DNA Camouflage

Supplementary Information

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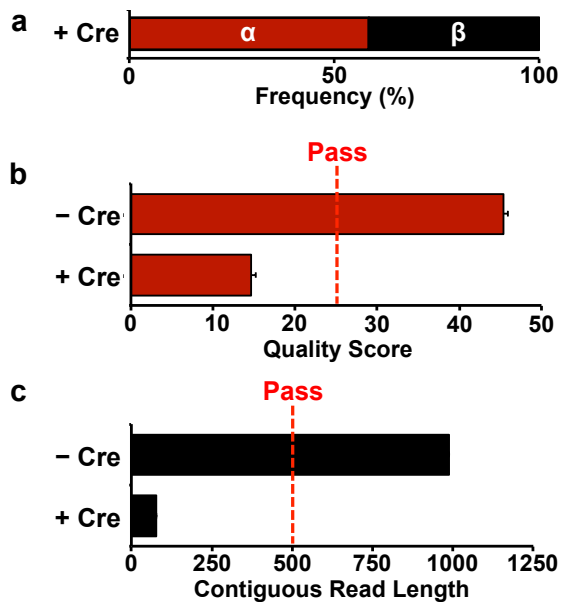
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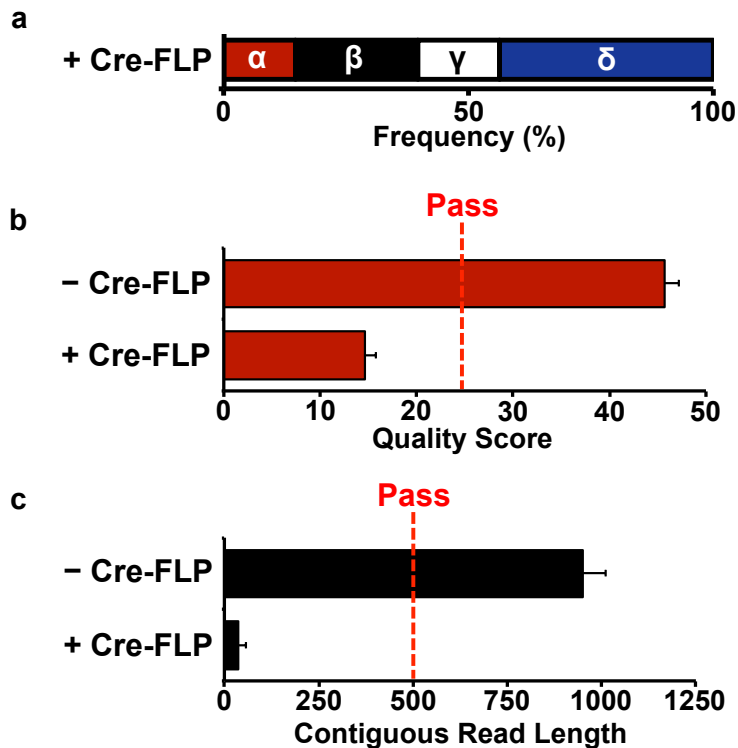
Supplementary Figure 1 DNA camouflage with the 2-state device. (**a**) In the presence of Cre, DSD-2[α] was randomly shuffled between α and β states within a cellular population. (**b**) Quality score (QS) values of sequencing reactions of DSD-2[α] maintained in the absence and presence of Cre. (**c**) Contiguous read length (CRL) scores of sequencing reactions of DSD-2[α] maintained in the absence and presence of Cre. All experiments were performed in triplicate, error bars represent ± 1 standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.
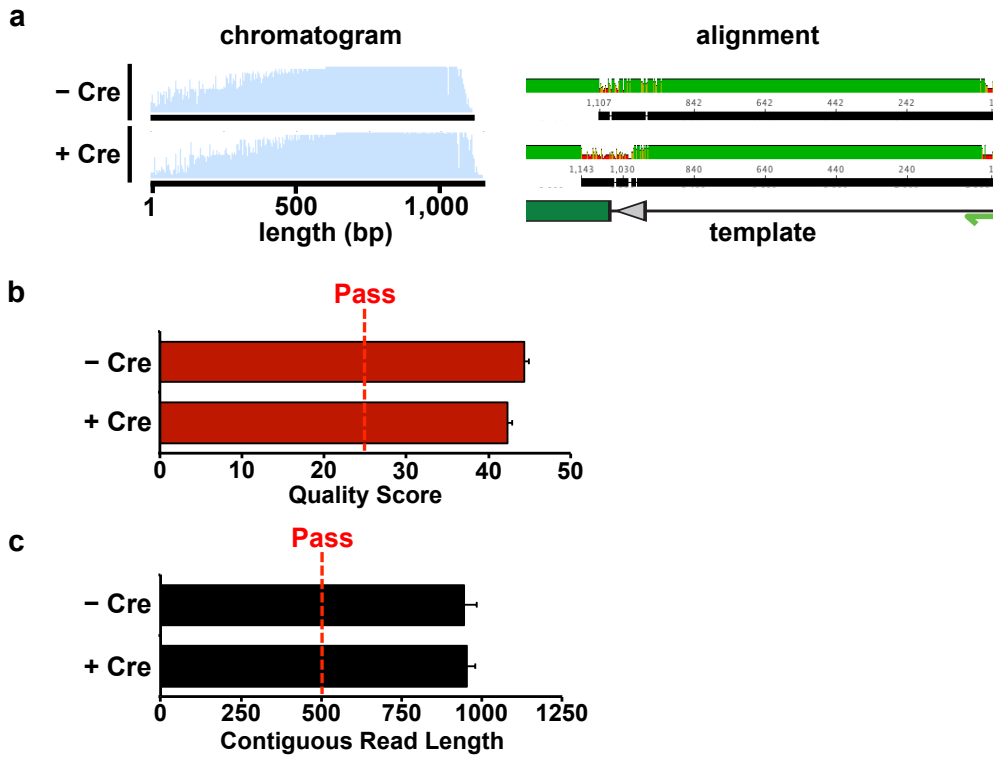
Supplementary Figure 2 DNA shuffling does not comprise sequencing outside of DSDs. (**a**) Sequencing of 1 kb downstream of DSD-2[α] produces high quality sequencing reads that align with the template in the absence and presence of Cre. (**b**) Quality score (QS) and (**c**) Contiguous read length (CRL) scores of sequencing reactions shown in **a**. All experiments were performed in triplicate, error bars represent ± 1 standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.
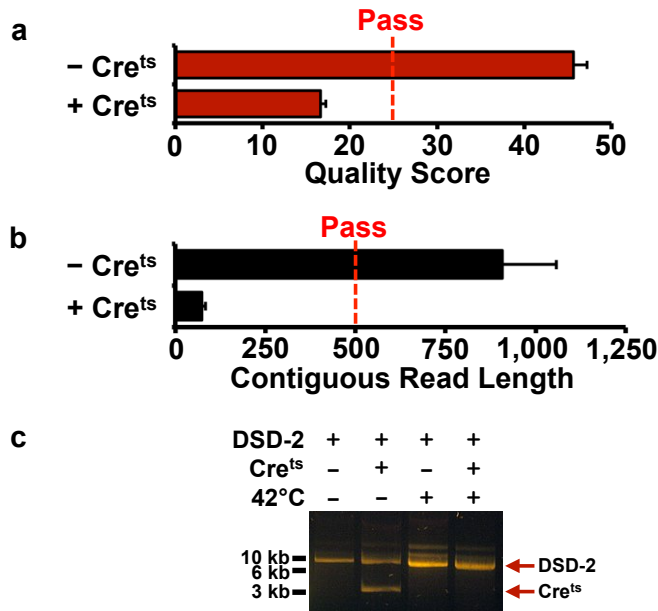
Supplementary Figure 3 DNA camouflage with a switchable 2-state device. (**a**) Quality score (QS) and (**b**) Contiguous read length (CRL) scores of sequencing reactions of DSD-2[α] maintained in the absence and presence of Cre[ts]. (**c**) The plasmid encoding Cre[ts] can be cured out of cells by growing cells at 42°C. All experiments were performed in triplicate, error bars represent ± 1 standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.

Supplementary Figure 4 DNA camouflage with the 4-state device. (**a**) In the presence of Cre and Flp, DSD-4[α] was randomly shuffled between α, β, γ, and δ states within a cellular population. (**b**) Quality score (QS) values of sequencing reactions of DSD-4[α] maintained in the absence and presence of Cre and Flp. (**c**) Contiguous read length (CRL) scores of sequencing reactions of DSD-4[α] maintained in the absence and presence of Cre and Flp. All experiments were performed in triplicate, error bars represent ± 1 standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.
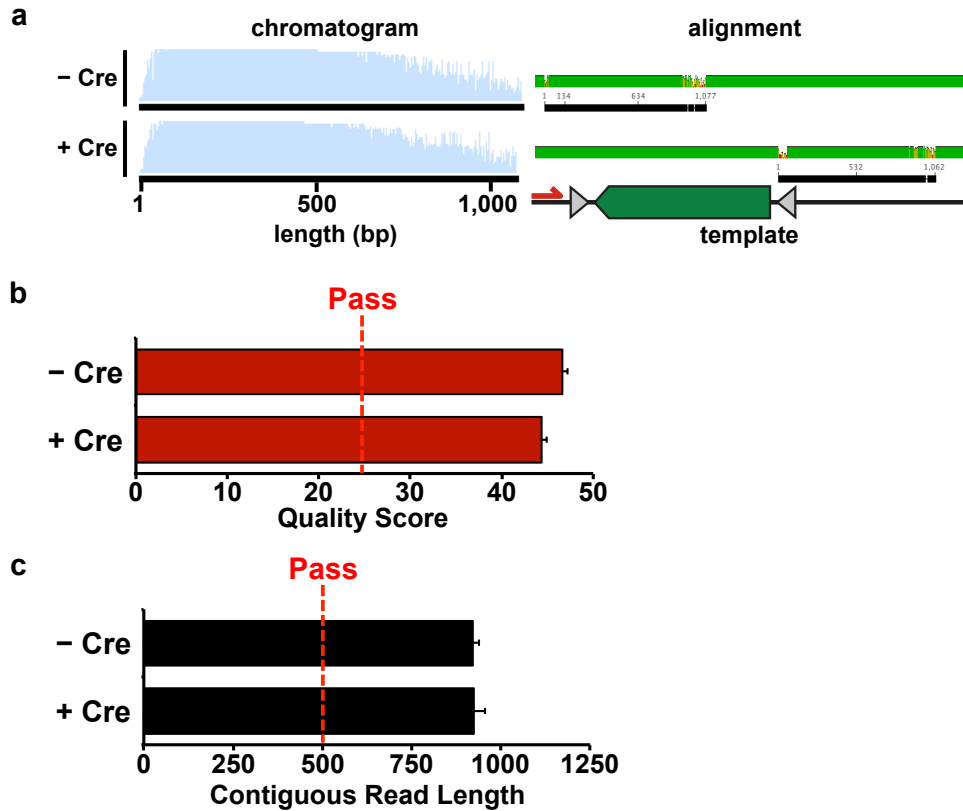
Supplementary Figure 5 Shuffling of DSD-2[α]^{p15A} leads to data excision. (**a**) When DSD-2[α] is placed on a multi-copy plasmid containing a p15A origin (DSD-2[α]^{p15A}), data is maintained in the absence of Cre but excised in the presence of Cre. (**b**) Quality score (QS) and (**c**) Contiguous read length (CRL) scores for sequence reactions shown in **a**. All experiments were performed in triplicate, error bars represent ± 1 standard deviation, and all sequencing reactions and QS/CRL measurements were performed by GENEWIZ Inc. under blind experimental conditions. QS scores below 24 and CRL scores below 500 indicate problems with sequencing results.
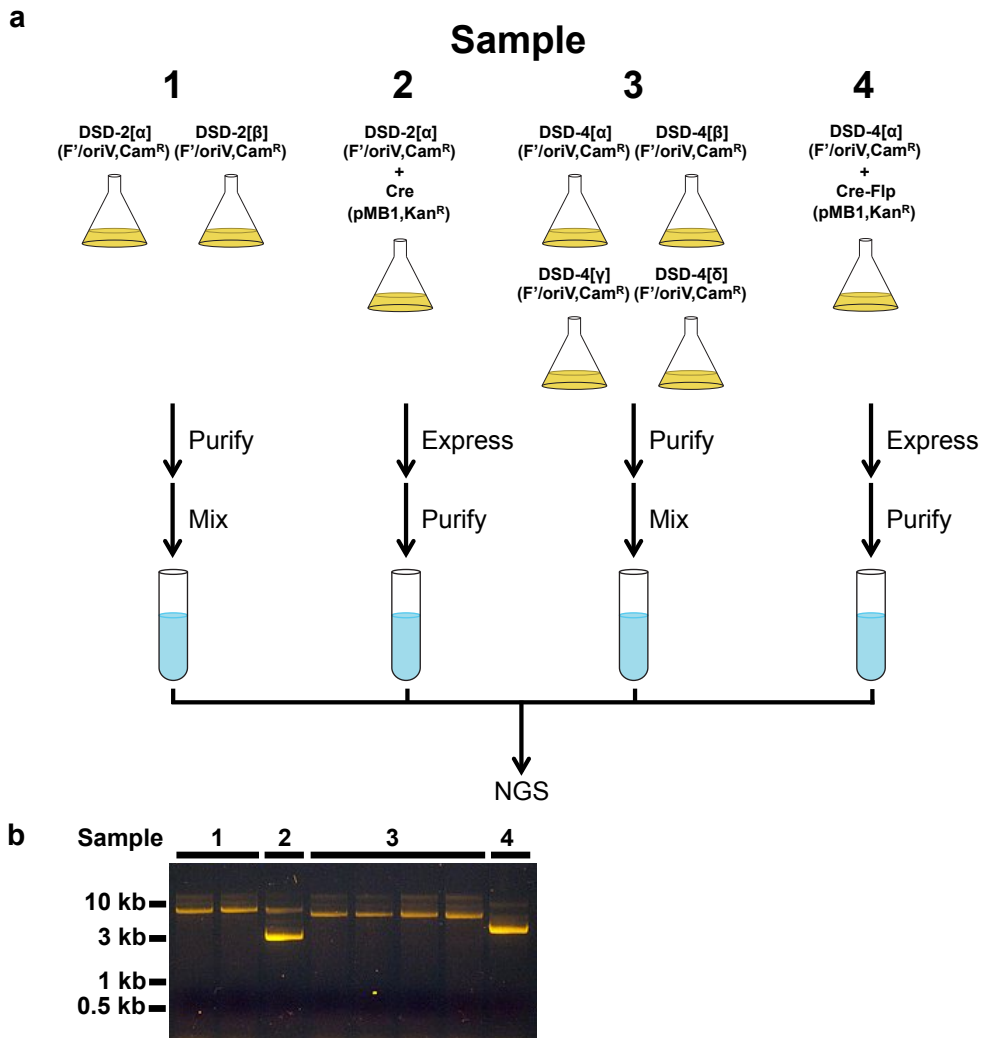
Supplementary Figure 6 Next-generation sequencing (NGS) of 2-state and 4-state devices. (**a**) Samples 1 and 3: DSD-2[α/β] and DSD4-[α/β/γ/δ] were each separately prepared, purified, and mixed at equal concentration in dH_2O. Sample 2 and 4: DSD-2[α] and DSD-4[α] were shuffled with Cre and Cre-Flp recombinases respectively, and then purified, and stored in in dH_2O. (**b**) Samples from (**a**) run on an agarose gel to demonstrate the purity.
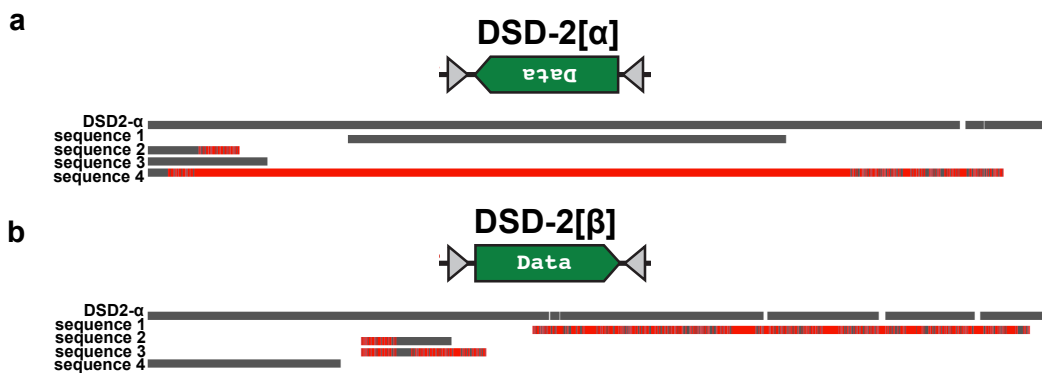
Supplementary Figure 7 NGS identified sequences for Sample 1. Sequences identified by the outside party for Sample 1 (**Supplementary Table 4**) aligned against (**a**) DSD-2[α] and (**b**) DSD-2[β] templates. Gray bars represent areas of perfect sequence alignment and red bars represent areas of sequence misalignment.
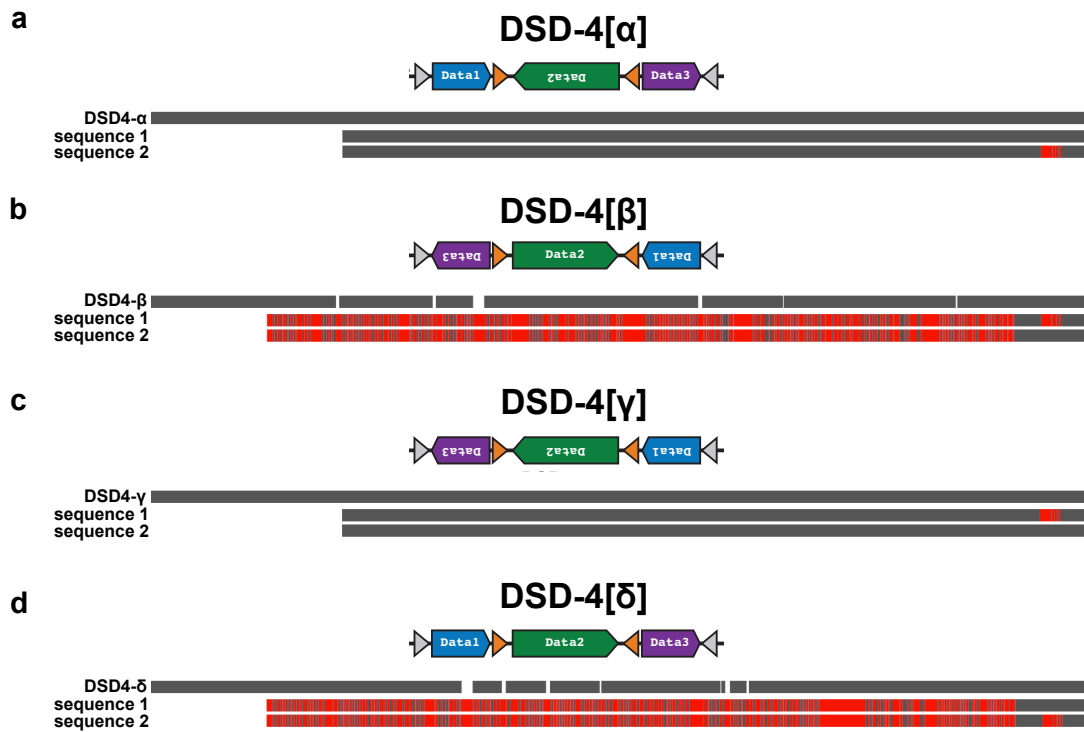
Supplementary Figure 8 NGS identified sequences for Sample 3. Sequences identified by the outside party for Sample 3 (**Supplementary Table 4**) aligned against (**a**) DSD-4[α], (**b**) DSD-4[β], (**c**) DSD4-[γ], and (**d**) DSD4-[δ] templates. Gray bars represent areas of perfect sequence alignment and red bars represent areas of sequence misalignment.
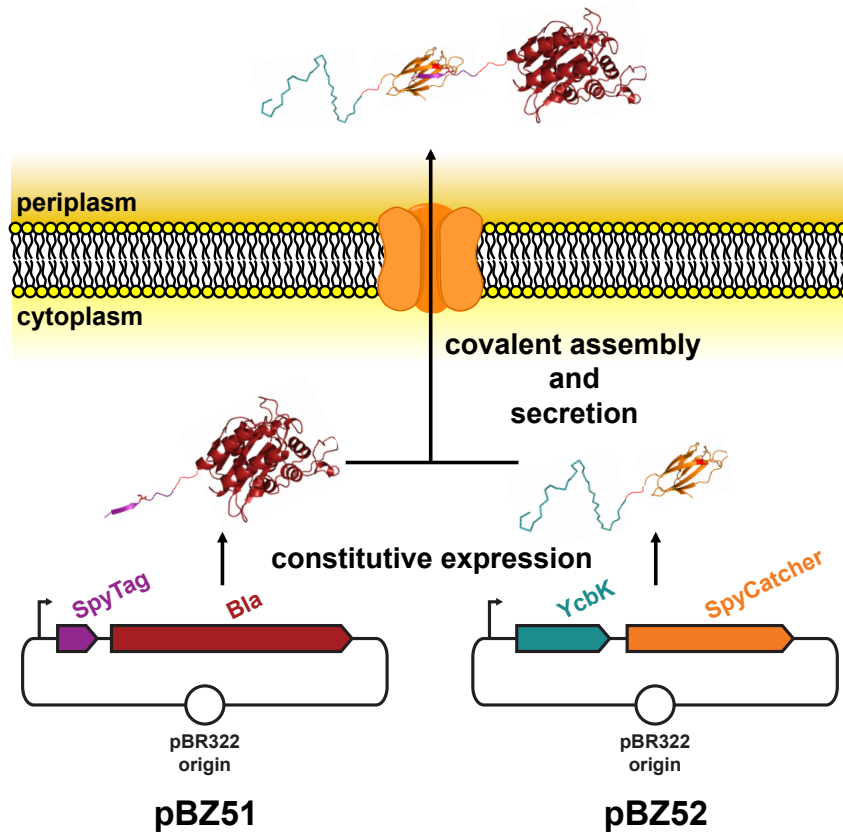
Supplementary Figure 9 Schematic of the addiction module.
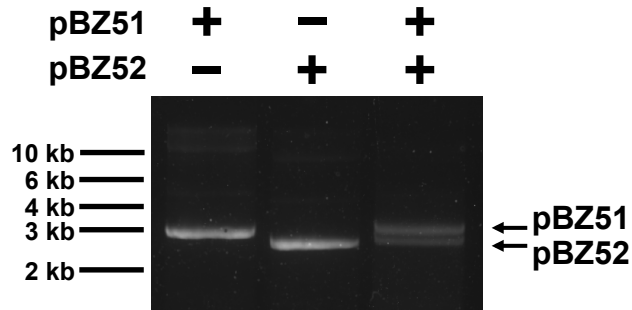
Supplementary Figure 10 pBZ51 and pBZ52 are stably maintained in *E. coli*. Cells transformed with pBZ51 (selected on Kan), pBZ52 (selected on Kan), and pBZ51 + pBZ52 (selected on Amp) were grown overnight, and plasmid DNA was extracted and run on a 1% agarose gel. Cells co-transformed with pBZ51 and pBZ52 were able to stably maintain both plasmids under Amp selection.

Sample	1	2	3	4
Total Sequences	2,035,696	2,827,422	3,762,818	2,665,635
% GC	48	49	47	46

Supplementary Table 1 NGS analysis of samples 1-4. Over 2 million ~300 bp reads were produced from NGS sequencing of samples 1-4 (**Supplementary Fig. 6**), with GC contents similar to expected values. DSD-2[α/β]: 9,549 bp/47.8% GC, Cre: 4,452 bp/49.8% GC, DSD4-[α/β/γ/δ]: 8,204 bp/46.8% GC, Cre-Flp: 5,769 bp/46.9% GC.

Sample	1	2	3	4
Sequence size	4,484,782	109,143	4,575,261	238,314
Number of scaffolds	711	248	500	536
% GC	50.7	49.3	50.7	50.1
Shortest contig size	301	300	306	300
Median sequence size	3,897	360	3,943	390
Mean sequence size	6,307.7	440.1	9,150.5	444.6
Longest contig size	51,023	5,385	93,737	5,397
Number of subsystems	564	2	576	2
Number of coding sequences	4,300	64	4,410	190
Number of RNAs	34	0	30	0

Supplementary Table 2 Assembly of NGS reads from samples 1-4. Here, the statistics of the assembled scaffolds from are shown.

Sample	Total Scaffolds	Aligned Scaffolds	% Aligned	Identified Vectors
1	711	12	1.7	• pBluescriptR (Amp^R) • pDONR221 (Kan^R) • pOTB7 (Cam^R)
2	248	3	1.2	• pBluescriptR (Amp^R) • pDONR221 (Kan^R) • pOTB7 (Cam^R)
3	500	10	2.0	• pBluescriptR (Amp^R) • pDONR221 (Kan^R) • pOTB7 (Cam^R)
4	536	6	1.1	• pBluescriptR (Amp^R) • pDONR221 (Kan^R) • pOTB7 (Cam^R) • pK7-GFP (Amp^R)

Supplementary Table 3 Identification of annotated and assembled samples 1-4. Since there was no prior information provided regarding samples 1-4, the assembled scaffolds (**Supplementary Table 2**) were blasted against a plasmid database (http://plasmid.med.harvard.edu/) by the outside party. Identified hits were based on >90% sequence identity and a minimum of 100 bp alignment length.

Sample	Sequence Number	Identified Insert
1	1	TTCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTG TGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGG GCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCA CTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACGCA TGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTAAATTG
	2	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAGCGGAT ACAATTGTGAGCGGATAACAATTTACATTGTGAGCGGATAACAAGATACTGAGCACATCAGCAGGACGCACTGACC
	3	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAGCGGAT ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTAGGTATCTGGCACTACG TTCAGGTAACCTGAAGCTCGAATCCAGTACTCGACGTC
	4	ATAACTTCGTATAATGTATGCTATACGAAGTTATGCTAGCTATGGAGAAACAGTAGAGAGTTGCGATAAAAAGCGTCAGGTAGGATCC GCTAATCTTATGGATAAAAATGCTATGGCATAGCAAAGTGTGACGCCGTGCAAATAATCAATGTGGACTTTTCTGCCGTGATTATAGA CACTTTTGTTACGCGTTTTTGTCATGGCTTTGGTCCCGCTTTGTTACAGAATGCTTTTAATAAGCGGGGTTACCGGTTTGGTTAGCGA GAAGAGCCAGTAAAAGACGCAGTGACGGCAATGTCTGATGCAATATGGACAATTGGTTTCTTCGTCGGTCTGATTATTAGTCTGGG
2		no insert sequence identified
3	1	TTCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTG TGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGG GCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCA CTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACGCA TGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTAAATTGTTATCCGC TCACAATTGTATCCGCTCATGAATTAATTCTTAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCAGGCATTGATGGAATCGTAGT CTCAATAACTTCGTATAGCATACATTATACGAAGTTAT
	2	TTCATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCTCTTTTCGTTGGGATCTTTCGAAAGG GCAGATTGTGTGGACAGGTAATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTCTGCTAGTT GAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTTGATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTG TGAGTTATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTTAACTCGATTCTATTAACAAGG GTATCACCTTCAAACTTGACTTCAGCACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTCGG GCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAG TGTTGGCCATGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTGCATCACCTTCACCCTCTCCA CTGACAGAAAATTTGTGCCCATTAACATCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACGCA TGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCTTGTTATCCGCTCACAATGTAAATTGTTATCCGC TCACAATTGTATCCGCTCATGAATTAATTCTTAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCTCATCGAGCATCAAATGAAA CTGCATAACTTCGTATAGCATACATTATACGAAGTTAT
4		no insert sequence identified

Supplementary Table 4 Identified sequences by the outside party following NGS analysis and sequence assembly. These sequences were assembled once the sequence of the backbone vectors were provided to the outside party.

Construct	Plasmid Name	Plasmid Backbone	Sequence	Legend
Cre	pBZ14	pET28a (pBR322 origin and Kan^R only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATTTACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACGTAATCTGGCATTTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACTTAGCCTGGGGGTAACTAAACTGGTCGAGCGATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATTAAGTCGACAACCTAGGAAAAACCTGAGGAAAATGCATAGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGCTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAA	$P_{LtetO-1}$ Cre Terminator Spacer
Cre^{ts}	pBZ20	pKD46 (origin and Amp^R only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATTTACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACGTAATCTGGCATTTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACTTAGCCTGGGGGTAACTAAACTGGTCGAGCGATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATTAAGTCGACAACCTAGGAAAAACCTGAGGAAAATGCATAGCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGCTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAA	$P_{LtetO-1}$ Cre Terminator Spacer
Cre-Flp	pBZ17	pET28a (pBR322 origin and Kan^R only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCACATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCAATTTACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGGACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAAGTTGAATAACCGGAAATGGTTTCCCGCAGAACCTGAAGATGTTCGCGATTATCTTCTATATCTTCAGGCGCGCGGTCTGGCAGTAAAAACTATCCAGCAACATTTGGGCCAGCTAAACATGCTTCATCGTCGGTCCGGGCTGCCACGACCAAGTGACAGCAATGCTGTTTCACTGGTTATGCGGCGGATCCGAAAAGAAAACGTTGATGCCGGTGAACGTGCAAAACAGGCTCTAGCGTTCGAACGCACTGATTTCGACCAGGTTCGTTCACTCATGGAAAATAGCGATCGCTGCCAGGATATACGTAATCTGGCATTTCTGGGGATTGCTTATAACACCCTGTTACGTATAGCCGAAATTGCCAGGATCAGGGTTAAAGATATCTCACGTACTGACGGTGGGAGAATGTTAATCCATATTGGCAGAACGAAAACGCTGGTTAGCACCGCAGGTGTAGAGAAGGCACTTAGCCTGGGGGTAACTAAACTGGTCGAGCGATGGATTTCCGTCTCTGGTGTAGCTGATGATCCGAATAACTACCTGTTTTGCCGGGTCAGAAAAAATGGTGTTGCCGCGCCATCTGCCACCAGCCAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGGCTAAGGATGACTCTGGTCAGAGATACCTGGCCTGGTCTGGACACAGTGCCCGTGTCGGAGCCGCGCGAGATATGGCCCGCGCTGGAGTTTCAATACCGGAGATCATGCAAGCTGGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCTGGAAGATGGCGATTAATAAAAGCTTCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGAGCCAATTTGATATATTATGTAAAACACCACCTAAGGTCCTGGTTCGTCAGTTTGTGGAAAGGTTTGAAAGACCTTCAGGGGAAAAAATAGCATCATGTGCTGCTGAACTAACCTATTTATGTTGGATGATTACTCATAACGGAACAGCAATCAAGAGAGCCACATTCATGAGCTATAATACTATCATAAGCAATTCGCTGAGTTTCGATATTGTCAACAAATCACTCCAGTTTAAATACAAGACGCAAAAAGCAACAATTCTGGAAGCCTCATTAAAGAAATTAATTCCTGCTTGGGAATTTACAATTATTCCTTACAATGGACAAAAACATCAATCTGATATCACTGATATTGTAAGTAGTTTGCAATTACAGTTCGAATCATCGGAAG	$P_{LtetO-1}$ Cre Flp Terminator Spacer

			AAGCAGATAAGGGAAATAGCCACAGTAAAAAAATGCTTAAAGCACTTCTAAGT GAGGGTGAAAGCATCTGGGAGATCACTGAGAAAATACTAAATTCGTTTGAGT ATACCTCGAGATTTACAAAAACAAAAACTTTATACCAATTCCTCTTCCTAGCTA CTTTCATCAATTGTGGAAGATTCAGCGATATTAAGAACGTTGATCCGAAATCA TTTAAATTAGTCCAAAATAAGTATCTGGGAGTAATAATCCAGTGTTTAGTGACA GAGACAAAGACAAGCGTTAGTAGGCACATATACTTCTTTAGCGCAAGGGGTA GGATCGATCCACTTGTATATTTGGATGAATTTTTGAGGAACTCTGAACCAGTC CTAAAACGAGTAAATAGGACCGGCAATTCTTCAAGCAACAAACAGGAATACCA ATTATTAAAAGATAACTTAGTCAGATCGTACAACAAGGCTTTGAAGAAAAATG CGCCTTATCCAATCTTTGCTATAAAGAATGGCCCAAAATCTCACATTGGAAGA CATTTGATGACCTCATTTCTGTCAATGAAGGGCCTAACGGAGTTGACTAATGT TGTGGGAAATTGGAGCGATAAGCGTGCTTCTGCCGTGGCCAGGACAACGTAT ACTCATCAGATAACAGCAATACCTGATCACTACTTCGCACTAGTTTCTCGGTA CTATGCATATGATCCAATATCAAAGGAAATGATAGCATTGAAGGATGAGACTA ATCCAATTGAGGAGTGGCAGCATATAGAACAGCTAAAGGGTAGTGCTGAAGG AAGCATACGATACCCCGCATGGAATGGGATAATATCACAGGAGGTACTAGAC TACCTTTCATCCTACATAAATTAA**TAAGTCGACAACCTAGGAAAAACCTGAGG** **AAAATGCATA**GCTAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT GGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACA AATCCGCCGGGAGCGGATTTGAACGCTGCGAAGCAACGGCCCGGAGGGTG GCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGC CATCCTGACGGATGGCCTTTTTGCGTTTCTACAAA	
DSD-2[α]	pBZ22	pBAC-LacZ (F'/oriV origins and Cam^R)	ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GCAGTTTCATTTGATGCTCG** **ATGAGTTTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGTATTTAG** **AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTA** **GGTATCTGGCACTACGTTCAGGTAACCTGAAGCTCGAATCCAGTACTCGACG** **TCTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAA** **CAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATG** **CCTCTAGCACGCGTACCTGGTGGCGCGGCCTTATTTGTATAGTTCATCCATGC** **CATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCT** **CTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTG** **TCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTC** **TGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTT** **GATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTT** **GTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTT** **AACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGT** **CTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTC** **GGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCG** **CAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAAC** **AGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTG** **CATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAACATCACCA** **TCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACG** **CATGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTGCTCAGT** **ATCTTGTTATCCGCTCACAATGTAAATTGTTATCCGCTCACAATTGTATCCGCT** **CATGAATTAATTCTTAGGCATATTCAAATCGTTTTCGTTACCGCTTGCAGGCAT** **CATGACAGAACACTACTTCCTATAAACGCTACACAGGCTCCTGAGATTAATAA** **TGCGGATCTGTCCCAGACTAATAATCAGACCGACGAAGAAACCAATTGTCCA** **TATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAAC** **CAAACCGGTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAA** **GCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCC** **ACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCA** **TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCT** **CCATAGCTAGC**ATAACTTCGTATAGCATACATTATACGAAGTTAT	loxP Data
DSD-2[α]^p15A	pBZ19	p15A (origin and Cam^R only)	ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GCAGTTTCATTTGATGCTCG** **ATGAGTTTTTCTAAGAATTAATTCATGAGCGGATACATATTTGAATGTATTTAG** **AAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTA** **GGTATCTGGCACTACGTTCAGGTAACCTGAAGCTCGAATCCAGTACTCGACG** **TCTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAA** **CAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTTTATTTGATG** **CCTCTAGCACGCGTACCTGGTGGCGCGGCCTTATTTGTATAGTTCATCCATGC** **CATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGTGGTCTCT** **CTTTTCGTTGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTAATGGTTG** **TCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGATAATGGTC** **TGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGTTAACTTT** **GATTCCATTCTTTTGTTTGTCTGCCATGATGTATACATTGTGTGAGTTATAGTT** **GTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAATACCTTTT** **AACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGCACGTGT** **CTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATAACCTTC** **GGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGTATCTCG** **CAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCATGGAAC** **AGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTG** **CATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAACATCACCA** **TCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCCTTTACG** **CATGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTGCTCAGT** **ATCTTGTTATCCGCTCACAATGTAAATTGTTATCCGCTCACAATTGTATCCGCT** **CATGAATTAATTCTTAGGCATATTCAAATCGTTTTCGTTACCGCTTGCAGGCAT** **CATGACAGAACACTACTTCCTATAAACGCTACACAGGCTCCTGAGATTAATAA** **TGCGGATCTGTCCCAGACTAATAATCAGACCGACGAAGAAACCAATTGTCCA** **TATTGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAAC** **CAAACCGGTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACCAAA** **GCCATGACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCC** **ACATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATAGCATTTTTATCCA** **TAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCT** **CCATAGCTAGC**ATAACTTCGTATAGCATACATTATACGAAGTTAT	loxP Data
DSD-4[α]	pBZ23	pBAC-LacZ (F'/oriV	ATAACTTCGTATAATGTATGCTATACGAAGTTAT**GCAGTTTCATTTGATGCTCG** **ATGAG****GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC****AAGCTCGAATCCAG** **TACTCGACGTCTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACC** **GACAAACAACAGATAAAACGAAAGGCCCAGTCTTTCGACTGAGCCTTTCGTTT** **TATTTGATGCCTCTAGCACGCGTACCTGGTGGCGCGGCCTTATTTGTATAGTTC**	loxP FRT Data1

		origins and Cam^R)	ATCCATGCCATGTGTAATCCCAGCAGCTGTTACAAACTCAAGAAGGACCATGT GGTCTCTCTTTTCGTTGGGGATCTTTCGAAAGGGCAGATTGTGTGGACAGGTA ATGGTTGTCTGGTAAAAGGACAGGGCCATCGCCAATTGGAGTATTTTGTTGAT AATGGTCTGCTAGTTGAACGCTTCCATCTTCAATGTTGTGTCTAATTTTGAAGT TAACTTTGATTCCATTCTTTGTTTGTCTGCCATGATGTATACATTGTGTGAGT TATAGTTGTATTCCAATTTGTGTCCAAGAATGTTTCCATCTTCTTTAAAATCAAT ACCTTTTAACTCGATTCTATTAACAAGGGTATCACCTTCAAACTTGACTTCAGC ACGTGTCTTGTAGTTCCCGTCATCTTTGAAAAATATAGTTCTTTCCTGTACATA ACCTTCGGGCATGGCACTCTTGAAAAAGTCATGCTGTTTCATATGATCTGGGT ATCTCGCAAAGCATTGAACACCATAACCGAAAGTAGTGACAAGTGTTGGCCA TGGAACAGGTAGTTTTCCAGTAGTGCAAATAAATTTAAGGGTAAGTTTTCCGT ATGTTGCATCACCTTCACCCTCTCCACTGACAGAAAATTTGTGCCCATTAACA TCACCATCTAATTCAACAAGAATTGGGACAACTCCAGTGAAAAGTTCTTCTCC TTTACGCATGGTATATCTCCTTCTTAAAGTGGTCAGTGCGTCCTGCTGATGTG CTCAGTATCTTGTTATCCGCTCACAATGTAAATTGTTATCCGCTCACAATTGTA TCCGCTCATGAATTAATTCTTAGAAGTTCCTATACTTTCTAGAGAATAGGAACT TCAGGCATTGATGGAATCGTAGTCTCAATAAACTTCGTATAGCATACATTATAC GAAGTTAT	Data2 Data3
SpyTag-Bla	pBZ51	pET28a (pBR322 origin and Kan^R only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA CATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGCCCACA TCGTGATGGTGGACGCCTACAAGCCGACGAAGGGTTCAGGGGGTTCCGGTC ACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACG AGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTT CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGG CGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT ACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATC TTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAG TGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTG GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGAT GCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTT ACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTG CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAA ATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCC AGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCA ACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTA AGCATTGGTGATAAGTCGACAACCTAGGAAAAACCTGAGGAAAATGCATAGC TAGAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTT TTATCTGTTGTTTGTCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG AGCGGATTTGAACGCTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGAC GCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGA TGGCCTTTTTGCGTTTCTACAAA	P_{LtetO-1} SpyTag Linker Bla Terminator
YcbK-SpyCatcher	pBZ52	pET28a (pBR322 origin and Kan^R only)	TCCCTATCAGTGATAGAGATTGACATCCCTATCAGTGATAGAGATACTGAGCA CATCAGCAGGACGCACTGACCACTTTAAGAAGGAGATATACCATGGATAAATT TGATGCGAACCGCCGCAAACTGCTGGCGCTGGCGGCGTGGCGCTGGGCG CGGCGATTCTGCCGACCCCGGCGTTTGCGACCCTGAGCACCCCGCGCGGAA GCGGTAGTGGAAGTATGGGAGTTGATACCTTATCAGGTTTATCAAGTGAGCA AGGTCAGTCCGGTGATATGACAATTGAAGAAGATAGTGCTACCCATATTAAAT TCTCAAAACGTGATGAGGACGGCAAAGAGTTAGCTGGTGCAACTATGGAGTT GCGTGATTCATCTGGTAAAACTATTAGTACATGGATTTCAGATGGACAAGTGA AAGATTTCTACCTGTATCCAGGAAAATATACATTTGTCGAAACCGCAGCACCA GACGGTTATGAGGTAGCAACTGCTATTACCTTTACAGTTAATGAGCAAGGTCA GGTTACTGTAAATGGCAAAGCAACTAAAAGGTGACGCTCATATTTAATGATAAG TCGACAACCTAGGAAAAACCTGAGGAAAATGCATAGCTAGAGGCATCAAATA AAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTC GGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGC TGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATAAACTG CCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTT TCTACAAA	P_{LtetO-1} YcbK SpyCatcher Terminator
MIT Message 1	pBZ63	pET28a (pBR322 origin and Kan^R only)	GACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACCTCG AGCTGGTGGCGCGCCTTATTTGTATAGTGGCCACGATCCATGCTAACGTCTC TGCGTAGGGATGAATCCCGTTTTGAACTCGTTCCTACTGACGGACGAGCTGA TAGGTAGCCGAAGTAGTGATACGATCCACACATGCCATCATTGCATACTCGT GCATTCAATGATGCATAGTCACGTAGTCCATATGGTAATGGTGATGTCAAGTC ACATGTCAATACTCGTCACTAGAACTGAGCGCGATGACTGGCGAGCTGGTGC GCTCCCGAGGCTGGTCGAGCGACTAAGTTGAATGCGCAGACCGATCGAGAC GACTCTAGCGCTGGAATAAATCAGAATAAAGACCTAGGGATATATTCCGCTTC GCATGTTCATCATCAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGG TATCATTACCCCCATGAACAGAAATCCCCCTTACACGG	Forward primer MIT message 1 Reverse primer
MIT Message 2	pBZ64	pET28a (pBR322 origin and Kan^R only)	GACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACCTCG AGCTGGTGGCGCGCCTTATTTGTATAGCCCACCAATACTGCCAATAGACGGT ACTGTACACCCTGTTTTACAGCAACGGGAAAGGAGGATCACTTTCTACAATTG TGTGCTGGACTGACAGTCGCATATCCACACATGCCATCATTGCATACTCGTG CATTCAATGATGCATCTACACGTAGTCCATATGGTAATGGTGATGTCACTACA CATGTCAATACTCGTCACTAGAACTGAGCGCGATACGACTCGCCCATAGGGT TCGCCGGCTCGCACTGACTACCTTACGCTCTGACCCAGATCGGAGCCGGCC GCATGACCCCTGTGATATAATACCGTTCATCCCTAGGGATATATTCCGCTTCG CATGTTCATCATCAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGGT ATCATTACCCCCATGAACAGAAATCCCCCTTACACGG	Forward primer MIT message 2 Reverse primer

Supplementary Table 5 Identity, plasmid, and sequence information of all constructs used in this study.