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**Antibody Design LABORATORIES**
*Your Partner in antibody development™*

# TGEX-HC-hG4[SPLE-PG]-Zeo

Mammalian Expression Vector for Fc-Engineered Constant Region of Human IgG1 Heavy Chain
Catalog #: MX045
Version: A1.2 – October 2023
*For Research Use Only*

## Product Information

**Contents:** 10 μg of TGEX-HC-hG4[SPLE-PG]-Zeo plasmid in 20 μl of DNA Conservation Buffer (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5) (0.5 μg/μl).

**Storage:** The plasmid is shipped on ice. Upon receipt, store at the plasmid at -20°C.

**Quality control:** The plasmid was confirmed by sequencing and certified to meet all specifications.

## General Product Use

The **TGEX™** vector series is designed for the rapid expression of antibody molecules by transient gene expression in mammalian cells in suspension culture. Transfection of **TGEX™** expression vectors harboring variable region inserts typically yields antibody titers between 50 – 250 mg/L in serum-free conditions in just a few days.

**TGEX™-HC** family vectors are designed for the expression of an antibody heavy chain variable region with the constant heavy region of an isotype of choice (**Antibody Design Labs** offers many different isotypes in both wild-type and engineered forms, see **Related Products**). Expression of full-length antibodies is achieved with co-transfection with a light chain variable region cloned in to one of the **TGEX™-LC** family vectors.

**Antibody Design Labs** provides a variety of engineered **TGEX™-HC-hG** vectors, which are designed to express human heavy chains with specific amino acid substitutions in their constant region known to modulate antibody half-life or increase/decrease antibody effector functions (i.e., antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)).

### References

1. **Logan J., & Shenk T. 1984.** Adenovirus tripartite leader sequence enhances translation of mRNAs late after infection. *Proc Natl Acad Sci*, *81*(12):3655–9.
2. **Mariati, H. *et al.* 2010.** Evaluating post-transcriptional regulatory elements for enhancing transient gene expression levels in CHO K1 and HEK293 cells. *Protein Expr Purif*, *69*(1):9–15.
3. **Valadon P. *et al.* 2006.** Screening phage display libraries for organ-specific vascular immunotargeting in vivo. *Proc Natl Acad Sci*, 103(2):407–12.
4. **Lin-Chao S. *et al.* 1992**. High copy number of the PUC plasmid results from a ROM/ROP-suppressible point mutation in RNA II. *Mol Microbiol*, 6(22):3385–93.
5. **Schlothauer, T. et al. 2016.** Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. *Protein Eng Des Sel*, 29:457-466.

## Plasmid Features
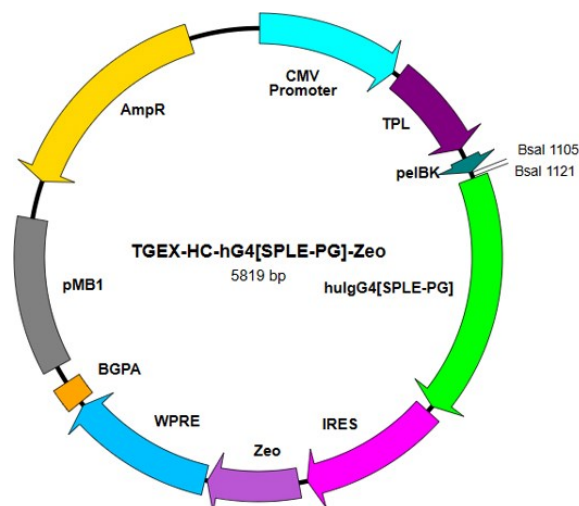


- ❖ **CMV promoter** and **adenovirus tripartite leader sequence**[1,2] for enhanced expression and translation of transgenes.
- ❖ **Composite pelBK leader**[3]: a hybrid between mammalian kappa leader and bacterial leader for secretion of the immunoglobulin chain.
- ❖ Double-inverted BsaI **cloning site** optimized for homologous recombination using **FAST-Licase™** (Catalog #: MB101S, MB101L) or restriction enzyme cloning.
- ❖ **IRES** sequence for efficient co-expression of selective marker using the same promoter.
- ❖ **Zeo**: *Sh ble* gene from *Streptoalloteichus hindustanus* conferring resistance to Zeocin™ for selection of stable transformants in mammalian cell lines.
- ❖ **WPR:** Woodchuck hepatitis post-transcriptional regulatory element for enhanced expression of transgenes.
- ❖ **BGPA**: Rabbit β-globin polyadenylation signal for efficient polyadenylation.
- ❖ **pMB1:** Origin of replication for *E. coli* with a temperature-sensitive high copy-number phenotype.[4]
- ❖ **AmpR:** TEM1 β-lactamase gene conferring resistance to ampicillin for selection in *E. coli*.

## Specific Features

- ❖ **HC-hG4[SPLE-PG]:** human IgG4 heavy chain constant region with the S228P hinge stabilization mutation, and L235E/P329G mutations known to decrease ADCC and CDC *in vitro*.[5]

# Protocol

## Cloning Into TGEX™-HC-hG Vectors

A double-inverted BsaI cloning site separates the pelBK leader peptide sequence from the beginning of the antibody constant region. Cloning into a BsaI-digested TGEX™ vector may be accomplished by two different methods (see below). Both methods result in removal of BsaI sites, which results in the scarless insertion of the antibody variable domain into the plasmid.

### Homologous Recombination (Preferred Method):

Homologous recombination is the easiest and most efficient method to seamlessly clone antibody variable regions into TGEX™ vectors. We recommend the **FAST-LICASE™** (Catalog #: MB101S, MB101L) with the following overhangs:

**pelBK overhang**  5'– CGGCCCAGCCGGCCATGGCA

**hIgG1 overhang**  5'– GCATCCACCAAGGGCCCATC
(reverse complement for PCR primers)

For synthetic dsDNA constructs, the overhangs are added to each side of the VH domain. For PCR-amplified constructs, primers should contain the overhang followed by the antibody variable region priming area, see **FAST-LICASE™** manual for additional details.

### Restriction Cloning (Alternative Method):

Antibody variable regions can be cloned into TGEX™ vectors by restriction cloning by designing primers to amplify variable regions with the required BsaI sites with sufficient complementarity overhangs for in-frame ligation, see examples of compatible oligos below:

**Oligo1**  5'–NNGGTCTCTGGCA–VH-start

**Oligo2**  5'–NNGGTCTCGATGC–JH-end

If the antibody variable region insert contains BsaI sites, which prevents cloning using this method, the BsaI sites can be replaced with sites for another type IIS restriction enzyme that generates identical 4-base long 5' overhangs (e.g., BsmBI).

## Recombinant Antibody Production & Purification

Commonly used cell lines (e.g., HEK293, CHO) adapted for culture in suspension and serum-free conditions are recommended. Many transfection reagents designed for transient transfection are commercially available.

We recommend testing transfections conditions with a fluorescent reporter plasmid first (i.e., **TGEX™-eGFP-Zeo**, Cat# MX022) to evaluate transfection efficiency and determine optimal transfection conditions.

Expression of full-length antibodies requires cotransfection with heavy chain (i.e., **TGEX™-HC** family) and light chain (i.e., **TGEX™-LC**) vectors containing variable regions of interest. We recommend starting with a 1:1 light chain to heavy chain ratio during transfection. In our experience, a 2:1 light chain to heavy chain ratio often exhibits an increased expression, although each antibody may require fine tuning of the ratio for optimal expression.

Stably-transfected cells can be produced by selection using Zeocin™. We recommend culturing transfected cells for 6 days before supplementing growth media with 100 µg/ml Zeocin™. Continue passaging cells at regular intervals and densities while maintaining Zeocin™ concentration for an additional 2-3 weeks before increasing selective pressure to up to 1 mg/ml Zeocin™ for an additional 1-2 weeks. Following selection, proceed to single-cell subcloning and analysis of stable transformants for antibody expression.

Antibody production may be assessed by SDS-PAGE, ELISA, or another quantitative assay. Recombinant antibodies may be purified directly from culture supernatant using Protein A/G/L affinity chromatography or coated magnetic beads.

## Legal and Disclaimers

## Related Products

| VECTOR | CATALOG | ISOTYPE/USE | DESCRIPTION |
|---|---|---|---|
| **Antibody Heavy Chain Expression Vectors** | | | |
| TGEX™-HC-hG1-Zeo | MX026 | Human IgG1 | Heavy chain expression plasmid for human IgG1 |
| TGEX™-FH-hG1-Zeo | MX023 | Human IgG1 CH1 | Expression plasmid for human IgG1 Fab fragments |
| TGEX™-FC-hG1-Zeo | MX025 | Human IgG1 Fc | Expression plasmid for human IgG1 Fc fusions |
| TGEX™-HC-hG2-Zeo | MX027 | Human IgG2 | Heavy chain expression plasmid for human IgG2 |
| TGEX™-HC-hG3-Zeo | MX028 | Human IgG3 | Heavy chain expression plasmid for human IgG3 |
| TGEX™-HC-hG4[S228P]-Zeo | MX029 | Human IgG4[S228P] | Heavy chain expression plasmid for human IgG4 |
| TGEX™-HC-mG1-Zeo | MX032 | Murine IgG1 | Heavy chain expression plasmid for murine IgG1 |
| TGEX™-HC-mG2a-Zeo | MX033 | Murine IgG2a | Heavy chain expression plasmid for murine IgG2a |
| TGEX™-HC-mG2b-Zeo | MX034 | Murine IgG2b | Heavy chain expression plasmid for murine IgG2b |
| TGEX™-HC-mG3-Zeo | MX035 | Murine IgG3 | Heavy chain expression plasmid for murine IgG3 |
| TGEX™-HC-rbG-Zeo | MX039 | Rabbit IgG | Heavy chain expression plasmid for rabbit IgG |
| **Fc-engineered Antibody Heavy Chain Expression Vectors** | | | |
| TGEX™-HC-hG1[EA]-Zeo | MX041 | Human IgG1 | IgG1 heavy chain with mutated Fc to increase ADCC/CDC |
| TGEX™-HC-hG1[NA]-Zeo | MX042 | Human IgG1 | IgG1 heavy chain with mutated Fc for aglycosylated antibody |
| TGEX™-HC-hG1[LALA-PG]-Zeo | MX043 | Human IgG1 | IgG1 heavy chain with mutated Fc to reduce ADCC/CDC |
| TGEX™-HC-hG1[YTE-KF]-Zeo | MX044 | Human IgG1 | IgG1 heavy chain with mutated Fc to increase serum half-life |
| TGEX™-HC-hG4[SPLE-PG]-Zeo | MX045 | Human IgG4[S228P] | IgG4 heavy chain with mutated Fc to reduce ADCC/CDC |
| **Antibody Light Chain Expression Vectors** | | | |
| TGEX™-LC-hK-Zeo | MX030 | Human Kappa | Light chain expression plasmid for human Kappa |
| TGEX™-LC-hL-Zeo | MX031 | Human Lambda 2 | Light chain expression plasmid for human Lambda 2 |
| TGEX™-LC-mK-Zeo | MX036 | Murine Kappa | Light chain expression plasmid for murine Kappa |
| TGEX™-LC-mL1-Zeo | MX037 | Murine Lambda 1 | Light chain expression plasmid for murine Lambda 1 |
| TGEX™-LC-mL2-Zeo | MX038 | Murine Lambda 2 | Light chain expression plasmid for murine Lambda 2 |
| TGEX™-LC-rbKb4 | MX040 | Rabbit Kappa b4 | Light chain expression plasmid for rabbit Kappa b4 |
| **Engineering Vectors** | | | |
| TGEX™-AC- Zeo | MX020 | Any expressions | Universal mammalian expression vector |
| TGEX™-eGFP- Zeo | MX022 | Transfection control | Control plasmid for monitoring transient transfections |
| TGEX™-SCblue-Zeo | MX024 | scFv cloning vector | For the transfer of scFv from any pADL phagemid vector and expression as scFv-Fc fusion |

## Examples for Desired Antibody Format

| COMBINATION | FORMAT | PURIFICATION METHOD[1] |
|---|---|---|
| TGEX™-HC-hG1-Zeo + TGEX™-LC-hK-Zeo | Full-length human or chimeric IgG1/K | Protein A or G |
| TGEX™-FH-hG1-Zeo + TGEX™-LC-hK-Zeo | Human or chimeric IgG1/K Fab fragment | Protein L, G or IMAC |
| TGEX™-FC-hG1 | Human IgG1 Fc fusion | Protein A or G |
| TGEX™-SCblue-Zeo | Human IgG1 scFv-Fc fusion | Protein A or G |

[1]Purification by Protein A, G, and L may require additional testing.

```
                        SpeI(19)   AseI(27)
   1 gttgacattgattattgactagttattaataagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcc

 106 cgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttccctagtaacgccaatagggactttccattgacgtcaatgggtggagt
                                            NdeI(254)
 211 atttacggtaaaactgcccacttggcagtacatcaagtgtatcatatgccaagtccgcccccctattgacgtcaatgacggtaaatggcccgcctggcattatgccc
                                         SnaBI(360)
 316 agtacatgaccttacgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacaccaatgggcgtggat

 421 agcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaataacc
                                                                              SacI (588)
 526 ccgcccccgttgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagatcctcactctcttccgcatcgctg
            PvuII (645)
 631 tctgcgagggccagctgttggggtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgaggaggatttgatattcacc

 736 tggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatctttccttgatgatgtcatacttatcctgtcccttttttttttccacagct

 841 cgcggttgaggacaaaactcttcgcggtctttccagtactcttggatcggaaacccgtcggcctccgaacggtactccgccaccgagggacctgagcgagtccgca
                  XhoI (967)                              PmeI (1004)    EcoRI (1020)
 946 tcgaccggatcggaaaacctctcgagaaaggcgtctaaccagtcacagtcgcaagtttaaacggatctctagcgaattcggcttgggccgccaccatggagacag
                                            BsaI (1110)  BsaI (1121)
                                               MluI (1114)        ApaI (1144)
                                           hIgG4[SPLE-PG]»1 A  S  T  K  G  P  S  V  F  P
1051 acacactcctgctatgggtactgctgctcttagcggcccagccggccatggcag gagacctaacgcgtatggtctca gcatccaccaagggcccatccgtcttcc
              BbeI (1165)
          EgeI (1163)                                                                     EgeI (1260)
          NarI (1162)                                                                     NarI (1259)
          KasI (1161)                                            AgeI (1233)              KasI (1258)
  11  L  A  P  C  S  R  S  T  S  E  S  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A
1156 ccctggcgccctgctccaggagcacctccgagagcacagccgccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcg

           BbeI (1262)
  46  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  K  T
1261 ccctgaccagcggcgtgcacaccttccccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacgaaga

  81  Y  T  C  N  V  D  H  K  P  S  N  T  K  V  D  K  R  V  E  S  K  Y  G  P  P  C  P  P  C  P  A  P  E  F  E
1366 cctacacctgcaacgtagatcacaagcccagcaacaccaaggtggacaagagagttgagtccaaatatggtcccccatgcccaccttgcccagcacctgagttcg
                                                                     PmlI (1547)
 116  G  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  Q  E  D
1471 aggggggaccatcagtcttcctgttccccccaaaacccaaggacactctcatgatctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccaggaag

 151  P  E  V  Q  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  F  N  S  T  Y  R  V  V  S  V
1576 accccgaggtccagttcaactggtacgtggatggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagttcaacagcacgtaccgtgtggtcagcg
                                                         AatI (1748)
 186  L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  G  L  G  S  S  I  E  K  T  I  S  K  A  K
1681 tcctcaccgtcctgcaccaggactggctgaacggcaaggagtacaagtgcaaggtgtccaacaaaggcctcgggtcctccatcgagaaaaccatctccaaagcca
              Bsp1407I (1811)
 221  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  Q  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S
1786 aagggcagccccgagagccacaggtgtacacccctgcccccatcccaggaggagatgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctacccca

 256  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S  R  L
1891 gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaggc
                                                                      NsiI (2054)
                                                                   BfrBI (2052)
 291  T  V  D  K  S  R  W  Q  E  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  L
1996 taaccgtggacaagagcaggtggcaggagggggaatgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacacagaagagcctctccctgtctc

 326  G  K  *         NheI (2116)
2101 tgggtaaataa acagctagcggttccaaccttggttcccctctccctttcccccccctaacgttactggccgaagccgcttggaataaggccggtgtgcgtttgt
                                               ApaI (2255)                            AvrII (2289)
2206 ctatatgttattttccaccatattgccgtctttttggcaatgtgagggcccggaaacctggccctgtcttcttgacgagcattcctaggggtctttcccctctcgc

2311 caaaggaatgcaaggtctgttgaatgtcgtgaaggaagcagttcctctgggagcttcttgaagacaaacaacgtctgtagcgaccctttgcaggcagcggaaccc
                                             PmlI (2454)
2416 cccacctggcgacaggtgcctctgcggccaaaagccacgtgtataagatacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtgga
                                                             KpnI (2583)
                                                          Asp718I (2579)
2521 aagagtcaaatggctctcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtacccattgtatgggatctgatctggggcctcggtgcacatgctt
                                                                            BamHI (2727)
                                                                              SmaI (2725)
                                                                             XmaI (2723)
                                                         HindIII (2710)
            PciI (2628)
2626 tacatgtgtttagtcgaggttaaaaaaacgtctaggcccccccgaaccacgggggacgtggtttttcctttgaaaaacacgatgataagcttgccacaacccgggatc
         SalI (2739)                                                                         SmaI (2835)
   XbaI (2733)         MscI (2748)                           BsePI (2781)                      XmaI (2833)
          Zeo»1 M  A  K  L  T  S  A  V  P  V  L  T  A  R  D  V  A  G  A  V  E  F  W  T  D  R  L  G  F  S  R
2731 ctctagagtcgac atggccaagttgaccagtgccgttccggtgctcaccgcgcgcgacgtcgccggagcggtcgagttctggaccgaccggctcgggttctcccg

  32  D  F  V  E  D  D  F  A  G  V  V  R  D  D  V  T  L  F  I  S  A  V  Q  D  Q  V  V  P  D  N  T  L  A  W  V
2836 ggacttcgtggaggacgacttcgccggtgtggtccgggacgacgtgaccctgttcatcagcgcggtccaggaccaggtggtgccggacaacaccctggcctgggt

                                                                   FseI (3021)
  67  W  V  R  G  L  D  E  L  Y  A  E  W  S  E  V  V  S  T  N  F  R  D  A  S  G  P  A  M  T  E  I  G  E  Q  P
2941 gtgggtgcgcggcctggacgagctgtacgccgagtggtcggaggtcgtgtccacgaacttccgggacgcctccgggccggccatgaccgagatcggcgagcagcc
```

```
                                 EagI (3076)                                    EagI (3121)
  102   W   G   R   E   F   A   L   R   D   P   A   G   N   C   V   H   F   V   A   E   E   Q   D   *       NotI (3121)
 3046 gtggggcgggagttcgccctgcgcgacccggccggcaactgcgtgcactttgtggcagaggagcaggactgagcggccgcaatcaacctctggattacaaatt

 3151 tgtgaaagattgactggtattcttaactatgttgctccttttacgctatgtggatacgctgctttaatgcctttgtatcatgctattgcttcccgtatggctttc

 3256 attttctcctccttgtataaatcctggttgctgtctctttatgaggagttgtggcccgttgtcaggcaacgtggcgtggtgtgcactgtgtttgctgacgcaacc

 3361 cccactggttggggcattgccaccacctgtcagctcctttccgggactttcgctttccccctccctattgccacggcggaactcatcgccgcctgccttgcccgc

 3466 tgctggacaggggctcggctgttgggcactgacaattccgtggtgttgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgccacctggattctg

 3571 cgcgggacgtccttctgctacgtcccttcggccctcaatccagcggaccttccttcccgcggcctgctgccggctctgcggcctcttccgcgtcttcgccttcgc

 3676 cctcagacgagtcggatctcccctttgggccgcctccccgcagatcccccgacctcgacctctggctaataaaggaaatttattttcattgcaatagtgtgttgga
                                                                                        HindIII (3853)
                     NdeI (3810)                                           BglII (3847)
 3781 atttttttgtgtctctcactcggaaggacatatggggaggcaaatcatttggtcgagatccctcggagatctaagcttcatgtgagcaaaaggccagcaaaaggcc

 3886 aggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgaca

 3991 ggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcggga

 4096 agcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttcagcccgaccgc

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 4516 ttgatctttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatcttcacctagatcctttaaat

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 4726 cgttcatccatagttgcctgactccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgtgacccacgctca
                                                                                              AseI (4923)
 4831 ccggctccagatttatcagcaataaaccagccagccggaagcgccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaattgttgccgg
                           AviII (4973)
 4936 gaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattcagc
                                                                                               PvuI (5121)
 5041 tccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccgca

 5146 gtgttatcactcatggttatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctga

 5251 gaatagtgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttct

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 5566 atttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaatagggggttccgcgcacatttccccgaaaagtgccacctgaa
             BglII (5671)                                                                             MscI (5750)
 5671 gatctatacattgaatcaatattggcaattagccatattagtcattggtttatatagcataaatcaatattggctattggccattgcatacgttgtatctatatca
                Bsp1407I (5782)
 5776 taatatgtacatttatattggctcatgtccaatatgaccgc
```