Figure 1

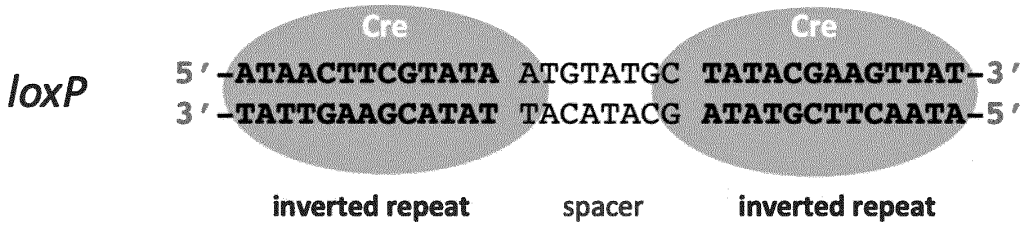


Figure 2

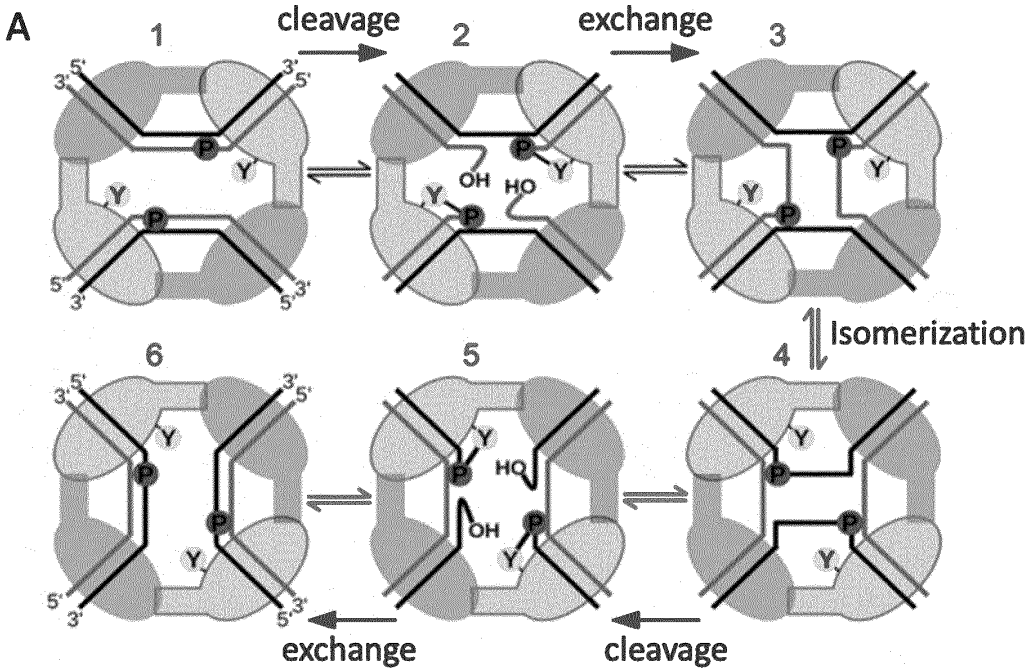


Figure 3

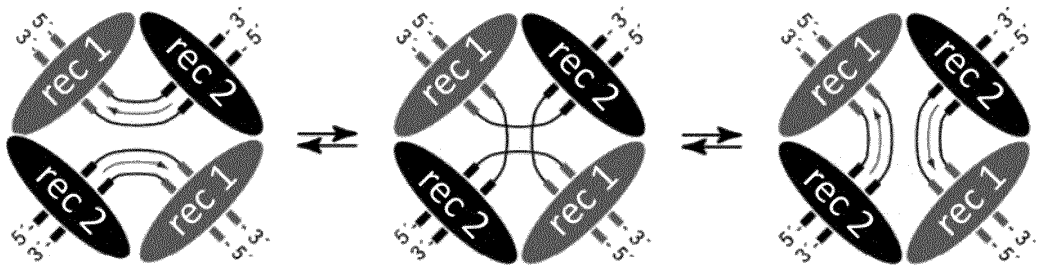


Figure 4

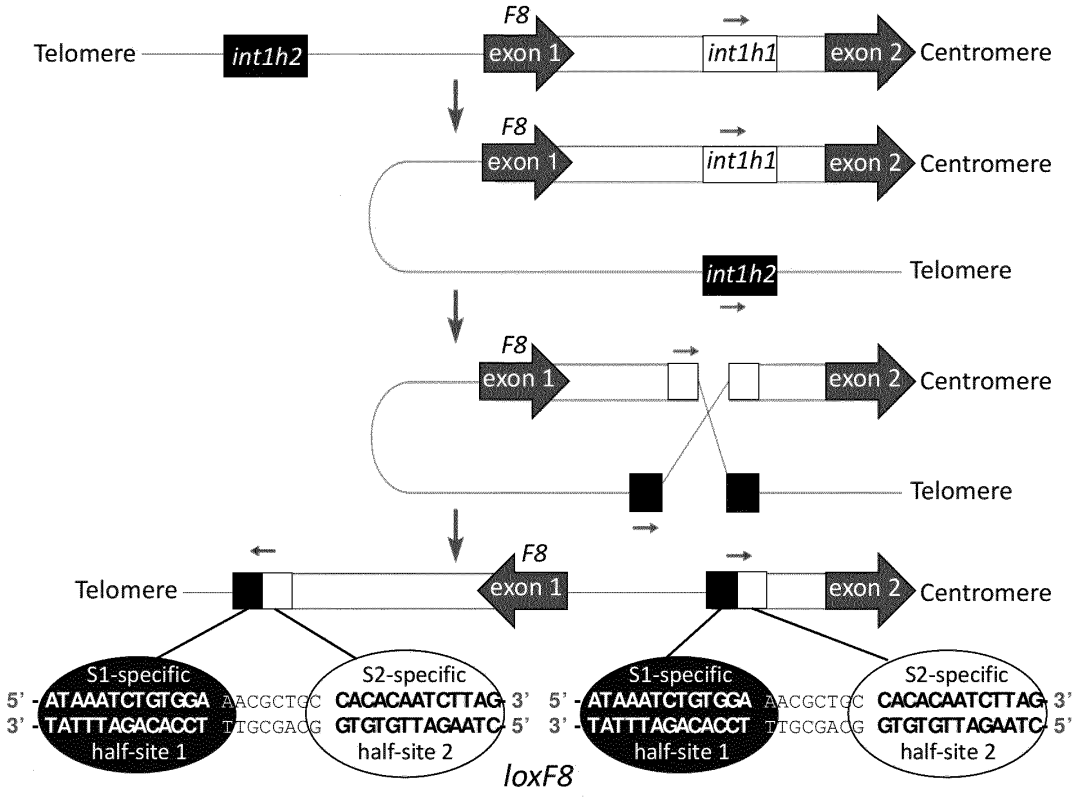


Figure 5

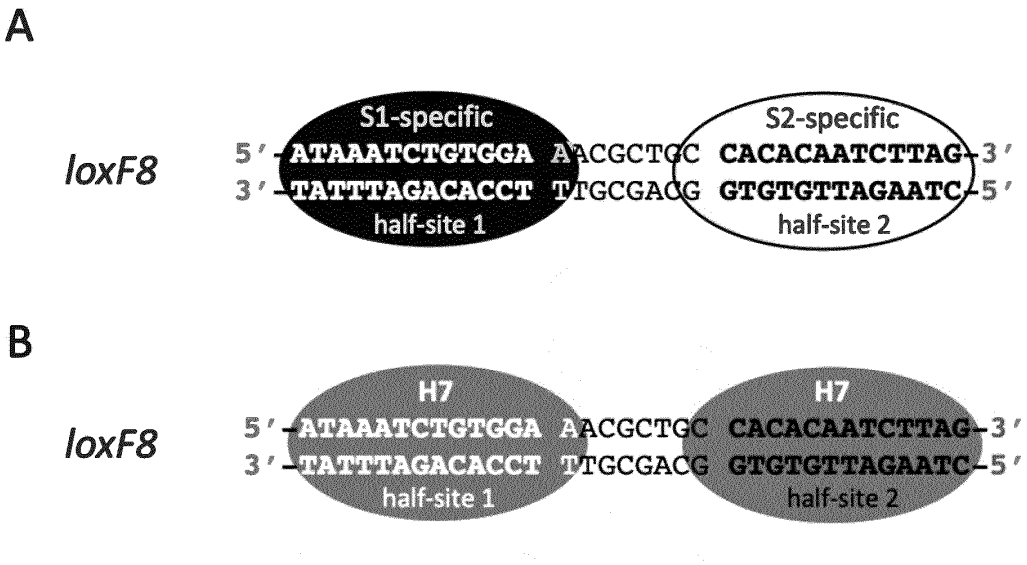


Figure 6

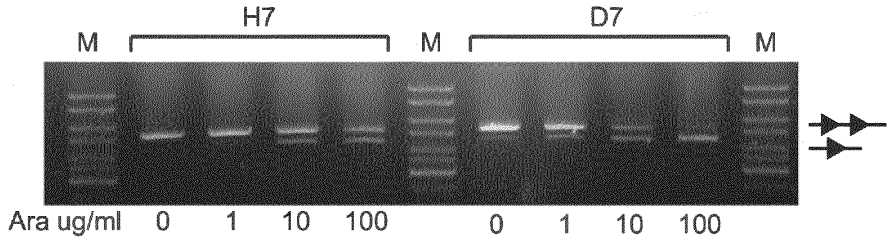


Figure 7

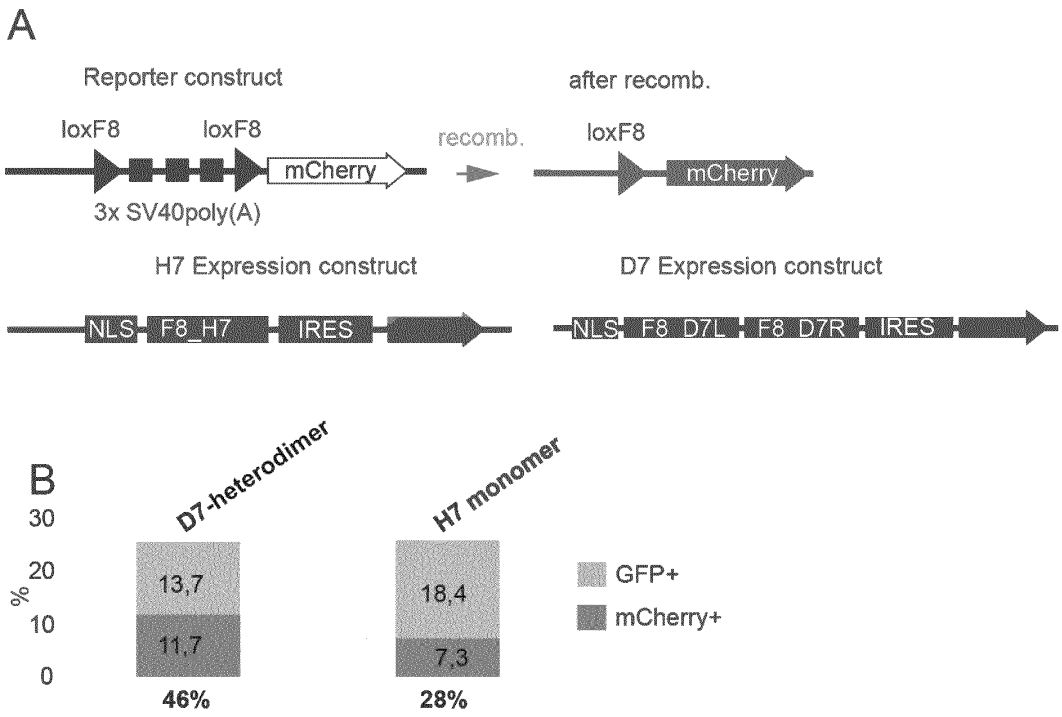


Figure 8

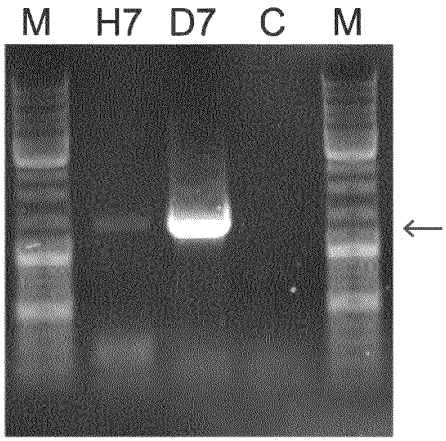


Figure 9

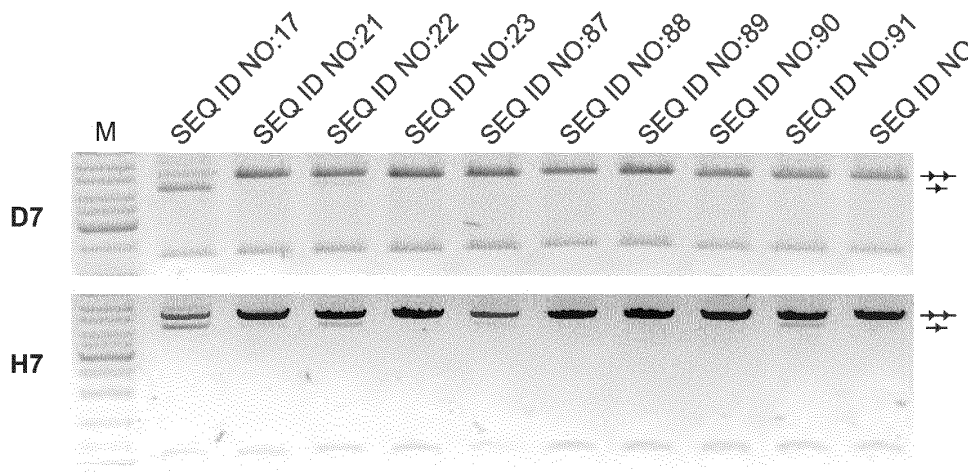


Figure 10

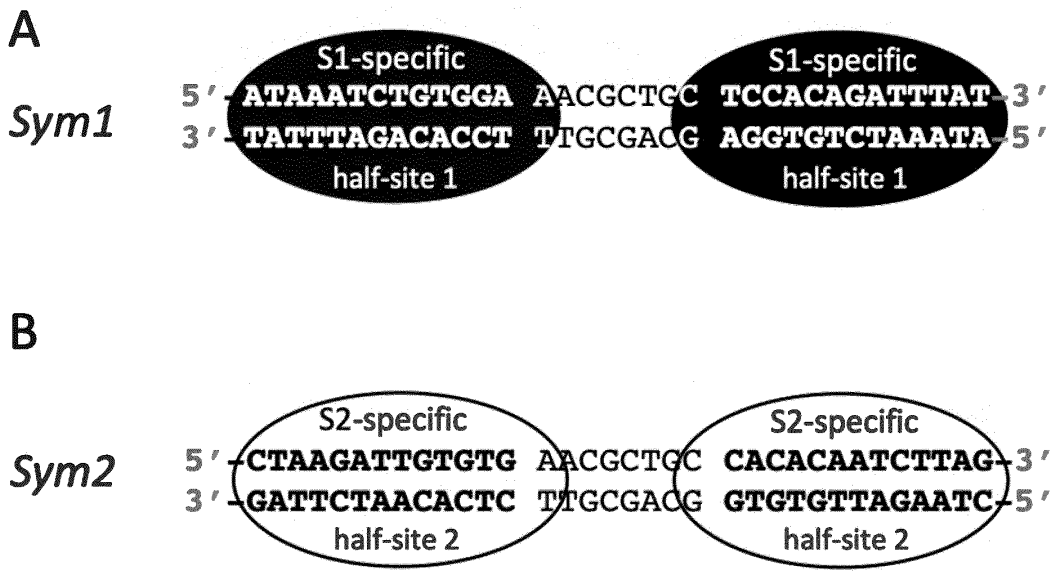


Figure 11

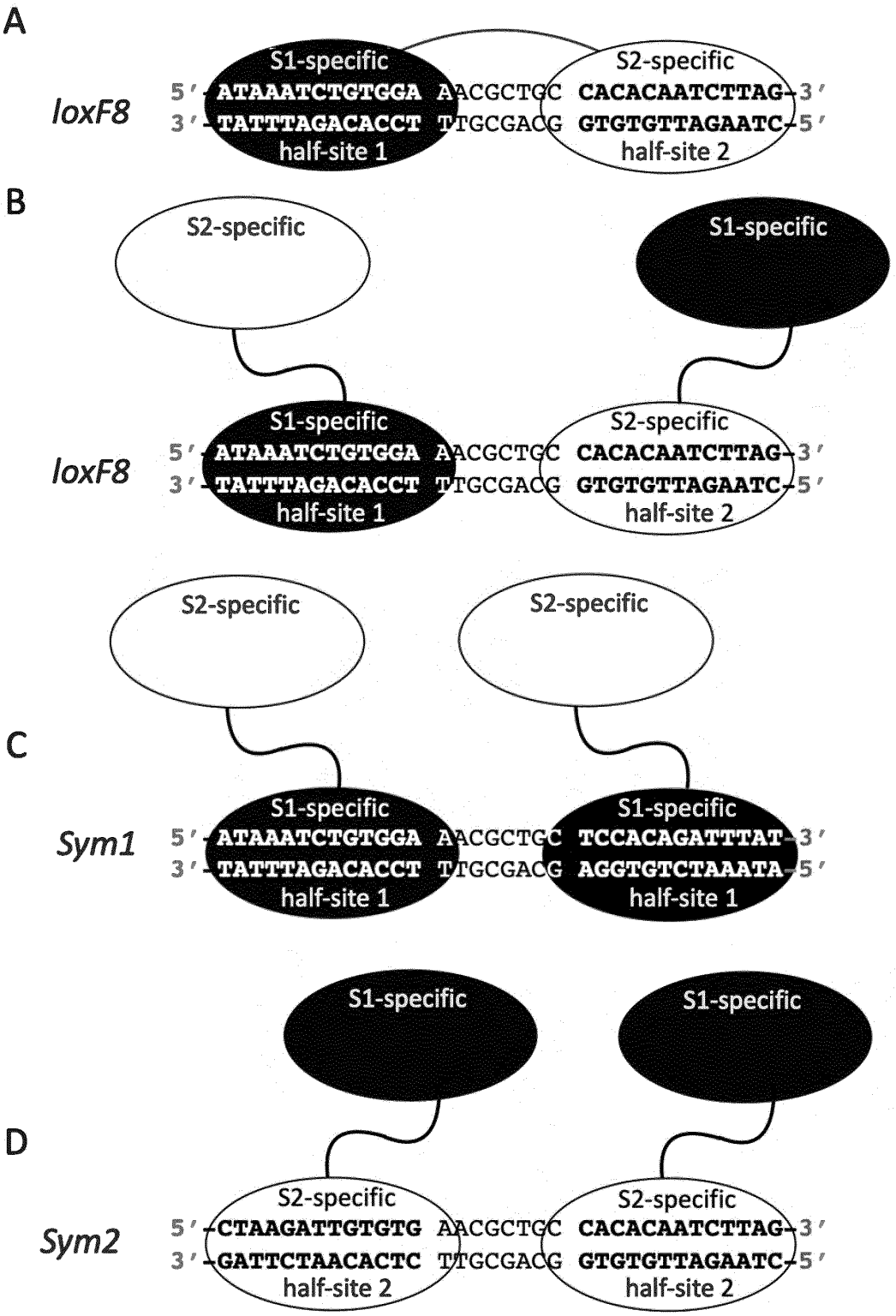

A
loxF8
S1-specific
S2-specific
5′-ATAAATCTGTGGA AACGCTGC CACACAATCTTAG-3′
3′-TATTTAGACACCT TTGCGACG GTGTGTTAGAATC-5′
half-site 1
half-site 2
B
S2-specific
S1-specific
loxF8
S1-specific
S2-specific
5′-ATAAATCTGTGGA AACGCTGC CACACAATCTTAG-3′
3′-TATTTAGACACCT TTGCGACG GTGTGTTAGAATC-5′
half-site 1
half-site 2
C
S2-specific
S2-specific
Sym1
S1-specific
S1-specific
5′-ATAAATCTGTGGA AACGCTGC TCCACAGATTTAT-3′
3′-TATTTAGACACCT TTGCGACG AGGTGTCTAAATA-5′
half-site 1
half-site 1
D
S1-specific
S1-specific
Sym2
S2-specific
S2-specific
5′-CTAAGATTGTGTG AACGCTGC CACACAATCTTAG-3′
3′-GATTCTAACACTC TTGCGACG GTGTGTTAGAATC-5′
half-site 2
half-site 2

Figure 12

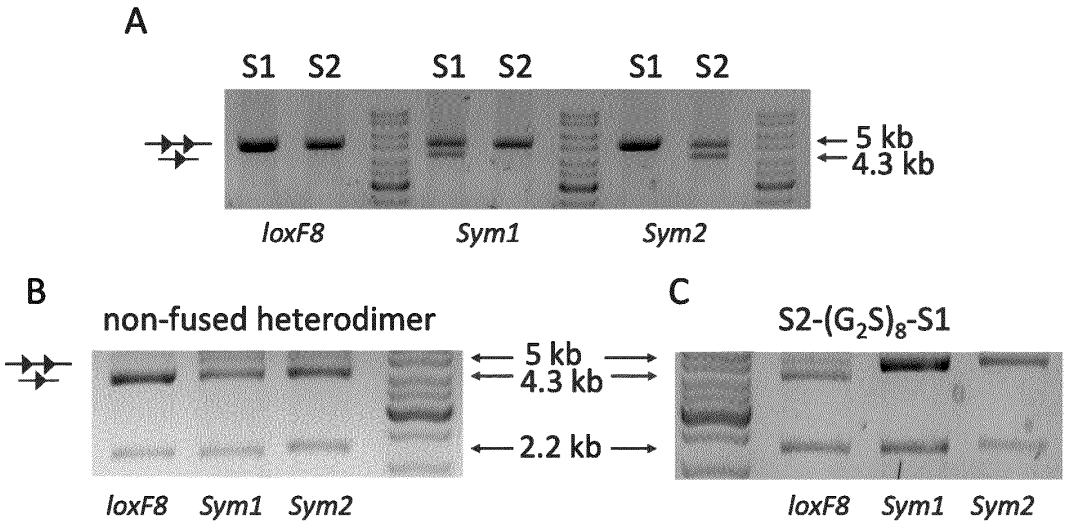


Figure 13

Linker library 1: Adapter1-XhoI-$(RVM)_6$-$(G_2S)_4$-$(RVM)_6$-BsrGI-Adapter2
Nucleotides Amino acids Nucleotides

Linker library 2: Adapter1-XhoI-$(G_2S)_2$-$(RVM)_{12}$-$(G_2S)_2$-BsrGI-Adapter2
Amino acids Nucleotides Amino acids

Linker library 3: Adapter1-XhoI- $(RVM)_{24}$-BsrGI-Adapter2
Nucleotides

Figure 14

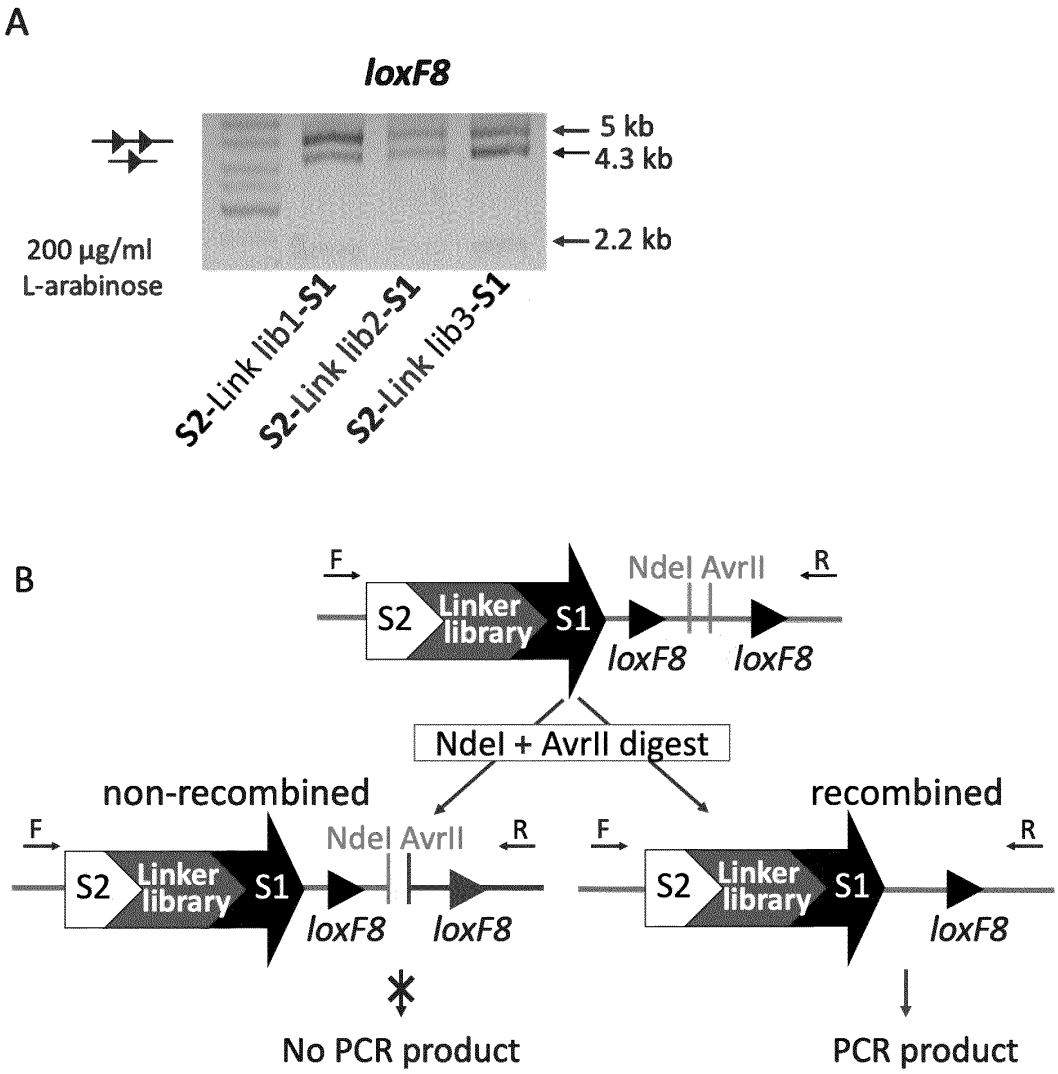

A
loxF8
5 kb
4.3 kb
2.2 kb
200 µg/ml
L-arabinose
S2-Link lib1-S1
S2-Link lib2-S1
S2-Link lib3-S1
B
F
S2
Linker library
S1
NdeI AvrII
R
loxF8
loxF8
NdeI + AvrII digest
non-recombined
recombined
No PCR product
PCR product

Figure 15

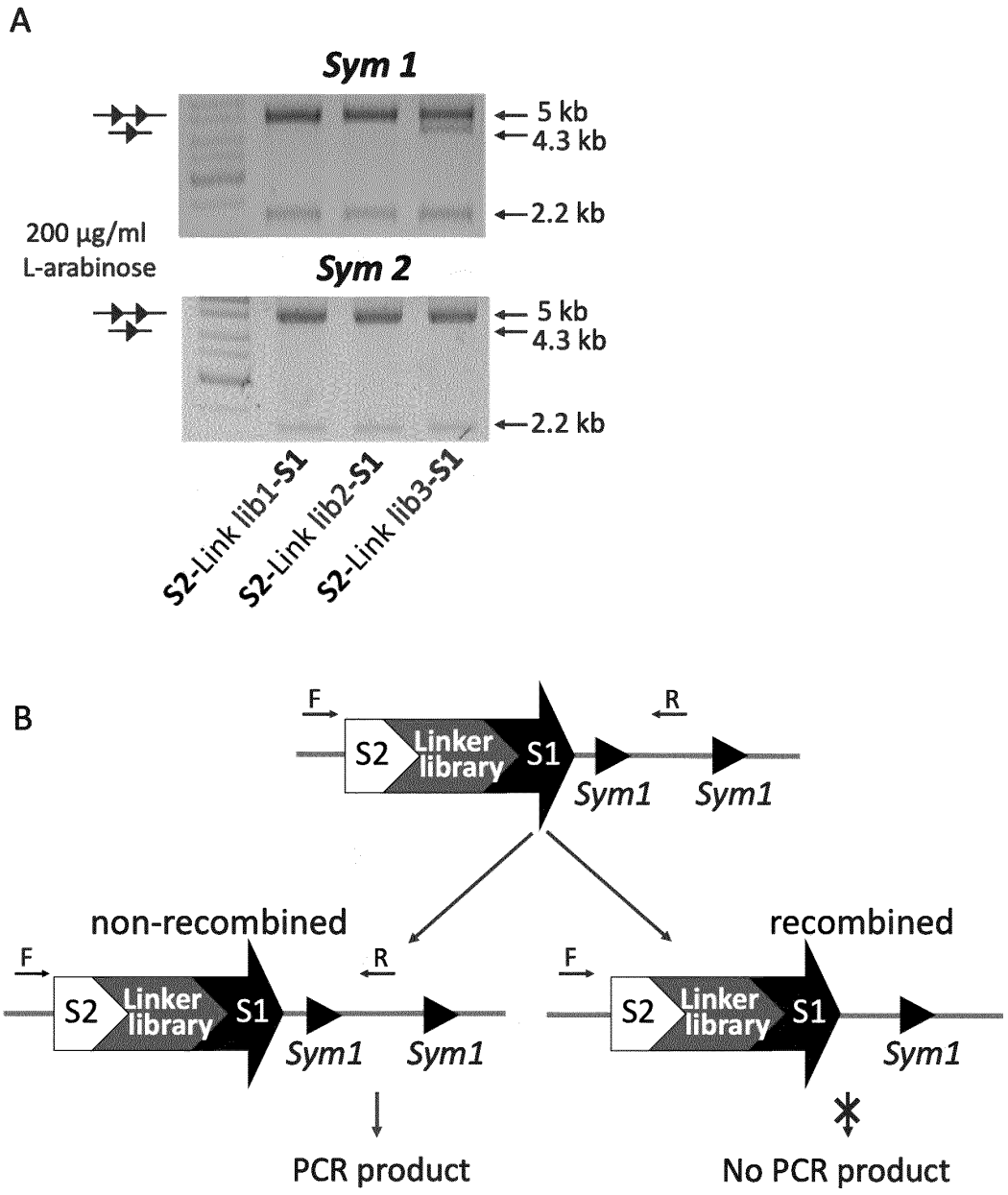

A
Sym 1
5 kb
4.3 kb
2.2 kb
200 µg/ml
L-arabinose
Sym 2
5 kb
4.3 kb
2.2 kb
S2-Link lib1-S1
S2-Link lib2-S1
S2-Link lib3-S1
B
F
R
S2
Linker library
S1
Sym1
Sym1
non-recombined
recombined
PCR product
No PCR product

Figure 16

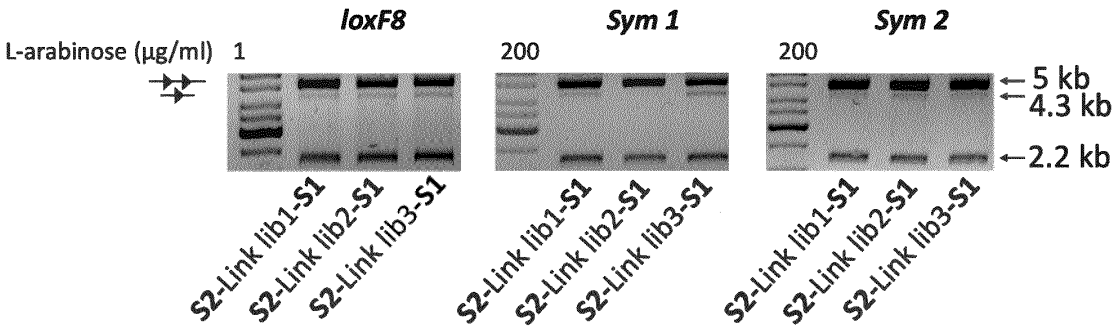


Figure 17

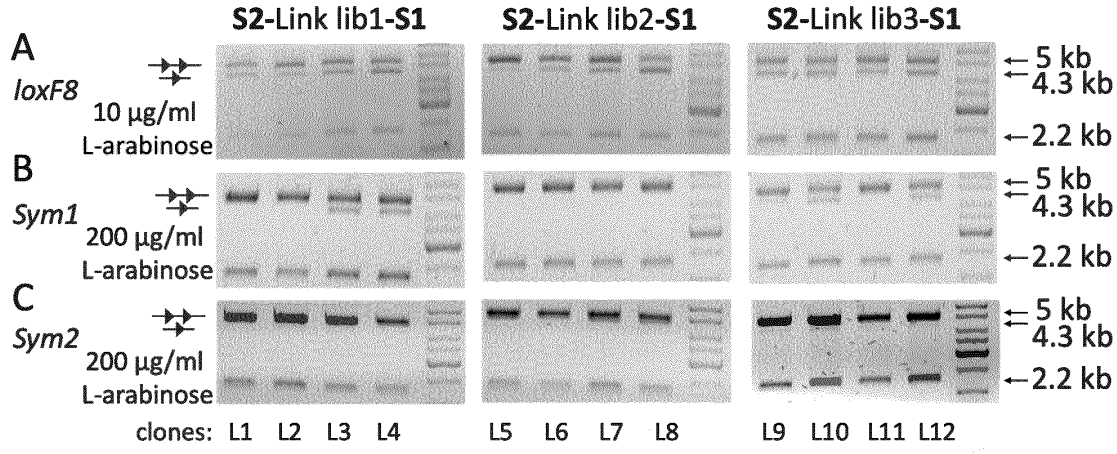


Figure 18

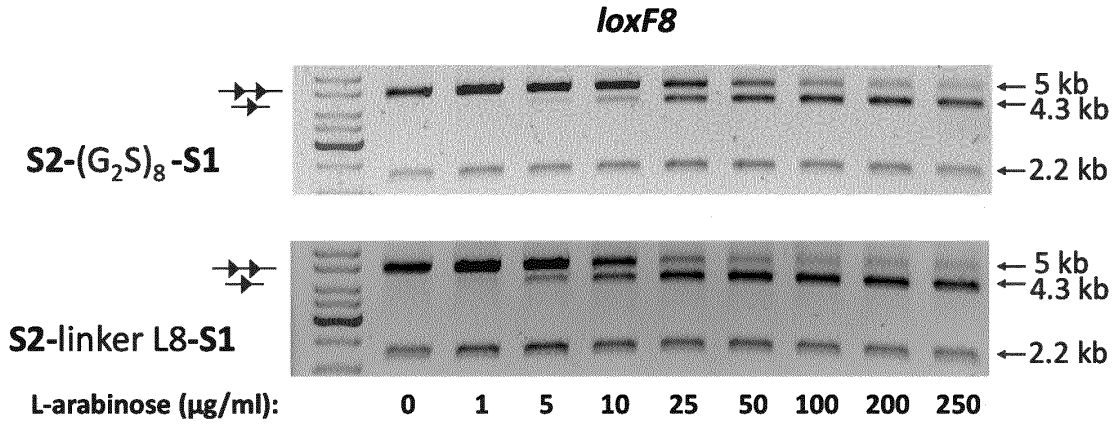


Figure 19

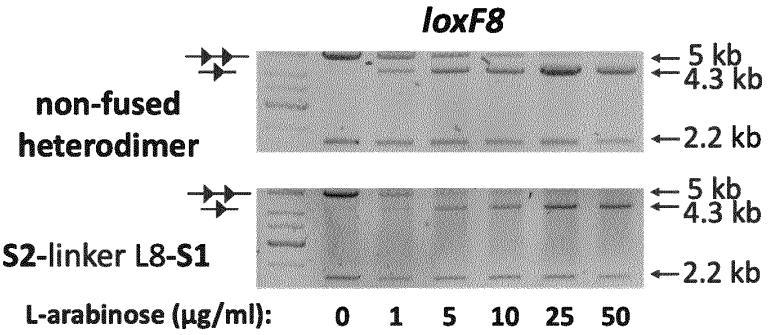


Figure 20

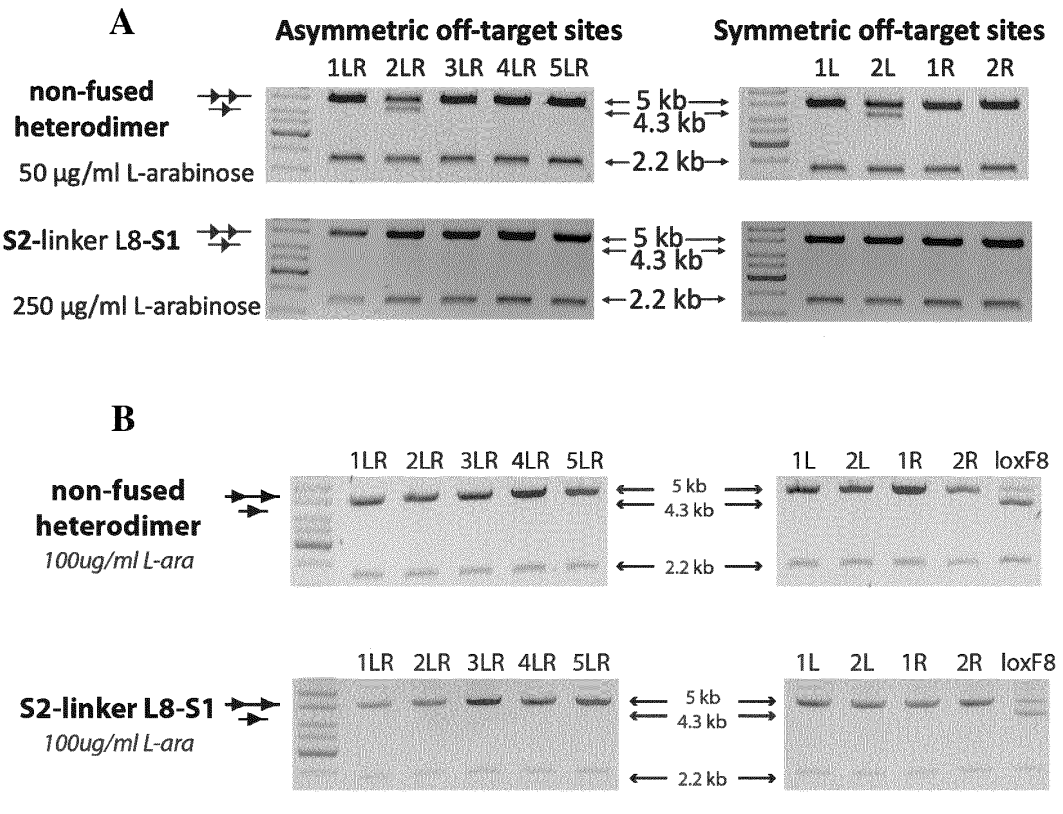


Figure 21

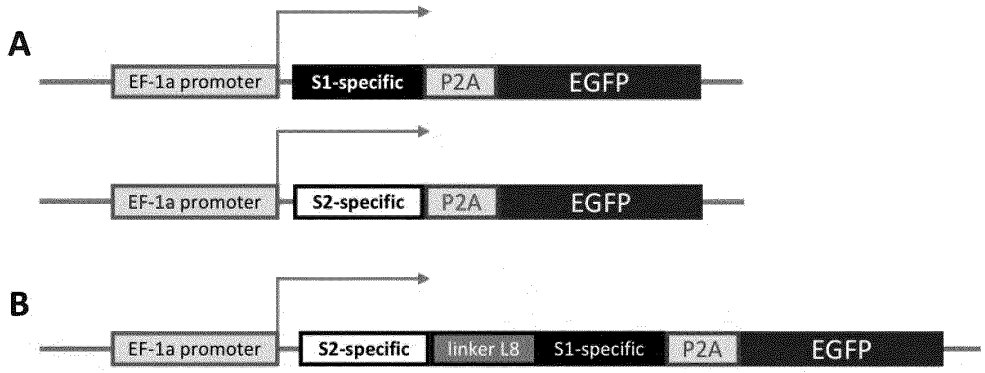


Figure 22

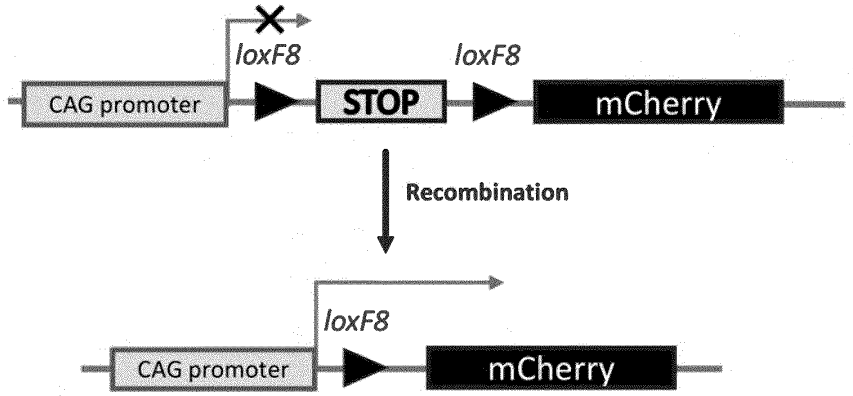


Figure 23

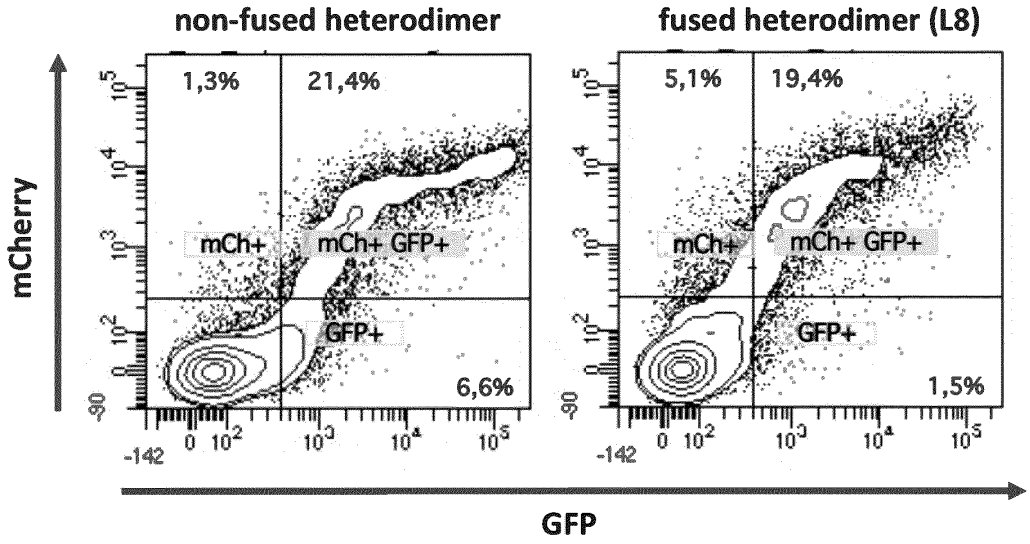


Figure 24

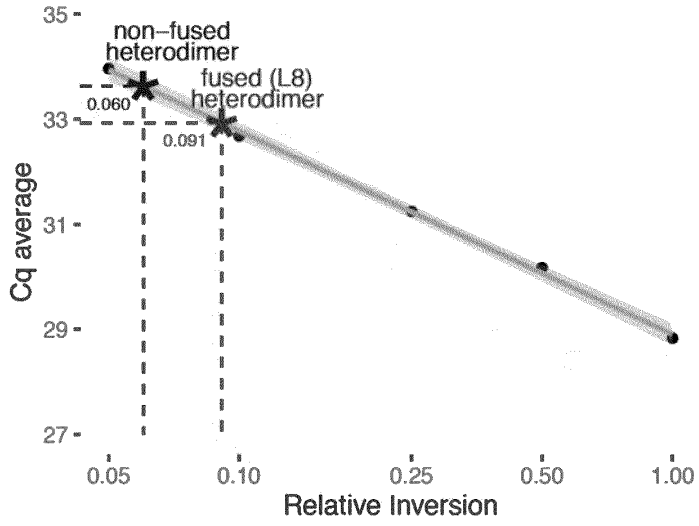
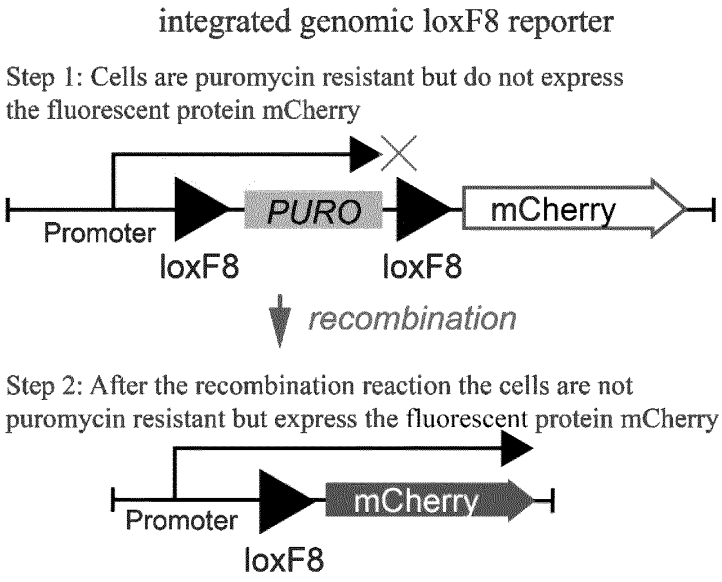


Figure 25

A


integrated genomic loxF8 reporter
Step 1: Cells are puromycin resistant but do not express the fluorescent protein mCherry
Promoter
PURO
mCherry
loxF8
loxF8
recombination
Step 2: After the recombination reaction the cells are not puromycin resistant but express the fluorescent protein mCherry
Promoter
mCherry
loxF8

B

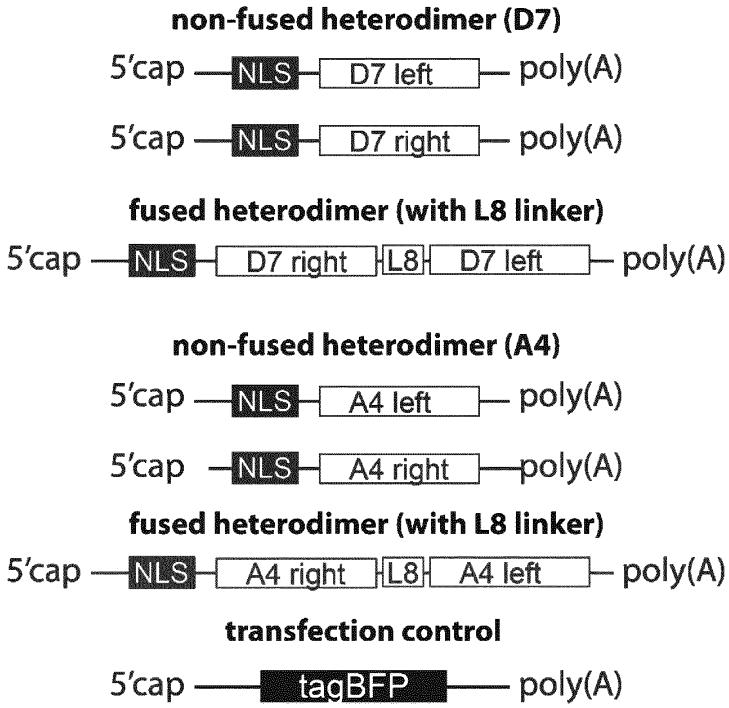

non-fused heterodimer (D7)
5'cap NLS D7 left poly(A)
5'cap NLS D7 right poly(A)
fused heterodimer (with L8 linker)
5'cap NLS D7 right L8 D7 left poly(A)
non-fused heterodimer (A4)
5'cap NLS A4 left poly(A)
5'cap NLS A4 right poly(A)
fused heterodimer (with L8 linker)
5'cap NLS A4 right L8 A4 left poly(A)
transfection control
5'cap tagBFP poly(A)

Figure 25 continued

C

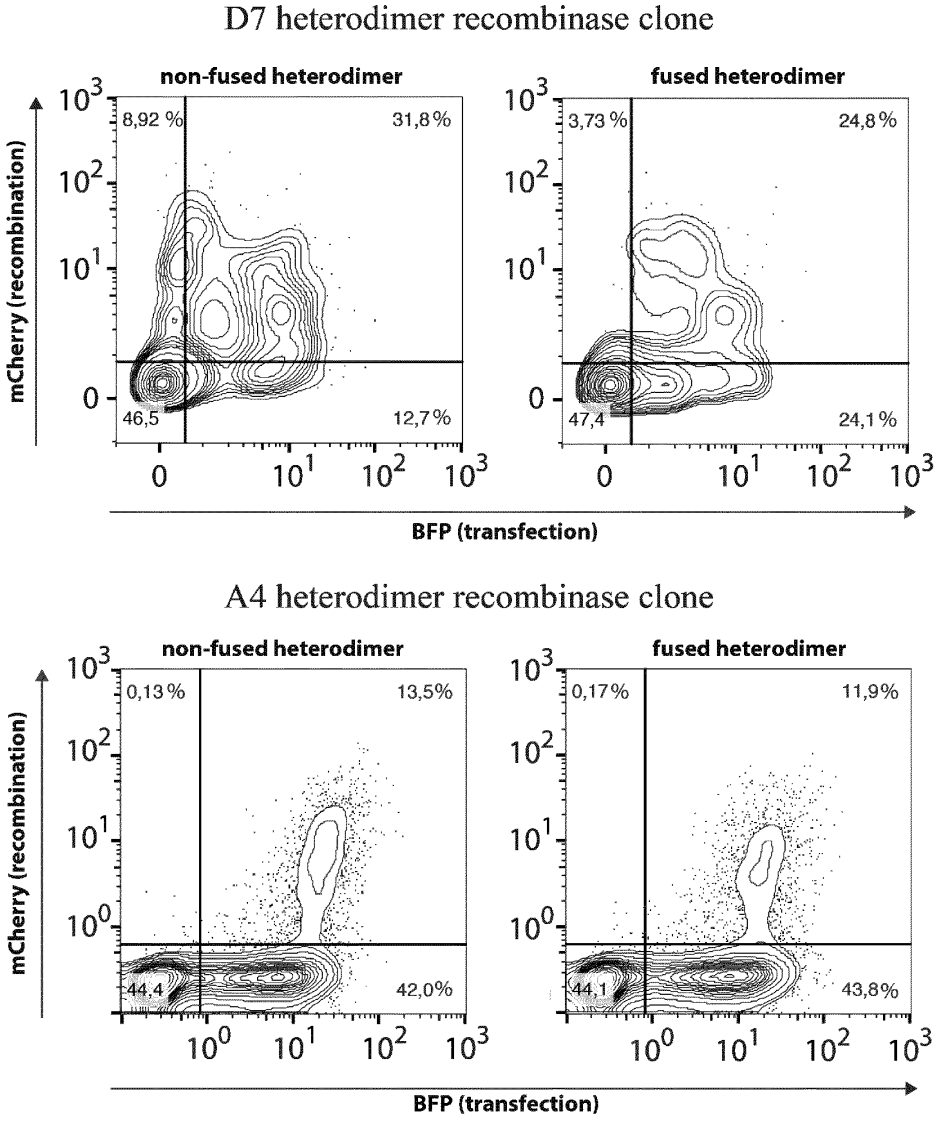
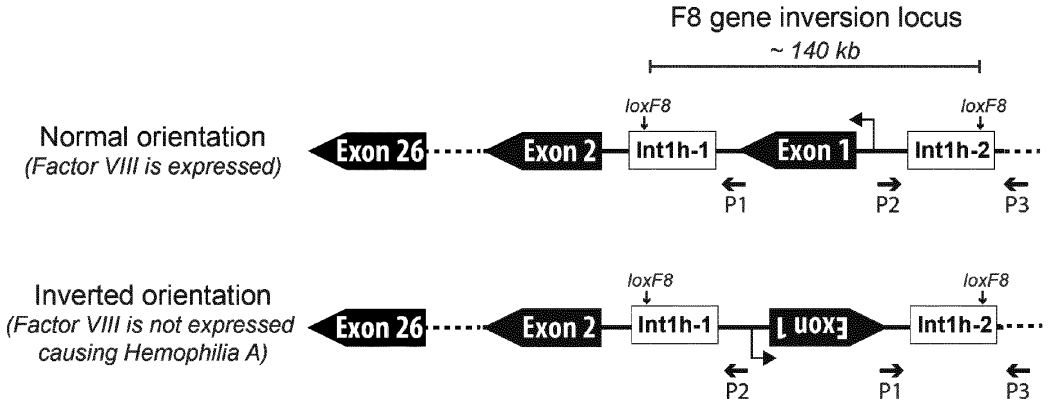


Figure 26

A


F8 gene inversion locus
~ 140 kb
Normal orientation
(Factor VIII is expressed)
loxF8
loxF8
Exon 26
Exon 2
Int1h-1
Exon 1
Int1h-2
P1
P2
P3
Inverted orientation
(Factor VIII is not expressed causing Hemophilia A)
loxF8
loxF8
Exon 26
Exon 2
Int1h-1
Exon 1
Int1h-2
P2
P1
P3

B

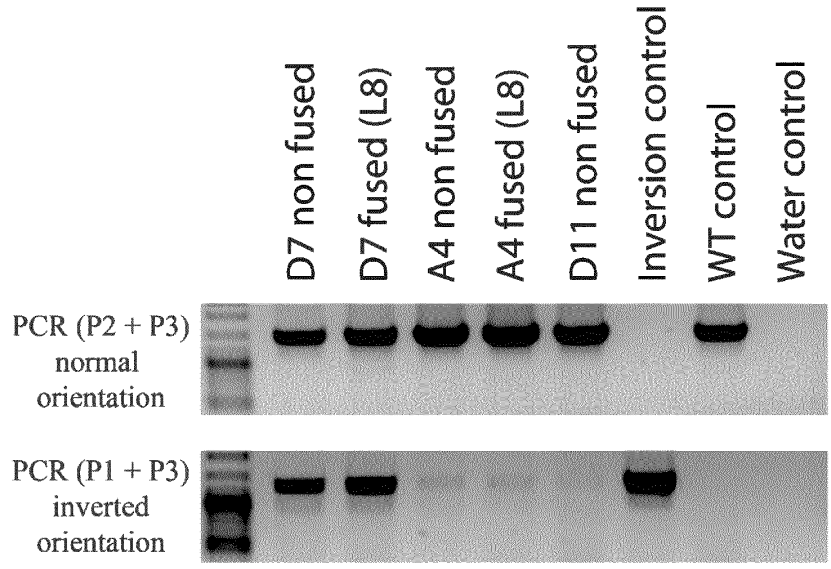
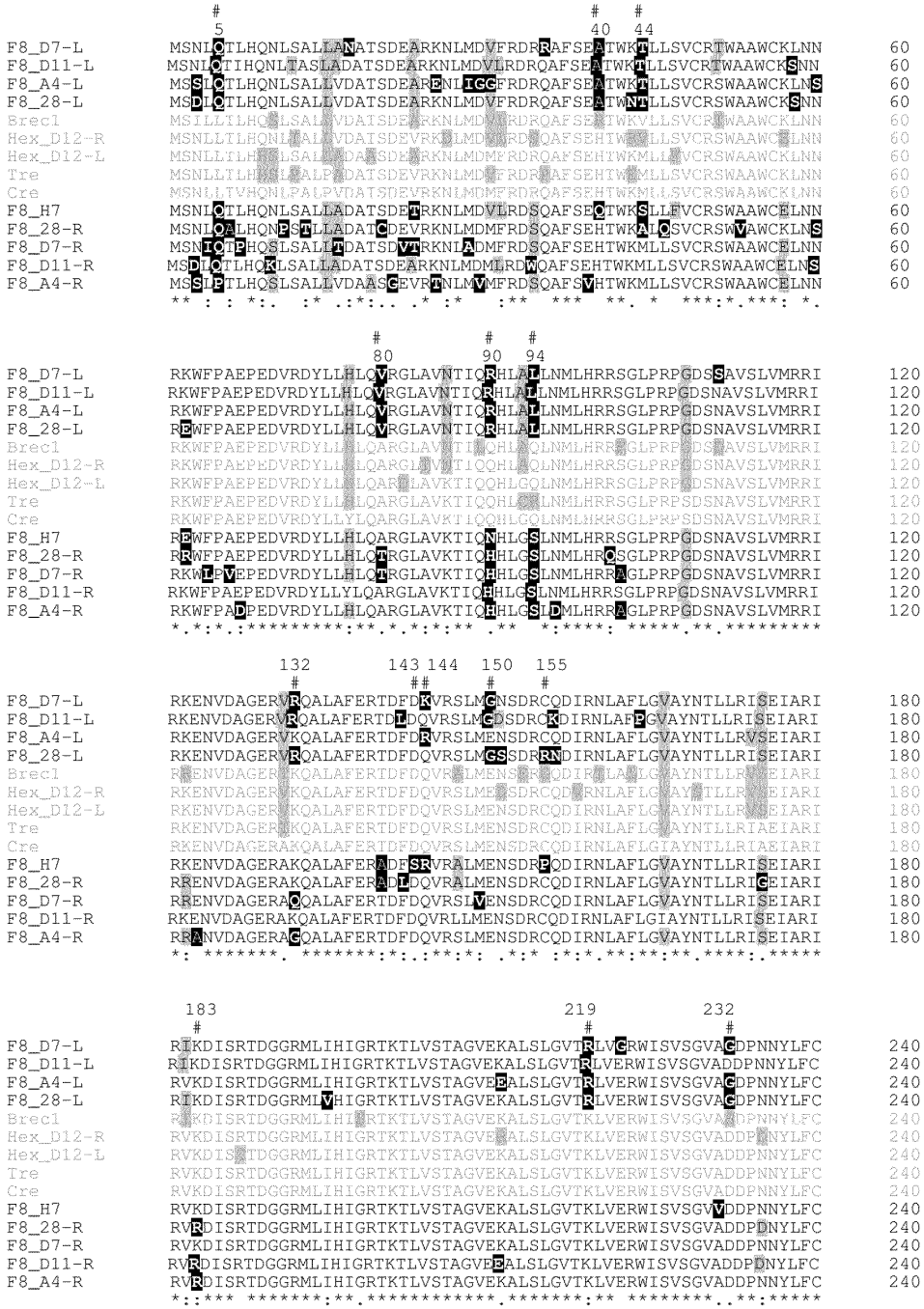

D7 non fused
D7 fused (L8)
A4 non fused
A4 fused (L8)
D11 non fused
Inversion control
WT control
Water control
PCR (P2 + P3) normal orientation
PCR (P1 + P3) inverted orientation

Figure 27


F8_D7-L
F8_D11-L
F8_A4-L
F8_28-L
Brec1
Hex_D12-R
Hex_D12-L
Tre
Cre
F8_H7
F8_28-R
F8_D7-R
F8_D11-R
F8_A4-R

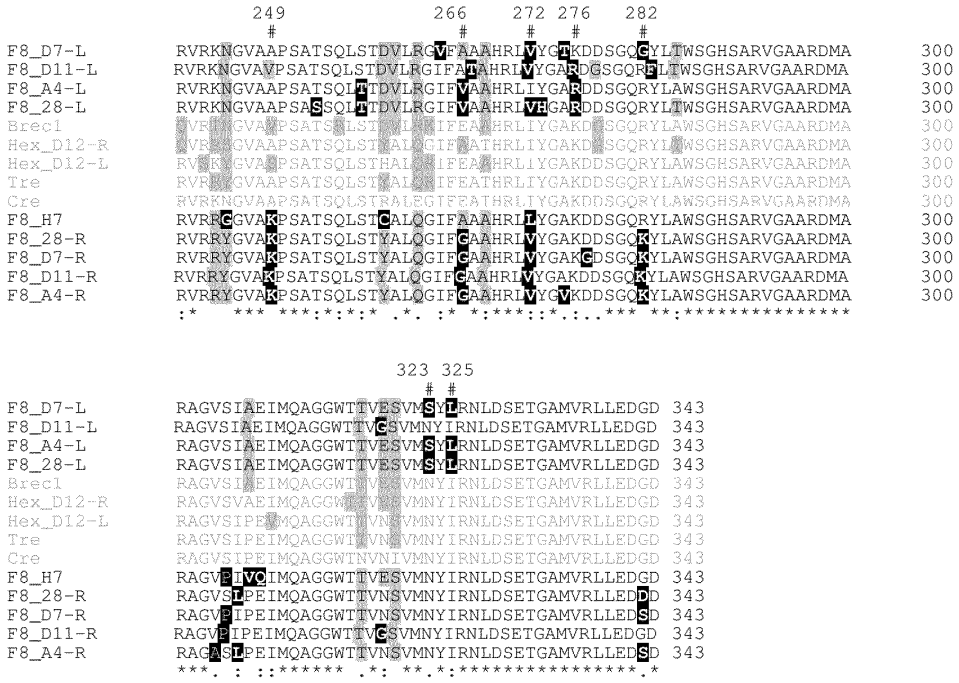


Figure 27 (cont.)