisks (**), and expected fragment sizes are given in **(b)**. DNA marker (Mk) is indicated and expected insert bands are circled.

a



b

sub-fragment	expected sizes for internal cuts (**)	sub-fragment	expected sizes for internal cuts (**)
A-D		E-H	15, 1734, 1763, 2743
I-L		M-P	
Q-T		U-X	not pictured
Y-AB	862, 1008, 1230, 2360	AC-AF	10, 1104, 2208
AG-AJ		AK-AN	3347, 3608
AO-AR	1561, 3796	AS-AV	
AW-AZ	1388, 2711, 3809	BA-BD	2669, 3681
BE-BH	87, 1865, 5436	BI-BL	1402, 3653