SAND2016-5411R

NMSBA: Sandia Biotech 2016

PI: Anne Ruffing
Sandia Biotech POC: Amanda Portillo

Description: A report generated by Sandia National Laboratories for work performed under the New Mexico Small Business Assistance program (requestor: Sandia Biotech LLC).

Project Objective

To modify the FluorAbody plasmid previously developed by Sandia Biotech to include a binding site for biotin by introducing the biotin carboxyl carrier protein (BCCP) and a gold binding protein (GBP) into a loop of the red fluorescent protein (mRFP).

Plasmid Design

Previous work has demonstrated that protein fragments, such as regions of light-chain antibody fragments, can be inserted into loop 9 of green fluorescent protein (GFP) to enable binding without loss of the fluorescent properties [1, 2]. Therefore, we will target the corresponding loop 9 of mRFP for insertion of BCCP and GBP.

As BCCP (86 amino acids) is much longer than the previously inserted light-chain fragment (11 amino acids), functional insertion of the biotin binding site may be more difficult. To reduce this risk, we propose 3 different constructs:

- BCCP directly inserted into loop 9
- Fusion of BCCP with flexible linkers flanking BCCP: $(GGGGS)_2$
- Fusion of BCCP with rigid linkers flanking BCCP: $(EAAAK)_2$

Both flexible and rigid linkers have been shown to improve the activity of fusion proteins [3], thus these linkers may provide additional space needed to enable both functional biotin binding and mRFP fluorescence. Plasmid sequences for the proposed constructs are included in Appendix A.

The GBP domain was computationally derived [4] and 3 repeats of this 14 amino acid sequence was experimentally demonstrated to bind gold nanoparticles [5]. This binding site will also be introduced into loop 9 of mRFP in the FluorAbody plasmid with the following constructs:

- Fusion of three GBP repeats with two flexible linkers flanking GBP: $(GGGGS)_2$
- Fusion of four GBP repeats with two flexible linkers flanking GBP: $(GGGGS)_2$
- Fusion of four GBP repeats with three flexible linkers flanking GBP: $(GGGGS)_3$

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The FluorAbody plasmid (pFluorAbody) was provided by Sandia Biotech, and the three BCCP and three GBP inserts (BCCP, BCCPflex, BCCPrigid, GBP3flex2, GBP4flex2, and GBP4flex3) were codon optimized for expression in *Escherichia coli*, modified with overlap regions for Gibson Assembly, and synthesized by Integrated DNA Technologies (IDT). The plasmid was amplified using the primers in Table 1 (primers ordered from IDT). After gel purification of the linear plasmid (Zymo DNA gel isolation kit), the plasmid (0.1 pmol) and associated BCCP or GBP fragment (0.2 pmol) were combined for Gibson Assembly (NEB Gibson Assembly master mix). Gibson Assembly mixes were then transformed into *E. coli* DH5α competent cells (NEB) using heat shock transformation.

Table 1: Primers used in Gibson Assembly of modified FluorAbody plasmids, PCR screening, and DNA sequencing

Primer Name	Sequence	Description
pFluorAbodyF	GGCGGCCACTACGACGC	Primers for amplification of pFluorAbody for Gibson Assembly
pFluorAbodyR	GTCCTTCAGCTTCAGCCTCATC	
InsertF	CCTTCCCCGAGGGCTTC	Primers for PCR screening of inserts; the forward primer was used for DNA sequencing
InsertR	GTTGCTGACAGTAATAAGTTGCG	

Construct Confirmation

Transformants were screened using PCR amplification of the inserted fragment (Figure 1). The plasmids from three positive transformants were isolated and sent for DNA sequencing at Eurofins Genomics. DNA sequencing confirmed successful integration of the BCCP and GBP fragments (with some mutations in the GBP constructs). Plasmids with no sequence mutations were selected for the BCCP constructs and with minimal mutations in the GBP constructs (see Appendix B for DNA sequencing results). Mutations in the GBP constructs were due to the repetitive nature of the GBP, which led to difficulties in both DNA synthesis and Gibson assembly. The sequenced transformants were provided to Sandia Biotech for functional testing.

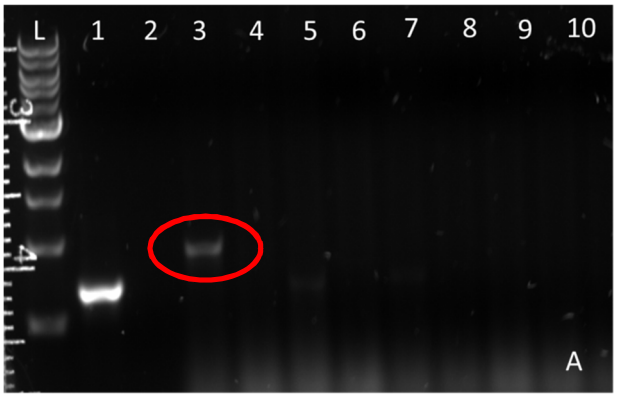

Figure 1: PCR screening of BCCPrigid inserts in pFluorAbody. Positive bands are expected at 1.011 kb (circled in red). L – 1kb ladder, 1 – pFluorAbody control, 2 – DH5α gDNA control, 3 – 10 candidates for screening.

References

1. Chen, Y., et al., *The structure of a GFP-based antibody (fluorobody) to TLH, a toxin from Vibrio parahaemolyticus.* Acta Crystallographica Section F, 2015. **71**(7): p. 913-918.
2. Wang, R., et al., *Development of a Functional Antibody by Using a Green Fluorescent Protein Frame as the Template.* Applied and Environmental Microbiology, 2014. **80**(14): p. 4126-4137.
3. Chen, X., J. Zaro, and W.-C. Shen, *Fusion Protein Linkers: Property, Design and Functionality.* Advanced drug delivery reviews, 2013. **65**(10): p. 1357-1369.
4. Braun, R., M. Sarikaya, and K. Schulten, *Genetically engineered gold-binding polypeptides: structure prediction and molecular dynamics.* Journal of Biomaterials Science, Polymer Edition, 2002. **13**(7): p. 747-757.
5. Ko, S., et al., *Directed self-assembly of gold binding polypeptide-protein A fusion proteins for development of gold nanoparticle-based SPR immunosensors.* Biosensors and Bioelectronics, 2009. **24**(8): p. 2592-2597.

Appendix A: Plasmid Sequences and Maps

pFluorAbody

Description: Plasmid provided by Sandia Biotech.

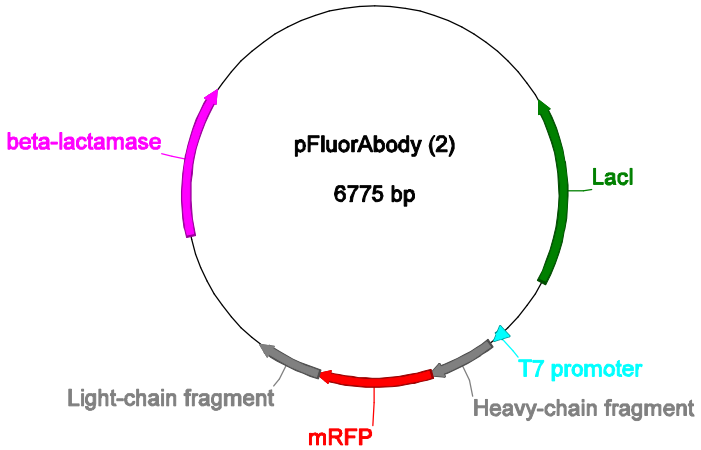

Figure 2. Plasmid map for pFluorAbody

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2016
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VERSION
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  ORGANISM  .
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pFluorAbody-BCCP

Description: pFluorAbody with BCCP inserted into loop 9 of mRFP.

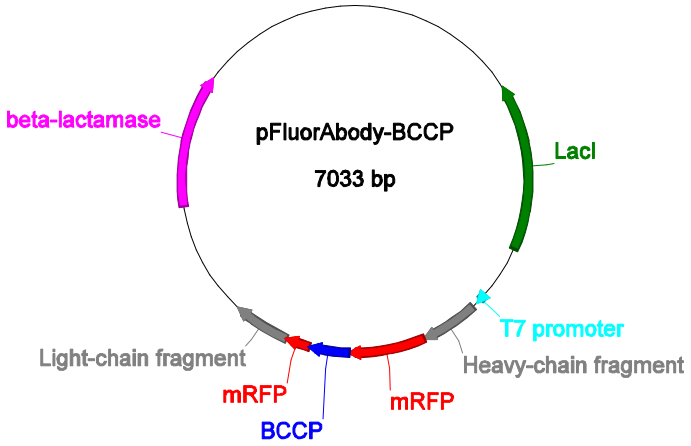

Figure 3. Plasmid map for pFluorAbody-BCCP

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//

pFluorAbody-BCCPflex

Description: pFluorAbody with BCCP flanked by flexible linkers and inserted into loop 9 of mRFP.

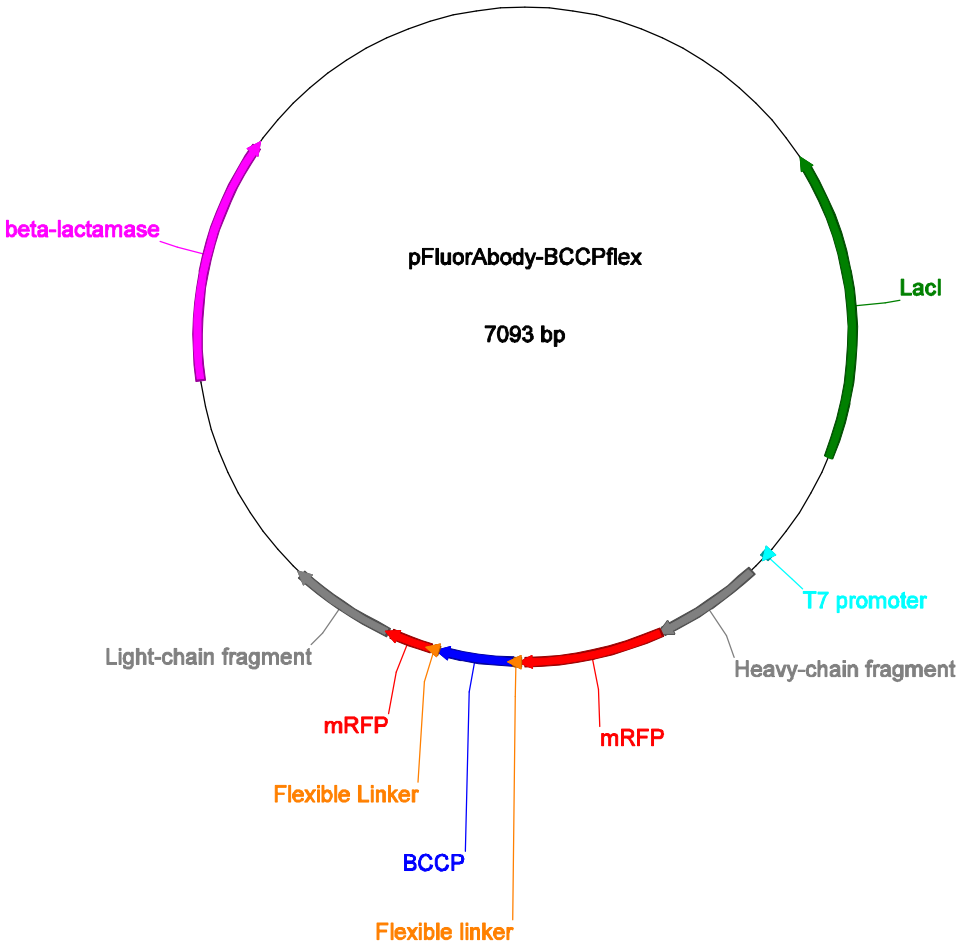

Figure 4. Plasmid map for pFluorAbody-BCCPflex.

```
LOCUS       pFluorAbody_BCCP        7093 bp ds-DNA     circular     07-MAR-
2016
DEFINITION  .
ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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       61 GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT
      121 GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGCTG CGGTAAAGCT
      181 CATCAGCGTG GTCGTGAAGC GATTCACAGA TGTCTGCCTG TTCATCCGCG TCCAGCTCGT
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     7081 TACACTCCGC TAT
//
```

pFluorAbody-BCCPrigid

Description: pFluorAbody with BCCP flanked by rigid linkers and inserted into loop 9 of mRFP.

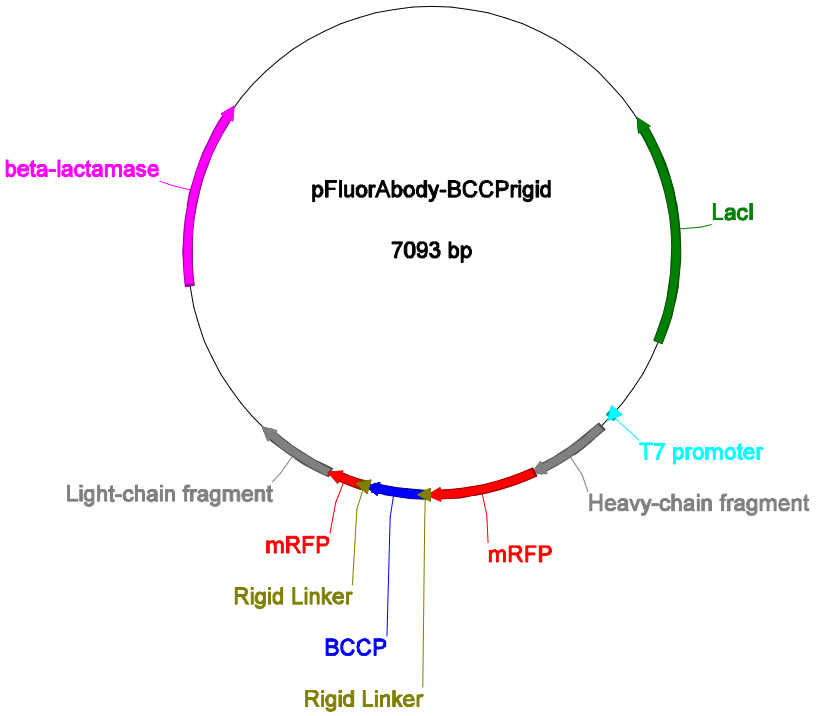

Figure 5. Plasmid map for pFluorAbody-BCCPrigid.

```
LOCUS       pFluorAbody_BCCP        7093 bp ds-DNA     circular     07-MAR-
2016
DEFINITION  .
ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
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      181 CATCAGCGTG GTCGTGAAGC GATTCACAGA TGTCTGCCTG TTCATCCGCG TCCAGCTCGT
      241 TGAGTTTCTC CAGAAGCGTT AATGTCTGGC TTCTGATAAA GCGGGCCATG TTAAGGGCGG
      301 TTTTTTCCTG TTTGGTCACT GATGCCTCCG TGTAAGGGGG ATTTCTGTTC ATGGGGGTAA
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481 AAATCACTCA GGGTCAATGC CAGCGCTTCG TTAATACAGA TGTAGGTGTT CCACAGGGTA
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601 TTTCCAGACT TTACGAAACA CGGAAACCGA AGACCATTCA TGTTGTTGCT CAGGTCGCAG
661 ACGTTTTGCA GCAGCAGTCG CTTCACGTTC GCTCGCGTAT CGGTGATTCA TTCTGCTAAC
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1801 TCCACAGCAA TGGCATCCTG GTCATCCAGC GGATAGTTAA TGATCAGCCC ACTGACGCGT
1861 TGCGCGAGAA GATTGTGCAC CGCCGCTTTA CAGGCTTCGA CGCCGCTTCG TTCTACCATC
1921 GACACCACCA CGCTGGCACC CAGTTGATCG GCGCGAGATT TAATCGCCGC GACAATTTGC
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2101 TTTTCCCGCG TTTTCGCAGA AACGTGGCTG GCCTGGTTCA CCACGCGGGA AACGGTCTGA
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2821 GGTAAGGGCC TGGAATGGGT TGCAAGGATT TATCCTACGA ATGGTTATGC TAGATATGCC
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3001 GACGGCTTCT ATGCTATGGA CTACTGGGGT CAAGGAACCC TGGTCACCGT CTCCTCGGGC
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pFluorAbody-GBP3flex2 (designed)

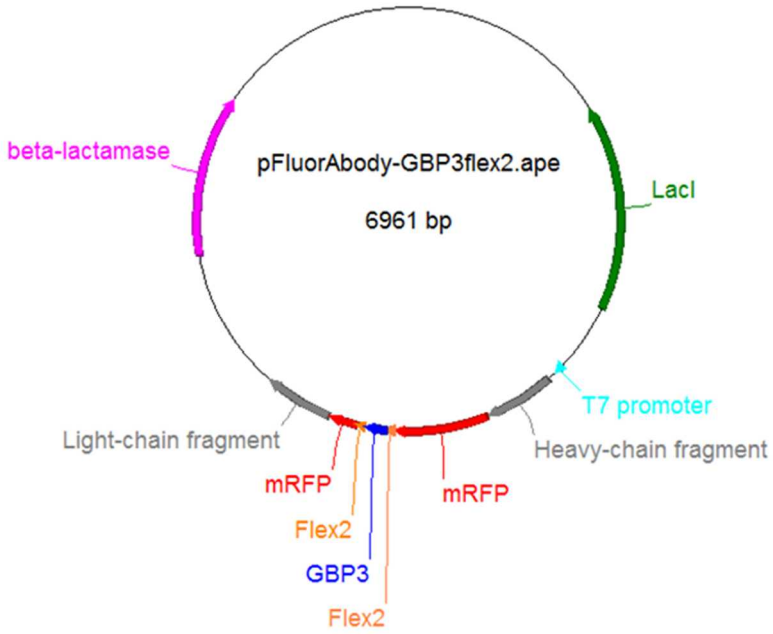

Figure 6. Plasmid map for pFluorAbody-GBP3flex2

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2016
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ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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ORIGIN
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1681 AATGCGACCA GATGCTCCAC GCCCAGTCGC GTACCGTCTT CATGGGAGAA AATAATACTG
1741 TTGATGGGTG TCTGGTCAGA GACATCAAGA AATAACGCCG GAACATTAGT GCAGGCAGCT
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2461 GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC
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2581 CGATCTCGAT CCCGCGAAAT TAATACGACT CACTATAGGG GAATTGTGAG CGGATAACAA
2641 TTCCCCTCTA GAAATAATTT TGTTTAACTT TAAGAAGGAG ATATACCATG GATGGCCGAG
2701 GTTCAGCTGG TGGAGTCTGG CGGTGGCCTG GTGCAGCCAG GGGGCTCACT CCGTTTGTCC
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pFluorAbody-GBP4flex2 (designed)

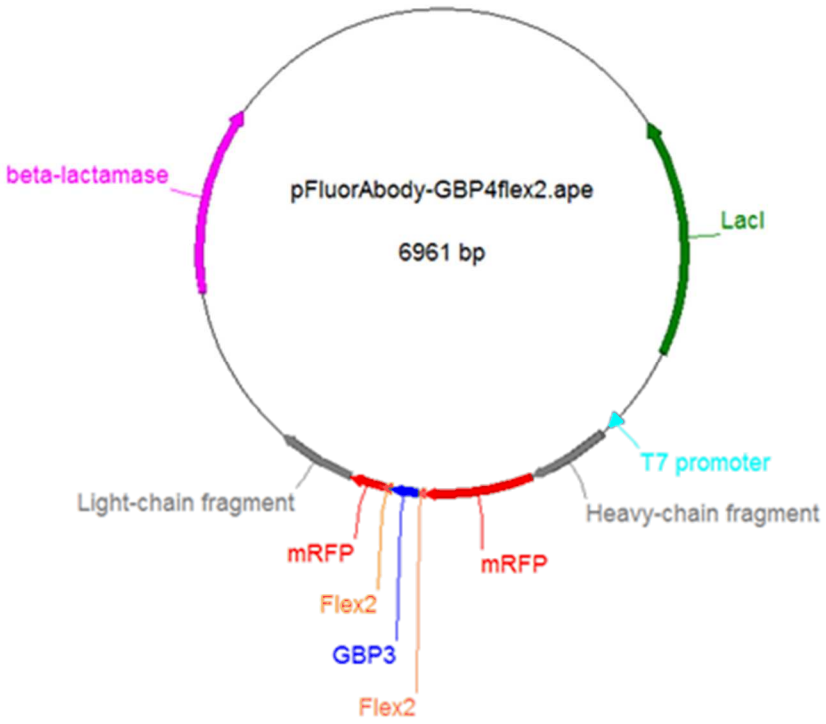

Figure 7. Plasmid map for pFluorAbody-GBP4flex2

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VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT
COMMENT     ApEinfo:methylated:1
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pFluorAbody-GBP4flex3 (designed)

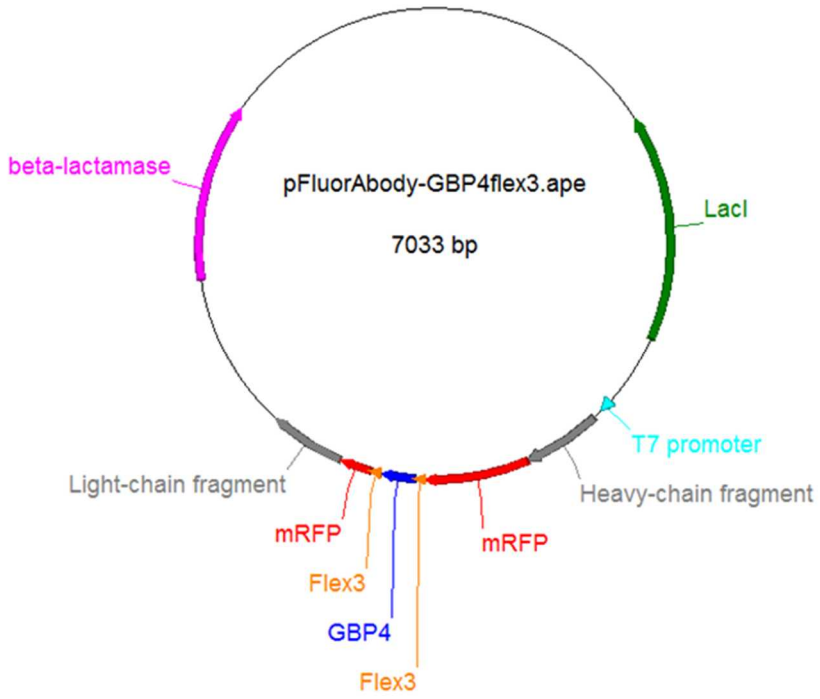

Figure 8. Plasmid map for pFluorAbody-GBP4flex3

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VERSION
SOURCE      .
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Appendix B: DNA Sequencing Results

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                      ****************************** ** *************************

pFluorAbody_BCCP      ACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGT
BCCP10F_PREMIX        ACGGATTTCACTCTGNGCATCNNNANNCNGCACCCGGNGGACTTCNNAACTNNNNNNTGN
                      ***************  ****   *  * *** **** *******  ****      **

Transformant #1 was selected for pFA-BCCP
```

pFluorAbody-BCCPflex

```
>Flex3F_PREMIX Sample_Name=Flex3F_PREMIX Chromat_id=4770415 Read_id=4718432 Version=1 Length=541
NNNNNNNNNNACTTCNNNNACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCA
CCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGG
CGAGATCAAGATGAGGCTGAAGCTGAAGGACAGAGGTGGTGGTTCTGGTGGAGGAGGATCAGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCGCATC
GTACGTTCCCCGATGGTTGGTACTTTCTACCGCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGACAACGTGGGCGATACCC
TGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGA
ATTCGACGAACCGCTTGTTGTTATCGAAGGAGGTGGTGGTTCTGGTGGAGGAGGAT
```

```
pFluorAbody_BCCPflex      GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG
Flex3F_PREMIX             ----------------------------------------NNNNNNNNNNACTTCNNN
                                                                          *****

pFluorAbody_BCCPflex      GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Flex3F_PREMIX             NACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                           ***********************************************************

pFluorAbody_BCCPflex      AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Flex3F_PREMIX             AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                          ************************************************************

pFluorAbody_BCCPflex      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Flex3F_PREMIX             ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                          ************************************************************

pFluorAbody_BCCPflex      ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGTGGTTCTGGTGGAGGAGGATCAgaa
Flex3F_PREMIX             ATCAAGATGAGGCTGAAGCTGAAGGACAGAGGTGGTGGTTCTGGTGGAGGAGGATCAGAA
                          *************************** ******************************

pFluorAbody_BCCPflex      gcgccagcagcagcggaaatcagtggtcacatcgtacgttccccgatggttggtactttc
Flex3F_PREMIX             GCGCCAGCAGCAGCGGAAATCAGTGGTCGCATCGTACGTTCCCCGATGGTTGGTACTTTC
                          *************************** ******************************

pFluorAbody_BCCPflex      taccgcaccccaagcccggacgcaaaagcgttcatcgaagtgggtcagaaagtcaacgtg
Flex3F_PREMIX             TACCGCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGACAACGTG
                          ***************************************************  *******

pFluorAbody_BCCPflex      ggcgataccctgtgcatcgttgaagccatgaaaatgatgaaccagatcgaagcggacaaa
Flex3F_PREMIX             GGCGATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAA
                          ************************************************************
```

```
pFluorAbody_BCCPflex      tccggtaccgttaaagctatccttgttgaatctggtcagccggttgaattcgacgaaccg
Flex3F_PREMIX             TCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAATTCGACGAACCG
                          ************************************************************

pFluorAbody_BCCPflex      cttgttgttatcgaaGGAGGTGGTGGTTCTGGTGGAGGAGGATCAGGCGGCCACTACGAC
Flex3F_PREMIX             CTTGTTGTTATCGAAGGAGGTGGTGGTTCTGGTGGAGGAGGAT-----------------
                          *****************************************
```

Possible mutations in flex #3, need to do additional sequencing, isolate new plasmids

Flex6 - poor results
Flex7 - some results with reverse primer
Flex8 - some results with reverse primer

```
>pBCCPflex7R_PREMIX Sample_Name=pBCCPflex7R_PREMIX Chromat_id=4840270 Read_id=4788052 Version=1
Length=329
NNNNNNNNNNNNNNGNNACTGCTGATGGTCAGAGTGAAATCCGTCCCAGATCTGGACCCAGAGAAGCGAGAAAGGACTCCAGAGTAGAGGAAGGATGC
CGAGTAAATCAGTAGTTTCGGAGCTTTTCCTGGTTTCTGTTGATACCAGGCTACAGCAGTATTCACATCCTGACTGGCACGGCAGGTGATGGTGACC
CTATCGCCCACAGAGGCGGACAGGGAGCTCGGGGACTGGGTCATCTGGATATCGGATCCGCCTCCGCCTGTGCGGCCCTCGGCGCGCTCGTACTGTT
CCACGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCC

template       CGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGT
flex7R_RC      -------------------------GGACATCACCTCCCACAACGAGGACTACACCATCGT
                                         ************************************

template       GGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGAC
flex7R_RC      GGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGAC
               ************************************************************

template       CCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGC
flex7R_RC      CCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGC
               ************************************************************

template       CAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAA
flex7R_RC      CAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAA
               ************************************************************

template       ACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTC
flex7R_RC      ACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTC
               ************************************************************

template       CAGATCTGGGACGGATTT-----------------------------------
flex7R_RC      CAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTNNCNNNNNNNNNNNNN
               ******************
```

Sequence is good but not long enough to cover BCCPflex region

```
>pBCCPflex8R_PREMIX Sample_Name=pBCCPflex8R_PREMIX Chromat_id=4840271 Read_id=4788058 Version=1
Length=677
GNNNNNNNNNNNCNACTGCTGATGGTCNGAGTGNNTCCGTCCCAGATCTGGACCCAGAGAAGCGAGAAAGGACTCCAGAGTAGAGGAAGGATGCCGA
GTAAATCAGTAGTTTCGGAGCTTTTCCTGGTTTCTGTTGATACCAGGCTACAGCAGTATTCACATCCTGACTGGCACGGCAGGTGATGGTGACCCTA
TCGCCCACAGAGGCGGACAGGGAGCTCGGGGACTGGGTCATCTGGATATCGGATCCGCCTCCGCCTGTGCGGCCCTCGGCGCGCTCGTACTGTTCCA
CGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCCAGCTTGATGTCGGTCTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGGCCATGTAGGTGGT
CTTGACCTCGGCGTCGTAGTGGCCGCCTGATCCTCCTCCACCAGAACCACCACCTCCTTCGATAACAACAAGCGGTTCGTCGAATTCAACCGGCTGA
CCAGATTCAACAAGGATAGCTTTAACGGTACCGGATTTGTCCGCTTCGATCTGGTTCATCATTTTCATGGCTTCAACGATGCACAGGGTATCGCCCA
CGTTGACTTTCTGACCCACTTCGATGAACGCTTTTGCGTCCGGGCTTTGGGGTGCGGTANAAAGTACNNNNCNTCGGGGGAACGTACGATGTGNN

template       AGGATCAgaagcgccagcagcagcggaaatcagtggtcacatcgtacgttcc-ccgatgg
flex8R_RC      -----------------------------------NNCACATCGTACGTTCCCCCGANGN
                                                   *************** **** *

template       ttggtactttcta-ccgcaccccaagcccggacgcaaaagcgttcatcgaagtgggtcag
flex8R_RC      NNNGTACTTTNTACCGCACCCCAAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAG
                  ******* ** *    *** ************************************

template       aaagtcaacgtgggcgataccctgtgcatcgttgaagccatgaaaatgatgaaccagatc
flex8R_RC      AAAGTCAACGTGGGCGATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATC
               ************************************************************

template       gaagcggacaaatccggtaccgttaaagctatccttgttgaatctggtcagccggttgaa
flex8R_RC      GAAGCGGACAAATCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAA
```

```
                ************************************************************

template        ttcgacgaaccgcttgttgttatcgaaGGAGGTGGTGGTTCTGGTGGAGGAGGATCAGGC
flex8R_RC       TTCGACGAACCGCTTGTTGTTATCGAAGGAGGTGGTGGTTCTGGTGGAGGAGGATCAGGC
                ************************************************************

template        GGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
flex8R_RC       GGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
                ************************************************************

template        GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATC
flex8R_RC       GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATC
                ************************************************************

template        GTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATG
flex8R_RC       GTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATG
                ************************************************************

template        ACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGT
flex8R_RC       ACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGT
                ************************************************************

template        GCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCG
flex8R_RC       GCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCG
                ************************************************************

template        AAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGG
flex8R_RC       AAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGG
                ************************************************************

template        TCCAGATCTGGGACGGATTT--------------------------------
flex8R_RC       TCCAGATCTGGGACGGANNCACTCNGACCATCAGCAGTNGNNNNNNNNNNNC
                *****************
```

Sequence looks good - covers most of BCCPflex region, but not all of it - send for sequencing with forward primer and also try purifying some more

Flex8

```
>BCCPflex8_2R_PREMIX Sample_Name=BCCPflex8_2R_PREMIX Chromat_id=4861625 Read_id=4809351 Version=1
Length=1576
NNNNNNTCNNNNTGCNACTGCTGATGGTCAGAGTGAANTCCGTCCCAGATCTGGACCCAGAGAAGCGAGAAAGGACTCCAGAGTAGAGGAAGGATGC
CGAGTAAATCAGTAGTTTCGGAGCTTTTCCTGGTTTCTGTTGATACCAGGCTACAGCAGTATTCACATCCTGACTGGCACGGCAGGTGATGGTGACC
CTATCGCCCACAGAGGCGGACAGGGAGCTCGGGGACTGGGTCATCTGGATATCGGATCCGCCTCCGCCTGTGCGGCCCTCGGCGCGCTCGTACTGTT
CCACGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCCAGCTTGATGTCGGTCTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGGCCATGTAGGT
GGTCTTGACCTCGGCGTCGTAGTGGCCGCCTGATCCTCCTCCACCAGAACCACCACCTCCTTCGATAACAACAAGCGGTTCGTCGAATTCAACCGGC
TGACCAGATTCAACAAGGATAGCTTTAACGGTACCGGATTTGTCCGCTTCGATCTGGTTCATCATTTTCATGGCTTCAACGATGCACAGGGTATCGC
CCACGTTGACTTTCTGACCCACTTCGATGAACGCTTTTGCGTCCGGGCTTGGGGTGCGGTAGAAAGTACCAACCATCGGGGAACGTACGATGTGACC
ACTGATTTCCGCTGCTGCTGGCGCTTCTGATCCTCCTCCACCAGAACCACCACCTCCGTCCTTCAGCTTCAGCCTCATCTTGATCTCGCCCTTCAGG
GCACCGTCCTCGGGGTACATCCGCTCGGTGGAGGCCTCCCAGCCCATAGTCTTCTTCTGCATTACGGGGCCGTCGGAGGGGAAGTTGGTGCCGCGCA
GCTTCACCTTGTAGATGAACTCGCCGTCCTGCAGGGAGGAGTCCTGGGTCACGGTCACCACGCCGCCGTCCTCNANTTCATCACGCGNTCCNACTTG
AAGCCNTCGGGGAANNCANCTTCANNAATCGGGNANGTNGGCGGGGT

>Flex8RC
ACCCCGCCNACNTNCCCGATTNNTGAAGNTGNNTTCCCCGANGGCTTCAAGTNGGANCGCGTGATGAANTNGAGGACGGCGGCGTGGTGACCGTGAC
CCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGTG
GTTCTGGTGGAGGAGGATCAGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGTTGGTACTTTCTACCGCACCCCAAG
CCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAA
GCGGACAAATCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAATTCGACGAACCGCTTGTTGTTATCGAAGGAGGTGGTGGTT
CTGGTGGAGGAGGATCAGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGA
CATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATC
CAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCT
GGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATC
TGGGACGGANTTCACTCTGACCATCAGCAGTNGCANNNNGANNNNNN
```

```
Flex8RC         ----------------------------------------------------ACCCCGC
template        CTGGGACATCCTGTCCCCTCAGTTCCAGTACGGCTCCAAGGCCTACGTGAAGCACCCCGC
                                                                  *******

Flex8RC         CNACNTNCCCGAT--TNNTGAAGNTGNNTTCCCCGANGGCTTCAAGTNGGANCGCGTGAT
template        CGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGAT
```

```
             * ** * *****        *       ******** ********** *** ********

Flex8RC      G-AANTNGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGA
template     GAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGA
             * *  * ****************************************************

Flex8RC      GTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCA
template     GTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCA
             ************************************************************

Flex8RC      GAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCT
template     GAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCT
             ************************************************************

Flex8RC      GAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGTGGTTCTGGTGGAGG
template     GAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGTGGTTCTGGTGGAGG
             ************************************************************

Flex8RC      AGGATCAGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGT
template     AGGATCAgaagcgccagcagcagcggaaatcagtggtcacatcgtacgttccccgatggt
             ************************************************************

Flex8RC      TGGTACTTTCTACCGCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAA
template     tggtactttctaccgcaccccaagcccggacgcaaaagcgttcatcgaagtgggtcagaa
             ************************************************************

Flex8RC      AGTCAACGTGGGCGATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGA
template     agtcaacgtgggcgataccctgtgcatcgttgaagccatgaaaatgatgaaccagatcga
             ************************************************************

Flex8RC      AGCGGACAAATCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAATT
template     agcggacaaatccggtaccgttaaagctatccttgttgaatctggtcagccggttgaatt
             ************************************************************

Flex8RC      CGACGAACCGCTTGTTGTTATCGAAGGAGGTGGTGGTTCTGGTGGAGGAGGATCAGGCGG
template     cgacgaaccgcttgttgttatcgaaGGAGGTGGTGGTTCTGGTGGAGGAGGATCAGGCGG
             ************************************************************

Flex8RC      CCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGG
template     CCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGG
             ************************************************************

Flex8RC      CGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGT
template     CGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGT
             ************************************************************

Flex8RC      GGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGAC
template     GGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGAC
             ************************************************************

Flex8RC      CCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGC
template     CCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGC
             ************************************************************

Flex8RC      CAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAA
template     CAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAA
             ************************************************************

Flex8RC      ACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTC
template     ACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTC
             ************************************************************

Flex8RC      CAGATCTGGGACGGANTTCACTCTGACCATCAGCAGTNGCANNNNGANNNNNN-------
template     CAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAAC
             *************** *********************        *
```

Transformant pFA-BCCPflex #8 selected.

<u>pFluorAbody-BCCPrigid</u>

Sequenced rigid_BCCP candidates #10 and #12, only #12 had good sequences:

```
>Rigid12F_PREMIX Sample_Name=Rigid12F_PREMIX Chromat_id=4770420 Read_id=4718412 Version=1
Length=1250
NNNNNNNNNNNNNCTTCNNGGANNNGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGG
GCGAGATCAAGATGAGGCTGAAGCTGAAGGACGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGAAGCGCCAGCAGCAGCGGAAATCAGTGGTCACAT
CGTACGTTCCCCGATGGTTGGTACTTTCTACCGCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCGATACC
CTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTG
AATTCGACGAACCGCTTGTTGTTATCGAAGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACAT
GGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGGCATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC
GAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCA
TCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTT
CCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCGGCAGNNTGCAGCCGGAGGACTTCNAACTT
ATTACTGTCAGCAACATTATACTACTCCTCCNACGTTCGGACAGGGTACCAAGGNGNNGATCAANCGCGCGGATGCGGCNGCCCNGNGNANNCNNCC
CNACCACTNNANTCCGGNNNNNNNANNNCCNAANGNANNNNAATTGGNNNNNCCCCCNGTAGAANNNNNNNNNNNNCCNTNGGNNNNNCNAANGGGN
TTNGNNNNNTTTTNNNNNNNGANNANCNNANNNNNNTNNNNNNNGNAGCNNNCNNNNNNCGCTATTACNNGGNGNNNNNTNNNNN

pFluorAbody_BCCPrigid      GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Rigid12F_PREMIX            GGANNNGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                           *     ****************** ************************************

pFluorAbody_BCCPrigid      AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Rigid12F_PREMIX            AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                           ************************************************************

pFluorAbody_BCCPrigid      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Rigid12F_PREMIX            ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                           ************************************************************

pFluorAbody_BCCPrigid      ATCAAGATGAGGCTGAAGCTGAAGGACGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGgaa
Rigid12F_PREMIX            ATCAAGATGAGGCTGAAGCTGAAGGACGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGAA
                           ************************************************************

pFluorAbody_BCCPrigid      gcgccagcagcagcggaaatcagtggtcacatcgtacgttccccgatggttggtactttc
Rigid12F_PREMIX            GCGCCAGCAGCAGCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGTTGGTACTTTC
                           ************************************************************

pFluorAbody_BCCPrigid      taccgcaccccaagcccggacgcaaaagcgttcatcgaagtgggtcagaaagtcaacgtg
Rigid12F_PREMIX            TACCGCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTG
                           ************************************************************

pFluorAbody_BCCPrigid      ggcgataccctgtgcatcgttgaagccatgaaaatgatgaaccagatcgaagcggacaaa
Rigid12F_PREMIX            GGCGATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAA
                           ************************************************************

pFluorAbody_BCCPrigid      tccggtaccgttaaagctatccttgttgaatctggtcagccggttgaattcgacgaaccg
Rigid12F_PREMIX            TCCGGTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAATTCGACGAACCG
                           ************************************************************

pFluorAbody_BCCPrigid      cttgttgttatcgaaGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGGCGGCCACTACGAC
Rigid12F_PREMIX            CTTGTTGTTATCGAAGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGGCGGCCACTACGAC
                           ************************************************************

pFluorAbody_BCCPrigid      GCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAG
Rigid12F_PREMIX            GCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAG
                           ************************************************************

pFluorAbody_BCCPrigid      ACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC
Rigid12F_PREMIX            ACCGGCATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC
                           **** *******************************************************

pFluorAbody_BCCPrigid      GAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCG
Rigid12F_PREMIX            GAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCG
                           ************************************************************

pFluorAbody_BCCPrigid      AGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGAT
Rigid12F_PREMIX            AGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGAT
                           ************************************************************

pFluorAbody_BCCPrigid      GTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATT
```

```
Rigid12F_PREMIX            GTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATT
                           ************************************************************

pFluorAbody_BCCPrigid      TACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGG
Rigid12F_PREMIX            TACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGG
                           ************************************************************

pFluorAbody_BCCPrigid      ACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGT
Rigid12F_PREMIX            ACGGATTTCACTCTGACCATCGGCAGNNTGCAGCCGGAGGACTTC-NAACTTATTACTGT
                           ********************* ****  *****************  *************

pFluorAbody_BCCPrigid      CAGCAACATTATACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGC
Rigid12F_PREMIX            CAGCAACATTATACTACTCCTCCNACGTTCGGACAGGGTACCAAGGNGNNGATCAANCGC
                           ********************** ********************** *  ****** ***

pFluorAbody_BCCPrigid      GCGGATGCGGCCGCACTCGAGGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGAT
Rigid12F_PREMIX            GCGGATGCGGCNGCCCNGNGNANNCNNCCCNACCACTNNANTCCGGNNNNNNN-------
                           *********** ** *            *   * *       *
```

Need a reverse primer for screening:

BCCPseqR GTTGCTGACAGTAATAAGTTGCG

Rigid1R – very little sequence
Rigid13R – good sequence
Rigid25R – very little sequence

```
>pBCCPrigid13R_PREMIX Sample_Name=pBCCPrigid13R_PREMIX Chromat_id=4840274 Read_id=4788047
Version=1 Length=1536
NNNNNNNNNNNNNNNNNGNNACTGCTGNNGGTCNGAGTGAAATCCGTCCCAGATCTGGACCCAGAGAAGCGAGAAAGGACTCCAGAGTAGAGGAAGGAT
GCCGAGTAAATCAGTAGTTTCGGAGCTTTTCCTGGTTTCTGTTGATACCAGGCTACAGCAGTATTCACATCCTGACTGGCACGGCAGGTGATGGTGA
CCCTATCGCCCACAGAGGCGGACAGGGAGCTCGGGGACTGGGTCATCTGGATATCGGATCCGCCTCCGCCTGTGCGGCCCTCGGCGCGCTCGTACTG
TTCCACGATGGTGTAGTCCTCGTTGTGGGAGGTGATGTCCAGCTTGATGTCGGTCTTGTAGGCGCCGGGCAGCTGCACGGGCTTCTTGGCCATGTAG
GTGGTCTTGACCTCGGCGTCGTAGTGGCCGCCCTTGGCGGCGGCTTCTTTAGCAGCCGCTTCTTCGATAACAACAAGCGGTTCGTCGAATTCAACCG
GCTGACCAGATTCAACAAGGATAGCTTTAACGGTACCGGATTTGTCCGCTTCGATCTGGTTCATCATTTTCATGGCTTCAACGATGCACAGGGTATC
GCCCACGTTGACTTTCTGACCCACTTCGATGAACGCTTTTGCGTCCGGGCTTGGGGTGCGGTAGAAAGTACCAACCATCGGGGAACGTACGATGTGA
CCACTGATTTCCGCTGCTGCTGGCGCTTCCTTGGCGGCGGCTTCTTTAGCAGCCGCTTCGTCCTTCAGCTTCAGCCTCATCTTGATCTCGCCCTTCA
GGGCACCGTCCTCGGGGTACATCCGCTCGGTGGAGGCCTCCCAGCCCATAGTCTTCTTCTGCATTACGGGGCCGTCGGAGGGGAAGTTGGTGCCGCG
CAGCTTCACCTTGTAGATGAACTCGCCGTCCTGCAGGGAGGAGTCCTGGGTCACGGTCACCACGCCGCCGTCCTCGAAGTTCATCACGCGCTCCNAC
TTGAAGCCCTCGGGGAAGGACAGCTTCAAGTAGTCGGGGATGTCGGCGGGGTGCTNNNNGTAGGCCTTGGAGCCGTACTGGAACTGAGGGNNNAGGA
TGNCCNAGNNNAAGGNANGGGACCNCCNTTGGNNNTTNCNNNTTGGNGGNNGGN

template         ----------------------------------------------CGACATCCCCGACT
Rigid13R_RC      NCCCTCAGTTCCAGTACGGCTCCAAGGCCTACNNNNAGCACCCCGCCGACATCCCCGACT
                                                              **************

template         ACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG
Rigid13R_RC      ACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTNGGAGCGCGTGATGAACTTCGAGGACG
                 ********************************* *************************

template         GCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGG
Rigid13R_RC      GCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGG
                 ************************************************************

template         TGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGG
Rigid13R_RC      TGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGG
                 ************************************************************

template         GCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCA
Rigid13R_RC      GCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCA
                 ************************************************************

template         AGATGAGGCTGAAGCTGAAGGACGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGgaagcgc
Rigid13R_RC      AGATGAGGCTGAAGCTGAAGGACGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGAAGCGC
                 ************************************************************

template         cagcagcagcggaaatcagtggtcacatcgtacgttccccgatggttggtactttctacc
Rigid13R_RC      CAGCAGCAGCGGAAATCAGTGGTCACATCGTACGTTCCCCGATGGTTGGTACTTTCTACC
                 ************************************************************
```

```
template         gcaccccaagcccggacgcaaaagcgttcatcgaagtgggtcagaaagtcaacgtgggcg
Rigid13R_RC      GCACCCCAAGCCCGGACGCAAAAGCGTTCATCGAAGTGGGTCAGAAAGTCAACGTGGGCG
                 ************************************************************

template         ataccctgtgcatcgttgaagccatgaaaatgatgaaccagatcgaagcggacaaatccg
Rigid13R_RC      ATACCCTGTGCATCGTTGAAGCCATGAAAATGATGAACCAGATCGAAGCGGACAAATCCG
                 ************************************************************

template         gtaccgttaaagctatccttgttgaatctggtcagccggttgaattcgacgaaccgcttg
Rigid13R_RC      GTACCGTTAAAGCTATCCTTGTTGAATCTGGTCAGCCGGTTGAATTCGACGAACCGCTTG
                 ************************************************************

template         ttgttatcgaaGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGGCGGCCACTACGACGCCG
Rigid13R_RC      TTGTTATCGAAGAAGCGGCTGCTAAAGAAGCCGCCGCCAAGGGCGGCCACTACGACGCCG
                 ************************************************************

template         AGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCG
Rigid13R_RC      AGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCG
                 ************************************************************

template         ACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGC
Rigid13R_RC      ACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGC
                 ************************************************************

template         GCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCT
Rigid13R_RC      GCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCT
                 ************************************************************

template         CCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGA
Rigid13R_RC      CCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGA
                 ************************************************************

template         ATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACT
Rigid13R_RC      ATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACT
                 ************************************************************

template         CGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGG
Rigid13R_RC      CGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGG
                 ************************************************************

template         ATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGTCAGC
Rigid13R_RC      ATTTCACTCNGACCNNCAGCAGTNNCNNNNNNNNNNNNNNN-------------------
                 ********* ****  *******

Transformant #13 was selected for pFA-BCCPrigid
```

pFluorAbody-GBP3Flex2

Best sequence actually had 5 repeats of GBP (#7 renamed as Au5Flex2):

```
>Au3F2_7_PREMIX Sample_Name=Au3F2_7_PREMIX Chromat_id=5053750
Read_id=5000661 Version=1 Length=1195

NNNNNNNNNNNNNNANNNNNAGGANGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGC
GAGTTCATCTACAAGGTGAGGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGA
CTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGAT
GAGGCTGAAGCTGAAGGACAGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATGCACGGTAAAACGCAGGCC
ACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACG
GGAAGACGCAAGCCACCTCCGGGACAATTCAATCTATGCACGGCAAGACACAGGCTACCTCTGGCACCAT
CCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGC
GGTGGTTCTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGC
```

CCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACA
GTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCTCC
CTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAG
CCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGG
AGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCG
GAGGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCNACGTTCGGACAGGGTACCAAGG
TGGAGNTNAANNNNGCGGATGCNNCCGCCTNNAGCACCNNNCTACCCNACTGNNNTCCGNNNNNANNAAG

```
pFA-Au3flex2        GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Au3F2_7_PREMIX      GANGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                    ** ********************* ************************************

pFA-Au3flex2        AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Au3F2_7_PREMIX      AAGGTGAGGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                    ******* ****************************************************

pFA-Au3flex2        ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Au3F2_7_PREMIX      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                    ************************************************************

pFA-Au3flex2        ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
Au3F2_7_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACAGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
                    *************************** ********************************

pFA-Au3flex2        CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
Au3F2_7_PREMIX      CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
                    ************************************************************

pFA-Au3flex2        ACCTCTGGCACC-----------------------------------------------
Au3F2_7_PREMIX      ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
                    ************

pFA-Au3flex2        -------------------------------------ATCCAGTCGATGCACGGGAAGACG
Au3F2_7_PREMIX      TCTATGCACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACGGGAAGACG
                                                         ************************

pFA-Au3flex2        CAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGC
Au3F2_7_PREMIX      CAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGC
                    ************************************************************

pFA-Au3flex2        GGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
Au3F2_7_PREMIX      GGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
                    ************************************************************

pFA-Au3flex2        GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATC
Au3F2_7_PREMIX      GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATC
                    ************************************************************

pFA-Au3flex2        GTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATG
Au3F2_7_PREMIX      GTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATG
                    ************************************************************

pFA-Au3flex2        ACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGT
Au3F2_7_PREMIX      ACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGT
                    ************************************************************

pFA-Au3flex2        GCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCG
Au3F2_7_PREMIX      GCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCG
```

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                        ************************************************************

pFA-Au3flex2            AAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGG
Au3F2_7_PREMIX          AAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGG
                        ************************************************************

pFA-Au3flex2            TCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCA
Au3F2_7_PREMIX          TCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCA
                        ************************************************************

pFA-Au3flex2            ACTTATTACTGTCAGCAACATTATACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTG
Au3F2_7_PREMIX          ACTTATTACTGTCAGCAACATTATACTACTCCTCCNACGTTCGGACAGGGTACCAAGGTG
                        ********************************** ************************

pFA-Au3flex2            GAGATCAAACGCGCGGATGCGGCCGCACTCGAGGCGGCCGCACTCGAGCACCACCACCAC
Au3F2_7_PREMIX          GAGNTNAANNNNGCGGATGCNNCCGCCTNNAGCACCNNNCTACCCNACTGNNNTCCGNNN
                        *** * **    ********  ****        *      ** * *       *

pFA-Au3flex2            CACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACC
Au3F2_7_PREMIX          NNANNAAG--------------------------------------------------
                               *
```

#7 has extra sequence and a point mutation

CAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATG
TACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACAGAGGCGGGGGTTC
AGGTGGTGGAGGCAGCATGCACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACAC
AGGCTACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAATCTATG
CACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACA
ATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTA
CATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGA
GGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATC

```
pFA-Au3flex2            ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
Au3F2_7_PREMIX          ATCAAGATGAGGCTGAAGCTGAAGGACAGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
                        ************************** ******************************

pFA-Au3flex2            CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
Au3F2_7_PREMIX          CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
                        ************************************************************

pFA-Au3flex2            ACCTCTGGCACC-----------------------------------------------
Au3F2_7_PREMIX          ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
                        ************
```

48+36 = 84 nt, 28 AA … look for frame shift

```
pFA-Au3flex2            ------------------------------------ATCCAGTCGATGCACGGGAAGACG
Au3F2_7_PREMIX          TCTATGCACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACGGGAAGACG
                                                             ************************

pFA-Au3flex2            CAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGC
Au3F2_7_PREMIX          CAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGC
                        ************************************************************
```

Point mutation and extra sequence:

RGGGSGGGGSMHGKTQATSGTIQSMHGKTQATSGTIQSMHGKTQATSGTIQSMHGKTQAT

SGTIQSMHGKTQATSGTIQSGGGGSGGGGS

GGGGSGGGGSMHGKTQATSGTIQSMHGKTQATSGTIQSMHGKTQATSGTIQSGGGGSGGGGS

- This has 5 repeats of gold binding motif Au5Flex2

Candidate #6 sequence

>Au3F2_6_PREMIX Sample_Name=Au3F2_6_PREMIX Chromat_id=5053751
Read_id=5000642 Version=1 Length=1412

NNNNNNNNNNNNACTTNNAGGANGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGA
GTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGA
GGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATGCACGGTAAAACGCAGGCCAC
CAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACGGG
AAGACGCAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGCGGCC
ACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGAC
CGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAG
GGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCTGTCCCCGAGCTCCCTGTCCGCCTCTGTGG
GCGATAGGGTCACCATCACCTGCCGTGCCGGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAA
ACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTC
TCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTT
ATTACTGTCAGCAACATTATACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGCGC
GGATGCGGCCGCACTCGAGCACCACNNCCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAA
GCTGAATTGGCTGCTGCNNCCGCTGANNANNAACTAGCATAACCCCTTGGGGNTCTAANCGGNNNNNGAG

```
pFA-Au3flex2        GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG
Au3F2_6_PREMIX      ---------------------------------------NNNNNNNNNNNNACTTNNAG
                                                                   ****  **

pFA-Au3flex2        GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Au3F2_6_PREMIX      GANGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                    ** *****************************************************

pFA-Au3flex2        AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Au3F2_6_PREMIX      AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                    ************************************************************

pFA-Au3flex2        ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Au3F2_6_PREMIX      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                    ************************************************************

pFA-Au3flex2        ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
Au3F2_6_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
                    ************************************************************

pFA-Au3flex2        CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
Au3F2_6_PREMIX      CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
                    ************************************************************

pFA-Au3flex2        ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
Au3F2_6_PREMIX      ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
                    ************************************************************

pFA-Au3flex2        TCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGCGGCCACTACGACGCCGAGGTCAAG
Au3F2_6_PREMIX      TCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGCGGCCACTACGACGCCGAGGTCAAG
                    ************************************************************
```

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pFA-Au3flex2        ACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAG
Au3F2_6_PREMIX      ACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAG
                    ************************************************************

pFA-Au3flex2        CTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAG
Au3F2_6_PREMIX      CTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAG
                    ************************************************************

pFA-Au3flex2        GGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCC
Au3F2_6_PREMIX      GGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCTGTCCCCGAGCTCCCTGTCC
                    ************************************** *******************

pFA-Au3flex2        GCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCT
Au3F2_6_PREMIX      GCCTCTGTGGGCGATAGGGTCACCATCACCTGCCGTGCCGGTCAGGATGTGAATACTGCT
                    ************************************** *******************

pFA-Au3flex2        GTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCC
Au3F2_6_PREMIX      GTAGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCC
                    ************************************************************

pFA-Au3flex2        TTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACT
Au3F2_6_PREMIX      TTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACT
                    ************************************************************

pFA-Au3flex2        CTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTAT
Au3F2_6_PREMIX      CTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTAT
                    ************************************************************

pFA-Au3flex2        ACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCC
Au3F2_6_PREMIX      ACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCC
                    ************************************************************

pFA-Au3flex2        GCACTCGAGGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAAC
Au3F2_6_PREMIX      GCAC---------------TCGAGCACCACNNCCACCACCACTGAGATCCGGCTGCTAAC
                    ****               ***********  ****************************

pFA-Au3flex2        AAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCC
Au3F2_6_PREMIX      AAAGCCCGAAAGGAAGCTGAATTGGCTGCTGCNNCCGCTGANNANNAACTAGCATAACCC
                    ******************** ***********  *******  *  **************

pFA-Au3flex2        CTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGA
Au3F2_6_PREMIX      CTTGGGGNTCTAANCGGNNNNNGAG----------------------------------
                    *******     *   *
```

#6 has a few point mutations and missing sequence (note: missing sequence is in the antibody part of the plasmid and not the insert)

Candidate #5 sequence

>Au3F2_5_PREMIX Sample_Name=Au3F2_5_PREMIX Chromat_id=5082808 Read_id=5029715 Version=1 Length=519

NNNNNNNNNNNNNNNNNNNGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCAT
CTACNAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGG
AGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAG
GACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCGTGCACGGTAAAACGCAGGCCACCAGCGGTACAATCCAAT
CTATGCACGGCAAGACACAGGCTACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGA
CAATTCAATCGGGAGGCGGAGGATCAGGCGGCTGTGGTTCTGGAGGNCACTACGACTTNNNAGGTCNNNACCA
CCNNNATGGNCNAGAAACCCGTGTAGGTGNCCGGCGCCTACTAGACCGACATCCACCTGGATATCACNTCCCATN
NTG

```
Au3F2_5_PREMIX      ---------------------------------------------NNNNNNNNNNNNNNNNN
```

```
pFA-Au3flex2        GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG

Au3F2_5_PREMIX      NNNGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
pFA-Au3flex2        GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                       ********************************************************

Au3F2_5_PREMIX      NAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
pFA-Au3flex2        AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                     ********************************************************

Au3F2_5_PREMIX      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
pFA-Au3flex2        ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                    ************************************************************

Au3F2_5_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCGTG
pFA-Au3flex2        ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGCAGCATG
                    ******************************************************** **

Au3F2_5_PREMIX      CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
pFA-Au3flex2        CACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCT
                    ************************************************************

Au3F2_5_PREMIX      ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
pFA-Au3flex2        ACCTCTGGCACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAA
                    ************************************************************

Au3F2_5_PREMIX      TCGGGAGGCGGAGGATCAGGCGGCTGTGGTTCTGGAGGNCACTACGACTTNNNAGGTCNN
pFA-Au3flex2        TCGGGAGGCGGAGGATCAGGCGGCGGTGGTTCTGGCGGCCACTACGACGCCG-AGGTCAA
                    ************************ ********** ** *********     *****

Au3F2_5_PREMIX      NACCACCNNNATGGNCNAGAAACCCGTGTAGGTGNCCGGCGCCTACTAGACCGACATCCA
pFA-Au3flex2        GACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAA
                     ******   **** * **** ****** ** ** *********** *********** *

Au3F2_5_PREMIX      CCTGGATATCACNTCCCATNNTG-------------------------------------
pFA-Au3flex2        GCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGA
                     ***** ***** *****    *
```

GTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTC
CCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGA
CGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGAGGCGGGGGTTCAGGTGGTGGAGGC
AGCATGCACGGTAAAACGCAGGCCACCAGCGGTACAATCCAATCTATGCACGGCAAGACACAGGCTACCTCTGG
CACCATCCAGTCGATGCACGGGAAGACGCAAGCCACCTCCGGGACAATTCAATCGGGAGGCGGAGGATCAGGCG
GCGGTGGTTCTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAG
CGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATC

2 point mutations ATG -> GTG methionine -> valine and GGT -> TGT glycine -> cysteine

<u>pFluorAbody-GBP4Flex2</u>

Candidate #10

>Au4F2_10_PREMIX Sample_Name=Au4F2_10_PREMIX Chromat_id=5053752 Read_id=5000636 Version=1 Length=1440

NNNNNNNNNNANTTNNAGAANGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTC
ATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGG
GAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAA
GGACGGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATGCACGGAAAGACCCAAGCAACAAGCGGGACTATCCA
AAGTATGCACGGAAAGACGCAGGCAACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTG
GAACTATTCAGAGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCGGGA
GGAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCC
CGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGA
GCGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTC
TGTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAA
ACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTC
CAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGTCGGAGGACTTCGCAACTTATTACTGTCAGCAA
CATTATACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCNGCACTCGAN
NACCACCACCACCACCACTGAGATCCGGCTGCTAACAAANCCNGAAAGGANNNNGAATTGGCTGCTGCCACCGC
TGAGCANNAACTAGCNNANCCCCTTGGGGCCNTAANCGGNTCTTGAGGGNTTTTNNCTGANNNNNNNNNNNN
NCNGGNTTGGCNNTGGNACCGCCNTNANNNNNNNNAANCN

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pFA-Au4flex2         GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Au4F2_10_PREMIX      AANGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                      * ********************* ************************************

pFA-Au4flex2         AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Au4F2_10_PREMIX      AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                     ************************************************************

pFA-Au4flex2         ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Au4F2_10_PREMIX      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                     ************************************************************

pFA-Au4flex2         ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATG
Au4F2_10_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATG
                     ************************************************************

pFA-Au4flex2         CACGGAAAGACCCAAGCAACAAGCGGGACTATCCAAAGTATGCACGGAAAGACGCAGGCA
Au4F2_10_PREMIX      CACGGAAAGACCCAAGCAACAAGCGGGACTATCCAAAGTATGCACGGAAAGACGCAGGCA
                     ************************************************************

pFA-Au4flex2         ACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTGGAACTATTCAG
Au4F2_10_PREMIX      ACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTGGAACTATTCAG
                     ************************************************************

pFA-Au4flex2         AGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCG
Au4F2_10_PREMIX      AGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCG
                     ************************************************************

pFA-Au4flex2         GGAGGAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAG
Au4F2_10_PREMIX      GGAGGAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAG
                     ************************************************************

pFA-Au4flex2         AAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCAC
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Au4F2_10_PREMIX      AAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCAC
                     ************************************************************

pFA-Au4flex2         AACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGC
Au4F2_10_PREMIX      AACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGC
                     ************************************************************

pFA-Au4flex2         GGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGG
Au4F2_10_PREMIX      GGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGG
                     ************************************************************

pFA-Au4flex2         GTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAG
Au4F2_10_PREMIX      GTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAG
                     ************************************************************

pFA-Au4flex2         AAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTC
Au4F2_10_PREMIX      AAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTC
                     ************************************************************

pFA-Au4flex2         CTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTG
Au4F2_10_PREMIX      CTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTG
                     ************************************************************

pFA-Au4flex2         CAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCCACGTTC
Au4F2_10_PREMIX      CAGTCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCCACGTTC
                     *** ********************************************************

pFA-Au4flex2         GGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCCGCACTCGAGGCGGCCGCA
Au4F2_10_PREMIX      GGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCNGCAC--------------
                     **************************************** ****

pFA-Au4flex2         CTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCT
Au4F2_10_PREMIX      -TCGANNACCACCACCACCACCACTGAGATCCGGCTGCTAACAAANCCNGAAAGGANNNN
                      ****  ************************************** ** *******

pFA-Au4flex2         GAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGG
Au4F2_10_PREMIX      GAATTGGCTGCTGCCACCGCTGAGCANNAACTAGCNNANCCCCTTGGGGCCNTAANCGGN
                     ** ***********************  *******  * ************   *   *

pFA-Au4flex2         GTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGC
Au4F2_10_PREMIX      TC-TTGAGGGNTTTTNNCTGANNNNNNNNN---NNNNCNGGNTTGGCNNTGGNACCGCCN
                        ******* ****                      * ** *****    *   ***
```

#10 looks good except for a point mutation in the plasmid

Nt #409 – CCG is now TCG – proline to serine

Candidate #9

>Au4F2_9_PREMIX Sample_Name=Au4F2_9_PREMIX Chromat_id=5082810 Read_id=5029738 Version=1 Length=1505

NNNNNNNNNNANTTNNANGNNGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTC
ATCTACAAGGTGAAGCTGCGCGGCACCAACCTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACTATGGGCTGG
GAGGCCTCCACCCAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAA
GGACAGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATGCACGGAAAGACCCAAGCAACAAGCGGGACTATCCAA
AGTATGCACGGAAAGACGCAGGCAACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTGG

AACTATTCAGAGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCGGGAG
GAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAGAAGCCCGTGCAGCTGCCC
GGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAG
CGCGCCGAGGGCCGCACAGGCGGAGGCGGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCT
GTGGGCGATAGGGTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAA
CCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCTTTCTCGCTTCTCTGGGTCC
AGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAAC
ATTATACTACTCCTCCCACGTTCGGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCCGCACTCGAGN
ACCCNANNNCCCNACTGAGATCCGGCTGCTNNCAAGCCCGAAAGGAANNNNAGTTGGCTGCTGCCCNNCTGAA
CANANCTAGNNNACCCNTNGGGG

```
pFA-Au4flex2        GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAG
Au4F2_9_PREMIX      ------------------------------------------NNNNNNNNNNANTTNNAN
                                                                    * **  *

pFA-Au4flex2        GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Au4F2_9_PREMIX      GNNGGCGGCGTGGTGACCGTGACCNAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                    *  ********************* ***********************************

pFA-Au4flex2        AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Au4F2_9_PREMIX      AAGGTGAAGCTGCGCGGCACCAACCTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
                    ************************* ***********************************

pFA-Au4flex2        ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Au4F2_9_PREMIX      ATGGGCTGGGAGGCCTCCACCCAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                    ********************* **************************************

pFA-Au4flex2        ATCAAGATGAGGCTGAAGCTGAAGGACGGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATG
Au4F2_9_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACAGAGGTGGGGGAAGCGGAGGCGGTGGTAGCATG
                    *************************** ********************************

pFA-Au4flex2        CACGGAAAGACCCAAGCAACAAGCGGGACTATCCAAAGTATGCACGGAAAGACGCAGGCA
Au4F2_9_PREMIX      CACGGAAAGACCCAAGCAACAAGCGGGACTATCCAAAGTATGCACGGAAAGACGCAGGCA
                    ************************************************************

pFA-Au4flex2        ACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTGGAACTATTCAG
Au4F2_9_PREMIX      ACATCTGGGACCATTCAAAGCATGCACGGCAAGACCCAGGCTACCTCTGGAACTATTCAG
                    ************************************************************

pFA-Au4flex2        AGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCG
Au4F2_9_PREMIX      AGTATGCATGGGAAGACGCAGGCAACGTCCGGTACAATTCAATCTGGGGGGGGTGGCTCG
                    ************************************************************

pFA-Au4flex2        GGAGGAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAG
Au4F2_9_PREMIX      GGAGGAGGAGGAAGTGGCGGCCACTACGACGCCGAGGTCAAGACCACCTACATGGCCAAG
                    ************************************************************

pFA-Au4flex2        AAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCAC
Au4F2_9_PREMIX      AAGCCCGTGCAGCTGCCCGGCGCCTACAAGACCGACATCAAGCTGGACATCACCTCCCAC
                    ************************************************************

pFA-Au4flex2        AACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGC
Au4F2_9_PREMIX      AACGAGGACTACACCATCGTGGAACAGTACGAGCGCGCCGAGGGCCGCACAGGCGGAGGC
                    ************************************************************

pFA-Au4flex2        GGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGG
Au4F2_9_PREMIX      GGATCCGATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGG
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                    ************************************************************

pFA-Au4flex2        GTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAG
Au4F2_9_PREMIX      GTCACCATCACCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAG
                    ************************************************************

pFA-Au4flex2        AAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTC
Au4F2_9_PREMIX      AAACCAGGAAAAGCTCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTC
                    ************************************************************

pFA-Au4flex2        CTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTG
Au4F2_9_PREMIX      CTTTCTCGCTTCTCTGGGTCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTG
                    ************************************************************

pFA-Au4flex2        CAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCCACGTTC
Au4F2_9_PREMIX      CAGCCGGAGGACTTCGCAACTTATTACTGTCAGCAACATTATACTACTCCTCCCACGTTC
                    ************************************************************

pFA-Au4flex2        GGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCCGCACTCGAGGCGGCCGCA
Au4F2_9_PREMIX      GGACAGGGTACCAAGGTGGAGATCAAACGCGCGGATGCGGCCGCACTCGAGNACCCNANN
                    **************************************************    *

pFA-Au4flex2        CTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCT
Au4F2_9_PREMIX      NCCCNAC-----------------TGAGATCCGGCTGCTNNCAAG-CCCGAAAGGAANNN
                      *   *                 ***************  ***  ***********

pFA-Au4flex2        GAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGG
Au4F2_9_PREMIX      NAGTTGGCTGCTGCCCNNCTGAACANANC--TAGNN-NACCCNTNGGGG-----------
                     **************       *   *    ***    **** * ****
```

T-> C, G-> C, G-> A

Mutations: TTC -> CTC serine -> leucine, GAG -> CAG glutamic acid -> glutamine, GGA -> AGA glycine -> arginine

<u>pFluorAbody-GBP4Flex3</u>

Only one candidate from PCR screening (#1):

```
>Au4F3_1_PREMIX Sample_Name=Au4F3_1_PREMIX Chromat_id=5053754
Read_id=5000641 Version=1 Length=477

NNNNNNNNNNNNNNNNNNNANGNNGGCGGCGNGGTGACCGTGACCCAGNNCTCCTCCCTGCAGGACGGCG
AGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGAC
TATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAGATCAAGATG
AGGCTGAAGCTGAAGGACAGGGGTGGAGGCTCAGGAGGGGGTGGAAGCGGGGGCGGGGGCTCCATGCACG
GCAAAACCCAGGCTACTTCCGGGACGATTCAATCAATGCACGGCAAAACGCAGGCAACGTCTGGAACTAT
TCAAAGTATGCACGGCAAGACACAGGCCACAAGTGGCACAATCCAATCTATGCATGGCAAGACGCAAGCG
ACCTCTGGGACTATCCAGTCCGGGGGAGGAGGATCTGGTGGTGGCGGGAGTGGTGGA
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```
pFA-Au4flex3        GACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTAC
Au4F3_1_PREMIX      GNNGGCGGCGNGGTGACCGTGACCCAGNNCTCCTCCCTGCAGGACGGCGAGTTCATCTAC
                    *  ******* ****************  ******************************

pFA-Au4flex3        AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
Au4F3_1_PREMIX      AAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACT
```

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                    ************************************************************

pFA-Au4flex3        ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
Au4F3_1_PREMIX      ATGGGCTGGGAGGCCTCCACCGAGCGGATGTACCCCGAGGACGGTGCCCTGAAGGGCGAG
                    ************************************************************

pFA-Au4flex3        ATCAAGATGAGGCTGAAGCTGAAGGACGGGGGTGGAGGCTCAGGAGGGGGTGGAAGCGGG
Au4F3_1_PREMIX      ATCAAGATGAGGCTGAAGCTGAAGGACAGGGGTGGAGGCTCAGGAGGGGGTGGAAGCGGG
                    *************************** ******************************

pFA-Au4flex3        GGCGGGGGCTCCATGCACGGCAAAACCCAGGCTACTTCCGGGACGATTCAATCAATGCAC
Au4F3_1_PREMIX      GGCGGGGGCTCCATGCACGGCAAAACCCAGGCTACTTCCGGGACGATTCAATCAATGCAC
                    ************************************************************

pFA-Au4flex3        GGCAAAACGCAGGCAACGTCTGGAACTATTCAAAGTATGCACGGCAAGACACAGGCCACA
Au4F3_1_PREMIX      GGCAAAACGCAGGCAACGTCTGGAACTATTCAAAGTATGCACGGCAAGACACAGGCCACA
                    ************************************************************

pFA-Au4flex3        AGTGGCACAATCCAATCTATGCATGGCAAGACGCAAGCGACCTCTGGGACTATCCAGTCC
Au4F3_1_PREMIX      AGTGGCACAATCCAATCTATGCATGGCAAGACGCAAGCGACCTCTGGGACTATCCAGTCC
                    ************************************************************

pFA-Au4flex3        GGGGGAGGAGGATCTGGTGGTGGCGGGAGTGGTGGAGGCGGGTCAGGCGGCCACTACGAC
Au4F3_1_PREMIX      GGGGGAGGAGGATCTGGTGGTGGCGGGAGTGGTGGA------------------------
                    ************************************
```

Mutation in linker sequence – GGT -> GAT glycine to aspartic acid