# Figure S1

```
>tr|A9M500|A9M500_9VIBR Phage integrase family protein.
          Length = 380

 Score =  115 bits (288), Expect = 5e-24,   Method: Compositional matrix adjust.

 Identities = 94/321 (29%), Positives = 155/321 (48%), Gaps = 15/321 (4%)

Cre : 25  KNLMDMFRDRQA-FSEHTWKMLLSVCRSWAAWCKLNNRKWFPAEPEDVRDYLLYLQARGL 83
          ++L++ F+ R+  +S +T + L S     +  WC  N+R   PA P+ V  + +  +A  L
VCre: 45  EHLLNEFKKREERYSPNTLRRLESAWTCFVDWCLANHRHSLPATPDTVEAFFIE-RAEEL 103

Cre : 84  AVKTIQQHLGQLNMLHRRSGLPRPSDSNAVSLVMRRIRKENVDAGERAKQALAFERTDFD 143
             T+  +   ++ +HR +G P P     V   ++ I ++ V  GE  KQA  F
VCre: 104 HRNTLSVYRWAISRVHRVAGCPDPCLDIYVEDRLKAIARKKVREGEAVKQASPFNEQHLL 163

Cre : 144 QVRSLMENSDRCQDIRNLAFLGIAYNTLLRIAEIARIRVKDISRTDGGRMLIHIGRTKTL 203
          ++ SL   SD+    RNLA L +AY ++LR +E+A IRV D+     G  ++ I  TKT
VCre: 164 KLTSLWYRSDKLLLRRNLALLAVAYESMLRASELANIRVSDMELAGDGTAILTIPITKTN 223

Cre : 204 VSTAGVEKALSLGVTKLVERWISVSGVADDPNNYLFCRVRK-NGVAAPSATSQ------- 255
           S       LS  V  L+  +     +    + +LF  V K N    P    Q
VCre: 224 HSGEPDTCILSQDVVSLLMDYTEAGKLDMSSDGFLFVGVSKHNTCIKPKKDKQTGEVLHK 283

Cre : 256 -LSTRALEGIFEATHRLIYGAKDDSGQRYLAWSGHSARVGAARDMARAGVSIPEIMQAGG 314
           ++T+ +EG+F +    +    D   Q    ++ HSARVGAA+D+ + G +  +I Q+G
VCre: 284 PITTKTVEGVFYSAWETL----DLGRQGVKPFTAHSARVGAAQDLLKKGYNTLQIQQSGR 339

Cre : 315 WTNVNIVMNYIRNLDSETGAM 335
          W++  +V  Y R + +  GAM
VCre: 340 WSSGAMVARYGRAILARDGAM 360
```

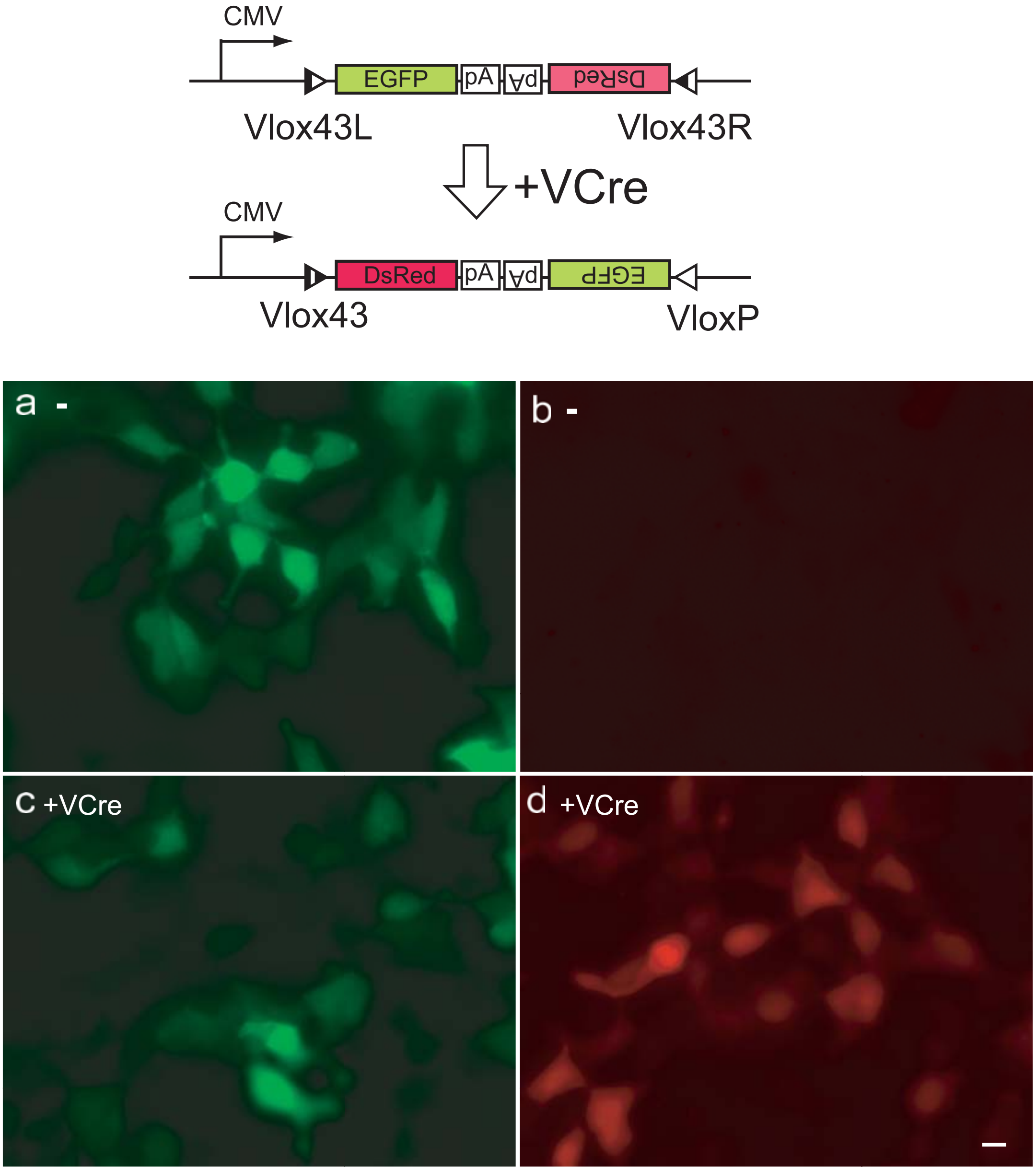

CMV
EGFP
pA
pA
DsRed
Vlox43L
Vlox43R
+VCre
CMV
DsRed
pA
pA
EGFP
Vlox43
VloxP
a -
b -
c +VCre
d +VCre

Figure S2

# Figure S3

```
>tr|Q7M0H5|Q7M0H5_CRIGR SubName: Full=Cre recombinase protein;
         Length = 350

 Score =  144 bits (362), Expect = 2e-32,   Method: Compositional matrix adjust.
 Identities = 104/330 (31%), Positives = 174/330 (52%), Gaps = 23/330 (6%)

SCre: 58   EITQAKLKHFLDNGHRTRRA---NTWRALMSRWAKFESWCLTNNLTPLPATPEVVATFIE 114
           + T  +++  L +  R R+A   +TW+ L+S    + +WC  NN    PA PE V  ++
Cre : 24   DATSDEVRKNLMDMFRDRQAFSEHTWKMLLSVCRSWAAWCKLNNRKWFPAEPEDVRDYLL 83

SCre: 115  YYQAS--SYTTLSQYAWAINSFHVECGLLSPVSSKTVQDKQNEIRIVKLESGGLAQEQAT 172
           Y QA   +  T+ Q+   +N  H   GL  P  S  V      IR   +++G  A+ QA
Cre : 84   YLQARGLAVKTIQQHLGQLNMLHRRSGLPRPSDSNAVSLVMRRIRKENVDAGERAK-QAL 142

SCre: 173  PFRLHHLQMLIESYGESERLLDKRNLALLNIAYESLLRESELLRIKVGHLKSTFEGDYVL 232
            F       +      S+R  D RNLA L IAY +LLR +E+ RI+V  +  T  G  ++
Cre : 143  AFERTDFDQVRSLMENSDRCQDIRNLAFLGIAYNTLLRIAEIARIRVKDISRTDGGRMLI 202

SCre: 233  SVPYTKT--NDSGEEEVVNITPLGF-KLIQRYIQGAGLTKE--DYLFQPIGRSNKVSVQA 287
            +  TKT  + +G E+ ++   LG  KL++R+I  +G+  +  +YLF  + R N V+  +
Cre : 203  HIGRTKTLVSTAGVEKALS---LGVTKLVERWISVSGVADDPNNYLFCRV-RKNGVAAPS 258

SCre: 288  KP--MSTRTVDRVF------LWAFESLGIDRHSAWSGHSARIGAAQDLLAAGYSIAQIQE 339
               +STR ++ +F      ++  +     R+ AWSGHSAR+GAA+D+  AG SI +I +
Cre : 259  ATSQLSTRALEGIFEATHRLIYGAKDDSGQRYLAWSGHSARVGAARDMARAGVSIPEIMQ 318

SCre: 340  NGRWKSPMMVLRYGKDIKAKESAMAKMLAE 369
            G W +  +V+ Y +++ ++  AM ++L +
Cre : 319  AGGWTNVNIVMNYIRNLDSETGAMVRLLED 348
```

```
>tr|A9M500|A9M500_9VIBR SubName: Full=Phage integrase family
           protein;
          Length = 380

 Score =  186 bits (471), Expect = 5e-45,   Method: Compositional matrix adjust.
 Identities = 122/302 (40%), Positives = 170/302 (56%), Gaps = 16/302 (5%)

SCre : 78  NTWRALMSRWAKFESWCLTNNLTPLPATPEVV-ATFIEYYQASSYTTLSQYAWAINSFHV 136
           NT R L S W  F  WCL N+   LPATP+ V A FIE  +     TLS Y WAI+  H
VCre : 61  NTLRRLESAWTCFVDWCLANHRHSLPATPDTVEAFFIERAEELHRNTLSVYRWAISRVHR 120

SCre : 137 ECGLLSPVSSKTVQDKQNEIRIVKLESGGLAQEQATPFRLHHLQMLIESYGESERLLDKR 196
             G   P     V+D+   I   K+  G  A +QA+PF   HL  L   +  S++LL +R
VCre : 121 VAGCPDPCLDIYVEDRLKAIARKKVREGE-AVKQASPFNEQHLLKLTSLWYRSDKLLLRR 179

SCre : 197 NLALLNIAYESLLRESELLRIKVGHLKSTFEGDYVLSVPYTKTNDSGEEEVVNITPLGFK 256
           NLALL +AYES+LR SEL  I+V  ++   +G  +L++P TKTN SGE +   ++
VCre : 180 NLALLAVAYESMLRASELANIRVSDMELAGDGTAILTIPITKTNHSGEPDTCILSQDVVS 239

SCre : 257 LIQRYIQGAGL--TKEDYLFQPIGRSNKV----------SVQAKPMSTRTVDRVFLWAFE 304
           L+  Y +   L  + + +LF  + + N             V  KP++T+TV+ VF  A+E
VCre : 240 LLMDYTEAGKLDMSSDGFLFVGVSKHNTCIKPKKDKQTGEVLHKPITTKTVEGVFYSAWE 299

SCre : 305 SLGIDRHSA--WSGHSARIGAAQDLLAAGYSIAQIQENGRWKSPMMVLRYGKDIKAKESA 362
           +L + R     ++ HSAR+GAAQDLL  GY+  QIQ++GRW S  MV RYG+ I A++ A
VCre : 300 TLDLGRQGVKPFTAHSARVGAAQDLLKKGYNTLQIQQSGRWSSGAMVARYGRAILARDGA 359

SCre : 363 MA 364
           MA
VCre : 360 MA 361
```

Figure S4

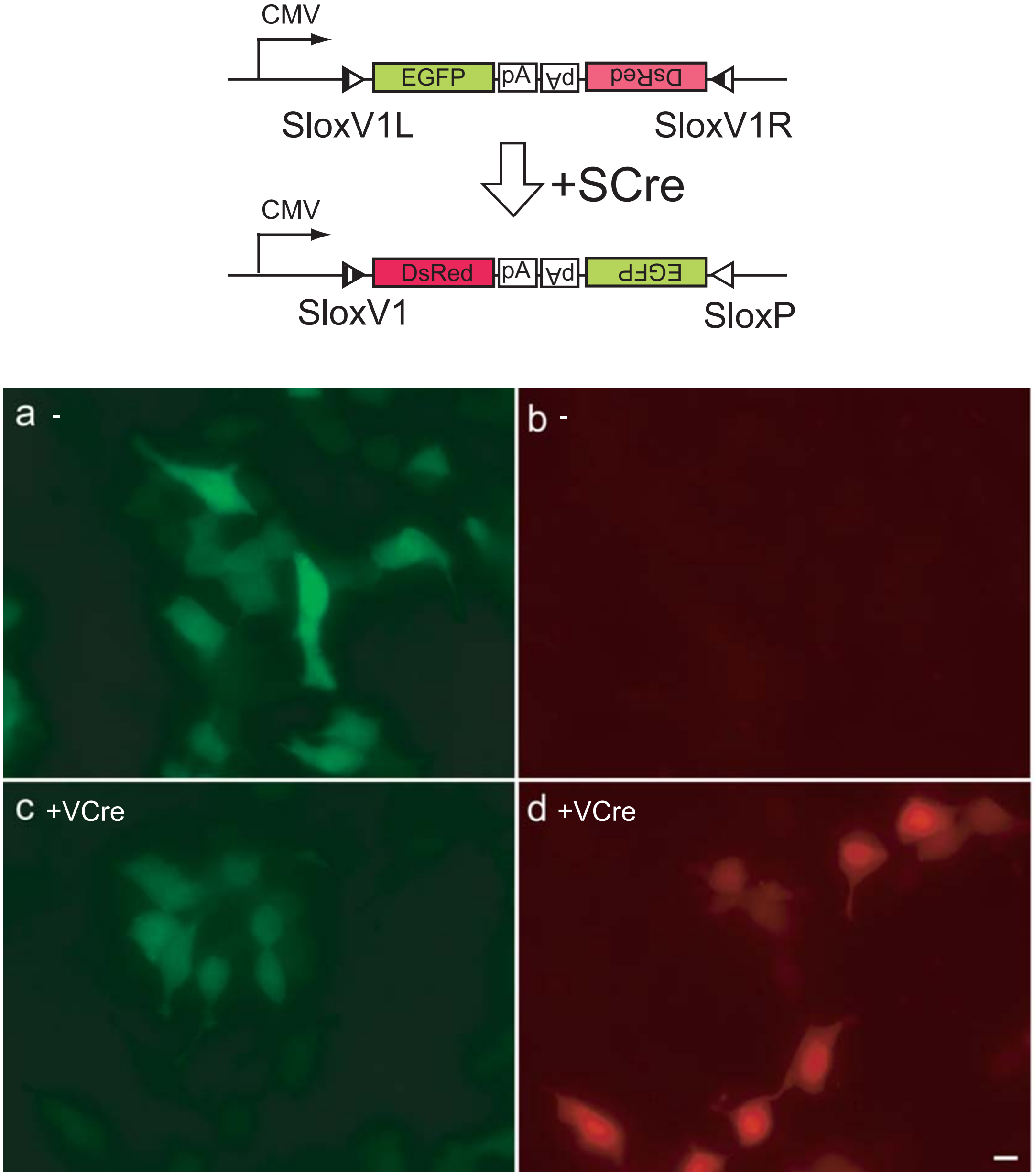

CMV
EGFP
pA
pA
DsRed
SloxV1L
SloxV1R
+SCre
CMV
DsRed
pA
pA
EGFP
SloxV1
SloxP
a -
b -
c +VCre
d +VCre

Figure S5

# Genetic modification of both alleles in human cells through four kinds of site-specific recombination systems

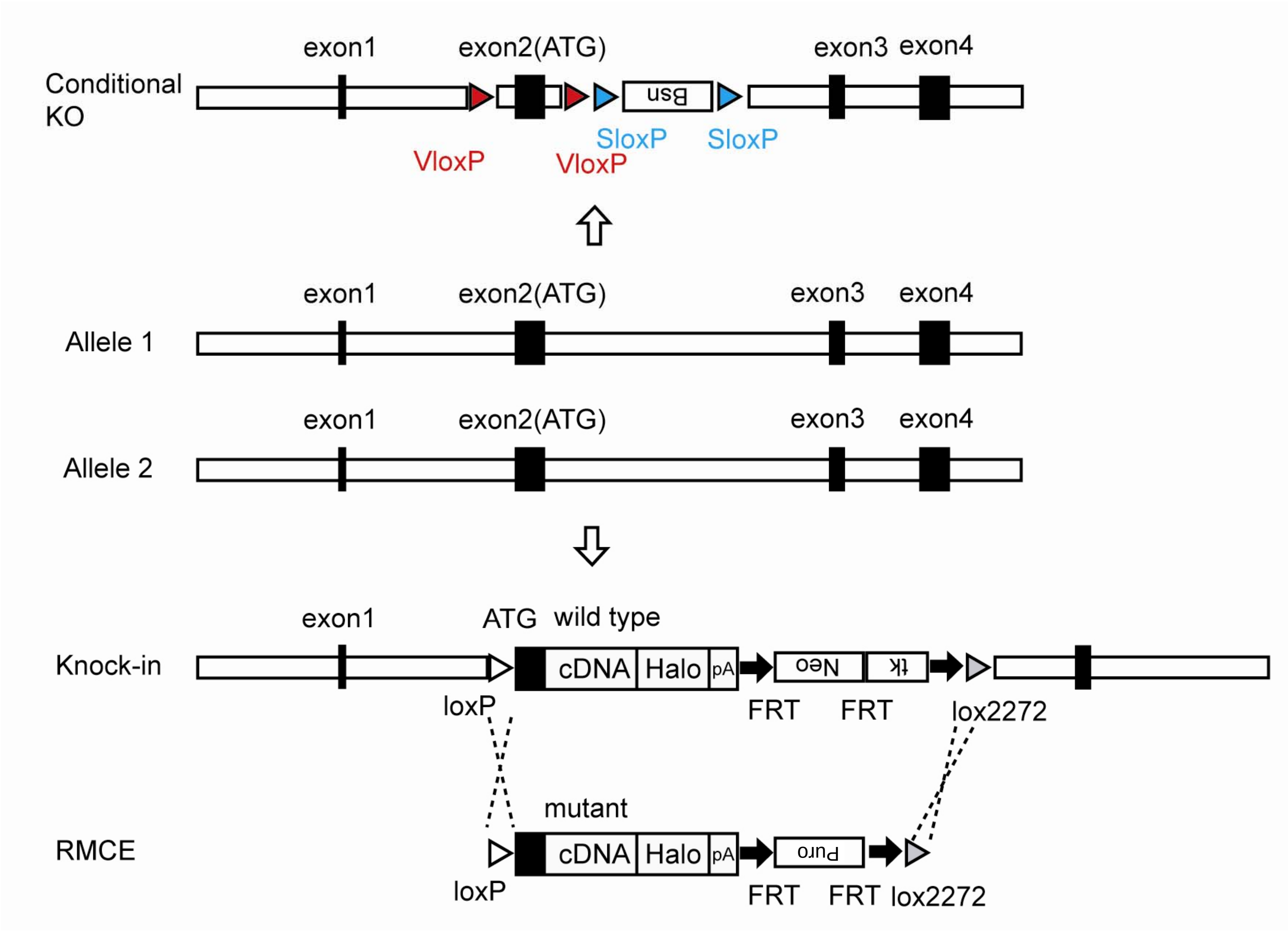


Figure S6

Exchange of mutant exon or C-terminal tag via multiple site-specific recombination

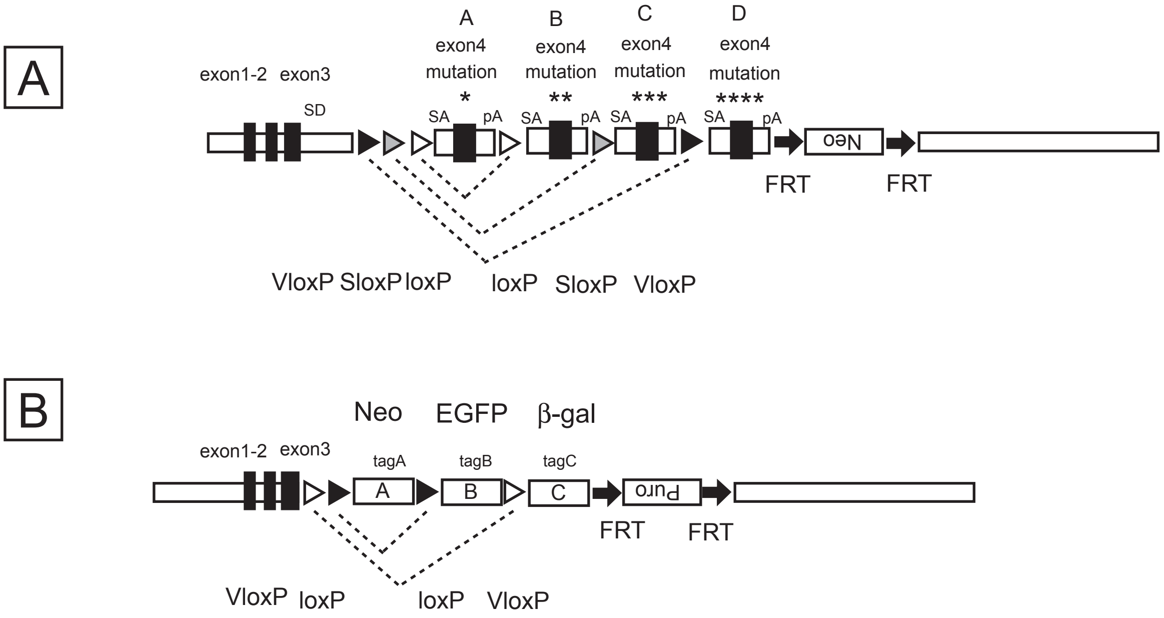
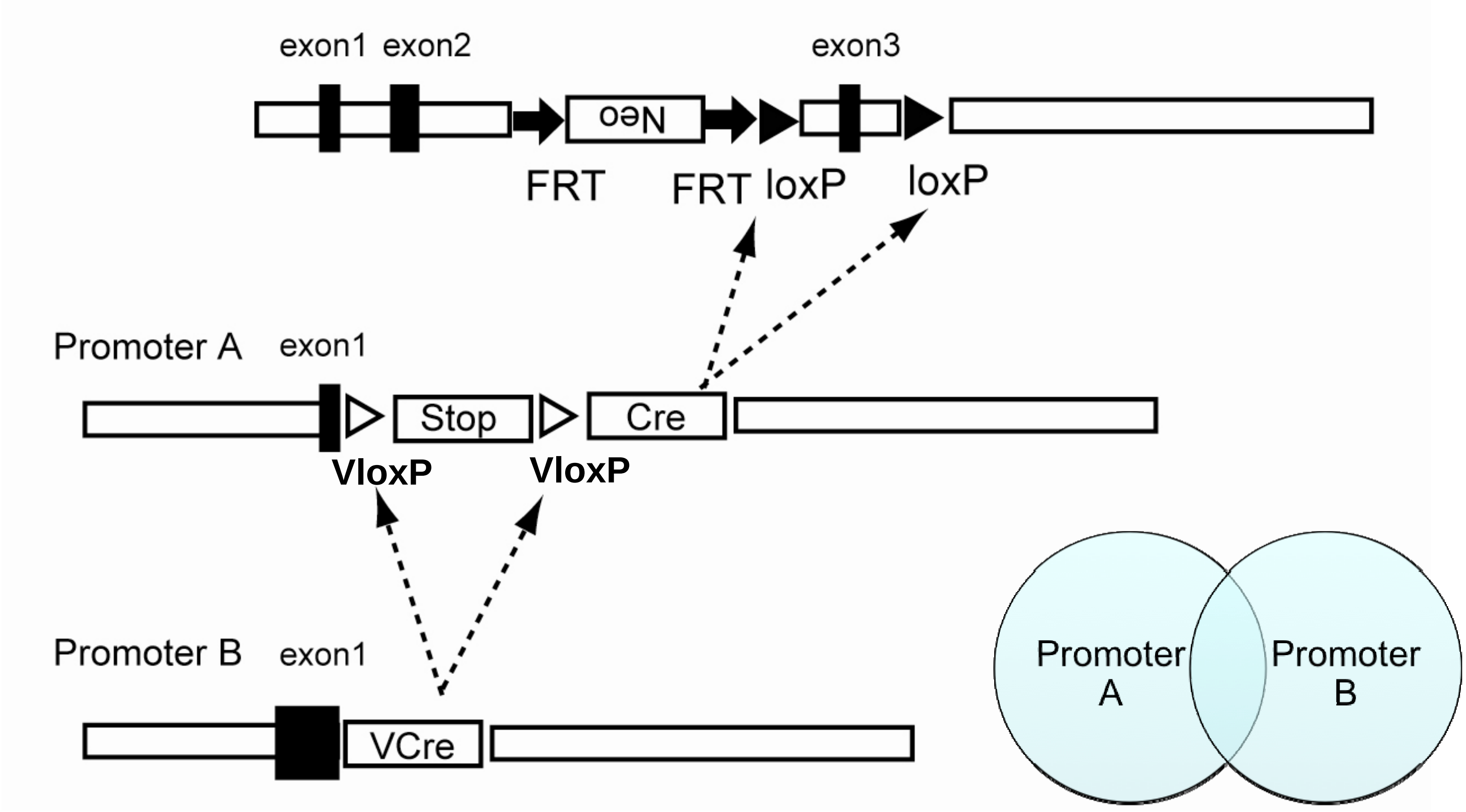


Figure S7

Figure S8

# Improving cell or tissue specificity in deleter mice by using a combination of two promoters



Introducing a foreign expression cassette into a human artificial chromosome (HAC) vector via the VCre/VloxP system and removing the HAC vector via the SCre/CloxP system

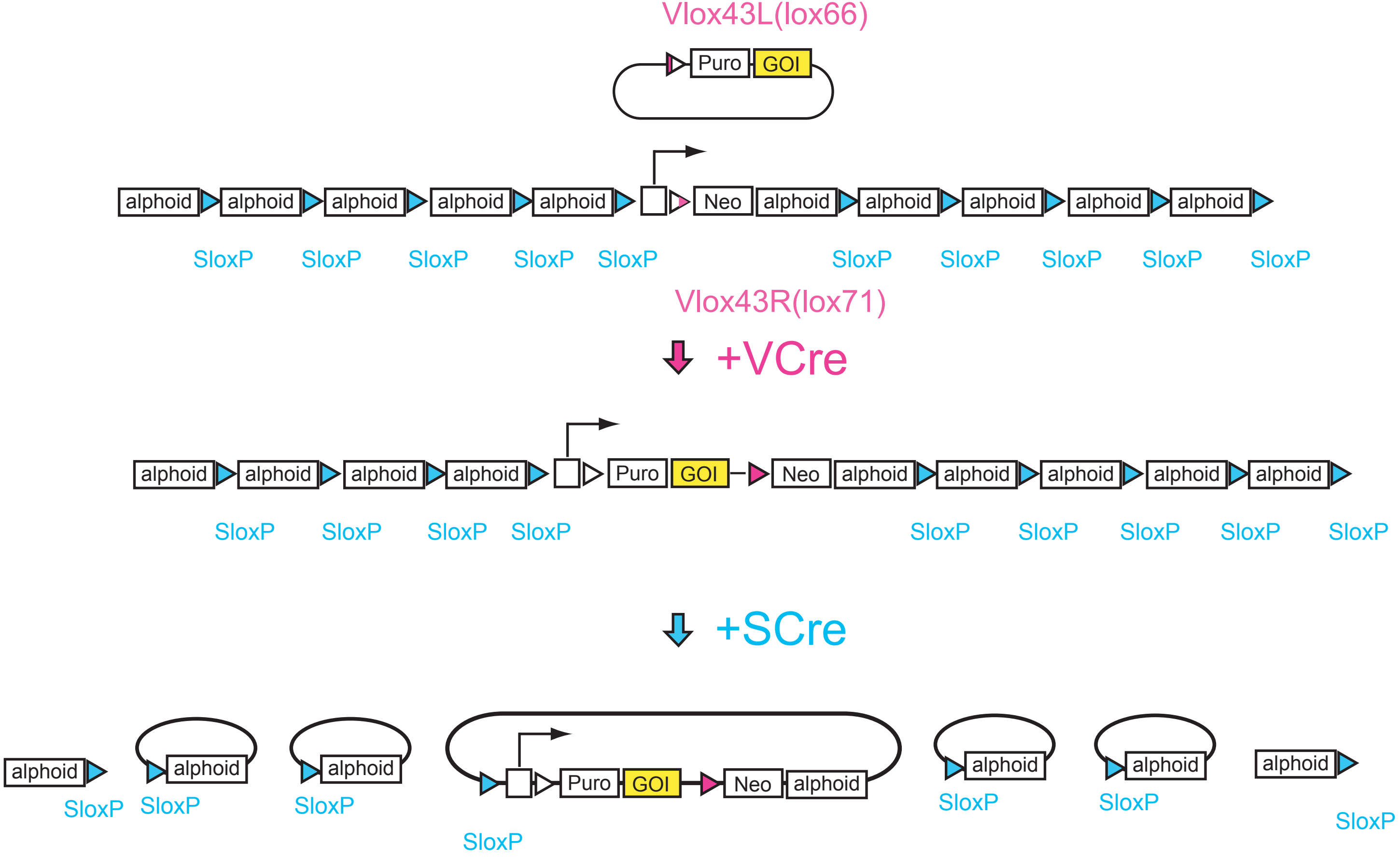


Figure S9

Double conditional knockout of paralogous or related genes

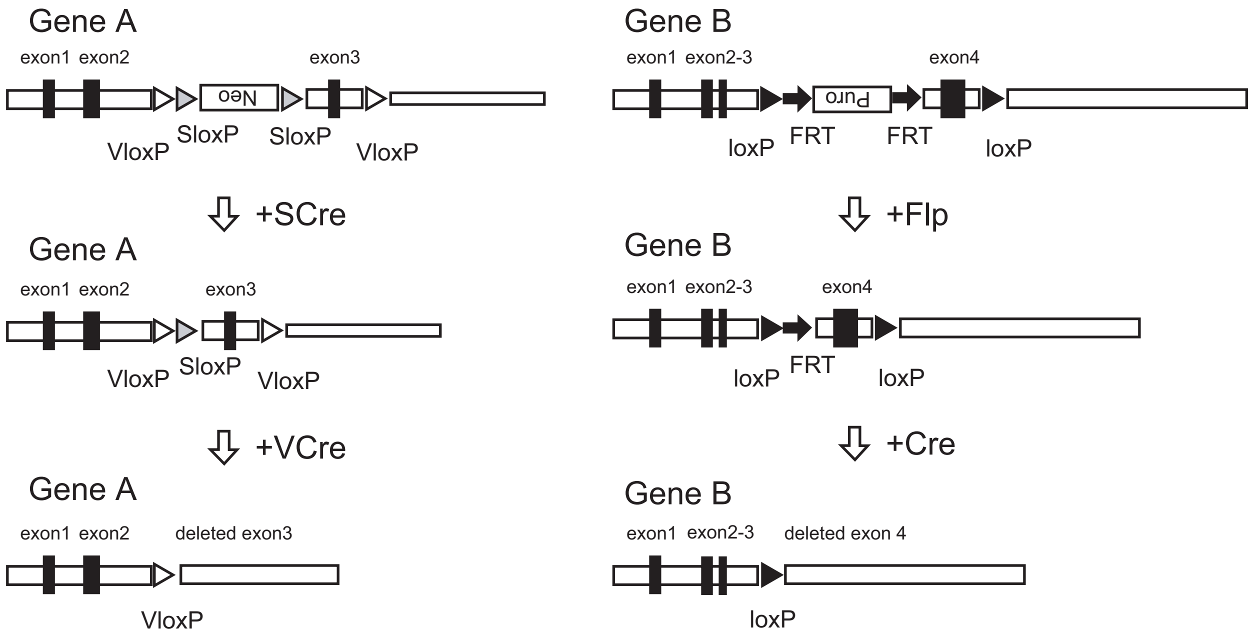


Figure S10