R4L1 GATEWAY Binary Vector (R4L1pGWB)

1. R4L1pGWB vector consists of 4 kind of resistance marker seriese
   R4L1pGWB4xx (Pnos:NPTII), kanamycin resistance
   R4L1pGWB5xx (Pnos:HPT), hygromycin resistance
   R4L1pGWB6xx (Pnos:bar), BASTA resistance
   R4L1pGWB7xx (Pnos:GPT), tunicamycin resistance
2. C-terminal tags are automatically fused subsequent to the LR reaction.
3. Please read the GATEWAY instruction manual (Invitrogen) for procedures, etc.

   Please note that our R4L1pGWB seriese are available only for basic research. If you plan to use these R4L1pGWBs for commercial research, please contact Invitrogen about licencing of GATEWAY^{TM}.
   Please do not distribute to other researchers without permission.

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The R4L1pGWB Series

"n" indicate selection markers, 4 (401 etc, kanamycin resistance)、5 (501 etc, hygromycin resistance)、6 (601 etc, BASTA resistance), 7 (701 etc, tunicamycin resistance).

(32) R4L1pGWBn32 : [--R4- CmR-*ccd*B-L1-G3GFP-GUS--]

(33) R4L1pGWBn33 : [--R4- CmR-*ccd*B-L1-GUS--]

(35) R4L1pGWBn35 : [--R4- CmR-*ccd*B-L1-LUC--]

(40) R4L1pGWBn40 : [--R4- CmR-*ccd*B-L1-EYFP--]

(43) R4L1pGWBn43 : [--R4- CmR-*ccd*B-L1-ECFP--]

(50) R4L1pGWBn50 : [--R4- CmR-*ccd*B-L1-G3GFP--]

(59) R4L1pGWBn59 : [--R4- CmR-*ccd*B-L1-tagRFP--]

Notes:

1. DNA sequences enclosed in [ ] are introduced into the *Hin*dIII-*Sac*I site of the binary vector pPZP221 [Hajdukiewicz et al. (1994) Plant Mol. Biol. **25**: 989-994; see next page].   R = the *att*R site.

Construction of pGWBs

The binary vector used as the starting plasmid for the construction of R4L1pGWBs was pPZP221 [Hajdukiewicz et al. (1994) Plant Mol. Biol. **25**: 989-994].   The aacC1 selection marker of pPZP221 was replaced with Pnos:NPTII:Tnos (R4L1pGWB4xx, kanamycin resistance), Pnos:HPT:Tnos (R4L1pGWB5xx, hygromycin resistance), Pnos:bar:Tnos (R4L1pGWB6xx, BASTA resistance), or Pnos:GPT:Tnos (R4L1pGWB7xx, tunicamycin resistance).   The *Hin*dIII-*Sac*I region in pPZP221 was replaced by the GATEWAY cassette and tag in the R4L1pGWB as shown below.

```
RB-(HindIII)-GATEWAY-(SacI)-Tnos-[Tnos-marker-Pnos]-LB
```

The DNA fragment diagrammed above is present between LB and RB of binary vector.   Note that the marker unit is in reverse orientation.

Antibiotics can be used for selection in bacteria are as below.
    R4L1pGWBs: spectinomycin, chloramphenicol
    recombinant R4L1pGWB (after LR reaction) :   spectinomycin.

The princple behind the GATEWAY technology and the R4L1pGWB vector series could be generalized as follow.
(1) Subsequent to the LR reaction with promoter Entry Clone and a R4L1pGWB vector, the *att*R4-(CmR, *ccd*B)-*att*L1 cassette in the R4L1pGWB will be replaced by the *att*B4-(Promoter)-*att*B1.
(2) N indicates the nucleotide that shold be included in *Your Clone*.
(3)

Important Note
(1) Linearization of R4L1pGWBs by digestion before performing the LR reaction is not necessary.   The efficiency of the LR reaction depends on the topology of the plasmids in the following order (see instruction manual of Invitrogen).

Either or both plasmids linear > both plasmids relaxed >> both plasmids supercoiled

(2)Select with spectinomycin (100 ug/ml) after the LR reaction.

(3)Use the *Escherichia coli* strain DH5alpha for cloning the product of the LR (BP) reactions, since negative selection with *ccd*B does not work in the *E.coli* which has F' episome (see instruction manual of Invitrogen).

(4)Sometimes, *E.coli* with both the Entry Clone and recombinant R4L1pGWB are obtained subsequent to the cloning.   I recommend that only the *E. coli* colonies with only the recombinant R4L1pGWB be used.

R4L1pGWBn32, n33, n35, n40, n43, n50, n59 (--R4-CmR-ccdB-L1-tag---)

(AAGCTT) GTGGATCCCCCATC
<u>ACA ACT TTG TAT AGA AAA</u> --(CmR,*ccd*B)—ATG CCA ACT TTG TAC AAA AAA GCA GGC
<u>T</u>CA AGC <mark>tag</mark> GCT <u>TA(G</u> AGCTC)

(1) The *Hin*dIII (AAGCTT) and *Sac*I (GAGCTC) sites are shown.
(2) The *att*R4-CmR-*ccd*B-*att*L1 cassette is underlined.
(3) The sequence of each <mark>tag</mark> is indicated in the "Tags" section.

After LR reaction, the following sequence will be obtained.

(AAGCTT) GTGGATCCCCCATC
<u>ACA ACT TTG TAT AGA AAA GTT G</u>NN (promoter) <u>N</u>CA AGT TTG TAC AAA AAA GCA GGC
<u>T</u>CA AGC <mark>tag</mark> GCT <u>TA(G</u> AGCTC)

(1) Translation will stop at the termination codon TAG (double-underlined) after the <mark>tag</mark>
   if tag itself does not include termination codon.

Tags

R4L1pGWBn32 (G3GFP-GUS)   2529bp

```
ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAAT
 M  S  K  G  E  E  L  F  T  G  V  V  P  I  L  V  E  L  D  G  D  V  N
GGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTT
 G  H  K  F  S  V  S  G  E  G  E  G  D  A  T  Y  G  K  L  T  L  K  F
ATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTTACTACTTTCGCTTACGGTGTGCAG
 I  C  T  T  G  K  L  P  V  P  W  P  T  L  V  T  T  F  A  Y  G  V  Q
TGCTTCTCAAGATACCCAGATCATATGAAGCGGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGATAC
 C  F  S  R  Y  P  D  H  M  K  R  H  D  F  F  K  S  A  M  P  E  G  Y
GTGCAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAG
 V  Q  E  R  T  I  F  F  K  D  D  G  N  Y  K  T  R  A  E  V  K  F  E
GGAGACACCCTCGTCAACAGGATCGAGCTTAAGGGAATCGATTTCAAGGAGGACGGAAACATCCTCGGC
 G  D  T  L  V  N  R  I  E  L  K  G  I  D  F  K  E  D  G  N  I  L  G
CACAAGTTGGAATACAACTTCAACTCCCACAACGTGTACATCATGGCAGACAAACAAAAGAATGGAATC
 H  K  L  E  Y  N  F  N  S  H  N  V  Y  I  M  A  D  K  Q  K  N  G  I
AAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAA
 K  V  N  F  K  I  R  H  N  I  E  D  G  S  V  Q  L  A  D  H  Y  Q  Q
AATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTT
 N  T  P  I  G  D  G  P  V  L  L  P  D  N  H  Y  L  S  T  Q  S  A  L
TCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACA
 S  K  D  P  N  E  K  R  D  H  M  V  L  L  E  F  V  T  A  A  G  I  T
CATGGCATGGATGAACTATACAAAgctagtTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAA
 H  G  M  D  E  L  Y  K  A  S  L  R  P  V  E  T  P  T  R  E  I  K  K
CTCGACGGCCTGTGGGCATTCAGTCTGGATCGCGAAAACTGTGGAATTGATCAGCGTTGGTGGGAAAGC
 L  D  G  L  W  A  F  S  L  D  R  E  N  C  G  I  D  Q  R  W  W  E  S
GCGTTACAAGAAAGCCGGGCAATTGCTGTGCCAGGCAGTTTTAACGATCAGTTCGCCGATGCAGATATT
 A  L  Q  E  S  R  A  I  A  V  P  G  S  F  N  D  Q  F  A  D  A  D  I
CGTAATTATGCGGGCAACGTCTGGTATCAGCGCGAAGTCTTTATACCGAAAGGTTGGGCAGGCCAGCGT
 R  N  Y  A  G  N  V  W  Y  Q  R  E  V  F  I  P  K  G  W  A  G  Q  R
ATCGTGCTGCGTTTCGATGCGGTCACTCATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTGATGGAG
 I  V  L  R  F  D  A  V  T  H  Y  G  K  V  W  V  N  N  Q  E  V  M  E
CATCAGGGCGGCTATACGCCATTTGAAGCCGATGTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGT
 H  Q  G  G  Y  T  P  F  E  A  D  V  T  P  Y  V  I  A  G  K  S  V  R
ATCACCGTTTGTGTGAACAACGAACTGAACTGGCAGACTATCCCGCCGGGAATGGTGATTACCGACGAA
 I  T  V  C  V  N  N  E  L  N  W  Q  T  I  P  P  G  M  V  I  T  D  E
AACGGCAAGAAAAAGCAGTCTTACTTCCATGATTTCTTTAACTATGCCGGAATCCATCGCAGCGTAATG
 N  G  K  K  K  Q  S  Y  F  H  D  F  F  N  Y  A  G  I  H  R  S  V  M
CTCTACACCACGCCGAACACCTGGGTGGACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGTAAC
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L   Y   T   T   P   N   T   W   V   D   D   I   T   V   V   T   H   V   A   Q   D   C   N
CACGCGTCTGTTGACTGGCAGGTGGTGGCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGATCAA
H   A   S   V   D   W   Q   V   V   A   N   G   D   V   S   V   E   L   R   D   A   D   Q
CAGGTGGTTGCAACTGGACAAGGCACTAGCGGGACTTTGCAAGTGGTGAATCCGCACCTCTGGCAACCG
Q   V   V   A   T   G   Q   G   T   S   G   T   L   Q   V   V   N   P   H   L   W   Q   P
GGTGAAGGTTATCTCTATGAACTGTGCGTCACAGCCAAAAGCCAGACAGAGTGTGATATCTACCCGCTT
G   E   G   Y   L   Y   E   L   C   V   T   A   K   S   Q   T   E   C   D   I   Y   P   L
CGCGTCGGCATCCGGTCAGTGGCAGTGAAGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTT
R   V   G   I   R   S   V   A   V   K   G   E   Q   F   L   I   N   H   K   P   F   Y   F
ACTGGCTTTGGTCGTCATGAAGATGCGGACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCAC
T   G   F   G   R   H   E   D   A   D   L   R   G   K   G   F   D   N   V   L   M   V   H
GACCACGCATTAATGGACTGGATTGGGGCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAG
D   H   A   L   M   D   W   I   G   A   N   S   Y   R   T   S   H   Y   P   Y   A   E   E
ATGCTCGACTGGGCAGATGAACATGGCATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTAACCTC
M   L   D   W   A   D   E   H   G   I   V   V   I   D   E   T   A   A   V   G   F   N   L
TCTTTAGGCATTGGTTTCGAAGCGGGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGG
S   L   G   I   G   F   E   A   G   N   K   P   K   E   L   Y   S   E   E   A   V   N   G
GAAACTCAGCAAGCGCACTTACAGGCGATTAAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTG
E   T   Q   Q   A   H   L   Q   A   I   K   E   L   I   A   R   D   K   N   H   P   S   V
GTGATGTGGAGTATTGCCAACGAACCGGATACCCGTCCGCAAGGTGCACGGGAATATTTCGCGCCACTG
V   M   W   S   I   A   N   E   P   D   T   R   P   Q   G   A   R   E   Y   F   A   P   L
GCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGACGCT
A   E   A   T   R   K   L   D   P   T   R   P   I   T   C   V   N   V   M   F   C   D   A
CACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGGATGGTATGTCCAA
H   T   D   T   I   S   D   L   F   D   V   L   C   L   N   R   Y   Y   G   W   Y   V   Q
AGCGGCGATTTGGAAACGGCAGAGAAGGTACTGGAAAAAGAACTTCTGGCCTGGCAGGAGAAACTGCAT
S   G   D   L   E   T   A   E   K   V   L   E   K   E   L   L   A   W   Q   E   K   L   H
CAGCCGATTATCATCACCGAATACGGCGTGGATACGTTAGCCGGGCTGCACTCAATGTACACCGACATG
Q   P   I   I   I   T   E   Y   G   V   D   T   L   A   G   L   H   S   M   Y   T   D   M
TGGAGTGAAGAGTATCAGTGTGCATGGCTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTC
W   S   E   E   Y   Q   C   A   W   L   D   M   Y   H   R   V   F   D   R   V   S   A   V
GTCGGTGAACAGGTATGGAATTTCGCCGATTTTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAAC
V   G   E   Q   V   W   N   F   A   D   F   A   T   S   Q   G   I   L   R   V   G   G   N
AAGAAAGGGATCTTCACTCGCGACCGCAAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACT
K   K   G   I   F   T   R   D   R   K   P   K   S   A   A   F   L   L   Q   K   R   W   T
GGCATGAACTTCGGTGAAAAACCGCAGCAGGGAGGCAAACAATGA
G   M   N   F   G   E   K   P   Q   Q   G   G   K   Q   *
```

Junction of G3GFP and GUS

```
---GAT GAA CTA TAC AAA gct agt tta cgt cct ---
    D   E   L   Y   K   A   S   L   R   P
```

Capital letters: G3GFP
Small letters: GUS

R4L1pGWBn33 (GUS)   1812bp

```
ATGTTACGTCCTGTAGAAACCCCAACCCGTGAAATCAAAAAACTCGACGGCCTGTGGGCATTCAGTCTG
 M  L  R  P  V  E  T  P  T  R  E  I  K  K  L  D  G  L  W  A  F  S  L
GATCGCGAAAACTGTGGAATTGATCAGCGTTGGTGGGAAAGCGCGTTACAAGAAAGCCGGGCAATTGCT
 D  R  E  N  C  G  I  D  Q  R  W  W  E  S  A  L  Q  E  S  R  A  I  A
GTGCCAGGCAGTTTTAACGATCAGTTCGCCGATGCAGATATTCGTAATTATGCGGGCAACGTCTGGTAT
 V  P  G  S  F  N  D  Q  F  A  D  A  D  I  R  N  Y  A  G  N  V  W  Y
CAGCGCGAAGTCTTTATACCGAAAGGTTGGGCAGGCCAGCGTATCGTGCTGCGTTTCGATGCGGTCACT
 Q  R  E  V  F  I  P  K  G  W  A  G  Q  R  I  V  L  R  F  D  A  V  T
CATTACGGCAAAGTGTGGGTCAATAATCAGGAAGTGATGGAGCATCAGGGCGGCTATACGCCATTTGAA
 H  Y  G  K  V  W  V  N  N  Q  E  V  M  E  H  Q  G  G  Y  T  P  F  E
GCCGATGTCACGCCGTATGTTATTGCCGGGAAAAGTGTACGTATCACCGTTTGTGTGAACAACGAACTG
 A  D  V  T  P  Y  V  I  A  G  K  S  V  R  I  T  V  C  V  N  N  E  L
AACTGGCAGACTATCCCGCCGGGAATGGTGATTACCGACGAAAACGGCAAGAAAAAGCAGTCTTACTTC
 N  W  Q  T  I  P  P  G  M  V  I  T  D  E  N  G  K  K  K  Q  S  Y  F
CATGATTTCTTTAACTATGCCGGAATCCATCGCAGCGTAATGCTCTACACCACGCCGAACACCTGGGTG
 H  D  F  F  N  Y  A  G  I  H  R  S  V  M  L  Y  T  T  P  N  T  W  V
GACGATATCACCGTGGTGACGCATGTCGCGCAAGACTGTAACCACGCGTCTGTTGACTGGCAGGTGGTG
 D  D  I  T  V  V  T  H  V  A  Q  D  C  N  H  A  S  V  D  W  Q  V  V
GCCAATGGTGATGTCAGCGTTGAACTGCGTGATGCGGATCAACAGGTGGTTGCAACTGGACAAGGCACT
 A  N  G  D  V  S  V  E  L  R  D  A  D  Q  Q  V  V  A  T  G  Q  G  T
AGCGGGACTTTGCAAGTGGTGAATCCGCACCTCTGGCAACCGGGTGAAGGTTATCTCTATGAACTGTGC
 S  G  T  L  Q  V  V  N  P  H  L  W  Q  P  G  E  G  Y  L  Y  E  L  C
GTCACAGCCAAAAGCCAGACAGAGTGTGATATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTG
 V  T  A  K  S  Q  T  E  C  D  I  Y  P  L  R  V  G  I  R  S  V  A  V
AAGGGCGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCG
 K  G  E  Q  F  L  I  N  H  K  P  F  Y  F  T  G  F  G  R  H  E  D  A
GACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGGATTGGG
 D  L  R  G  K  G  F  D  N  V  L  M  V  H  D  H  A  L  M  D  W  I  G
GCCAACTCCTACCGTACCTCGCATTACCCTTACGCTGAAGAGATGCTCGACTGGGCAGATGAACATGGC
 A  N  S  Y  R  T  S  H  Y  P  Y  A  E  E  M  L  D  W  A  D  E  H  G
ATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTAACCTCTCTTTAGGCATTGGTTTCGAAGCGGGC
 I  V  V  I  D  E  T  A  A  V  G  F  N  L  S  L  G  I  G  F  E  A  G
AACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTCAGCAAGCGCACTTACAGGCG
 N  K  P  K  E  L  Y  S  E  E  A  V  N  G  E  T  Q  Q  A  H  L  Q  A
ATTAAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGTGATGTGGAGTATTGCCAACGAACCG
 I  K  E  L  I  A  R  D  K  N  H  P  S  V  V  M  W  S  I  A  N  E  P
GATACCCGTCCGCAAGGTGCACGGGAATATTTCGCGCCACTGGCGGAAGCAACGCGTAAACTCGACCCG
 D  T  R  P  Q  G  A  R  E  Y  F  A  P  L  A  E  A  T  R  K  L  D  P
ACGCGTCCGATCACCTGCGTCAATGTAATGTTCTGCGACGCTCACACCGATACCATCAGCGATCTCTTT
```

```
T   R   P   I   T   C   V   N   V   M   F   C   D   A   H   T   D   T   I   S   D   L   F
GATGTGCTGTGCCTGAACCGTTATTACGGATGGTATGTCCAAAGCGGCGATTTGGAAACGGCAGAGAAG
D   V   L   C   L   N   R   Y   Y   G   W   Y   V   Q   S   G   D   L   E   T   A   E   K
GTACTGGAAAAAGAACTTCTGGCCTGGCAGGAGAAACTGCATCAGCCGATTATCATCACCGAATACGGC
V   L   E   K   E   L   L   A   W   Q   E   K   L   H   Q   P   I   I   I   T   E   Y   G
GTGGATACGTTAGCCGGGCTGCACTCAATGTACACCGACATGTGGAGTGAAGAGTATCAGTGTGCATGG
V   D   T   L   A   G   L   H   S   M   Y   T   D   M   W   S   E   E   Y   Q   C   A   W
CTGGATATGTATCACCGCGTCTTTGATCGCGTCAGCGCCGTCGTCGGTGAACAGGTATGGAATTTCGCC
L   D   M   Y   H   R   V   F   D   R   V   S   A   V   V   G   E   Q   V   W   N   F   A
GATTTTGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGC
D   F   A   T   S   Q   G   I   L   R   V   G   G   N   K   K   G   I   F   T   R   D   R
AAACCGAAGTCGGCGGCTTTTCTGCTGCAAAAACGCTGGACTGGCATGAACTTCGGTGAAAAACCGCAG
K   P   K   S   A   A   F   L   L   Q   K   R   W   T   G   M   N   F   G   E   K   P   Q
CAGGGAGGCAAACAATGA
Q   G   G   K   Q   *
```

R4L1pGWBn35 (LUC) 1653bp

```
ATGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCGCCATTCTATCCGCTGGAAGATGGAACCGCTGGA
 M  E  D  A  K  N  I  K  K  G  P  A  P  F  Y  P  L  E  D  G  T  A  G

GAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACAT
 E  Q  L  H  K  A  M  K  R  Y  A  L  V  P  G  T  I  A  F  T  D  A  H

ATCGAGGTGGACATCACTTACGCTGAGTACTTCGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGA
 I  E  V  D  I  T  Y  A  E  Y  F  E  M  S  V  R  L  A  E  A  M  K  R

TATGGGCTGAATACAAATCACAGAATCGTCGTATGCAGTGAAAACTCTCTTCAATTCTTTATGCCGGTG
 Y  G  L  N  T  N  H  R  I  V  V  C  S  E  N  S  L  Q  F  F  M  P  V

TTGGGCGCGTTATTTATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATGAACGTGAATTGCTC
 L  G  A  L  F  I  G  V  A  V  A  P  A  N  D  I  Y  N  E  R  E  L  L

AACAGTATGGGCATTTCGCAGCCTACCGTGGTGTTCGTTTCCAAAAAGGGGTTGCAAAAAATTTTGAAC
 N  S  M  G  I  S  Q  P  T  V  V  F  V  S  K  K  G  L  Q  K  I  L  N

GTGCAAAAAAAGCTCCCAATCATCCAAAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTT
 V  Q  K  K  L  P  I  I  Q  K  I  I  I  M  D  S  K  T  D  Y  Q  G  F

CAGTCGATGTACACGTTCGTCACATCTCATCTACCTCCCGGTTTTAATGAATACGATTTTGTGCCAGAG
 Q  S  M  Y  T  F  V  T  S  H  L  P  P  G  F  N  E  Y  D  F  V  P  E

TCCTTCGATAGGGACAAGACAATTGCACTGATCATGAACTCCTCTGGATCTACTGGTCTGCCTAAAGGT
 S  F  D  R  D  K  T  I  A  L  I  M  N  S  S  G  S  T  G  L  P  K  G

GTCGCTCTGCCTCATAGAACTGCCTGCGTGAGATTCTCGCATGCCAGAGATCCTATTTTTGGCAATCAA
 V  A  L  P  H  R  T  A  C  V  R  F  S  H  A  R  D  P  I  F  G  N  Q

ATCATTCCGGATACTGCGATTTTAAGTGTTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACACTC
 I  I  P  D  T  A  I  L  S  V  V  P  F  H  H  G  F  G  M  F  T  T  L

GGATATTTGATATGTGGATTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTCTGAGGAGC
 G  Y  L  I  C  G  F  R  V  V  L  M  Y  R  F  E  E  E  L  F  L  R  S

CTTCAGGATTACAAGATTCAAAGTGCGCTGCTGGTGCCAACCCTATTCTCCTTCTTCGCCAAAAGCACT
 L  Q  D  Y  K  I  Q  S  A  L  L  V  P  T  L  F  S  F  F  A  K  S  T

CTGATTGACAAATACGATTTATCTAATTTACACGAAATTGCTTCTGGTGGCGCTCCCCTCTCTAAGGAA
 L  I  D  K  Y  D  L  S  N  L  H  E  I  A  S  G  G  A  P  L  S  K  E

GTCGGGGAAGCGGTTGCCAAGAGGTTCCATCTGCCAGGTATCAGGCAAGGATATGGGCTCACTGAGACT
 V  G  E  A  V  A  K  R  F  H  L  P  G  I  R  Q  G  Y  G  L  T  E  T

ACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTCCATTT
 T  S  A  I  L  I  T  P  E  G  D  D  K  P  G  A  V  G  K  V  V  P  F

TTTGAAGCGAAGGTTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAATCAAAGAGGCGAACTGTGT
 F  E  A  K  V  V  D  L  D  T  G  K  T  L  G  V  N  Q  R  G  E  L  C

GTGAGAGGTCCTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAG
 V  R  G  P  M  I  M  S  G  Y  V  N  N  P  E  A  T  N  A  L  I  D  K

GATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACACTTCTTCATCGTTGACCGC
 D  G  W  L  H  S  G  D  I  A  Y  W  D  E  D  E  H  F  F  I  V  D  R

CTGAAGTCTCTGATTAAGTACAAAGGCTATCAGGTGGCTCCCGCTGAATTGGAATCCATCTTGCTCCAA
```

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 L   K   S   L   I   K   Y   K   G   Y   Q   V   A   P   A   E   L   E   S   I   L   L   Q
CACCCCAACATCTTCGACGCAGGTGTCGCAGGTCTTCCCGACGATGACGCCGGTGAACTTCCCGCCGCC
 H   P   N   I   F   D   A   G   V   A   G   L   P   D   D   D   A   G   E   L   P   A   A
GTTGTTGTTTTGGAGCACGGAAAGACGATGACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTA
 V   V   V   L   E   H   G   K   T   M   T   E   K   E   I   V   D   Y   V   A   S   Q   V
ACAACCGCGAAAAAGTTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAA
 T   T   A   K   K   L   R   G   G   V   V   F   V   D   E   V   P   K   G   L   T   G   K
CTCGACGCAAGAAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAAAGATCGCCGTGTAA
 L   D   A   R   K   I   R   E   I   L   I   K   A   K   K   G   G   K   I   A   V   *
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R4L1pGWBn40 (EYFP) 717bp

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
M   V   S   K   G   E   E   L   F   T   G   V   V   P   I   L   V   E   L   D   G   D   V

AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
N   G   H   K   F   S   V   S   G   E   G   E   G   D   A   T   Y   G   K   L   T   L   K

TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGCTACGGCCTG
F   I   C   T   T   G   K   L   P   V   P   W   P   T   L   V   T   T   F   G   Y   G   L

CAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC
Q   C   F   A   R   Y   P   D   H   M   K   Q   H   D   F   F   K   S   A   M   P   E   G

TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC
Y   V   Q   E   R   T   I   F   F   K   D   D   G   N   Y   K   T   R   A   E   V   K   F

GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG
E   G   D   T   L   V   N   R   I   E   L   K   G   I   D   F   K   E   D   G   N   I   L

GGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGC
G   H   K   L   E   Y   N   Y   N   S   H   N   V   Y   I   M   A   D   K   Q   K   N   G

ATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG
I   K   V   N   F   K   I   R   H   N   I   E   D   G   S   V   Q   L   A   D   H   Y   Q

CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCC
Q   N   T   P   I   G   D   G   P   V   L   L   P   D   N   H   Y   L   S   Y   Q   S   A

CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC
L   S   K   D   P   N   E   K   R   D   H   M   V   L   L   E   F   V   T   A   A   G   I

ACTCTCGGCATGGACGAGCTGTACAAG
T   L   G   M   D   E   L   Y   K

R4L1pGWBn43 (ECFP) 717bp

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA

M V S K G E E L F T G V V P I L V E L D G D V

AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG

N G H K F S V S G E G E G D A T Y G K L T L K

TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTGGGGCGTG

F I C T T G K L P V P W P T L V T T L T W G V

CAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGC

Q C F S R Y P D H M K Q H D F F K S A M P E G

TACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC

Y V Q E R T I F F K D D G N Y K T R A E V K F

GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG

E G D T L V N R I E L K G I D F K E D G N I L

GGGCACAAGCTGGAGTACAACTACATCAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGC

G H K L E Y N Y I S H N V Y I T A D K Q K N G

ATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG

I K A N F K I R H N I E D G S V Q L A D H Y Q

CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC

Q N T P I G D G P V L L P D N H Y L S T Q S A

CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC

L S K D P N E K R D H M V L L E F V T A A G I

ACTCTCGGCATGGACGAGCTGTACAAG

T L G M D E L Y K

R4L1pGWBn50 (G3GFP) 717bp

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ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAAT
 M  S  K  G  E  E  L  F  T  G  V  V  P  I  L  V  E  L  D  G  D  V  N
GGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTT
 G  H  K  F  S  V  S  G  E  G  E  G  D  A  T  Y  G  K  L  T  L  K  F
ATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTTACTACTTTCGCTTACGGTGTGCAG
 I  C  T  T  G  K  L  P  V  P  W  P  T  L  V  T  T  F  A  Y  G  V  Q
TGCTTCTCAAGATACCCAGATCATATGAAGCGGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGATAC
 C  F  S  R  Y  P  D  H  M  K  R  H  D  F  F  K  S  A  M  P  E  G  Y
GTGCAGGAGAGGACCATCTTCTTCAAGGACGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAG
 V  Q  E  R  T  I  F  F  K  D  D  G  N  Y  K  T  R  A  E  V  K  F  E
GGAGACACCCTCGTCAACAGGATCGAGCTTAAGGGAATCGATTTCAAGGAGGACGGAAACATCCTCGGC
 G  D  T  L  V  N  R  I  E  L  K  G  I  D  F  K  E  D  G  N  I  L  G
CACAAGTTGGAATACAACTTCAACTCCCACAACGTGTACATCATGGCAGACAAACAAAAGAATGGAATC
 H  K  L  E  Y  N  F  N  S  H  N  V  Y  I  M  A  D  K  Q  K  N  G  I
AAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAA
 K  V  N  F  K  I  R  H  N  I  E  D  G  S  V  Q  L  A  D  H  Y  Q  Q
AATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTT
 N  T  P  I  G  D  G  P  V  L  L  P  D  N  H  Y  L  S  T  Q  S  A  L
TCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACA
 S  K  D  P  N  E  K  R  D  H  M  V  L  L  E  F  V  T  A  A  G  I  T
CATGGCATGGATGAACTATACAAATAA
 H  G  M  D  E  L  Y  K  *
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R4L1pGWBn59 (tagRFP)  714bp
ATGGTGTCTAAGGGCGAAGAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTG
M   V   S   K   G   E   E   L   I   K   E   N   M   H   M   K   L   Y   M   E   G   T   V
AACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGA
N   N   H   H   F   K   C   T   S   E   G   E   G   K   P   Y   E   G   T   Q   T   M   R
ATCAAGGTGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGC
I   K   V   V   E   G   G   P   L   P   F   A   F   D   I   L   A   T   S   F   M   Y   G
AGCAGAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCTGAGGGCTTC
S   R   T   F   I   N   H   T   Q   G   I   P   D   F   F   K   Q   S   F   P   E   G   F
ACATGGGAGAGAGTCACCACATACGAAGACGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAG
T   W   E   R   V   T   T   Y   E   D   G   G   V   L   T   A   T   Q   D   T   S   L   Q
GACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAG
D   G   C   L   I   Y   N   V   K   I   R   G   V   N   F   P   S   N   G   P   V   M   Q
AAGAAAACACTCGGCTGGGAGGCCAACACCGAGATGCTGTACCCCGCTGACGGCGGCCTGGAAGGCAGA
K   K   T   L   G   W   E   A   N   T   E   M   L   Y   P   A   D   G   G   L   E   G   R
AGCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACTTCAAGACCACATACAGATCC
S   D   M   A   L   K   L   V   G   G   G   H   L   I   C   N   F   K   T   T   Y   R   S
AAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACCACAGACTGGAAAGAATCAAG
K   K   P   A   K   N   L   K   M   P   G   V   Y   Y   V   D   H   R   L   E   R   I   K
GAGGCCGACAAAGAGACCTACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGC
E   A   D   K   E   T   Y   V   E   Q   H   E   V   A   V   A   R   Y   C   D   L   P   S
AAACTGGGGCACAAACTTAATTGA
K   L   G   H   K   L   N   *
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Acknowledgements