Fig. 1

loxP (Cre)	ATAACTTCGTATA**ATGTATGC**TATACGAAGTTAT	SEQ ID NO:3
loxH (Fre)	ATATATACGTATA**TAGACATA**TATACGTATATAT	SEQ ID NO:4
rox (Dre)	CTAACTTTAAATA**ATGCCAAT**TATTTAAAGTTAT	SEQ ID NO:5
zox (Zre)	ATAACTTCGTATA**ACACACAT**TATGCGAAGTTAT	SEQ ID NO:6
loxLTR (Tre)	ACAACATCCTATT**ACACCCTA**TATGCCAACATGG	SEQ ID NO:7
SEQ ID NO:1	AACCCACTGCTTA**AGCCTCAA**TAAAGCTTGCCTT	
SEQ ID NO:2	CTGGGCGGGACTG**GGGAGTGG**CGAGCCCTCAGAT	

Fig. 2

SEQ ID NO:1	AACCCACTGCTTA**AGCCTCAA**TAAAGCTTGCCTT	
Subsite 1	AACCCACTGCTTA**AGCCTCAA**TAAGCAGTGGGTT	SEQ ID NO:8
Subsite 1b+2	AACACACTGCTTA**AGCCTCAA**TAAGCAGTGTGTT	SEQ ID NO:27
Subsite 1b+1	ATCACACTGCTTA**AGCCTCAA**TAAGCAGTGTGAT	SEQ ID NO:28
Subsite 1b	ATAACACTGCTTA**AGCCTCAA**TAAGCAGTGTTAT	SEQ ID NO:29
Subsite 1a	AACCCATTGTATA**AGCCTCAA**TATACAATGGGTT	SEQ ID NO:30
Subsite 1A	AACACATTGTATA**AGCCTCAA**TATACAATGTGTT	SEQ ID NO:9
Subsite 1B	ATACCACTGTATA**AGCCTCAA**TATACAGTGGTAT	SEQ ID NO:10
Subsite 1C	ATAACATTGCTTA**AGCCTCAA**TAAGCAATGTTAT	SEQ ID NO:11
Subsite 2	AAGGCAAGCTTTA**AGCCTCAA**TAAAGCTTGCCTT	SEQ ID NO:12
Subsite 2a+2	AAGGCAAGGTTTA**AGCCTCAA**TAAACCTTGCCTT	SEQ ID NO:31
Subsite 2a+1	AAGGCTAGGTTTA**AGCCTCAA**TAAACCTAGCCTT	SEQ ID NO:32
Subsite 2a	AAGGCTAGGTATA**AGCCTCAA**TATACCTAGCCTT	SEQ ID NO:33
Subsite 2b	ATAACAAGCTTTA**AGCCTCAA**TAAAGCTTGTTAT	SEQ ID NO:34
Subsite 2A	AAGACTAGGTATA**AGCCTCAA**TATACCTAGTCTT	SEQ ID NO:13
Subsite 2B	ATAGCAAGGTATA**AGCCTCAA**TATACCTTGCTAT	SEQ ID NO:14
Subsite 2C	ATAACTAGCTTTA**AGCCTCAA**TAAAGCTAGTTAT	SEQ ID NO:15
ΔloxP	ATAACTTCGTTTA**ATGTATGC**TAAACGAAGTTAT	SEQ ID NO:35
SEQ ID NO:2	CTGGGCGGGACTG**GGGAGTGG**CGAGCCCTCAGAT	
Subsite 1	CTGGGCGGGACTG**GGGAGTGG**CAGTCCCGCCCAG	SEQ ID NO:16
Subsite 1A	CTGACTTCGACTA**GGGAGTGG**TAGTCGAAGTCAG	SEQ ID NO:17
Subsite 1B	ATAGGTTCGAATG**GGGAGTGG**CATTCGAACCTAT	SEQ ID NO:18
Subsite 1C	ATAACCGGGAATA**GGGAGTGG**TATTCCCGGTTAT	SEQ ID NO:19
Subsite 2	ATCTGAGGGCTCG**GGGAGTGG**CGAGCCCTCAGAT	SEQ ID NO:20
Subsite 2A	ATCTGTTGGCATG**GGGAGTGG**CATGCCAACAGAT	SEQ ID NO:21
Subsite 2B	ATATGATGGCTTA**GGGAGTGG**TAAGCCATCATAT	SEQ ID NO:22
Subsite 2C	ATATGTGGGCACA**GGGAGTGG**TGTGCCCACATAT	SEQ ID NO:23

Fig. 3

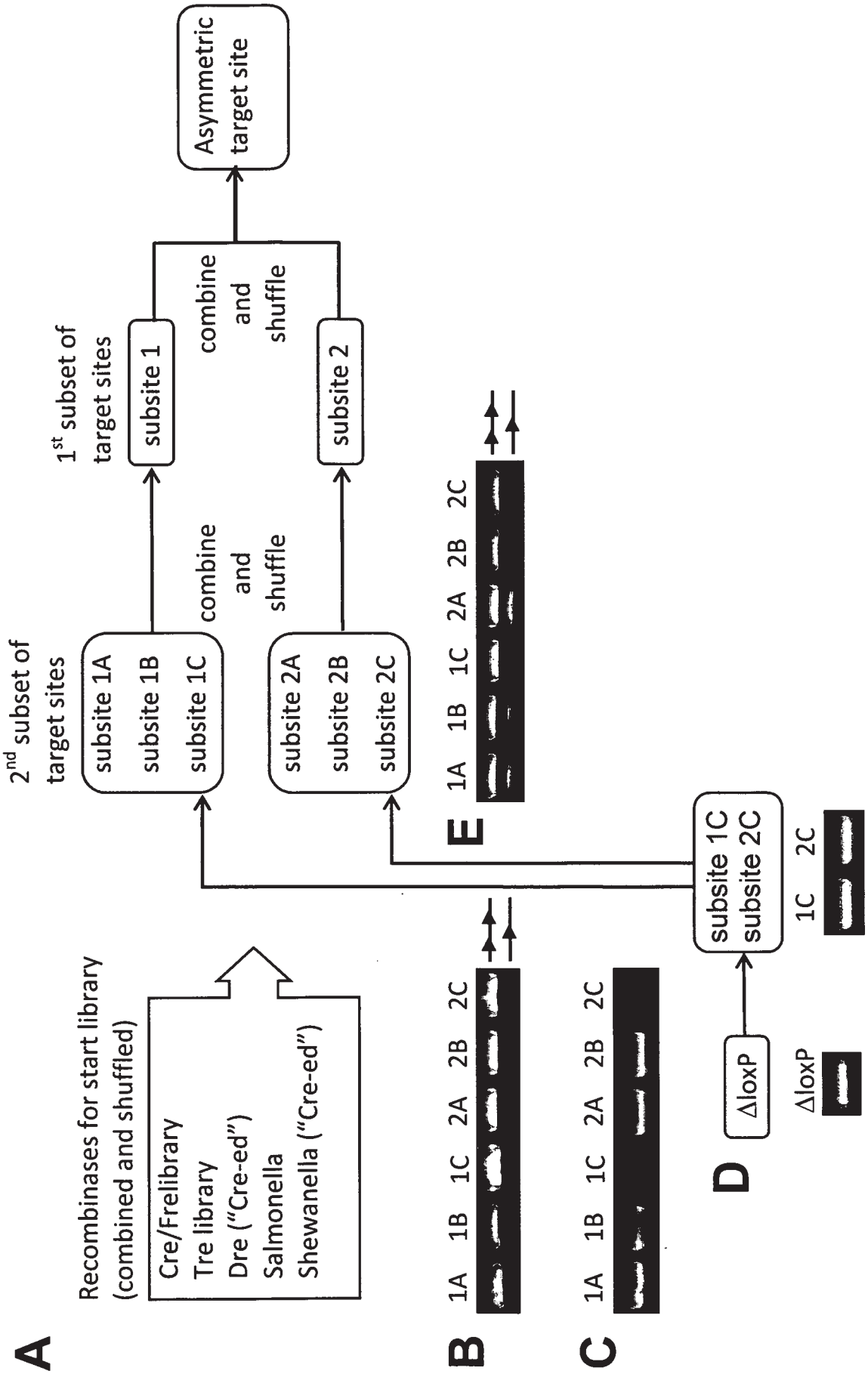

A
Recombinases for start library
(combined and shuffled)
Cre/Frelibrary
Tre library
Dre ("Cre-ed")
Salmonella
Shewanella ("Cre-ed")
2nd subset of target sites
subsite 1A
subsite 1B
subsite 1C
combine and shuffle
subsite 2A
subsite 2B
subsite 2C
1st subset of target sites
subsite 1
subsite 2
combine and shuffle
Asymmetric target site
B
1A 1B 1C 2A 2B 2C
C
1A 1B 1C 2A 2B 2C
D
ΔloxP
subsite 1C
subsite 2C
ΔloxP
1C 2C
E
1A 1B 1C 2A 2B 2C

Fig. 4

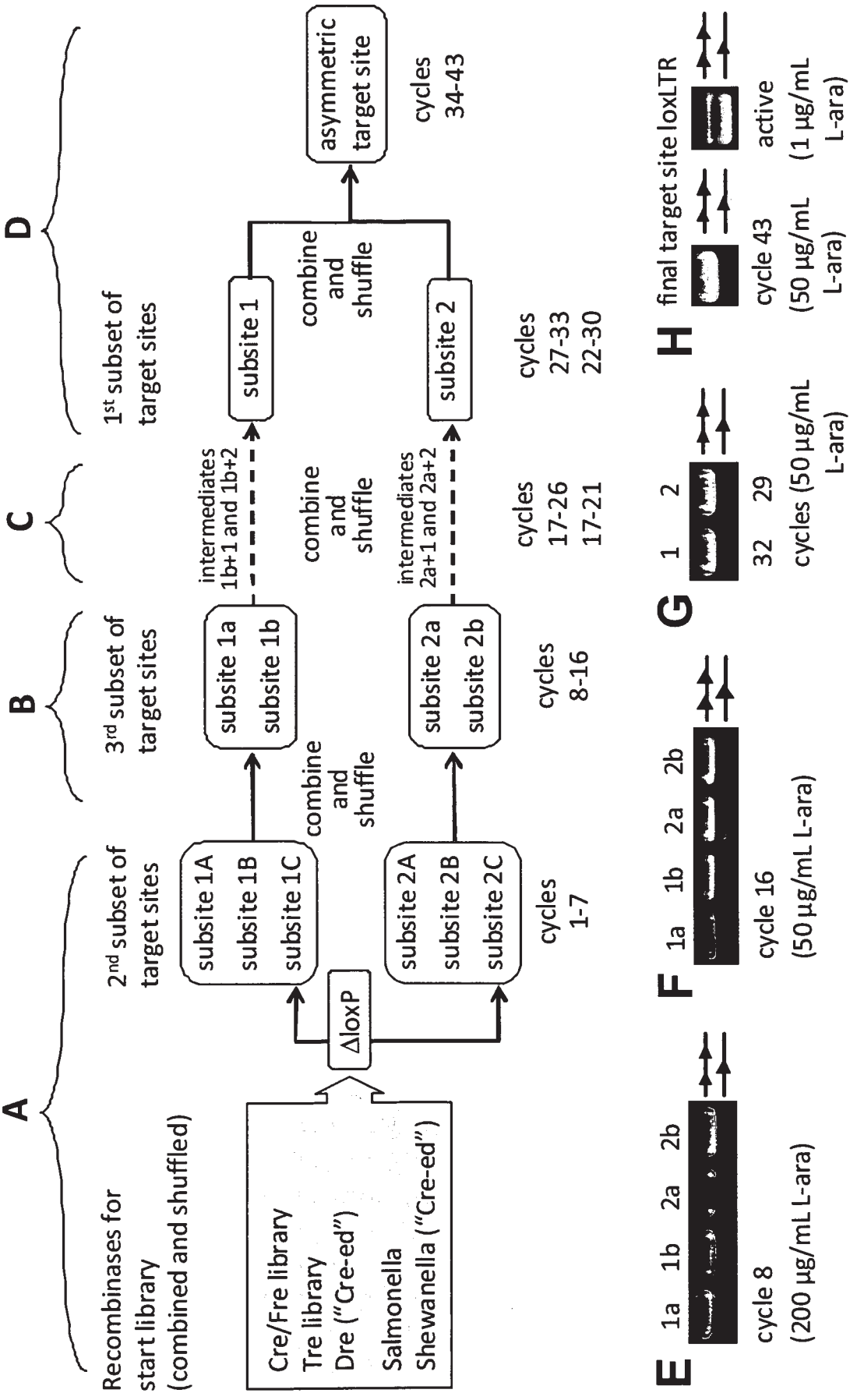

A
B
C
D
Recombinases for start library (combined and shuffled)
2nd subset of target sites
3rd subset of target sites
1st subset of target sites
Cre/Fre library
Tre library
Dre ("Cre-ed")
Salmonella
Shewanella ("Cre-ed")
ΔloxP
subsite 1A
subsite 1B
subsite 1C
subsite 2A
subsite 2B
subsite 2C
combine and shuffle
subsite 1a
subsite 1b
subsite 2a
subsite 2b
intermediates 1b+1 and 1b+2
intermediates 2a+1 and 2a+2
subsite 1
subsite 2
combine and shuffle
asymmetric target site
cycles 1-7
cycles 8-16
cycles 17-26
cycles 17-21
cycles 27-33
cycles 22-30
cycles 34-43
E
1a 1b 2a 2b
cycle 8
(200 µg/mL L-ara)
F
1a 1b 2a 2b
cycle 16
(50 µg/mL L-ara)
G
1 2
32 29
cycles (50 µg/mL L-ara)
H
final target site loxLTR
cycle 43
(50 µg/mL L-ara)
active
(1 µg/mL L-ara)

Fig. 5

a) Cre SEQ ID NO:36
b) Tre common consensus sequence 100% SEQ ID NO:37
c) Tre 3.0 consensus sequence 100% SEQ ID NO:38
d) Tre 3.0 consensus sequence 95% SEQ ID NO:39

```
 1          12 15                30              44
 MSNLLTVHQNLPALPVDATSDEVRKNLMDMFRDRQAFSEHTWKMLLSVCRSWAAWCKLN a
 MSXXXTXXXXLXALXXDXXSDXXXXXLXXXXRDXXAXSXXTWXXLLSXCRXWXAWCXXX b
 MSXXXTXXXXLSALLXDXXSDXXXXXLXXXXRDXXAXSXXTWXVLLSXCRXWXAWCXXX c
 MSXLXTXHXXLSALLXDXTSDEXRXNLMDVXRDXXAXSEHTWKVLLSVCRXWAAWCXLN d

60                            86      93
NRKWFPAEPEDVRDYLLYLQARGLAVKTIQQHLGQLNMLHRRSGLPRPSDSNAVSLVMRR a
XRXXFPXXPXXVRXYLLXLQXRGLXVXTXQQHLXXLNMXHRRXGLXRXXDXXXVSLXXRR b
XRXXFPXXPXXVRXYLLXLQXRGLXVNTXQQHLAXLNMXHRRXGLXRXXDSXXVSLXXRR c
NRKXFPAEPEDVRDYLLXLQXRGLAVNTIQQHLAXLNMLHRRXGLPRXXDSXAVSLVXRR d

120                                                          175
IRKENVDAGERAKQALAFERTDFDQVRSLMENSDRCQDIRNLAFLGIAYNTLLRIAEIAR a
IRXENVDAGERXKQALAFXRXDXXXXXXLXXXSXXXXDXRXLAXLGXAYNTLLRXXEXXX b
IRXENVDAGERXKQALAFXRXDXXXXXXLXXXSXXXXDXRXLAXLGXAYNTLLRXSEXXX c
IRXENVDAGERXKQALAFERTDXXQVRXLMXXSXRXQDIRXLAXLGXAYNTLLRISEIAR d

180
IRVKDISRTDGGRMLIHIGRTKTLVSTAGVEKALSLGVTKLVERWISVSGVADDPNNYLF a
XRXXDXSXTXGGRXLIHXXXTKTLVSTXGVEKALSLXXTXLXERWXSXSGVAXXXXXYLF b
XRXXDXSXTXGGRXLIHXXXTKTLVSTXGVEKALSLXXTXLXERWXSXSGVAXXXXXYLF c
IRXXDISXTXGGRMLIHIXRTKTLVSTAGVEKALSLXVTXLVERWISXSGVAXDPNNYLF d

240                  259 262
CRVRKNGVAAPSATSQLSTRALEGIFEATHRLIYGAKDDSGQRYLAWSGHSARVGAARDM a
CXXXXXGXAXPXAXXXLSXXXLXXIFXXXHXXXXGAKXXSGXRYXXWSGHSARVGAARDM b
CXXXXXGXAXPXAXXXLSXXXLXXIFXXXHXXXXGAKXXSGXRYXXWSGHSARVGAARDM c
CXVRXXGVAXPSATXQLSTSALQGIFXAXHXLIYGAKXXSGXRYLAWSGHSARVGAARDM d

300    307         317 320
ARAGVSIPEIMQAGGWTNVNIVMNYIRNLDSETGAMVRLLEDGD* a
ARAGVXIXEIMQAGGWXTVXXVMNYIRNLDSEXGAMVRLLEXXX* b
ARAGVXIAEIMQAGGWXTVXXVMNYIRNLDSEXGAMVRLLEXXX* c
ARAGVXIAEIMQAGGWTTVXSVMNYIRNLDSETGAMVRLLEXXD* d
```