

# Yeast ORF Collection

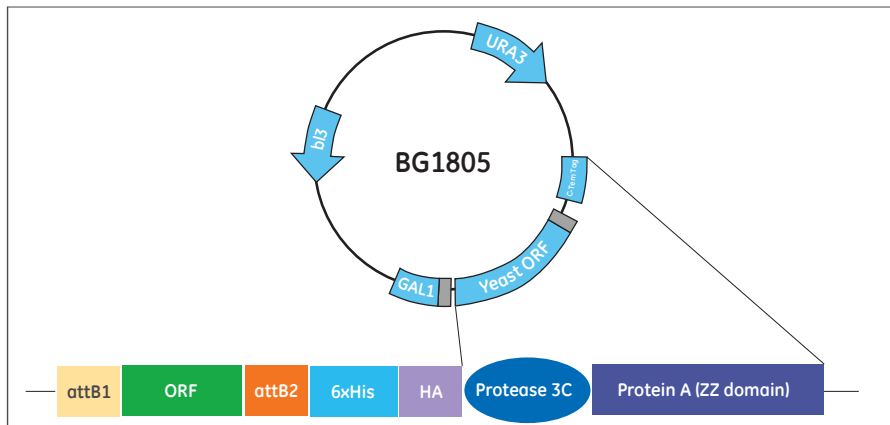
Cat. #YSC3867 (clone, *E. coli*), #YSC3868 (collection, *E. coli*)

Cat #YSC3869 (clone, yeast), #YSC3870 (collection, yeast)

## Introduction:

Developed by Eric Phizicky (University of Rochester) and Mike Snyder (Yale University)<sup>1</sup>, the Dharmacon™ Yeast ORF Collection enables robust protein expression and purification for over 4,900 *S. cerevisiae* genes. Each plasmid construct is available in yeast or *E. coli* format as a glycerol stock.

Yeast opens reading frames (ORFs) have been cloned into Gateway™ destination vectors, fully sequenced, and transformed into and yeast hosts. Each ORF is under the control of a GAL1 promoter and fused on the C-terminus to a tandem affinity tag that includes a hemagglutination (HA) tag. Successful yeast transformants have been verified to express protein of the correct length (including the 19-kDA tandem fusion tag) by western blotting.



**Figure 1.** Schematic map for vector BG1805. **Note:** When an individual ORF replaces the Gateway™ cassette, the size of the resulting vector will vary depending on the size of the ORF.

## Product Description:

### *E. coli*

Ampicillin resistant  
DH5[F-]80/lacZM15 (*lacZYA-argF*) U169 *deoR recA1 endA1 hsdR17* (rk-, mk+)  
*phoA supE44* - *thi-1 gyrA96 relA1*

### Yeast

URA3 (multicopy 2-micron) identical to yeast ChrV 116011-117048. The URA3 ORF in BG1805 is yeast ChrV:116167 to 116970. This means the vector also contains 155 base pairs upstream of the ATG translation start of URA3 and + 78 base pairs downstream of the translation termination site.

Y258 is MATa, *pep4-3, his4-580, ura3-52, leu2-3,112*

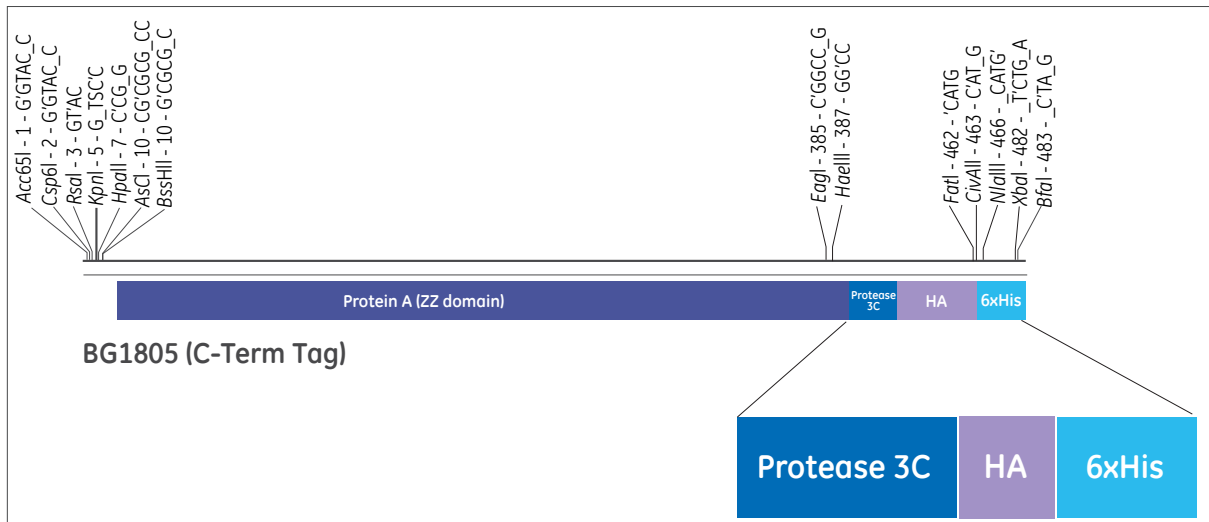
The vector is derived from pRSAB1234 from Erin O'Shea.

### Their vector was modified to BG1805 by Beth Grayhack as follows:

1. The C-terminal fusion tag was modified.
2. The vector was converted to a Gateway™ destination vector



**GAL1** promoter in BG1805 is identical to yeast **ChrII** bp 278,565-279048.



**Figure 2.** Information on the C-terminal fusion tag. Translation: A C-terminal fusion tag is present in the BG1805 vector. The Protein A ZZ domain is derived from *Staphylococcus aureus*. The amino acid sequence of the fusion tag is shown below: His6, HA epitope, protease 3C site, ZZ domain.

**Note:** This is a translation of the reverse complement of the vector sequence.



### Storage:

4 °C for up to one week  
-80 °C indefinitely

### Growth Medium:

Yeast ORF expression ready clones should be grown in SD-ura medium without any antibiotics. Long-term storage of strains should be stored in SD-ura medium containing 15% glycerol.

Grow a liquid culture in SD-ura overnight at 30 °C with shaking. Then, the cells are centrifuged:

SS34 rotor: 5,000 rpm for 5-10 minutes

Microcentrifuge: 2 minutes at top speed 3

Sorval 96-well deep-well plates: 1500-2000 for 10 minutes

The clear medium is removed and the cells are resuspended in SD-ura. The expression plasmid is selected for by growth in the absence of uracil. The URA3 gene is present on the plasmid and not in the chromosome. Thus, the yeast will not grow in medium lacking uracil if the plasmid is lost. Furthermore, expression of the ORF-fusion protein is repressed in glucose (D in SD and in YPD), so there should be little selection against any clone due to the identity of the ORF in the clone.

Make a concentrated mixture of solids including yeast nitrogen base, ammonium sulfate and selected amino acids and purine or pyrimidine bases.

**Table 1.** S powder - ura.

Component	15 Liters	60 Liters
YNB w/o a.a, w/o A.S.	25.1 g	100.4 g
Ammonium Sulfate	75.4 g	301.6 g
Isoleucine	450 mg	1.8 g
Valine	2.25 g	9.0 g
Adenine	300 mg	1.2 g
Arginine	300 mg	1.2 g
Histidine	300 mg	1.2 g
Leucine	450 mg	1.8 g
Lysine	450 mg	1.8 g
Methionine	300 mg	1.2 g
Phenylalanine	750 mg	3.0 g
Tryptophan	300 mg	1.2 g
Tyrosine	450 mg	1.8 g

YNB w/o a.a, w/o A.S = yeast nitrogen base without amino acids and without ammonium sulfate Difco: Ref 233520. All aminoacids are L amino acids and obtained from Sigma (98–99% purity).

### SD-ura Medium, 1 Liter:

S powder –ura	7.14 g
Dextrose	20 g
dH <sub>2</sub> O	to 1 L

### YPD Medium, 1 Liter:

Yeast extract	10 g
Peptone	20 g
Dextrose	20 g
dH <sub>2</sub> O	to 1 L
Agar (for plates)	20 g

The yeast ORF *E. coli* clones are grown in Terrific Broth (TB), with 0.1 mg/mL ampicillin, 30 °C with shaking. For long term storage, strains should be placed in TB medium containing 8% glycerol and stored at -80 °C.

### TB Medium, 1 Liter:

Tryptone	12 g
Yeast extract	24 g
Glycerol	4 mL
KH <sub>2</sub> PO <sub>4</sub>	2.31 g
K <sub>2</sub> HPO <sub>4</sub>	12.54 g
dH <sub>2</sub> O	to 1 L

### Western Blotting:

**Table 2.** Growth & Western Blot of PGAL- att-ORF-att-His6 HA 3C site ZZ clones.

Material	Vendor	Cat #
ECL Plus Western Blotting	Amersham Biosciences	RPN2132
0.5-mm Zirconia/Silica Beads	Biospec Products, INC.	11079105z
Calf Serum	Invitrogen (Gibco)	1601-0159
Goat anti-rabbit	Thermo Scientific	SAB1003
Rabbit anti-HA	Thermo Scientific	CAB3872
Complete Mini, EDTA-free Protease Inhibitor	Roche	1873580

### 3x YP + 6% Galactose: Per Liter:

Yeast extract	30 g
Peptone	60 g
H <sub>2</sub> O	700 mL
Autoclave	
Add 300 mL sterile 20% Galactose	

## Western Blot Lysis Buffer:

Tris-Cl pH 7.5	50 mM
EDTA	1 mM
Triton X- 100	0.5%
DTT	1 mM
NaCl	1 M
1X Complete Mini	pH 7
Pepstatin	2.5 ug/mL

## YP Galactose Induction MORF Growth Protocol:

1. Set up 5 mL SD-URA culture from single colony for overnight growth at 30 °C, shaking.
2. Inoculate a 25-mL -URA, 2% raffinose media with 1 mL of 5 mL overnight growth culture. Grow overnight at 30 °C shaking.
3. Dilute into 200 mL -URA, 2% raffinose media at starting OD<sub>600</sub> = 0.3. Grow at 30 °C, shaking.
4. At OD<sub>600</sub> = 1.2, add 100 mL 3x YP, 6% galactose, for a final concentration of 1x YP, 2% galactose.
5. Harvest after 6 hours\*.
  - Spin 5 K rpm for 10 minutes at 4 °C.
  - Take off supernatant and wash the pellets with 5 mL cold water.
  - Transfer to a new 14-mL tube and spin 5K for 10 minutes at 4 °C.
  - Take off supernatant, quick freeze, and store in the -80 °C freezer.

\* For Western Blot, harvest 2 mL culture separately.

**Note:** Protocol can be adjusted to 96-well format using a 1-2 mL culture.

## Western Blot - Lysis Buffer -> SDS Loading Buffer Method:

Method based on 96-well plate format

1. Add zirconium beads to a tube with cell pellets from 2 mL harvested cells.
2. Add 50 µL Lysis buffer.
3. Vortex at 4 °C for 10 × 30 sec with 1-minute ice water bath between vortex.
4. At room temp, add 50 µL boiling SDS 2x loading dye + BME.
5. Boil for 5 minutes at 100 °C.
6. Spin at 3 K at room temp for 5 minutes.
7. Load 10 µL on a SDS-PAGE gel.
8. Transfer proteins and Western Blot using Rabbit anti-HA (1°) and Goat anti-Rabbit (2°).
9. Detected with ECL Plus kit

## Sequence of BG1805 lacking an ORF:

1	ATGACGGTGA	AAACCTCTGA	CACATGCAGC	TCCCCGAGAC	GGTCACAGCT
51	TGTCGTAAAG	CGGATGCCGG	GAGCAGACAA	GCCCCGTCAGG	GCGCGTCAGC
101	GGGTGTTGGC	GGGTGTCGGG	GCTGGCTTAA	CTATGCGGCA	TCAGAGCAGA
151	TTGTACTGAG	AGTGCACCAT	ACCACAGCTT	TTCAATTCAA	TTCATCATT
201	TTTTTTTATT	CTTTTTTTTG	ATTTTCGGTTT	CTTTGAAATT	TTTTTGATT
251	GGTAATCTCC	GAACAGAAGG	AAGAACGAAG	GAAGGAGCAC	AGACTTAGAT
301	TGGTATATAT	ACGCATATGT	AGTGTGTAAG	AAACATGAAA	TGCCCCAGTA
351	TTCTTAACCC	AACTGCACAG	AACAAAACC	TGCAGGAAAC	GAAGATAAAT
401	CATGTCGAAA	GCTACATATA	AGGAACGTGC	TGCTACTCAT	CCTAGTCCTG
451	TTGCTGCCAA	GCTATTTAAT	ATCATGCACG	AAAAGCAAAC	AAACTTGTGT
501	GCTTCATTGG	ATGTTTCGTAC	CACCAAGGAA	TTACTGGAGT	TAGTTGAAGC
551	ATTAGGTCCC	AAAATTTGTT	TACTAAAAAC	ACATGTGGAT	ATCTTGACTG
601	ATTTTTCAT	GGAGGGCACA	GTTAAGCCGC	TAAAGGCATT	ATCCGCCAAG
651	TACAATTTTT	TACTCTTCGA	AGACAGAAAA	TTTGCTGACA	TTGGTAATAC
701	AGTCAAATTG	CAGTACTCTG	CGGGTGTATA	CAGAATAGCA	GAATGGGCAG
751	ACATTACGAA	TGCACACGGT	GTGGTGGGCC	CAGGTATTGT	TAGCGGTTTG
801	AAGCAGGCGG	CAGAAGAAGT	AACAAAGGAA	CCTAGAGGCC	TTTTGATGTT
851	AGCAGAATTG	TCATGCAAGG	GCTCCCTATC	TACTGGAGAA	TATACTAAGG
901	GTACTGTTGA	CATTGCGAAG	AGCGACAAAG	ATTTTGTAT	CGGCTTATT
951	GCTCAAAGAG	ACATGGGTGG	AAGAGATGAA	GGTTACGATT	GGTTGATTAT
1001	GACACCCGGT	GTGGGTTTAG	ATGACAAGGG	AGACGCATTG	GGTCAACAGT
1051	ATAGAACCCT	GGATGATGTG	GTCTCTACAG	GATCTGACAT	TATTATTGTT
1101	GGAAGAGGAC	TATTTGCAAA	GGGAAGGGAT	GCTAAGGTAG	AGGGTGAACG
1151	TTACAGAAAA	GCAGGCTGGG	AAGCATATTT	GAGAAGATGC	GGCCAGCAAA
1201	ACTAAAAAAC	TGTATTATATA	GTAAATGCAT	GTATACTAAA	CTCACAAATT
1251	AGAGCTTCAA	TTTAATTATA	TCAGTTATTA	CCCTATGCGG	TGTGAAATAC
1301	CGCACAGATG	CGTAAGGAGA	AAATACCGCA	TCAGGAAATT	GTAACGTTA
1351	ATATTTTGTT	AAAATTCGCG	TTAAATTTTT	GTTAAATCAG	CTCATTTTTT
1401	AACCAATAGG	CCGAAATCGG	CAAAATCCCT	TATAAATCAA	AAGAATAGAC

1451	CGAGATAGGG	TTGAGTGTG	TTCCAGTTG	GAACAAGAGT	CCACTATTAA
1501	AGAACGTGGA	CTCCAACGTC	AAAGGGCGAA	AAACCGTCTA	TCAGGGCGAT
1551	GGCCCACTAC	GTGAACCATC	ACCCTAATCA	AGTTTTTTGG	GGTCGAGGTG
1601	CCGTAAAGCA	CTAAATCGGA	ACCCTAAAGG	GAGCCCCGA	TTTAGAGCTT
1651	GACGGGGAAA	GCCGGCGAAC	GTGGCGAGAA	AGGAAGGGAA	GAAAGCGAAA
1701	GGAGCGGGCG	CTAGGCGGCT	GGCAAGTGTA	GCGGTCACGC	TGCGCGTAAC
1751	CACCACACCC	GCCGCGCTTA	ATGCGCCGCT	ACAGGGCGCG	TCGCGCCATT
1801	CGCCATTCAG	GCTGCGCAAC	TGTTGGGAAG	GGCGATCGGT	GCGGGCCTCT
1851	TCGCTATTAC	GCCAGCTGGC	GAAAGGGGGA	TGTGCTGCAA	GGCGATTAAG
1901	TTGGGTAACG	CCAGGGTTTT	CCCAGTCACG	ACGTTGTAAA	ACGACGGCCA
1951	GTGAGCGCGC	GTAATACGAC	TCACTATAGG	GCGAATGGG	TACCCCGCG
2001	CGCCTCACTG	ATGATTGCGG	TCTACTTTCG	GCGCCTGAGC	ATCATTTAGC
2051	TTTTTAGCTT	CTGCTAAAAG	GTTAGCGCTT	TGGCTTGGGT	CATCTTTTAA
2101	ACTTTGGATG	AAGCGTTTC	GTTGTTCTTC	GTTTAAGTTA	GGTAAATGTA
2151	AGATCTCATA	GAACGCGTTT	TGTTGTTCTT	TGTTGAATTT	GTTGTCTACT
2201	TTCGGCGCCT	GAGCATCATT	TAGCTTTTTA	GCTTCGCTA	AAAGGTTAGC
2251	GCTTTGGCTT	GGGTCATCTT	TTAAACTTTG	GATGAAGGCG	TTTCGTTGTT
2301	CTTCGTTTAA	GTTAGCTAAA	TGTAAGATCT	CATAGAACGC	GTTTTGTTGT
2351	TCTTTGTTGA	ATTTGTTGTC	CACGGCCGAT	GGTCCAGGAC	CTTGAAAAAG
2401	AACTTCTAAA	CCAGCATAAT	CAGGAACATC	GTATGGATAA	AAGATTCTAC
2451	CATGATGGTG	ATGATGATGT	CTAGACACAT	CAACCACTTT	GTACAAGAAA
2501	GCTGAACGAG	AAACGTAAAA	TGATATAAAT	ATCAATATAT	TAAATTAGAT
2551	TTTGCAATAA	AAACAGACTA	CATAATACTG	TAAAACACAA	CATATCCAGT
2601	CACTATGGTC	GACCTGCAGA	CTGGCTGTGT	ATAAGGGAGC	CTGACATTTA
2651	TATTCGCCAG	AACATCAGGT	TAATGGCGTT	TTTGATGTCA	TTTTCGCGGT
2701	GGCTGAGATC	AGCCACTTCT	TCCCCGATAA	CGGAGACCGG	CACACTGGCC
2751	ATATCGGTGG	TCATCATGCG	CCAGCTTTC	TCCCCGATAT	GCACCACCGG
2801	GTAAAGTTCA	CGGGAGACTT	TATCTGACAG	CAGACGTGCA	CTGGCCAGGG
2851	GGATCACCAT	CCGTCGCCCC	GGCGTGTCAA	TAATATCACT	CTGTACATCC
2901	ACAAACAGAC	GATAACGGCT	CTCTTTTTTA	TAGGTGTA	CCTTAACTG
2951	CATTCACCA	GTCCTGTTC	TCGTCAGCAA	AAGAGCCGTT	CATTTCAATA
3001	AACCGGGCGA	CCTCAGCCAT	CCCTTCCTGA	TTTTCCGCTT	TCCAGCGTTC
3051	GGCACGCAGA	CGACGGGCTT	CATCTGCAT	GGTTGTGCTT	ACCAGACCGG
3101	AGATATTGAC	ATCATATATG	CCTTGAGCAA	CTGATAGCTG	TCGCTGTCAA
3151	CTGTCACTGT	AATACGCTGC	TTCATAGCAC	ACCTCTTTTT	GACATACTTC
3201	GGGTATACAT	ATCAGTATAT	ATTCTTATAC	CGAAAAATC	AGCGCGCAA
3251	TACGCATACT	GTTATCTGGC	TTTAGTAAG	CCGGATCCAG	ATCTTTACGC
3301	CCCGCCCTGC	CACTCATCGC	AGTACTGTTG	TAATTCATTA	AGCATTCTGC
3351	CGACATGGAA	GCCATCACAG	ACGGCATGAT	GAACCTGAAT	CGCCAGCGGC
3401	ATCAGCACCT	TGTCGCCTTG	CGTATAATAT	TTGCCATGG	TGAAAACGGG
3451	GGCGAAGAAG	TTGTCCATAT	TGGCCACGTT	TAAATCAAAA	CTGGTGAAAC
3501	TCACCCAGGG	ATTGGCTGAG	ACGAAAAACA	TATTTCTCAAT	AAACCTTTA
3551	GGGAAATAGG	CAGGTTTTC	ACCGTAACAC	GCCACATCTT	GCGAATATAT
3601	GTGTAGAAAC	TGCCGGAAAT	CGTCGTGGTA	TTCACTCCAG	AGCGATGAAA
3651	ACGTTTCAGT	TTGCTCATGG	AAAACGGTGT	AACAAGGGTG	AACACTATCC
3701	CATATCACCA	GCTCACCGTC	TTTCATTGCC	ATACGGAATT	CCGGATGAGC
3751	ATTCATCAGG	CGGGCAAGAA	TGTGAATAAA	GGCCGGATAA	AACCTGTGCT
3801	TATTTTTCTT	TACGGTCTTT	AAAAAGGCCG	TAATATCCAG	CTGAACGGTC
3851	TGGTTATAGG	TACATTGAGC	AACTGACTGA	AATGCCTCAA	AATGTTCTTT
3901	ACGATGCCAT	TGGGATATAT	CAACGGTGGT	ATATCCAGTG	ATTTTTTTCT
3951	CCATTTTAGC	TTCTTTAGCT	CCTGAAAATC	TCGCCGGATC	CTAACTCAAA
4001	ATCCACACAT	TATACGAGCC	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC
4051	TAATGCGGCC	GCCATAGTGA	CTGGATATGT	TGTGTTTTAC	AGTATATATG
4101	AGTCTGTTTT	TCATGCAAAA	TCTAATTTAA	TATATTGATA	TTTATATCAT
4151	TTTACGTTTC	TCGTTACGCT	TTTTTGTAACA	AACTTGTTGA	TAATTCAGTC
4201	TCCTTGACGT	TAAAGTATAG	AGGTATATTA	ACAATTTTTT	GTTGATACTT
4251	TTATTACATT	TGAATAAGAA	GTAATACAAA	CCGAAAATGT	TGAAAGTATT
4301	AGTTAAAGTG	GTTATGCAGT	TTTTGCATTT	ATATATCTGT	TAATAGATCA
4351	AAAATCATCG	CTTCGCTGAT	TAATACCCC	AGAAATAAGG	CTAAAAACT
4401	AATCGCATT	TCATCCTATG	GTTGTTAATT	TGATTCGTTT	ATTTGAAGGT
4451	TTGTGGGGCC	AGGTTACTGC	CAATTTTTCC	TCTTCATAAC	CATAAAAGCT
4501	AGTATGTAG	AATCTTTTAT	GTTTCGGAGCA	GTGCGGCGCG	AGGCACATCT
4551	GCGTTTCAGG	AACGCGACCG	GTGAAGACGA	GGACGCACGG	AGGAGAGTCT
4601	TCCTTCGGAG	GGCTGTCAAC	CGCTCGGCGG	CTTCTAATCC	GTTCCCGCGG
4651	TGGAGCTCCA	GCTTTTGTTC	CCTTTAGTGA	GGGTTAATTG	CGCGCTTGCC

4701	GTAATCATGG	TCATAGCTGT	TTCCTGTGTG	AAATTGTTAT	CCGCTCACAA
4751	TTCCACACAA	CATACGAGCC	GGAAGCATAA	AGTGTAAGC	CTGGGGTGCC
4801	TAATGAGTGA	GGTAACTCAC	ATTAATTGCG	TTGCGCTCAC	TGCCCCGTTT
4851	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG
4901	CGGGGAGAG	CGGTTTGCGT	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT
4951	GACTCGCTGC	GCTCGGCTGT	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC
5001	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA
5051	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG
5101	TTGCTGGCGT	TTTTCATAG	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA
5151	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	GACAGGACTA	TAAAGATACC
5201	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCTG
5251	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT
5301	TTCTCATAGC	TCACGCTGTA	GGTATCTCAG	TTCGGTGTAG	GTCGTTGCGT
5351	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC
5401	TTATCCGGTA	ACTATCGTCT	TGAGTCCAAC	CCGGTAAAGC	ACGACTTATC
5451	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG
5501	GCGGTGCTAC	AGAGTCTTGG	AAGTGGTGGC	CTAACTACGG	CTACACTAGA
5551	AGGACAGTAT	TTGGTACTGT	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA
5601	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG
5651	GTTTTTTTTGT	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA
5701	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA
5751	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	ATCAAAAAGG	ATCTTCACTT
5801	AGATCCTTTT	AAATTAATAA	TGAAGTTTTA	AATCAATCTA	AAGTATATAT
5851	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT
5901	CTCAGCGATC	TGTCTATTTT	GTTTATCCAT	AGTTGCCTGA	CTCCCCGTCG
5951	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA
6001	ATGATACCGC	GAGACCCACG	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA
6051	CCAGCCAGCC	GGAAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG
6101	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTTCG
6151	CCAGTTAATA	GTTTGCSCAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGCT
6201	GTCACGCTCG	TCGTTTTGTA	TGGCTTCATF	CAGCTCCGGT	TCCCAACGAT
6251	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAGC	GGTTAGCTCC
6301	TTGGTCTCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGAG	TGTTATCACT
6351	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	TACTGTCATG	CCATCCGTAA
6401	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
6451	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCC	GCGTCAATAC	GGGATAATAC
6501	CGCGCCACAT	AGCAGAACTT	TAAAAGTGCT	CATCATTGGA	AAACGTCTTT
6551	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG
6601	TAACCCACTC	GTGCACCCAA	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG
6651	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA
6701	TAAGGGCGAG	ACGGAAATGT	TGAATACTCA	TACTCTTCTT	TTTTCAATAT
6751	TATTGAAGCA	TTTATCAGGG	TTATGTCTCT	ATGAGCGGAT	ACATATTTGA
6801	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCAGAA
6851	AAGTGCCACC	TGTATACTAG	GTTATAGTTT	CCTTTACTAT	CGTAACTTCC
6901	TACTCTGATT	AGGTTAACTC	CTCACCGTCG	TATATCTTGT	CGATTTCCCA
6951	TCACGACTTC	CTTCGTATGC	TATGGGGCGT	ACCTTACCC	ATTATAGTGT
7001	CCTCCATGAT	CTGATGGAAA	GTAGGATGTA	TTTATCTGCG	TATATTCATG
7051	CGTAAATTCG	TATTTGTGCG	TGATACGGCA	AGAAGAGTAC	TATATATAT
7101	ATGTCCGTTG	TGCGTCTATA	TCCACGCTGC	ACTTGTCACT	CGACATACAC
7151	GCGTCGAGCG	CAACGTAAAA	GCCTTCGCGA	GCAAAAGCCT	TTGCGAAACT
7201	TCAAGGATAA	GGCTTCAAGG	ATAAGAGATC	TTTCATATCC	TTGAAGTCTC
7251	GCGAAAACCT	TTGGTTTTCG	CGAGACTTCT	GCGTGAAAGT	TTTTTGGTTT
7301	TTGCGTGGCC	TGACATTTGCT	CGATGATTTT	ATAACGCTTA	TGGCGAAGGT
7351	GTTTGTAAACG	AGTTTTCATA	GAGAAACGAT	ATATAGAGAC	ACGATATAGG
7401	GATATATTGG	ATGGGTAGGT	GGAAAGCGAG	GAAC'TGAAAC	GTAGATTTGA
7451	GCTGGAGATG	TAAAAAATAC	AAATAGAGAT	CATAATGAGA	AATCTGTTTT
7501	TTTAACATCA	TTCTTGATAA	GTATCTCACT	TAGCTTTTGT	TATGCTTTTA
7551	CATTTGTAAA	GGATATGCAT	CATATATCTC	TGTTTATCT	TCTTTGGCAA
7601	GTATTTAAAAG	ACTGGTFACT	TCTTAGTAGT	TGCGATAGTG	AAAGACAAGT
7651	GTTTCATACG	CGTTAGGTGT	AGCCATATCT	TATATTAGCC	CCTACGGAAA
7701	TAGAACTTTT	TTACGTGGGC	GTCGAAGCGA	TCATTAGTCA	TTTGCGCCCT
7751	TCACCTCAGT	CCGAAAAAAA	TACCTTCTCT	TTTATCTGTG	GTTTCATCGG
7801	AAGAAGATTG	GAATTGCCTG	GATGTCACGT	TTTTCAATAG	TTCTCTGACG
7851	TAATATCTCG	CGTGT'TTCT	CTTTTTTCA	TTAGATTCTA	CGAAACAATC
7901	TTTTTATCGC	GAGAGCCCTA	CGTAAAAACA	TCTTGTTTTT	TCTTCATATC

7951	TAAGAAACAA	CCATTTTATC	GCGAGAGCGC	AACGTAAAGA	CAAGACATTT
8001	TTACGTCGAG	TCTAAGAAAC	AAACTTTTTA	ATCGCGAGAG	CGCAACGTAA
8051	AAACAAAATG	TTTTTACTTC	GTGTCTAAGA	AGCAACCATT	TTATCGCGAA
8101	AGCGCAACGT	AAAGACAAGA	CATTTTACG	TCGAGTCTAA	GAAACAAACT
8151	TTTTAATCGC	GAGAGCGCAA	CGTAAAAACA	AGATGTTTTA	CTTCGTGTCT
8201	ACGAAGCAAC	TAAGAAACCA	TTATTATCAT	GACATTAACC	TATAAAAATA

### Restriction Analysis Of BG1805 Lacking An ORF:

BG1805, 8274 bp. (CIRCULAR)

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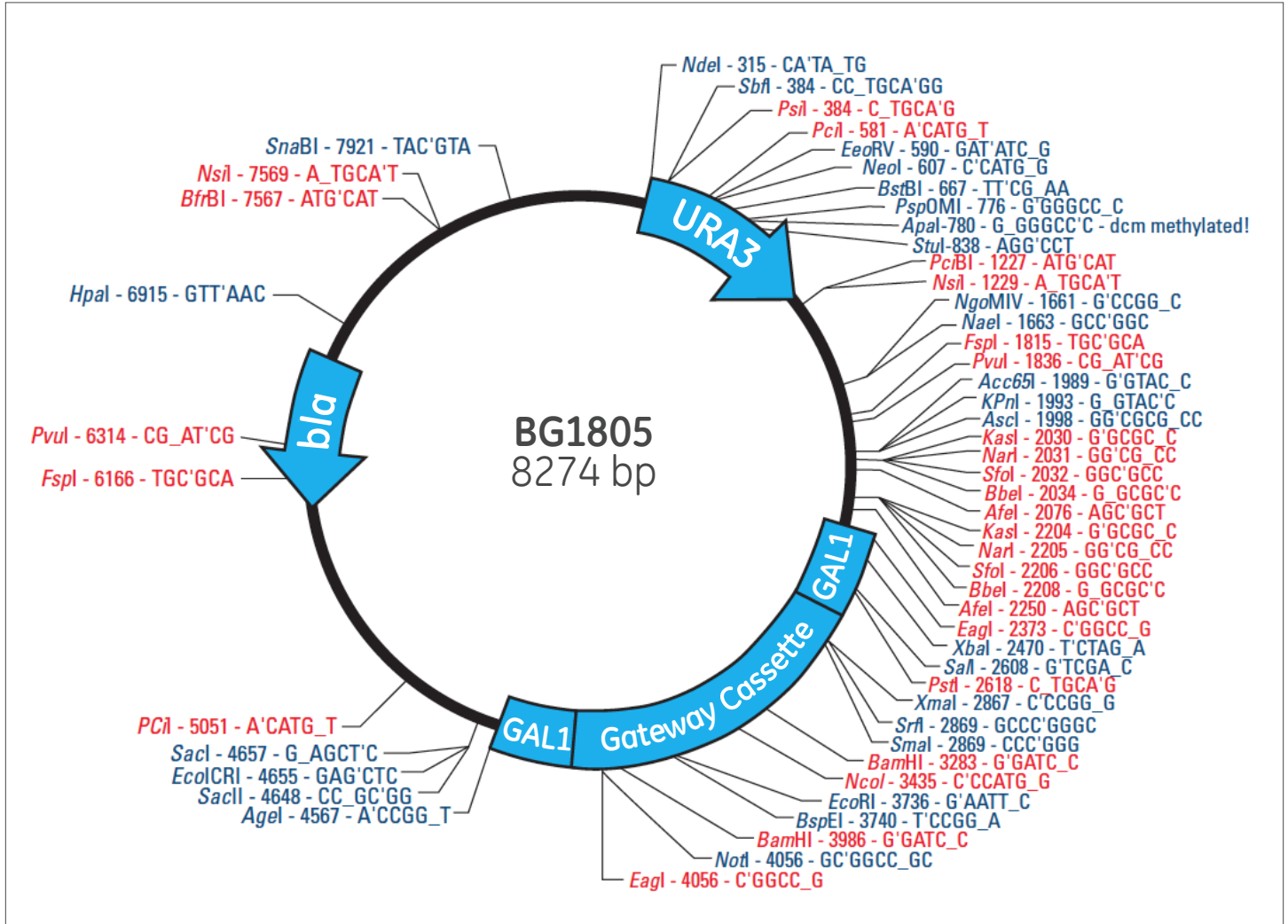


Figure 3. Restriction map for vector BG1805.

**AatII** (G\_ACGT^C) [ZraI]  
Enzyme does not cut.

**Acc65I** (G^GTAC\_C) [KpnI,Asp718I]  
[dcm methylated]  
Cuts 1 time.  
Cuts at position 1989.

**AcII** (AA^CG\_TT) [Psp1406I]  
Cuts 5 times.  
Cuts at positions 1148, 1345, 3651, 6170, 6543.  
Fragment sizes 197, 2306, 2519, 373, 2879.

**AfeI** (AGC^GCT) [Eco47III,Aor51HI,FunI]  
Cuts 2 times.  
Cuts at positions 2076, 2250.  
Fragment sizes 174, 8100.

**AflII** (C^TTAA\_G) [BfrI,BspTI,Bst98I,MspCI,Vha464I]  
Enzyme does not cut.

**AgeI** (A^CCGG\_T) [AsiAI,BshTI,CspAI,PinAI]  
Cuts 1 time.  
Cuts at position 4567.

**AluI** (AG^CT)  
Cuts 36 times.

**ApaI** (G\_GGCC^C) [Bsp120I,PspOMI]  
[dcm methylated] Cuts 1 time.  
Cuts at position [780].

**ApaLI** (G^TGCA\_C) [Alw44I,VneI]  
Cuts 4 times.  
Cuts at positions 162, 2836, 5365, 6611.  
Fragment sizes 2674, 2529, 1246, 1825.

**AscI** (GG^CGCG\_CC)  
Cuts 1 time.  
Cuts at position 1998.

**AseI** (AT^TA\_AT) [VspI,PshBI]  
Cuts 4 times.  
Cuts at positions 4370, 4822, 4881, 6116.  
Fragment sizes 452, 59, 1235, 6528.

**AsiSI** (GCG\_AT^CGC) [SgfI]  
Enzyme does not cut.

**AvrII** (C^CTAG\_G) [AspA2I,BlnI,XmaJI]  
Enzyme does not cut.

**BamHI** (G^GATC\_C)  
Cuts 2 times.  
Cuts at positions 3283, 3986.  
Fragment sizes 703, 7571.

**BbeI** (G\_GCGC^C) [NarI,EgeI,EheI,KasI,Mly113I,SfoI] Cuts 2 times.  
Cuts at positions 2034, 2208.  
Fragment sizes 174, 8100.

**BclI** (T^GATC\_A) [FbaI,Ksp22II]  
[dam methylated]  
Enzyme does not cut.



**BfaI** (C'TA\_G) [MaeI,FspBI,XspI]

Cuts 9 times.

Cuts at positions 442, 832, 1711, 2471, 4499, 5546, 5799, 6134, 6867.

Fragment sizes 390, 879, 760, 2028, 1047, 253, 335, 733, 1849.

**BfrBI** (ATG'CAT) [EcoT22I,Mph1103I,NsiI,Zsp2I]

Cuts 2 times.

Cuts at positions 1227, 7567.

Fragment sizes 6340, 1934.

**BglII** (A'GATC\_T)

Cuts 4 times.

Cuts at positions 2151, 2325, 3289, 7226.

Fragment sizes 174, 964, 3937, 3199.

**BmtI** (G\_CTAG'C) [NheI,AsuNHI]

Enzyme does not cut.

**BsiWI** (C'GTAC\_G) [Pfl123II,PspLI,SunI]

Enzyme does not cut.

**BspEI** (T'CCGG\_A) [AccIII,BlfI,BseAI,Bsp13I,Kpn2I,MroI]

[dam methylated]

Cuts 1 time.

Cuts at position 3740.

**BspHI** (T'CATG\_A) [PagI,RcaI]

[dam methylated] Cuts 4 times.

Cuts at positions 5771, 6779, 8227, 8273.

Fragment sizes 1008, 1448, 46, 5772.

**BsrGI** (T'GTAC\_A) [Bsp1407I,BstAUI,SspBI]

Cuts 3 times.

Cuts at positions 2490, 2892, 4175.

Fragment sizes 402, 1283, 6589.

**BssHII** (G'CGCG\_C) [BsePI,PauI]

Cuts 4 times.

Cuts at positions 1955, 1998, 3242, 4690.

Fragment sizes 43, 1244, 1448, 5539.

**BstBI** (TT'CG\_AA) [AsuII,Bpu14I,Bsp119I,BspT104I,Csp45I,NspV,SfuI]

Cuts 1 time.

Cuts at position 667.

**BstKTI** (G\_AT'C) [MboI,BfuCI,Bsp143I,BstMBI,DpnII,Kzo9I,NdeII,Sau3AI]

Cuts 27 times.

**BstUI** (CG'CG) [AccII,Bsh1236I,BstFNI,MvnI]

Cuts 36 times.

**BstZ17I** (GTA'TAC) [BssNAI,Bst1107I]

Cuts 4 times.

Cuts at positions 728, 1233, 3205, 6864.

Fragment sizes 505, 1972, 3659, 2138.

**ClaI** (AT'CG\_AT) [BanIII,Bsa29I,BseCI,Bsp106I,BspDI,BspXI,Bsu15I,BsuTUI,ZhoI] [dam methylated]

Enzyme does not cut.

**Csp6I** (G'TA\_C) [RsaI,AfaI]

Cuts 14 times.

Cuts at positions 153, 517, 650, 713, 901, 1990, 2491, 2893, 3322, 3860, 4176, 6423,

6979, 7087.

Fragment sizes 364, 133, 63, 188, 1089, 501, 402, 429, 538, 316, 2247, 556, 108,

1340.

**CviAII** (C'AT\_G) [NlaIII,FatI,Hin1II,Hsp92II]  
Cuts 28 times.

**DpnI** (GA'TC)  
Cuts 27 times.

**DraI** (TTT'AAA)  
Cuts 7 times.  
Cuts at positions 2098, 2272, 3481, 3820, 5810, 5829, 6521.  
Fragment sizes 174, 1209, 339, 1990, 19, 692, 3851.

**EagI** (C'GGCC\_G) [BseX3I,BstZI,EclXI,Eco52I]  
Cuts 2 times.  
Cuts at positions 2373, 4056.  
Fragment sizes 1683, 6591.

**EcoICRI** (GAG'CTC) [SacI,Ecl136II,Psp124BI,SstI]  
Cuts 1 time.  
Cuts at position 4655.

**EcoRI** (G'AATT\_C) [FunII]  
Cuts 1 time.  
Cuts at position 3736.

**EcoRV** (GAT'ATC) [Eco32I]  
Cuts 1 time.  
Cuts at position 590.

**FatI** ('CATG\_) [NlaIII,CviAII,Hin1II,Hsp92II]  
Cuts 28 times.

**FseI** (GG\_CCG'CC)  
Enzyme does not cut.

**FspI** (TGC'GCA) [Acc16I,AviIII,NsbI]  
Cuts 2 times.  
Cuts at positions 1815, 6166.  
Fragment sizes 4351, 3923.

**HaeIII** (GG'CC) [BshFI,BspANI,BsuRI,PalI,PhoI]  
Cuts 26 times.

**HhaI** (G\_CG'C) [AspLEI,BstHHI,CfoI,Hin6I,HinPII,HspAI]  
Cuts 42 times.

**HinPII** (G'CG\_C) [HhaI,AspLEI,BstHHI,CfoI,Hin6I,HspAI]  
Cuts 42 times.

**HindIII** (A'AGCT\_T)  
Enzyme does not cut.

**HpaI** (GTT'AAC) [KspAI]  
Cuts 1 time.  
Cuts at position 6915.

**HpaII** (C'CG\_G) [BsiSI,HapII,MspI]  
Cuts 27 times.

**HpyCH4IV** (A'CG\_T) [MaeII,TaiI,TscI]  
Cuts 29 times.

**HpyCH4V** (TG'CA) [CviRI]  
Cuts 30 times.

**KasI** (G'GCGC\_C) [NarI,BbeI,EgeI,EheI,Mly113I,SfoI]  
Cuts 2 times.  
Cuts at positions 2030, 2204.  
Fragment sizes 174, 8100.

**KpnI** (G\_GTAC'C) [Acc65I,Asp718I]

Cuts 1 time.  
Cuts at position 1993.

**MboI** ('GATC\_) [BfuCI,Bsp143I,BstKTI,BstMBI,DpnII,Kzo9I,NdeII,Sau3AI]  
[dam methylated] Cuts 27 times.

**MfeI** (C'AATF\_G) [MunI]  
Enzyme does not cut.

**MluI** (A'CGCG\_T)  
Cuts 4 times.  
Cuts at positions 2163, 2337, 7149, 7658.  
Fragment sizes 174, 4812, 509, 2779.

**MscI** (TGG'CCA) [Bali,MlsI,MluNI,Msp20I]  
[dcm methylated] Cuts 3 times.  
Cuts at positions 2748, [2844], 3473.  
Fragment sizes 96, 629, 7549.

**MseI** (T'TA\_A) [TruII,Tru9I]  
Cuts 47 times.

**NaeI** (GCC'GGC) [MroNI,NgoMIV,PdiI]  
Cuts 1 time.  
Cuts at position 1663.

**NarI** (GG'CG\_CC) [BbeI,EgeI,EheI,KasI,Mly113I,SfoI] Cuts 2 times.  
Cuts at positions 2031, 2205.  
Fragment sizes 174, 8100.

**NcoI** (C'CATG\_G) [Bsp19I]  
Cuts 2 times.  
Cuts at positions 607, 3435.  
Fragment sizes 2828, 5446.

**NdeI** (CA'TA\_TG) [FauNDI]  
Cuts 1 time.  
Cuts at position 315.

**NgoMIV** (G'CCGG\_C) [NaeI,MroNI,PdiI]  
Cuts 1 time.  
Cuts at position 1661.

**NheI** (G'CTAG\_C) [AsuNHI,BmtI]  
Enzyme does not cut.

**NlaIII** (\_CATG') [CviAII,FatI,Hin1II,Hsp92II]  
Cuts 28 times.

**NotI** (GC'GGCC\_GC) [CciNI]  
Cuts 1 time.  
Cuts at position 4056.

**NruI** (TCG'CGA) [Bsp68I]  
[dam methylated] Cuts 8 times.  
Cuts at positions 7177, 7251, 7270, 7909, 7971, 8034, 8096, 8159.  
Fragment sizes 74, 19, 639, 62, 63, 62, 63, 7292.

**NsiI** (A\_TGCA'T) [BfrBI,EcoT22I,Mph1103I,Zsp2I]  
Cuts 2 times.  
Cuts at positions 1229, 7569.  
Fragment sizes 6340, 1934.

**PacI** (TTA\_AT'TAA)  
Enzyme does not cut.

**PciI** (A'CATG\_T) [BspLU11I]

Cuts 2 times.

Cuts at positions 581, 5051.

Fragment sizes 4470, 3804.

**PmeI** (GTTT'AAAC) [MssI]

Enzyme does not cut.

**PmlI** (CAC'GTG) [PmaCI,AcvI,BbrPI,Eco72I,PspCI]

Enzyme does not cut.

**PsiI** (TTA'TAA)

Cuts 3 times.

Cuts at positions 1217, 1432, 7331.

Fragment sizes 215, 5899, 2160.

**PspOMI** (G'GGCC\_C) [ApaI,Bsp120I]

Cuts 1 time.

Cuts at position 776.

**PstI** (C\_TGCA'G) [BspMAI]

Cuts 2 times.

Cuts at positions 384, 2618.

Fragment sizes 2234, 6040.

**PvuI** (CG\_AT'CG) [BspCI,MvrI,Ple19I]

Cuts 2 times.

Cuts at positions 1836, 6314.

Fragment sizes 4478, 3796.

**PvuII** (CAG'CTG)

Cuts 3 times.

Cuts at positions 1865, 3840, 4875.

Fragment sizes 1975, 1035, 5264.

**RsaI** (GT'AC) [AfaI,Csp6I]

Cuts 14 times.

Cuts at positions 154, 518, 651, 714, 902, 1991, 2492, 2894, 3323, 3861, 4177, 6424, 6980, 7088.

Fragment sizes 364, 133, 63, 188, 1089, 501, 402, 429, 538, 316, 2247, 556, 108, 1340.

**SacI** (G\_AGCT'C) [Ecl136II,EcoICRI,Psp124BI,SstI]

Cuts 1 time.

Cuts at position 4657.

**SacII** (CC\_GC'GG) [Cfr42I,KspI,Sfr303I,SgrBI]

Cuts 1 time.

Cuts at position 4648.

**SalI** (G'TCGA\_C)

Cuts 1 time.

Cuts at position 2608.

**SbfI** (CC\_TGCA'GG) [Sse8387I,SdaI]

Cuts 1 time.

Cuts at position 384. ScaI (AGT'ACT) [AssI,ZrmI]

Cuts 3 times.

Cuts at positions 714, 3323, 6424.

Fragment sizes 2609, 3101, 2564.

**SfoI** (GGC'GCC) [NarI,BbeI,EgeI,EheI,KasI,Mly113I]

Cuts 2 times.

Cuts at positions 2032, 2206.

Fragment sizes 174, 8100.

**SmaI** (CCC'GGG) [Cfr9I,PspAI,XmaI,XmaCI]

Cuts 1 time.

Cuts at position 2869.

**SnaBI** (TAC'GTA) [BstSNI,Eco105I]

Cuts 1 time.  
Cuts at position 7921.

**SpeI** (A'CTAG\_T) [AhlI,BcuI]

Enzyme does not cut.

**SphI** (G\_CATG'C) [BbuI,PaeI,SpaHI]

Enzyme does not cut.

**SrfI** (GCCC'GGGC)

Cuts 1 time.  
Cuts at position 2869.

**SspI** (AAT'ATT)

Cuts 3 times.  
Cuts at positions 1352, 3428, 6748.  
Fragment sizes 2076, 3320, 2878.

**StuI** (AGG'CCT) [AatI,Eco147I,PceI,SseBI]

[dcm methylated] Cuts 1 time.  
Cuts at position 838.

**SwaI** (ATTT'AAAT) [SmiI]

Enzyme does not cut.

**TaiI** (\_ACGT') [MaeII,HpyCH4IV,TscI]

Cuts 29 times.

**TaqI** (T'CG\_A)

[dam methylated] Cuts 13 times.  
Cuts at positions 405, 667, 1593, 2609, 5151, 6595, 6940, 7140, 7154, 7320, 7722, 8006, 8131.  
Fragment sizes 262, 926, 1016, 2542, 1444, 345, 200, 14, 166, 402, 284, 125, 548.

**Tsp509I** ('AATT\_) [TspEI,Sse9I,TasI]

Cuts 37 times.

**XbaI** (T'CTAG\_A) [dam methylated]

Cuts 1 time.  
Cuts at position 2470.

**XhoI**

(C'TCGA\_G) [BssHI,Paer7I,Sfr274I,SlaI,StrI,TliI]  
Enzyme does not cut.

**XmaI** (C'CCGG\_G) [SmaI,Cfr9I,PspAI,XmaCI]

Cuts 1 time.  
Cuts at position 2867.

**ZraI** (GAC'GTC) [AatII]

Enzyme does not cut.

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