

P01502

P01503

P01504

DUALmembrane vector complementation sets

User Manual

Kit contents

The DUALmembrane vector complementation sets contain the following components:

Component	Supplied as	Complementation set N	Complementation set SUC	Complementation set STE
pBT3-C Bait vector	5 µg lyophilized plasmid	✓	✓	✓
pBT3-N Bait vector	5 µg lyophilized plasmid	-	✓	✓
pBT3-SUC Bait vector	5 µg lyophilized plasmid	✓	-	✓
pBT3-STE Bait vector	5 µg lyophilized plasmid	✓	✓	-
pPR3-C Prey/Library vector	5 µg lyophilized plasmid	✓	✓	✓
pPR3-SUC Prey/Library vector	5 µg lyophilized plasmid	✓	✓	✓
pPR3-STE Prey/Library vector	5 µg lyophilized plasmid	✓	✓	✓

Storage and handling of plasmids

Plasmids are supplied lyophilized. Upon receipt, add 50 µl water (final concentration 0.1 µg/µl) and incubate at 50°C for 5 minutes. Vortex for 1 minute and store at -20°C.

Vector information

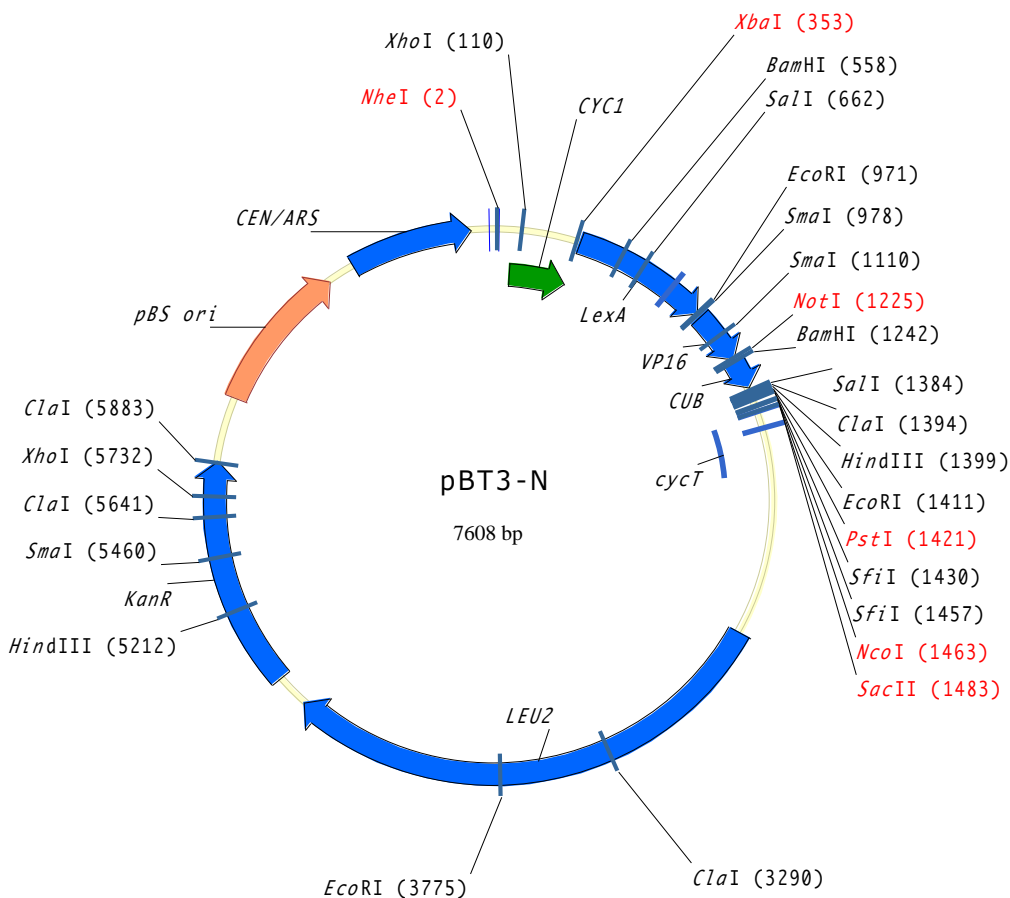
Vector maps and sequences

For PDF versions of all vector maps, as well as vector sequence files, please visit the support section of www.dualsystems.com.

Bait vectors				
Vector	Auxotrophic marker (yeast)	Origin of replication (yeast)	Resistance marker (E. coli)	Origin of replication (E. coli)
pBT3-N	LEU2	CEN/ARS (1-2 copies/cell)	Kan (select with 30 µg/ml kanamycine)	High copy
pBT3-C	LEU2	CEN/ARS (1-2 copies/cell)	Kan (select with 30 µg/ml kanamycine)	High copy
pBT3-SUC	LEU2	CEN/ARS (1-2 copies/cell)	Kan (select with 30 µg/ml kanamycine)	High copy
pBT3-STE	LEU2	CEN/ARS (1-2 copies/cell)	Kan (select with 30 µg/ml kanamycine)	High copy

Library vectors				
Vector	Auxotrophic marker (yeast)	Origin of replication (yeast)	Resistance marker (E. coli)	Origin of replication (E. coli)
pPR3-N	TRP1	2micron (20-50 copies/cell)	Amp (select with 100 µg/ml ampicillin)	High copy
pPR3-C	TRP1	2micron (20-50 copies/cell)	Amp (select with 100 µg/ml ampicillin)	High copy
pPR3-SUC	TRP1	2micron (20-50 copies/cell)	Amp (select with 100 µg/ml ampicillin)	High copy
pPR3-STE	TRP1	2micron (20-50 copies/cell)	Amp (select with 100 µg/ml ampicillin)	High copy

pBT3-N bait vector P03230



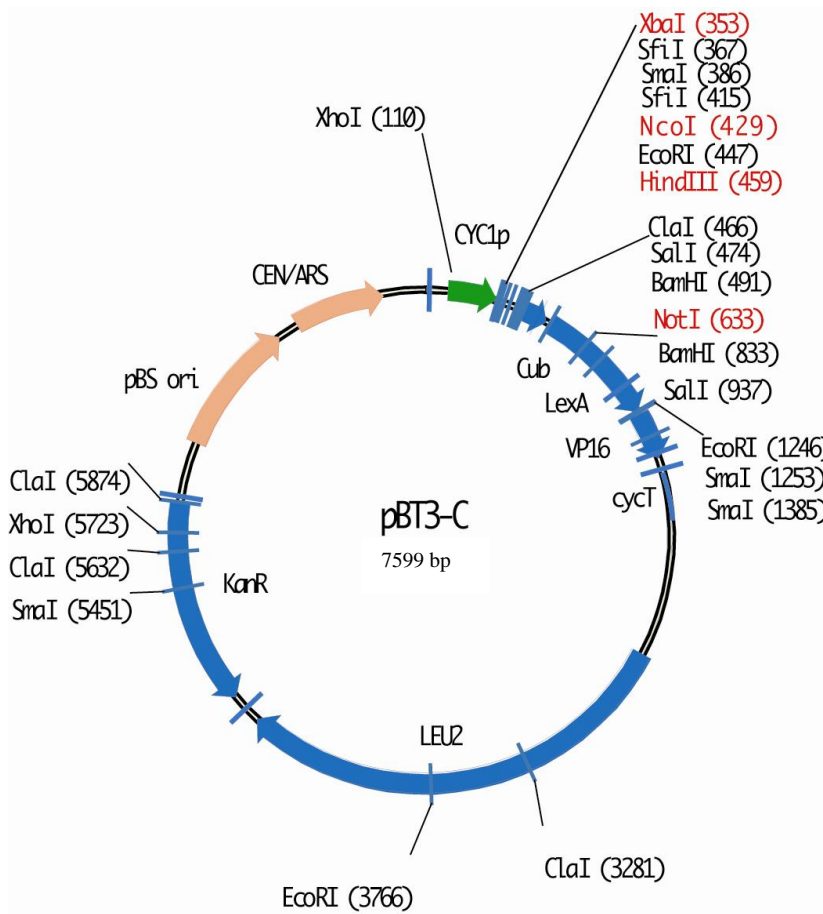
```

Pst I          Sfi I          Sfi I          Nco I          Sac II
-----
aat tcc tgc agg gcc att acg gcc agg cct tta att aag gcc gcc tcg gcc cca tgg taagta gct aac cgc gga
 N  S  C  R  A  I  T  A  R  P  L  I  K  A  A  S  A  P  W  *  V  A  N  R  G
----->
Cub reading frame
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 352	CYC1 promoter	Start: 2499 End: 4709	LEU2 auxotrophic marker
Start: 364 End: 969	LexA DNA binding domain	Start: 4858 End: 5891	KanR resistance gene
Start: 988 End: 1224	VP16 transactivation domain	Start: 6184 End: 6851	pBluescript origin of replication
Start: 1231 End: 1365	Cub, amino acids 34-76 of ubiquitin	Start: 6973 End: 7512	CEN/ARS origin of replication
Start: 1509 End: 1768	CYC1 terminator		

pBT3-C bait vector P03231



```

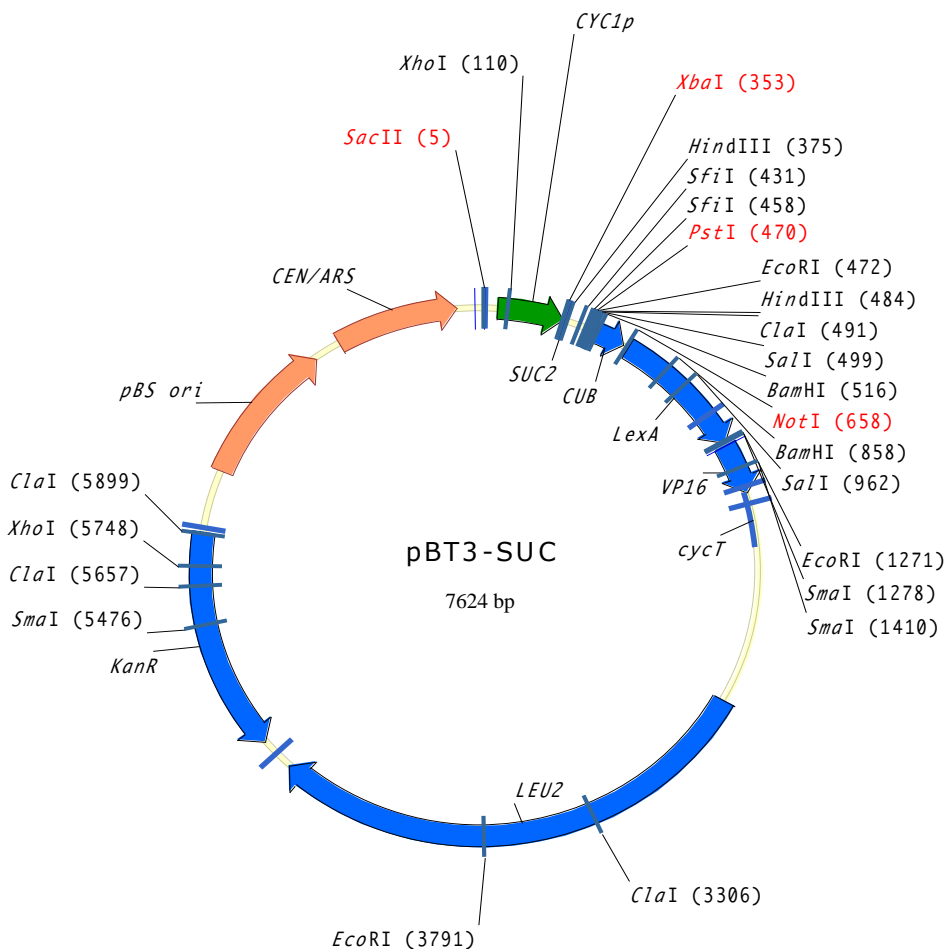
Xba I           Sfi I
-----
atc tag acg gcc att acg gcc aaa ctc gct cgc ccg ggc cga acc agt ggc tgc agg gcc gcc tcg gcc
I  *  T  A  I  T  A  K  L  A  R  P  G  R  T  S  G  C  R  A  A  S  A

           Nco I           Hind III
           -----
aaa ggc ctc cat ggg tat atc tgc agg aat tcg ata tca agc tta tcg ata ccg tcg acc atg tcg ggg
K  G  L  H  G  Y  I  C  R  N  S  I  S  S  L  S  I  P  S  T  M  S  G
                                     ----->
                                     Cub
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 352	CYC1 promoter	Start: 2490 End: 4700	LEU2 auxotrophic marker
Start: 480 End: 614	Cub, amino acids 34-76 of ubiquitin	Start: 4849 End: 5882	KanR resistance gene
Start: 639 End: 1250	LexA DNA binding domain	Start: 6175 End: 6842	pBluescript origin of replication
Start: 1263 End: 1499	VP16 transactivation domain	Start: 6964 End: 7503	CEN/ARS origin of replication
Start: 1501 End: 1758	CYC1 terminator		

pBT3-SUC bait vector P03232



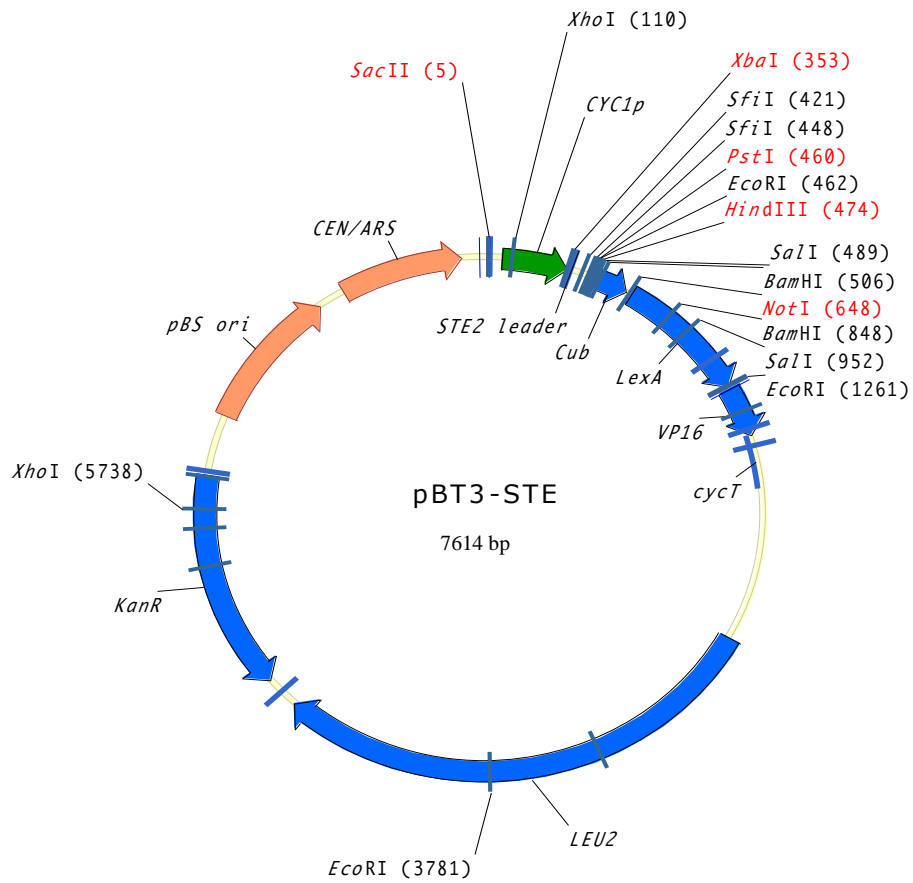
```

          Sfi I                Sfi I                Pst I
          -----                -----                -----
gca atg gcc att acg gcc agg cct tta att aag gcc gcc tcg gcc atc tgc agg aat
A  M  A  I  T  A  R  P  L  I  K  A  A  S  A  I  C  R  N
----->
SUC2 reading frame                                Cub reading frame
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 352	CYC1 promoter	Start: 1526 End: 1783	CYC1 terminator
Start: 361 End: 423	SUC2 cleavable signal sequence	Start: 2515 End: 4725	LEU2 auxotrophic marker
Start: 505 End: 639	Cub, amino acids 34-76 of ubiquitin	Start: 4874 End: 5907	KanR resistance gene
Start: 664 End: 1275	LexA DNA binding domain	Start: 6200 End: 6867	pBluescript origin of replication
Start: 1288 End: 1524	VP16 transactivation domain	Start: 6989 End: 7528	CEN/ARS origin of replication

pBT3-STE bait vector P03233



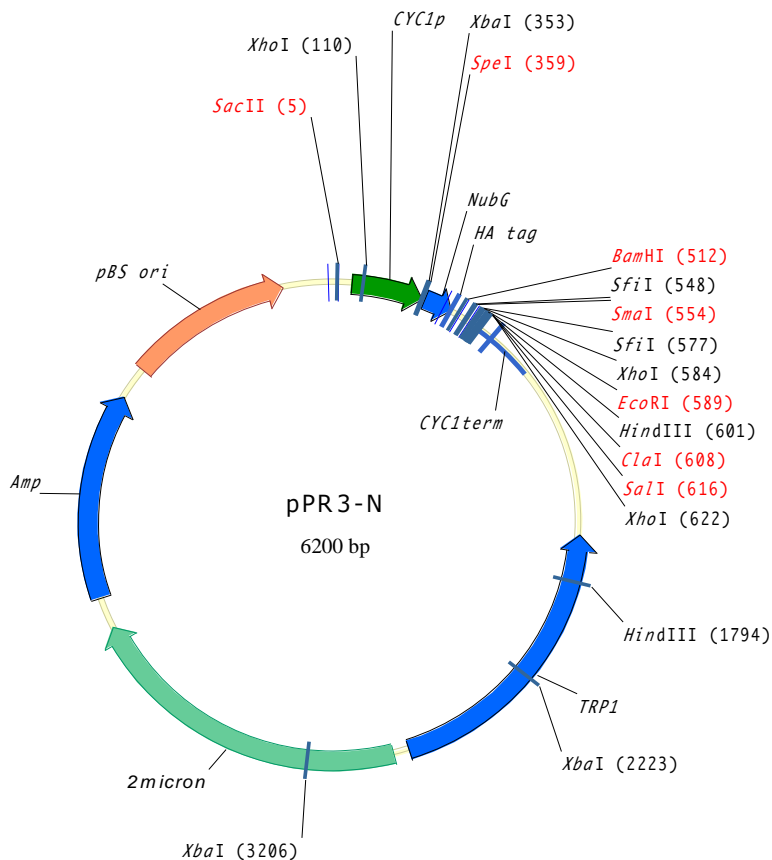
```

          Sfi I                Sfi I                Pst I                Hind III
          -----                -----                -----                -----
gta atg gcc att acg gcc agg cct tta att aag gcc gcc tcg gcc atc tgc agg aat tcg ata tca agc tta tcg
 V  M  A  I  T  A  R  P  L  I  K  A  A  S  A  I  C  R  N  S  I  S  S  L  S
----->
STE2 reading frame                                     Cub reading frame
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 352	CYC1 promoter	Start: 1516 End: 1773	CYC1 terminator
Start: 369 End: 407	STE2 leader sequence	Start: 2505 End: 4715	LEU2 auxotrophic marker
Start: 495 End: 629	Cub, amino acids 34-76 of ubiquitin	Start: 4864 End: 5897	KanR resistance gene
Start: 654 End: 1265	LexA DNA binding domain	Start: 6190 End: 6857	pBluescript origin of replication
Start: 1278 End: 1514	VP16 transactivation domain	Start: 6979 End: 7518	CEN/ARS origin of replication

pPR3-N NubG-X library vector P03234



```

                Sma I
                -----
          BamH I
          -----
gct gga tcc aag cag tgg tat caa cgc aga gtg gcc att acg gcc cgg gaa aaa aca tgt cgg cgg cct cgg cct ctc gag
A  G  S  K  Q  W  Y  Q  R  R  V  A  I  T  A  R  E  K  T  C  R  P  P  R  P  L  E
----->
NubG-HA reading frame
    
```

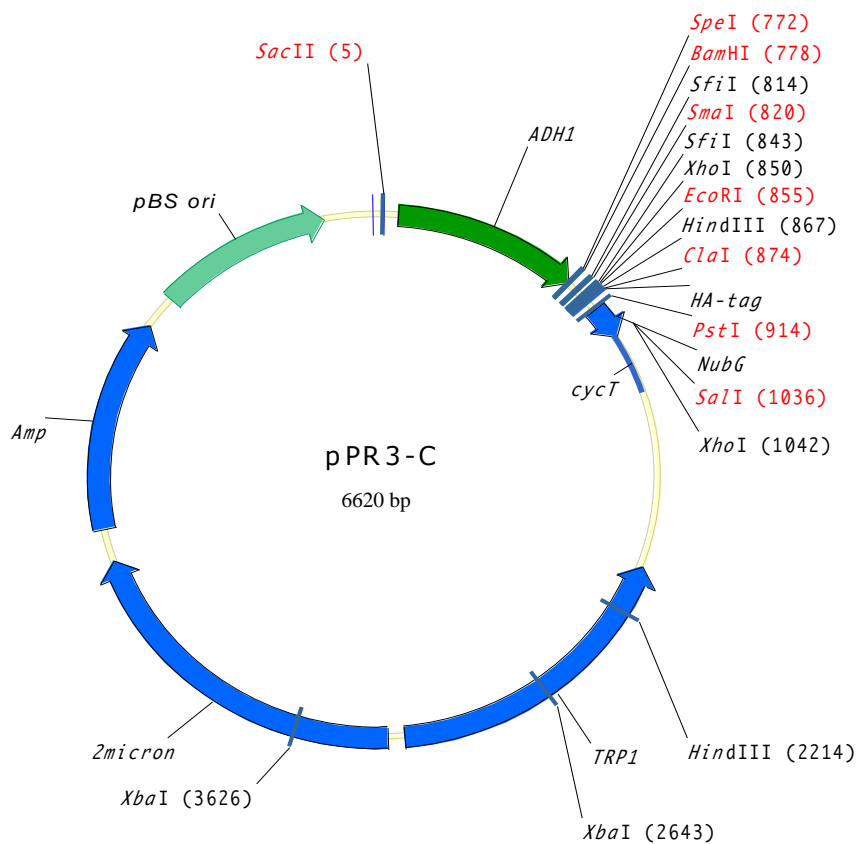
```

          EcoR I
          -----
          Cla I
          -----
          Sal I
          -----
aat tcg ata tca agc tta tcg ata ccg tcg acc
N  S  I  S  S  L  S  I  P  S  T
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 352	CYC1 promoter	Start: 1598 End: 2791	TRP1 auxotrophic marker
Start: 364 End: 480	NubG, ubiquitin amino acids 1-38	Start: 2855 End: 4202	2micron origin of replication
Start: 481 End: 510	HA epitope tag	Start: 4335 End: 5193	AmpR resistance gene
Start: 622 End: 883	CYC1 terminator	Start: 5328 End: 5995	pBluescript origin of replication

pPR3-C X-NubG library vector P03235



```

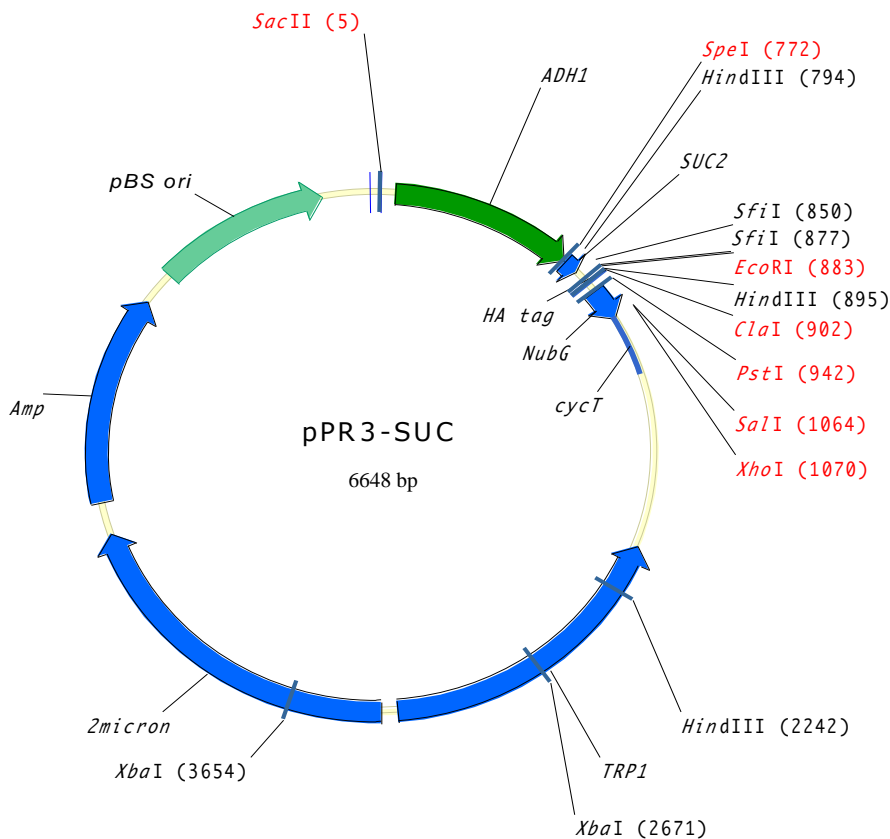
      BamH I          Sma I
      -----
Spe I  -----
-----
cac tag tgg atc caa gca gtg gta tca acg cag agt ggc cat tac ggc ccg gga aaa aac atg tcg gcc gcc tcg gcc tct

      EcoR I          Cla I
      -----
cga gaa ttc gat atc aag ctt atc gat atg tac
----->
      HA-NubG reading frame
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 771	ADH1 promoter	Start: 2018 End: 3211	TRP1 auxotrophic marker
Start: 878 End: 910	HA epitope tag	Start: 3275 End: 4622	2micron origin of replication
Start: 917 End: 1054	NubG, ubiquitin amino acids 1-38	Start: 4755 End: 5613	AmpR resistance gene
Start: 1047 End: 1304	CYC1 terminator	Start: 5748 End: 6415	pBluescript origin of replication

pPR3-SUC X-NubG library vector P03236



```

----- Sfi I ----- Sfi I ----- EcoR I ----- Cla I -----
gca atg gcc att acg gcc agg cct tta att aag gcc gcc tcg gcc gaa ttc gat atc aag ctt atc gat atg
----->
SUC2 reading frame                                     HA-NubG reading frame
    
```

Vector features

Position	Feature	Position	Feature
Start: 62 End: 771	ADH1 promoter	Start: 2046 End: 3239	TRP1 auxotrophic marker
Start: 780 End: 839	SUC2 cleavable signal sequence	Start: 3303 End: 4650	2micron origin of replication
Start: 906 End: 938	HA epitope tag	Start: 4783 End: 5641	AmpR resistance gene
Start: 945 End: 1079	NubG, ubiquitin amino acids 1-38	Start: 5776 End: 6443	pBluescript origin of replication
Start: 1075 End: 1332	CYC1 terminator		

Contact and support

Following the protocols in this manual you should be able to carry out an entire DUALmembrane pairwise interaction assay. If you have troubles at any point or feel that there is an error in one of the protocols, please take a look at the support section of our homepage www.dualsystems.com. If you don't find an answer to your question there, you may contact us at support@dualsystems.com and we will try to answer your questions as quickly as possible.

Please keep your license number ready when calling us by phone or mention your license number when writing an email to support@dualsystems.com. This will help us to answer your questions faster.

Dualsystems Biotech AG
Grabenstrasse 11a
8952 Schlieren
Switzerland
phone +41 (0)44 738 50 00
fax +41 (0)44 738 50 05

Notice to purchaser

DUALmembrane technology is subject to several granted and pending patents. Purchase of a DUALmembrane starter kit includes a limited, non-transferable license to practice the DUALmembrane system for non-commercial purposes only. Commercial entities who wish to purchase DUALmembrane reagents must obtain a separate license from Dualsystems Biotech.

Use of the *Sfi* I cloning strategy is licensed under U.S. Patent #5,595,895 issued to the National Institutes of Health.