Halotag

pXF89:   (Cassette 2)   GCTCTTCGATG_Halotag_GGAAGCGGCGGTCGAAGAGC
                        SapI   M   G S G G   SapI

pXF88:   (cassette 3)   GCTCTTCGGGTGGAAGCGGC_Halotag_TAACGAAGAGC
                        SapI   G G S G   *   SapI

pSDH51: (cassette 3a) GCTCTTCGGGTGGAAGCGGC_Halotag_ACGCGAAGAGC
                        SapI   G G S G   T   SapI

```
                    GCCGAGgtaacacttagttttttgttgctctgtttaaaaattaat
                    A   E
tttaagATCGGAACCGGATTCCCATTCGACCCACACTACGTCGAGGTCCTCGGAGAGCGTATGC
       I  G  T  G  F  P  F  D  P  H  Y  V  E  V  L  G  E  R  M
ACTACGTCGACGTCGGACCACGTGACGGAACCCAGTCCTCTTCCTCCACGGAAACCCAACCTC
 H  Y  D  V  G  P  R  D  G  T  P  V  L  F  L  H  G  N  P  T  S
CTCCTACGTCTGGCGTAACATCATCCCACACGTCGCCCCAACCCACCGTTGCATCGCCCCAGgt
  S  Y  V  W  R  N  I  I  P  H  V  A  P  T  H  R  C  I  A  P
aagttttcttatgggaaagaaggaaaaaaccgagattttacttgaaaaattgaatttttcgcgg
gattttcaccaaaaattgttgaatattcattatttcacgctgtaaaacaaaaaaaaaaaaaatc
aaaaactacgttgaaatcgcgttttttaagcgaattttcttcagaattgccagattttaacccca
aattttgcagtttttaaataaaatttcacctttttcggctcaaattgtagattttcctgaaaatt
tagtacaaaaaacaatttcctcgtaaattttttcaaatagattttcagACCTCATCGGAATGGGA
                                                 D  L  I  G  M  G
AAGTCCGACAAGCCAGACCTCGGATACTTCTTCGACGACCACGTCCGTTTCATGGACGCCTTCA
 K  S  D  K  P  D  L  G  Y  F  F  D  D  H  V  R  F  M  D  A  F
TCGAGGCCCTCGGACTCGAGGAGGTCGTCCTCGTCATCCACGACTGGGGATCGGCTCTCGGATT
 I  E  A  L  G  L  E  E  V  V  L  V  I  H  D  W  G  S  A  L  G  F
CCACTGGGCCAAGCGTAACCCAGAGCGTGTCAAGGGAATCGCCTTCATGGAGTTCATCCGTCCA
 H  W  A  K  R  N  P  E  R  V  K  G  I  A  F  M  E  F  I  R  P
ATCCCAACCTGGGACGAGTGGCCAGgtaagttatgtctacctgcctgcctaccgcctaaatttt
 I  P  T  W  D  E  W  P
ttgtgaagtttcttcaaaaaatccagaaaaaaaaacaattttcatacgatttttttcccttaaaa
ttgtgaattttcatgcttttttagccccaaaagtcattatttgagaaaaaatttcatacaaaaaa
gttttgagaaatacacaattttttaaatgtaattttcaaattttcaattttcaactagaaaatt
cacaaaacttgtaaattttggaccaaaacatttatacaattactttttttgaatctaataacta
caataactacaaatttaacattttcagAGTTCGCCCGTGAGACATTCCAAGCCTTCCGTACCAC
                            E  F  A  R  E  T  F  Q  A  F  R  T  T
CGACGTCGGACGTAAGCTCATCATCGACCAAAACGTCTTCATCGAGGGAACCCTCCCAATGGGA
 D  V  G  R  K  L  I  I  D  Q  N  V  F  I  E  G  T  L  P  M  G
GTCGTCCGTCCACTCACCGAGGTCGAGATGGACCACTACCGTGAGCCATTCCTCAACCCAGTCG
 V  V  R  P  L  T  E  V  E  M  D  H  Y  R  E  P  F  L  N  P  V
ACCGTGAGCCACTCTGGCGTTTCCCAAACGAGCTCCCAATCGCCGGAGAGCCAGCCAACATCGT
 D  R  E  P  L  W  R  F  P  N  E  L  P  I  A  G  E  P  A  N  I  V
CGCCCTCGTCGAGGAGTACATGGACTGGCTCCACCAATCCCCAGTCCCAAAGCTCCTCTTCTGG
  A  L  V  E  E  Y  M  D  W  L  H  Q  S  P  V  P  K  L  L  F  W
GGAACCCCAGgtaagttcttttttttgaaaagtgcagtttgtagtctaattttcatttattttct
 G  T  P
Ttttaaaaaacgcatcaattttttaaatattttgggacaaaatccgaaaactgtactaatttgta
gtttgtaaaattaaaaaaaaaacgcaaaaaaatgttttcaaaaatgcttagaataaaaataaaa
tttctaaaaaattgaacaaaataaacatttaaaaattcaaaaagtttgaaaaaatgcaaagttt
tttaatgcaaaaaatatttaatgtttaaacaaatttaaaaaatgttgtaagaatatgtttagag
acagttttttaaatgttaaattgcaaaaaaaaaaaagcaaaaaaaaatttgaaaaatgtaacaga
aaatttttaattaaaaaagtcgtgtttttttcaaacctgcaattctttgtaattttatttataaa
gagaaacaaatcaaattgtatattacgaaaaacgatactaaaattcgaaaatttgcggttttttt
gcgtaaaaaatacggttccgtaattttcagGAGTCCTCATCCCACCAGCCGAGGCCGCCCGTCT
                              G  V  L  I  P  P  A  E  A  A  R  L
CGCCAAGTCCCTCCCAAACTGCAAGGCCGTCGACATCGGACCAGGACTCAACCTCCTCCAAGAG
  A  K  S  L  P  N  C  K  A  V  D  I  G  P  G  L  N  L  L  Q  E
GACAACCCAGgtaagttcatagattttgaaaaaaagtttaagaactgaaaaatggaataaaaat
 D  N  P
Atttaagagcattttttaaatgtaaaattaacaaaaaaagcgcctaagaatgtttccaaaacagt
aaaaaaaggtttaaaaaaatgcaaaaaaaaaatttaagatgcttttaattacacaaaaaaatgaa
agtaaaaaaaattaaaatattgaaaaatgtatatttgtttgaaaaggtacattttttcaatgcaa
aaaatgtttctaaaaattaaaaaaaatatgtttaaattgtgtaaagtgtaaattaaaaaataaaa
ttatgaaaaagtattgtttcatactttttctaaattttggttgaaaaatttaaaattccagctt
tttattcacattatgtgttctaaaaatctttccccaattttcgggaccgtttttccagtatattt
ccatttttcaaaaagattttttaactgaaattcatgtttttcaatgctaaaaatcaataaaaaaag
aattttttcagACCTCATCGGTTCCGAGATCGCCCGTTGGCTCTCCACCCTCGAGATCTCCGGA
            D  L  I  G  S  E  I  A  R  W  L  S  T  L  E  I  S  G
```

Figure S1. Sequence of HaloTag donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

ceGFP with PATC introns

pJF5: (cassette 2) GCTCTTCGATG_ceGFP_GGAAGCGGCGGTCGAAGAGC
                    SapI   M   G S G G   SapI

pJF6: (cassette 3) GCTCTTCGGGTGGAAGCGGC_ceGFP_TAACGAAGAGC
                    SapI   G G S G   *   SapI

                    TCCAAGgtaacacttagttttttgttgctctgtttaaaaattaat
                    S K
        tttaagGGAGAGGAGCTGTTCACCGGAGTCGTCCCAATCCTCGTCGAGCTCGACGGGGACGTCA
              G E E L F T G V V P I L V E L D G D V
        ACGGACACAAGTTCTCCGTGTCCGGAGAGGGAGAGGGAGATGCCACCTACGGAAAGCTCACCCT
        N G H K F S V S G E G E G D A T Y G K L T L
        CAAGTTCATCTGCACCACCGGAAAGCTCCCAGTCCCATGGCCAACCCTCGTCACCACCTTCTGC
         K F I C T T G K L P V P W P T L V T T F C
        TACGGAGTCCAATGCTTCTCCCGTTACCCAGgtaagttttctgttttatttacttgtttctgag
         Y G V Q C F S R Y P
        Aattgggatttttagctgaaaaattggattttaagatgaaaaattgaattttcaactaaaaat
        ttcaattttagctgaaaagttaaattttttagctaaagagtgggattttttagttgaaaaatcga
        gatttctagctaaaaagtatgattttttagctgaaagattggaattttaagcagaaaaaattgta
        tttttagctgataaatcaaattttaagcggaaaaatttgaattttttagctaaaaaattccattt
        tcaactaaaaatcgtgattttttagctttaaaaattggattttttagctgaaaaatcggattttta
        gctgaaaattgaatttttttagttaaaaagtgagattttttaacagaaaagtgagattttttagt
        taaaaaataataattttttagctaaaaaatacaattttcaactaaagatcgtgattttttagcttta
        aaaattggattttttagctaaaaaatcggattttttagctgaaaattgaattttttttagttaaaaa
        gtgggattttttaagatgaaaagtgagatttttttagctgaaaagtgagatttttcagctaaaaaata
        caattttcaactgaaaaattgggattttttagctttaaaaattggattttcagctttaaaaattg
        gattttttagctgaaaagtgagattttttagttgaaaaattgaaatgtttagctgaaaaattggaa
        tttttaagtaaaaaagtgggattttttaagatgaaaagtgagatgtttttagctgaaaagtgagat
        tttttagctaaaaaatacaattttcaactgaaaaattgggattttttagctcaaaagtgggacgaa
        agacgaaaaaaaaaatcgattttttttaccaaaaattagattttcagACCACATGAAGCGTCACGA
                                                      D H M K R H D
        CTTCTTCAAGTCCGCCATGCCAGAGGGATACGTCCAAGAGCGTACCATCTTCTTCAAGGACGAC
         F F K S A M P E G Y V Q E R T I F F K D D
        GGAAACTACAAGgtaagttatgtacgcgatttctcggcgaaaaatggactttttttgctcaaaa
         G N Y K
        Actgaatttttcagctttttttgcactgaaaatgctgaaaattggtttttttttgctgaaaattca
        ccgaaaaaagctgaaaatattgcagaaatcgacgaaaaatgcctttttttgctgaaatttcagcc
        agaaaattgttcagaatcgcaatttcctgccaaaatcgacgtttttcggcgaaaattggtagat
        tttgaaccgaaaattgtgaaaaatggacttttttgctcaaaaacagactttttgagcttttttc
        acactgaaaatggtggaaaattggattttccagcagaaaattcggaaatttttcaattttttggcg
        gaaatttgacgaaaaatatatgaaaaacaccgatttttgtctgaaaaagttcaaaagccgaaaa
        attggccaaaaatctccaaaatcggacttttctggcgtttttcgtggaaaaattgaaattttttt
        tgaaaaattgagcaaaaatcgccgaaaaaccgctaaaaaaactgccgaaattggcaattttgag
        cttaaaaaaaacttgaaaaatgctcaaaattacccaattttcaccggaaattgagctaaaatcgt
        cgaaaaagactgaattttcaattttttagctgaaaatctgcctattttcggctaaaattgcttta
        aaaacattaaaattcaaatttttttggctaaaaatactcagattgcccttttttttttctccaaaaa
        tgacaaaaaaaaaatcaatcagaaaaatgcgatttttttttacaaaaattggcttcaaaacgtct
        aaattctccctaattttttgctgctgaaaattgtgcaaaaatcaccgaaaaatgacgattttagg
        ctgaaaatcttcaaaaaaagctcaaaattccccacttttcaccggaaaaagacgaaaaaaagctc
        caaatttatcgattaacattttcagACCCGTGCCGAGGTCAAGTTCGAGGGAGACACCCTCGTC
                                  T R A E V K F E G D T L V
        AACCGTATCGAGCTCAAGGGAATCGACTTCAAGGAGGACGGTAACATCCTGGGACACAAGCTGG
         N R I E L K G I D F K E D G N I L G H K L
        AGTACAACTACAACTCCCACAACGTCTACATCATGGCCGACAAGCAAAAGAACGGAATCAAGGT
        E Y N Y N S H N V Y I M A D K Q K N G I K V
        CAACTTCAAGgtaagttctttttttttgctcgttttttgagtgaaaaattgatggaattcacaaaa
         N F K
        aatggccaaagaatcctcaaaattggccgaaaattctgaaaaatgacctaaaaatcaccgaaaa
        acggccaaaaattatctgaaaatcgccgaaaatctcaaattttgaggaaattcccgctaaaaat
        taataaaaatcaccgaaaaaaggccaaaaattacctaataaaggccgaaaatcgcctaaaaagg
        ccaaaaattctgaaaaatggccaaatttcaattttcacacacaaaaaatccctgaattttcta
        tttttactcgaaaaactgtggatttacgggggtaaaaattcagtttttagtgctatttcacaag
        aaaaacacagcattttcgacaaaaactccgatttttaagccaaaaatttgaggtttttagtttt
        ttacgtgaaaacctgaactttttgccaaaaaaacacttttttttttagactaaaaatcaatttttcg
        atcaaaaatcactaattttgcctgaaaaaatatagatttttccactttttcactgaaaaaaaac
        tggattttcactagaaaatcaagttgtttttagaccaaaaattttattttttccgctgaaaatta
        tcgtttttcagtcaattttcagctaaaaatccctgaaattctggattttcagccaaaaaaatcg
        aaatttccaaaaaaaaccccgtttaaaaatgagtttagactgaaaattccgattttttgagccaa
        aaatttgattttttaatcgaaaaaaattggattttcaaccaaaaaatcagttttttcagtgattt
        tagagtaaaaatcactgattttaacacaaaattccagtttttcgtgattttttcactagaaaatc
        ccgattttttccccaaaaaatccattttttccgactaaaaatcccccaaagtaattttcagATCCGT
                                                                      I R
        CACAACATCGAGGACGGATCGGTCCAACTCGCCGACCACTACCAACAAAACACCCCAATCGGTG
         H N I E D G S V Q L A D H Y Q Q N T P I G
        ACGGACCAGTCCTCCTCCCAGACAACCACTACCTCTCCACCCAATCCGCCCTCTCCAAGGACCC
        D G P V L L P D N H Y L S T Q S A L S K D P
        AAACGAGAAGCGTGACCACATGGTCCTCCTCGAGTTCGTCACCGCCGCCGGAATCACCCACGGA
         N E K R D H M V L L E F V T A A G I T H G
        ATGGACGAGCTCTACAAG
        M D E L Y K

Figure S2. Sequence of ceGFP donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

mKate2

```
pXF222 (Cassette 2)    GCTCTTCGATG_mKate2_GGAGGTGGAGGTGGTCGAAGAGC
                       SapI   M             G G G G  G   SapI

pXF130 (Cassette 3)    GCTCTTCGGGTGGAGGTGGAGGT_mKate2_TAACGAAGAGC
                       SapI   G G G G               *   SapI

pSM04 (Cassette 3a)    GCTCTTCGGGTGGAGGTGGAGGT_mKate2_AGCCGAAGAGC
                       SapI   G G G G               S   SapI

pSM05 (Cassette 3b)    GCTCTTCGAGCGGAGGTGGAGGT_mKate2_TAACGAAGAGC
                       SapI   S G G G               *   SapI
```

```
       GTCTCCGAGCTCATTAAAGAAAACATGCATATGAAGCTCTACATGGAGGGAAC
        V  S  E  L  I  K  E  N  M  H  M  K  L  Y  M  E  G  T
CGTCAACAACCACCACTTCAAGTGCACCTCCGAGGGAGAGGGAAAGCCATACGAGGGAACCCAA
  V  N  N  H  H  F  K  C  T  S  E  G  E  G  K  P  Y  E  G  T  Q
ACCATGCGTATCAAGGCCGTCGAGGGAGGACCACTCCCATTCGCCTTCGACATCCTCGCCACCT
  T  M  R  I  K  A  V  E  G  G  P  L  P  F  A  F  D  I  L  A  T
CCTTCATGTACGGATCCAAGgtaagtttaaacatatatatactaactaaccctgattatttaaa
 S  F  M  Y  G  S  K
ttttcagACCTTCATCAACCACACCCAAGGAATCCCAGACTTCTTCAAGCAATCCTTCCCAGAG
        T  F  I  N  H  T  Q  G  I  P  D  F  F  K  Q  S  F  P  E
GGATTCACCTGGGAGCGTGTCACCACCTACGAGGACGGAGGAGTCCTCACCGCCACCCAAGACA
  G  F  T  W  E  R  V  T  T  Y  E  D  G  G  V  L  T  A  T  Q  D
CCTCCCTCCAAGACGGATGCCTCATCTACAACGTCAAGgtaagtttaaacagttcggtactaac
 T  S  L  Q  D  G  C  L  I  Y  N  V  K
taaccatacatatttaaattttcagATCCGTGGAGTCAACTTCCCATCCAACGGACCAGTCATG
                          I  R  G  V  N  F  P  S  N  G  P  V  M
CAAAAGAAGACCCTCGGATGGGAGGCCTCCACCGAGACCCTCTACCCAGCCGACGGAGGACTTG
 Q  K  K  T  L  G  W  E  A  S  T  E  T  L  Y  P  A  D  G  G  L
AGGGACGTGCCGACATGGCCCTCAAGCTCGTCGGAGGAGGACACCTCATCTGCAACCTCAAGgt
E  G  R  A  D  M  A  L  K  L  V  G  G  G  H  L  I  C  N  L  K
aagtttaaacatgattttactaactaactaatctgatttaaattttcagACCACCTACCGTTCC
                                                   T  T  Y  R  S
AAGAAGCCAGCCAAGAACCTCAAGATGCCAGGAGTCTACTACGTCGACCGTCGTCTTGAGCGTA
 K  K  P  A  K  N  L  K  M  P  G  V  Y  Y  V  D  R  R  L  E  R
TCAAGGAGGCCGACAAGGAGACCTACGTCGAGCAACACGAGGTCGCCGTCGCCCGTTACTGCGA
I  K  E  A  D  K  E  T  Y  V  E  Q  H  E  V  A  V  A  R  Y  C  D
CCTCCCATCCAAGCTCGGACACCGT
 L  P  S  K  L  G  H  R
```

Figure S3. Sequence of mKate2 donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

mScarlet

pSM10 (Cassette 2)   GCTCTTCGATG_mKate2_GGAGGTGGAGGTGGTCGAAGAGC
                     SapI   M   G G G G G   SapI

pSM08 (Cassette 3)   GCTCTTCGGGTGGAGGTGGAGGT_mScarlet_TAACGAAGAGC
                     SapI   G G G G G   *   SapI

pSDH57 (Cassette3a)

GCTCTTCGGGT_mScarlet_TTGGCTGAAGCGGCAGCTAAAGAAGCTGCCGCGAAGGAAGCAG
  SapI   G   L A E A A A A K E A A A K E A
CAGCCAAGGAGGCCGCAGCGAAGGAAGCCGCTGCAAAAGCCGCGGCCACGCGAAGAGC
 A A K E A A A K E A A A K A A A S   SapI

                      GTCTCCAAGGGCGAGGCAGTCATCAAGGAGTTCATGCGTTT
                       V S K G E A V I K E F M R F
CAAGGTGCACATGGAGGGATCCATGAACGGACACGAGTTCGAGATCGAGGGCGAGGGAGAGGGA
  K V H M E G S M N G H E F E I E G E G E G
CGCCCATACGAGGGAACCCAGACCGCCAAGCTCAAGGTGACCAAGgtaagtttaaacatatata
 R P Y E G T Q T A K L K V T K
tactaactaaccctgattatttaaattttcagGGTGGACCACTGCCATTCTCCTGGGACATCCT
                                  G G P L P F S W D I L
CTCCCCACAATTCATGTACGGCTCCCGTGCCTTCACCAAGCACCCAGCCGACATCCCCGACTAC
  S P Q F M Y G S R A F T K H P A D I P D Y
TACAAGCAATCCTTCCCCGAGGGATTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGAGGAG
 Y K Q S F P E G F K W E R V M N F E D G G
CCGTGACCGTCACCCAAGACACCTCCCTGGAGGACGGAACCCTCATCTACAAGGTGAAGCTCCG
 A V T V T Q D T S L E D G T L I Y K V K L R
TGGAACCAACTTCCCACCTGACGGCCCAGTCATGCAGAAGAAGACCATGGGATGGGAAGCCTCC
  G T N F P P D G P V M Q K K T M G W E A S
ACCGAGCGTTTGTACCCAGAGGACGGAGTGCTCAAGgtaagtttaaacagttcggtactaacta
 T E R L Y P E D G V L K
accatacatatttaaattttcagGGAGACATTAAGATGGCCCTCCGTCTGAAGGACGGAGGACG
                        G D I K M A L R L K D G G R
TTACCTGGCGGACTTCAAGACCACCTACAAGGCCAAGAAGCCAGTCCAGATGCCAGGAGCCTAC
  Y L A D F K T T Y K A K K P V Q M P G A Y
AACGTCGACCGCAAGCTCGACATCACCTCCCACAACGAGGACTACACCGTCGTGGAGCAATACG
  N V D R K L D I T S H N E D Y T V V E Q Y
AACGTTCCGAGGGACGCCACTCCACCGGAGGAATGGACGAGCTGTACAAG
 E R S E G R H S T G G M D E L Y K

Figure S4. Sequence of mScarlet donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

Dendra2

pSM12 (Cassette 2) GCTCTTCGATG_Dendra2_GGAAGCGGCGGTCGAAGAGC
                    SapI   M   G S G G   SapI

pSM03 (Cassette 3) GCTCTTCGGGTGGAAGCGGC_Dendra2_TAACGAAGAGC
                    SapI   G G S G   *   SapI


```
                   AACCTTATTAAGGAAGATATGAGAGTCAAAGTGCATATGGAAGG
                     N  L  I  K  E  D  M  R  V  K  V  H  M  E  G
AAACGTCAACGGTCATGCATTTGTTATTGAAGGTGAAGGTAAAGGAAAGCCATACGAAGGAACT
  N  V  N  G  H  A  F  V  I  E  G  E  G  K  G  K  P  Y  E  G  T
CAAACTGCTAACTTGACTGTCAAAGAAGGAGCACCACTACCATTTAGTTACgtaagtttaaaca
 Q  T  A  N  L  T  V  K  E  G  A  P  L  P  F  S  Y
tatatatactaactaaccctgattatttaaattttcagGATATTCTCACTACTGCCGTCCATTA
                                         D  I  L  T  T  A  V  H  Y
CGGAAACAGAGTTTTTACTAAATACCCAGAAGATATTCCTGATTACTTCAAGCAATCGTTTCCA
   G  N  R  V  F  T  K  Y  P  E  D  I  P  D  Y  F  K  Q  S  F  P
GAAGGATACTCGTGGGAAAGAACTATGACTTTCGAAGATAAAGGTATTTGCACTATTgtaagtt
 E  G  Y  S  W  E  R  T  M  T  F  E  D  K  G  I  C  T  I
taaacagttcggtactaactaaccatacatatttaaattttcagAGAAGTGATATTAGTCTCGA
                                              R  S  D  I  S  L  E
AGGTGATTGCTTCTTCCAAAATGTCAGATTTAAAGGTACTAACTTTCCTCCTAACGGACCAGTT
  G  D  C  F  F  Q  N  V  R  F  K  G  T  N  F  P  P  N  G  P  V
ATGCAAAAGAAGACTCTTAAGTGGGAACCATCGACTGAAAAACTACATGTTAGAGATGGACTAC
  M  Q  K  K  T  L  K  W  E  P  S  T  E  K  L  H  V  R  D  G  L
TTGTTGGAgtaagtttaaacttggacttactaactaacggattatatttaaattttcagAACAT
L  V  G                                                        N  I
TAACATGGCACTACTACTAGAAGGTGGAGGTCACTACCTTTGCGATTTTAAAACCACTTACAAA
   N  M  A  L  L  L  E  G  G  G  H  Y  L  C  D  F  K  T  T  Y  K
GCAAAGAAGGTCGTCCAACTTCCAGATGCACACTTTGTTGATCACAGAATTGAAATACTAGGAA
  A  K  K  V  V  Q  L  P  D  A  H  F  V  D  H  R  I  E  I  L  G
ACGATTCGGATTACAACAAAGTTAAGCTATACGAACACGCAGTTGCAAGATACAGTCCTCTACC
  N  D  S  D  Y  N  K  V  K  L  Y  E  H  A  V  A  R  Y  S  P  L  P
AAGTCAAGTATGG
  S  Q  V  W
```

Figure S5. Sequence of Dendra2 donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.

ePDZ

```
PSDH61 (Cassette 2)   GCTCTTCGATG_ePDZ_GGAGCTGGTCGAAGAGC
                        SapI    M        G  A  G    SapI


pSDH52 (Cassette 3b)

 GCTCTTCGAGCGGAGGTTCCGGAGGATCTGGCGGATCT_ePDZ_TAACGAAGAGC
   SapI   S  G  G  S  G  G  S  G  G          *    SapI
```

```
CCAGAGCTCGGATTCTCGATCTCCGGAGGTGTCGGAGGCCGTGGAAATCCATTCCGTCCTGACG
 P   E   L   G   F   S   I   S   G   G   V   G   G   R   G   N   P   F   R   P   D
ATGATGGAATTTTTGTTACTCGGGTCCAACCAGAAGGACCAGCTAGCAAACTTCTTCAACCTGG
 D   D   G   I   F   V   T   R   V   Q   P   E   G   P   A   S   K   L   L   Q   P   G
AGACAAGgtaagttaattaatttcatcgagagatcgtgcaatttctcattcatgaagactttc
 D   K
agATCATCCAAGCCAACGGTTACTCTTTCATTAATATTGAGCACGGTCAGGCTGTCAGCCTTCT
    I   I   Q   A   N   G   Y   S   F   I   N   I   E   H   G   Q   A   V   S   L   L
CAAGACCTTCCAGAACACAGTCGAGCTCATCATCGTCCGAGAGGTAGGAAACGGAGCTAAGCAG
   K   T   F   Q   N   T   V   E   L   I   I   V   R   E   V   G   N   G   A   K   Q
GAGATCCGTGTCCGCGTCGAAAAGGACGGAGGATCCGGAGgtaagttataatttctcctacaaa
   E   I   R   V   R   V   E   K   D   G   G   S   G
tttaaaactgacttacttttttctaattttcagGAGTTTCCAGTGTTCCAACCAACCTTGAGGT
                                       G   V   S   S   V   P   T   N   L   E   V
CGTTGCTGCCACACCAACAAGCCTTCTCATCTCCTGGGATGCTTACCGTGAACTTCCAGTCTCC
   V   A   A   T   P   T   S   L   L   I   S   W   D   A   Y   R   E   L   P   V   S
TACTATAGGATCACCTACGGAGAGACCGGAGGAAATTCTCCAGTCCAAGAATTCACGGTCCCAG
   Y   Y   R   I   T   Y   G   E   T   G   G   N   S   P   V   Q   E   F   T   V   P
gtaagttaattaattttatgagaatctaaaagtaatttggagagtacaatattttcagGAAGC
                                                                  G   S
AAGTCGACCGCCACAATTTCCGGATTGAAGCCAGGAGTCGACTACACCATCACTGTCTATGCTC
   K   S   T   A   T   I   S   G   L   K   P   G   V   D   Y   T   I   T   V   Y   A
ATTACAACTATCATTACTACTCATCACCAATCTCCATCAATTATAGAACGAGTAGATTGGAGCT
   H   Y   N   Y   H   Y   Y   S   S   P   I   S   I   N   Y   R   T   S   R   L   E   L
CAAGCTCCGTATTTTGCAATCGACAGTGCCACGCGCCCGTGATCCACCCGTCGCGACG
   K   L   R   I   L   Q   S   T   V   P   R   A   R   D   P   P   V   A   T
```

Figure S6. Sequence of ePDZ donor cassettes. Cassette-specific flanking sequences are shown at the top. The SapI recognition sites are in blue and the cleavages sites are in red.
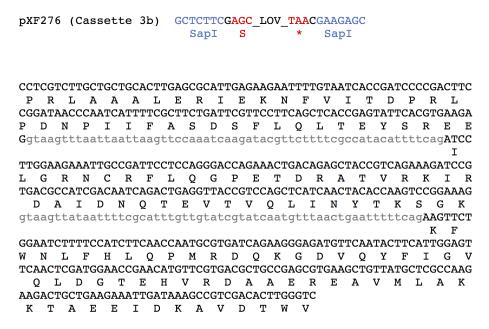
<u>LOV</u>

pXF276 (Cassette 3b)   GCTCTTCGAGC_LOV_TAACGAAGAGC
                         SapI   S   *   SapI

CCTCGTCTTGCTGCTGCACTTGAGCGCATTGAGAAGAATTTTGTAATCACCGATCCCCGACTTC
  P   R   L   A   A   A   L   E   R   I   E   K   N   F   V   I   T   D   P   R   L
CGGATAACCCAATCATTTTCGCTTCTGATTCGTTCCTTCAGCTCACCGAGTATTCACGTGAAGA
  P   D   N   P   I   I   F   A   S   D   S   F   L   Q   L   T   E   Y   S   R   E   E
GgtaagtttaattaattaagttccaaatcaagatacgttcttttcgccatacattttcagATCC
                                                                          I
TTGGAAGAAATTGCCGATTCCTCCAGGGACCAGAAACTGACAGAGCTACCGTCAGAAAGATCCG
  L   G   R   N   C   R   F   L   Q   G   P   E   T   D   R   A   T   V   R   K   I   R
TGACGCCATCGACAATCAGACTGAGGTTACCGTCCAGCTCATCAACTACACCAAGTCCGGAAAG
   D   A   I   D   N   Q   T   E   V   T   V   Q   L   I   N   Y   T   K   S   G   K
gtaagttataattttcgcatttgttgtatcgtatcaatgtttaactgaatttttcagAAGTTCT
                                                                      K   F
GGAATCTTTTCCATCTTCAACCAATGCGTGATCAGAAGGGAGATGTTCAATACTTCATTGGAGT
  W   N   L   F   H   L   Q   P   M   R   D   Q   K   G   D   V   Q   Y   F   I   G   V
TCAACTCGATGGAACCGAACATGTTCGTGACGCTGCCGAGCGTGAAGCTGTTATGCTCGCCAAG
   Q   L   D   G   T   E   H   V   R   D   A   A   E   R   E   A   V   M   L   A   K
AAGACTGCTGAAGAAATTGATAAAGCCGTCGACACTTGGGTC
  K   T   A   E   E   I   D   K   A   V   D   T   W   V

Figure S7. Sequence of LOVdonor cassette. Flanking sequences are shown at the top.
The SapI recognition sites are in blue and the cleavages sites are in red.