

Table S3. DNA substrates and primers using for phage engineering

Name	G-block coordinates ¹	Primers ²	Deletion Coordinates ³
SbashΔ30	26,704 – 26,953; 28,001 – 28,250	Sbash30KO FWD: GATGACTGACCGGCGAACCCATC Sbash30KO REV: CCACGGCTCGCTACCGCC	26,954 – 28,000
SbashΔ31	27,812 – 28,061; 28,488 – 28,702	Sbash31KO FWD: ACGATGACGATCGTGATGGATGGCG Sbash31KO REV: ACAGTGC GCGGCTGGACC	28,062 – 28,487
SbashΔ34	28,865 – 29,108; 29,223 – 29,472	Sbash34KO FWD: GCGACGCGGTTGCCGATCT Sbash34KO REV: CTACCGGGATACGCTACCGCCG	29,109 – 29,222
SbashΔ35	29,273 – 29,522; 30,810 – 31,059	Sbash35KO FWD: GACTTTACCCTAAGCAAGGGTGTAGTTC Sbash35KO REV: GATGTGATCGCGCCTCGAG	29,523 – 30,809
SbashΔ37	31,380 – 31,629; 31,765 – 32,014	Sbash37KO FWD: ACATCCGCGACGGAATCTCGTT Sbash37KO REV: GGGTGATGACTACTGCGCGC	31,630 – 31,764
SbashΔ38	31,586 – 31,835; 32,499 – 32,748	Sbash38KO FWD: CGAAAGGGTGTAGTCGTA CTCAAATCTCCT Sbash38KO REV: ACCACCATCGGCATCATCCAGC	31,836 – 32,498
SbashΔ39	32,352 – 32,601; 32,959 – 33,208	Sbash39KO FWD: CGCGATGGACGTGATCCAGAACG Sbash39KO REV: GATTCGAACCTGCGTAGGCGTAAGC	32,602-32,958
CrossroadsΔ132	68,020 – 68,269; 69,080 – 69,329	Crossroads132KO FWD: CCAGTCTGGCGGCTGGTCGG Crossroads132KO REV: TCAACCCTGGCGCTCGCGG	68,270 – 69,079
CrossroadsΔ141	72,778 – 73,027; 74,228 – 74,477	Crossroads141KO FWD: CCGTGGTTCGTGACCTTCATTTTCGT Crossroads141KO REV: CGCTGGCTTAAGCGTCGCTAGT	73,028 – 74,227
CrossroadsΔ140-141	71,743-71,942; 74,249-74,448	Crossroads140_141KO_Fwd_V2: GCCATGCGACGGGGACCG Crossroads140_141KO_Rev_V2: GATACCGACACATAACGAGACCTAAGCG	71,943-74,248
CrossroadsΔ139-141	70,510-70,766; 74,273-74,515	CrossroadsKO139_141_Fwd: CGTCGTCTGCCTCGGTGTC CrossroadsKO139_141_Rev: GACACGAACCAAGCAGCTC	70,767-74,272

¹Each G-block was designed to include ~250bp upstream and downstream of the deletion end points, and the coordinates for each are shown.

²Primers are designed to anneal to the flanks of the G-block substrate and were used to amplify the substrate prior to co-electroporation with phage genomic DNA.

³Deletion coordinates correspond to the wild-type phage sequence