Supporting information

Real-Time Femtomolar Detection of Cancer Biomarkers from Photoconjugated Antibody-Phage Constructs

Michael Brasino[1], Jennifer N. Cha*[1,2]
[1]Materials Science and Engineering Program and [2]Department of Chemical and Biological Engineering, University of Colorado, Boulder
*To whom correspondence is addressed: Jennifer.Cha@colorado.edu

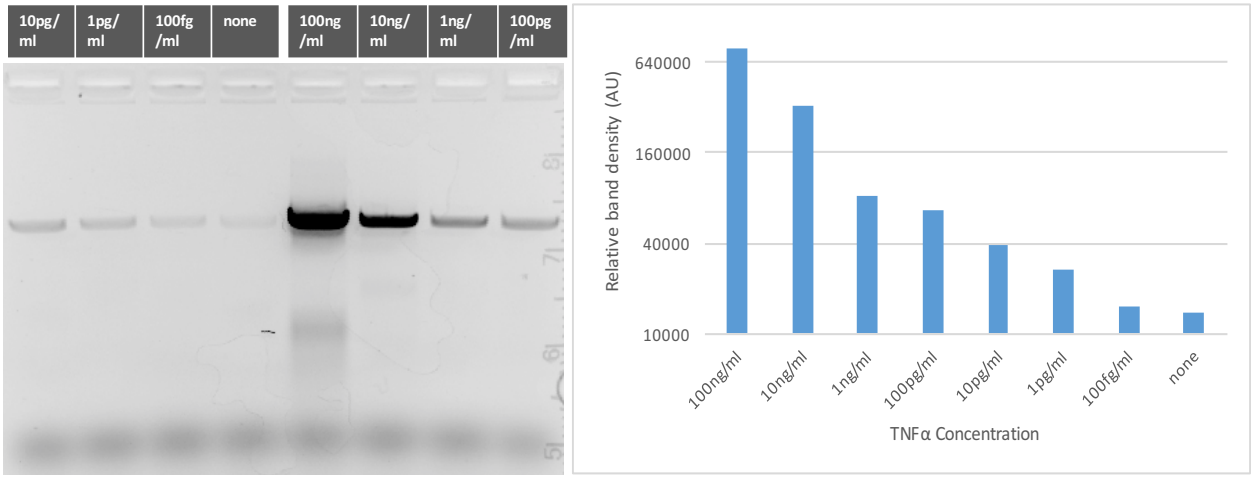

Figure S1: Gel electrophoresis based assay for detecting TNFα: Anti-TNFα-phage were used to detect various concentrations of TNFα diluted in 0.1%PBST solution with 5mg/ml BSA. Immunoassays were otherwise performed as reported in materials and methods. PCR was performed in a standard thermocycler for 25 cycles by adding assay beads directly to PCR reaction mixture containing primers, dNTPs and polymerase. After PCR reaction, reaction mixtures were added directly to a 0.75% agarose gels and electrophoresed for 30mins at 120V. Samples are placed in the gel out of order to avoid any gel effects.

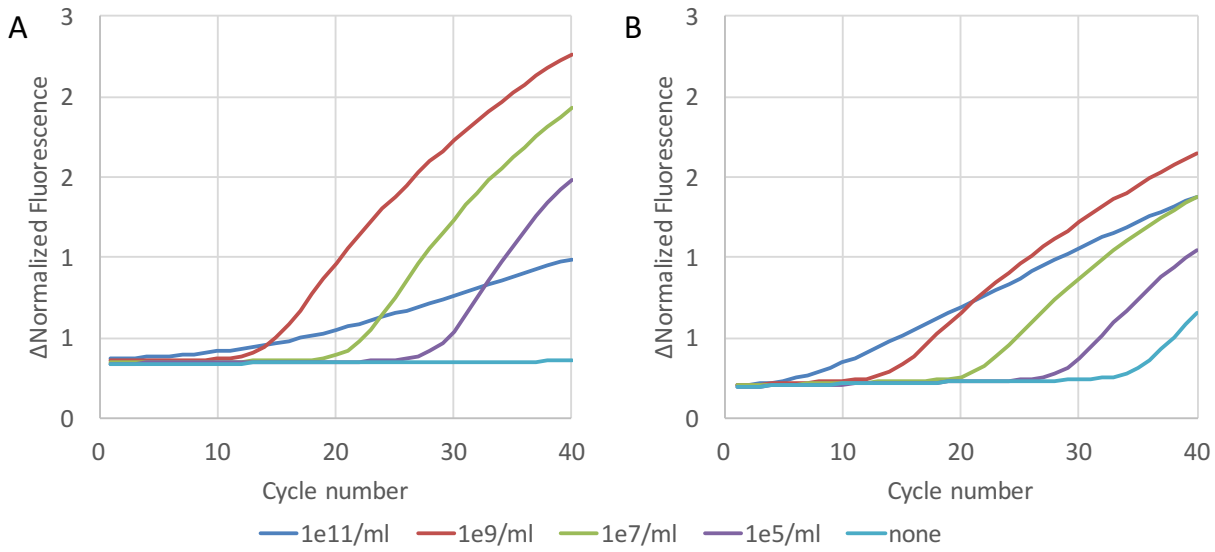

Figure S2: Validation of Taqman probes for real time PCR detection of phage in the presence of immunoassay magnetic beads: **A)** Indicated concentrations of phage are added to a real time PCR reaction mixture containing Taqman universal mastermix II (Life Technologies) along with phage specific primers (600nM) and Taqman probe (250nM) (Integrated DNA Technologies). **B)** Reactions were supplemented with 5ug of streptavidin functionalized magnetic beads (Dynabeads myONE streptavidin T1, Life Technologies).

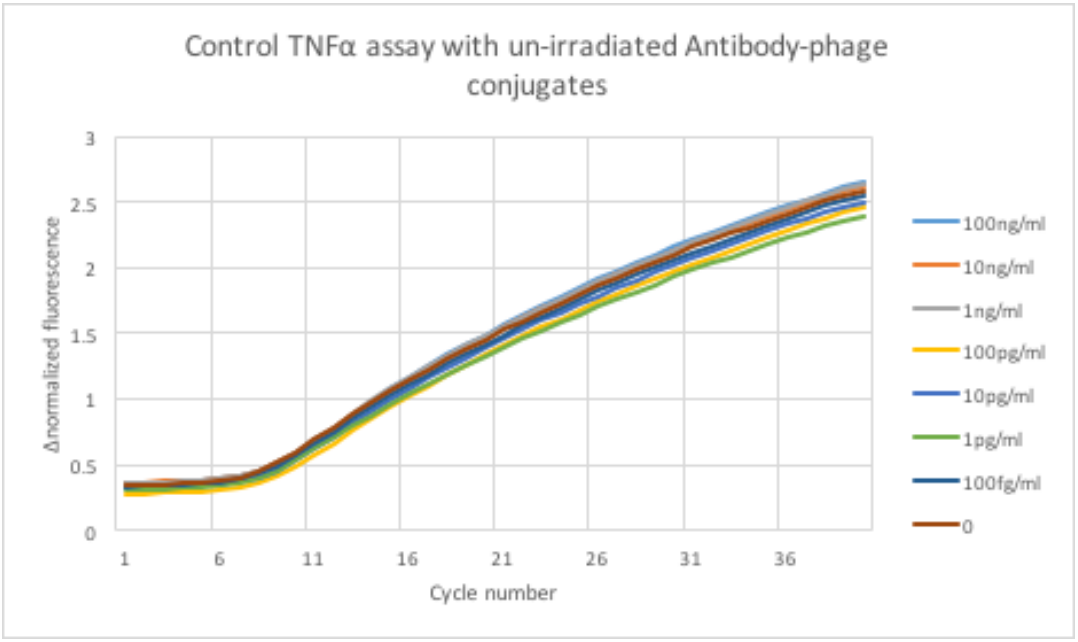

Figure S3: Control qPCR assay for TNFα using phage antibody conjugates without UV irradiation. Amplification curves for various TNFα concentrations in a TNFα assay performed as in Figure 3 but without irradiating the antibody-phage mixture prior to using it in the assay. Because of the lack of irradiation, phage in the mixture bind to the capture antibody present on the assay beads, causing an amplification at early cycles for all TNFα concentrations.

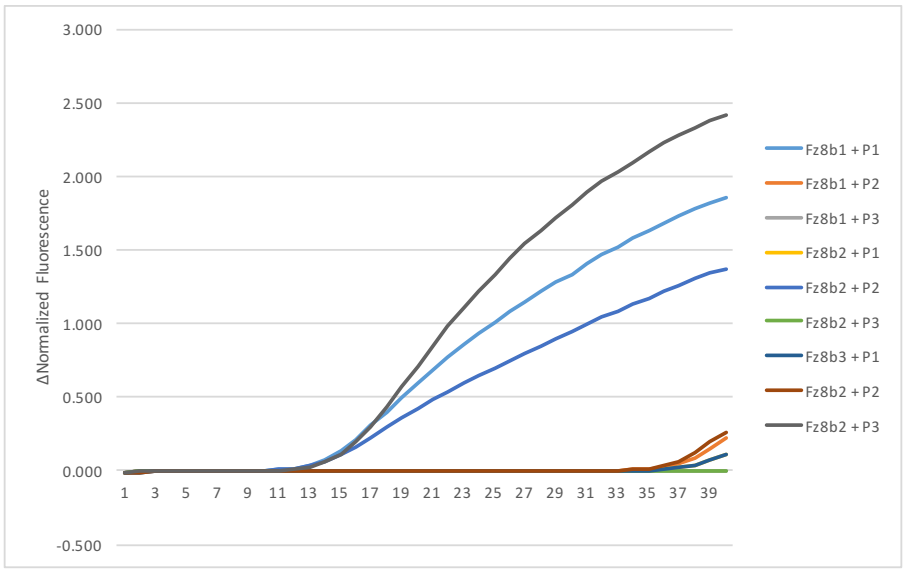

Figure S4: Sequence specific real time PCR amplification of various phage via various primers: Taqman probe based real time amplification curves of the three phage types (Fz8b1,2,3) used for multiplex immunoassays diluted to 1e9/ml in reactions with primer sets specific to each phage type (P1,2,3). In multiplex immunoassays, Fz8b1 was conjugated with TNFα, Fz8b2 with IL6 and Fz8b3 with IL-1ß

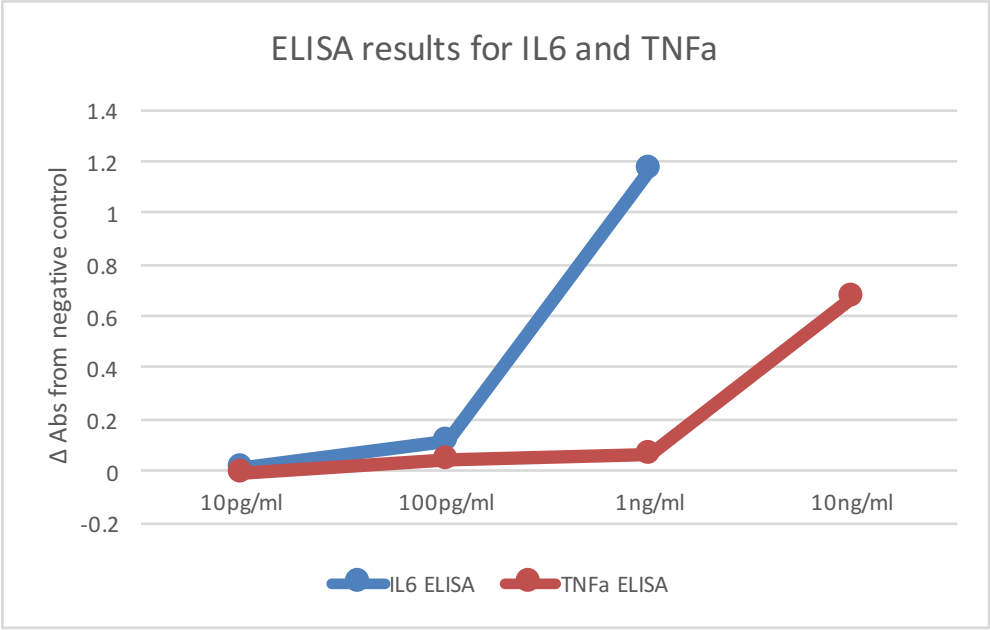

Figure S5: Comparative phage ELISAs of IL6 and TNFα: Antibody-phage conjugates prepared identically used to detect listed concentrations of IL-6 or TNFα immobilized on magnetic beads via Enzyme linked immunosobant assay and an Anti-M13-HRP conjugate.

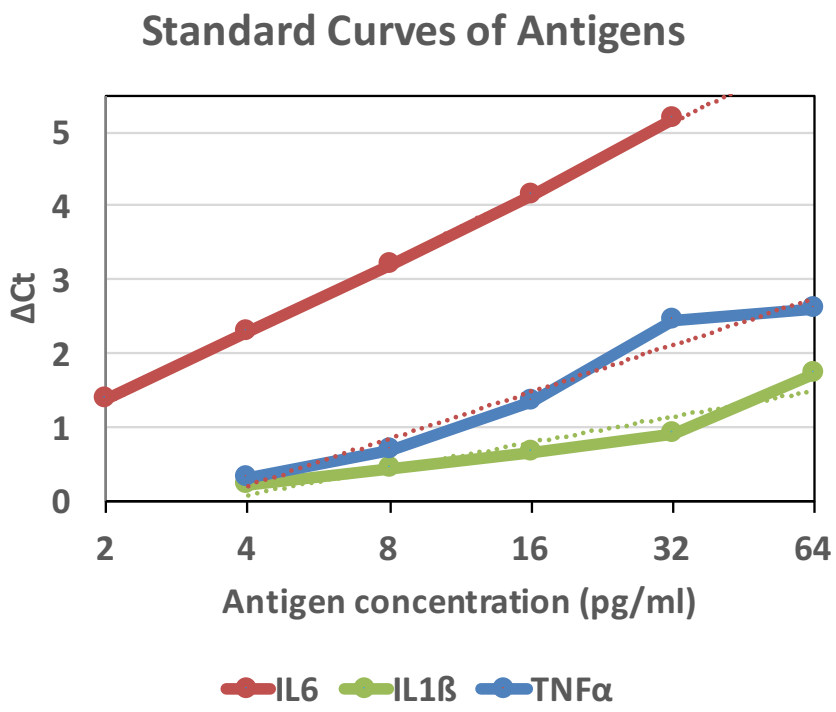

Figure S6: Standard curves at low concentrations of three antigens in multiplex assay format: Each antigen listed is diluted to the indicated concentrations and tested in multiplex immunoassay format. Resulting ΔCt values for each dilution series are analyzed for lines of best fit through the least square method. Resulting logarithmic formula are used to calculate the indicated concentration from ΔCt values attained in later multiplex assays testing multiple antigens at once.

Table S1: Sequence of primers used for Taqman probe based qPCR.

Name/description	Sequence
fwd primer 1	Tacccatgaacacaagttgcg
fwd primer 2	TcttgaaacaggttctagcggAA
fwd primer 3	CTTtcatgtgaacttgaggtacgAA
rev primer for all phage	AAgccgcggtaaatagcaataa
Universal Taqman probe	FAM-ATGCGAAAAACCTAAAAGAGCTTGCCGA-ZEN

Phage construct Fz8b-1 used in this study

Mon Mar 28, 2016 10:14 MDT
GenBank Fz8b-1.ape
Text Map

```
     1 aacgctactaccattagtagaattgatgccacctttcagctcgcgccccaaatgaaaatatagctaaacaggtt 75

    76 attgaccatttgcgaaatgtatctaatggtcaaactaaatctactcgttcgcagaattgggaatcaactgttaca 150

   151 tggaatgaaacttccagacaccgtactttagttgcatatttaaaacatgttgaactacagcaccagattcagcaa 225
                                                          >>>>>>>>>>>>>>>>>>>>>>>
                                                          PCR forward primer site

   226 ttaagctctaagccatccgcaaaaatgacctcttatcaaaaggagcaattaaaggtactgtctaatcctgacctg 300
       >>>>>>>
       PCR forward primer site

   301 ttggaatttgcttccggtctggttcgctttgaggctcgaattgaaacgcgatatttgaagtctttcgggcttcct 375

   376 cttaatctttttgatgcaattcgctttgcttctgactataatagacagggtaaagacctgatttttgatttatgg 450

   451 tcattctcgttttctgaactgtttaaagcatttgaggggattcaatgaatatttatgacgattccgcagtattg 525

   526 gacgctatccagtctaaacattttacaattacccccctctggcaaaacttcctttgcaaaagcctctcgctatttt 600

   601 ggtttctatcgtcgtctggttaatgagggttatgatagtgttgctcttaccatgcctcgtaattccttttggcgt 675

   676 tatgtatctgcattagttgagtgtggtattcctaaatctcaattgatgaatctttccacctgtaataatgttgtt 750

   751 ccgttagttcgttttattaacgtagatttttcctcccaacgtcctgactggtataatgagccagttcttaaaatc 825

   826 gcataaggtaattcaaaatgattaaagttgaaattaaaccgtctcaagcgcaatttactacccgttctggtgttt 900

   901 ctcgtcagggcaagccttattcactgaatgagcagctttgttacgttgatttgggtaatgaatatccggtgcttg 975

   976 tcaagattactctcgacgaaggtcagccagcgtatgcgcctggtctgtacaccgtgcatctgtcctcgttcaaag 1050

  1051 ttggtcagttcggttctcttatgattgaccgtctgcgcctcgttccggctaagtaacatggagcaggtcgcggat 1125

  1126 ttcgacacaatttatcaggcgatgatacaaatctccgttgtactttgtttcgcgcttggtataatcgctgggggt 1200

  1201 caaagatgagtgttttagtgtattctttcgcctctttcgttttaggttggtgccttcgtagtggcattacgtatt 1275

  1276 ttacccgtttaatggaaacttcctcatgaaaaagtctttagtcctcaaagcctccgtagccgttgctaccctcgt 1350
                               >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
```

```
                              pVIII sig

1351 tccgatgctgtctttcgctgcagagggtgaggatcccgcaaaagcggcctttgactccctgcaagcctcagcgac 1425

                        >>.>>>>>>>>>.>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                        pVIII
     >>>>>>>>>>>>>>>>>>>
     pVIII sig

1426 cgaatatatcggttatgcgtgggcgatggttgttgtcattgtcggcgcaactatcggtatcaagctgtttaagaa 1500

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     pVIII

1501 attcacctcgaaagcaagctgataaaccgatacaattaaaggctccttttggagcctttttttttggagattttc 1575

     >>>>>>>>>>>>>>>>>>>>>>
     pVIII

1576 aacgtgaaaaaattattattcgcaattcctttagttgttcctttctattctcactcggccgacgtggccatggta 1650

        >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>               >>>
        pIII sig                                                        Z domain

1651 gacaacaaattcaacaaagaacaacaaaacgcgttctatgagatcttacatttacctaacttaaacgaagaacaa 1725

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Z domain

1726 cgaaacgccttcatcTAGagtttaaaagatgacccaagccaaagcgctaaccttttagcagaagctaaaaagcta 1800
                    *
     >>>>>>>>>>>>>>>...>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     Z domain
                    >>>
                    Q32BPA

1801 aatgatgctcaggcgccgaaagcggccgcagaaactgttgaaagttgtttagcaaaacctcatacagaaaattca 1875

                                >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                pIII
     >>>>>>>>>>>>>>>>>>>>>
     Z domain

1876 tttactaacgtctggaaagacgacaaaactttagatcgttacgctaactatgagggctgtctgtggaatgctaca 1950

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     pIII

1951 ggcgttgtggtttgtactggtgacgaaactcagtgttacggtacatgggttcctattgggcttgctatccctgaa 2025

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     pIII

2026 aatgagggtggtggctctgagggtggcggttctgagggtggcggttctgagggtggcggtactaaacctcctgag 2100

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     pIII

2101 tacggtgatacacctattccgggctatacttatatcaaccctctcgacggcacttatccgcctggtactgagcaa 2175

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
```

pIII

2176 aaccccgctaatcctaatccttctcttgaggagtctcagcctcttaatactttcatgtttcagaataataggttc 2250

>>>
pIII

2251 cgaaataggcagggtgcattaactgtttatacgggcactgttactcaaggcactgaccccgttaaaacttattac 2325

>>>
pIII

2326 cagtacactcctgtatcatcaaaagccatgtatgacgcttactggaacggtaaattcagagactgcgctttccat 2400

>>>
pIII

2401 tctggctttaatgaagatccattcgtttgtgaatatcaaggccaatcgtctgacctgcctcaacctcctgtcaat 2475

>>>>>>>>>>>>>>.>>
pIII

2476 gctggcggcggctctggtggtggttctggtggcggctctgagggtggcggctctgagggtggcggttctgagggt 2550

>>>
pIII

2551 ggcggctctgagggtggcggttccggtggcggctccggttccggtgattttgattatgaaaaaatggcaaacgct 2625

>>>
pIII

2626 aataagggggctatgaccgaaaatgccgatgaaaacgcgctacagtctgacgctaaaggcaaacttgattctgtc 2700

>>>
pIII

2701 gctactgattacggtgctgctatcgatggtttcattggtgacgtttccggccttgctaatggtaatggtgctact 2775

>>>
pIII

2776 ggtgattttgctggctctaattcccaaatggctcaagtcggtgacggtgataattcacctttaatgaataatttc 2850

>>>
pIII

2851 cgtcaatatttaccttctttgcctcagtcggttgaatgtcgcccttatgtctttggcgctggtaaaccatatgaa 2925

>>>
pIII

2926 ttttctattgattgtgacaaaataaacttattccgtggtgtctttgcgtttcttttatatgttgccacctttatg 3000

>>>
pIII

3001 tatgtattttcgacgtttgctaacatactgcgtaataaggagtcttaatcatgccagttcttttgggtattccgt 3075

>>
pIII

3076 tattattgcgtttcctcggtttccttctggtaactttgttcggctatctgcttactttccttaaaaagggcttcg 3150

3151 gtaagatagctattgctatttcattgtttcttgctcttattattgggcttaactcaattcttgtgggttatctct 3225

3226 ctgatattagcgcacaattaccctctgattttgttcagggcgttcagttaattctcccgtctaatgcgcttccct 3300

3301 gtttttatgttattctctctgtaaaggctgctattttcatttttgacgttaaacaaaaaatcgtttcttatttgg 3375

3376 attgggataaataaatatggctgtttattttgtaactggcaaattaggctctggaaagacgctcgttagcgttgg 3450

3451 taagattcaggataaaattgtagctgggtgcaaaatagcaactaatcttgatttaaggcttcaaaacctcccgca 3525

3526 agtcgggaggttcgctaaaacgcctcgcgttcttagaataccggataagccttctatttctgatttgcttgctat 3600

3601 tggtcgtggtaatgattcctacgacgaaaataaaaacggtttgcttgttcttgatgaatgcggtacttggtttaa 3675

3676 tacccgttcatggaatgacaaggaaagacagccgattattgattggtttcttcatgctcgtaaattgggatggga 3750

3751 tattatttttcttgttcaggatttatctattgttgataaacaggcgcgttctgcattagctgaacacgttgttta 3825

3826 ttgtcgccgtctggacagaattactttacccttgtcggcactttatattctcttgttactggctcaaaaatgcc 3900

3901 tctgcctaaattacatgttggtgttgttaaatatggtgattctcaattaagccctactgttgagcgttggcttta 3975

3976 tactggtaagaatttatataacgcatatgacactaaacaggctttttccagtaattatgattcaggtgtttattc 4050

4051 atatttaacccctttatttatcacacggtcggtatttcaaaccattaaatttaggtcagaagatgaaattaactaa 4125

4126 aatatatttgaaaaagttttctcgcgttctttgtcttgcgataggatttgcatcagcatttacatatagttatat 4200

4201 aacccaacctaagccggaggttaaaaaggtagtctctcagacctatgattttgataaattcactattgactcttc 4275

4276 tcagcgtcttaatctaagctatcgctatgttttcaaggattctaagggaaaattaattaatagcgacgatttaca 4350

4351 gaagcaaggttattccatcacatatattgatttatgtactgtttcaattaaaaaaggtaattcaaatgaaattgt 4425

4426 taaatgtaattaattttgttttcttgatgtttgtttcatcatcttcttttgctcaagtaattgaaatgaataatt 4500

4501 cgcctctgcgcgatttcgtgacttggtattcaaagcaaacaggtgaatctgttattgtctcacctgatgttaaag 4575

```
4576 gtacagtgactgtatattcctctgacgttaagcctgaaaatttacgcaatttctttatctctgttttacgtgcta 4650

4651 ataattttgatatggttggctcaattccttccataattcagaaatataacccaaatagtcaggattatattgatg 4725

4726 aattgccatcatctgatattcaggaatatgatgataattccgctccttctggtggtttctttgttccgcaaaatg 4800

4801 ataatgttactcaaacatttaaaattaataacgttcgcgcaaaggatttaataagggttgtagaattgtttgtta 4875

4876 aatctaatacatctaaatcctcaaatgtattatctgttgatggttctaacttattagtagttagcgcccctaaag 4950

4951 atattttagataaccttccgcaatttctttctactgttgatttgccaactgaccagatattgattgaaggattaa 5025

5026 ttttcgaggttcagcaaggtgatgctttagatttttcctttgctgctggctctcagcgcggcactgttgctggtg 5100

5101 gtgttaatactgaccgtctaacctctgttttatcttctgcgggtggttcgttcggtatttttaacggcgatgttt 5175

5176 tagggctatcagttcgcgcattaaagactaatagccattcaaaaatattgtctgtgcctcgtattcttacgcttt 5250

          >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
          PCR reverse primer site

5251 caggtcagaagggttctatttctgttggccagaatgtccctttattactggtcgtgtaactggtgaatctgcca 5325

5326 atgtaaataatccatttcagacggttgagcgtcaaaatgttggtatttctatgagtgtttttcccgttgcaatgg 5400

5401 ctggcggtaatattgttttagatataaccagtaaggccgatagtttgagttcttctactcaggcaagtgatgtta 5475

5476 ttactaatcaaagaagtattgcgacaacggttaatttgcgtgatggtcagactcttttgctcggtggcctcactg 5550

5551 attacaaaaacacttctcaagattctggtgtgccgttcctgtctaaaatccctttaatcggcctcctgtttagct 5625

5626 cccgttctgattctaacgaggaaagcacgttgtacgtgctcgtcaaagcaaccatagtacgcgccctgtagcggc 5700

5701 gcattaagcgcggcgggtgtggtggttacgcgcagcgtgaccgctacacttgccagcgccctagcgcccgctcct 5775

5776 ttcgctttcttcccttcctttctcgccacgttctccggctttccccgtcaagctctaaatcgggggatctcggga 5850

5851 aaagcgttggtgaccaaaggtgccttttatcatcactttaaaaataaaaaacaattactcagtgcctgttataag 5925

5926 cagcaattaattatgattgatgcctacatcacaacaaaaactgatttaacaaatggttggtctgccttagaaagt 6000

6001 atatttgaacattatcttgattatattattgataataataaaaaccttatccctatccaagaagtgatgcctatc 6075
```

```
6076 attggttggaatgaacttgaaaaaattagccttgaatacattactggtaaggtaaacgccattgtcagcaaattg 6150


6151 atccaagagaaccaacttaaagcttacccatgaacacaagttgcgaagcatttatgatgatgatgtgcttaaaaa 6225

                           >>>>>>>>>>>>>>>>>>>
                           qPCR forward primer site

6226 cttactcaatggctggtttatgcatatcgcaatacatgcgaaaaacctaaaagagcttgccgataaaaaaggcca 6300

                                    >>>>>>>>>>>>>>>>>>>>>>>>>>>>>
                                    Taqman Probe site

6301 atttattgctatttaccgcggctttttattgagcttgaaagataaataaaatagataggttttatttgaagctaa 6375

       >>>>>>>>>>>>>>>>>>>
       qPCR reverse primer site

6376 atcttctttatcgtaaaaaatgccctcttgggttatcaagagggtcattatatttcgcggaataacatcatttgg 6450


6451 tgacgaaataactaagcacttgtctcctgtttactcccctgagcttgaggggttaacatgaaggtcatcgatagc 6525

               >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
               TetA

6526 aggataataatacagtaaaacgctaaaccaataatccaaatccagccatcccaaattggtagtgaatgattataa 6600

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6601 ataacagcaaacagtaatgggccaataacaccggttgcattggtaaggctcaccaataatccctgtaaagcacct 6675

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6676 tgctgatgactctttgtttggatagacatcactccctgtaatgcaggtaaagcgatcccaccaccagccaataaa 6750

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6751 attaaaacagggaaaactaaccaaccttcagatataaacgctaaaaaggcaaatgcactactatctgcaataaat 6825

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6826 ccgagcagtactgccgttttttcgccccatttagtggctattcttcctgccacaaaggcttggaatactgagtgt 6900

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6901 aaaagaccaagacccgctaatgaaaagccaaccatcatgctattccatccaaaacgattttcggtaaatagcacc 6975

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA

6976 cacaccgttgcgggaatttggcctatcaattgcgctgaaaaataaataatcaacaaaatgggcatcgttttaaat 7050

     >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>
     TetA
```

7051 aaagtgatgtataccgaattcgattgcgtctcaacccctacttcggtatctgtattatcacgtgtatttttggtt 7125

>>>
TetA

7126 tcacggaaccaaaacataaccacaaggaaagtgacaatatttagcaacgcagcgataaaaaagggactatgcggt 7200

>>>
TetA

7201 gaaatctctcctgcaaaaccaccaataataggccccgctattaaaccaagcccaaaacttgcccctaaccaaccg 7275

>>>
TetA

7276 aaccacttcacgcgttgagaagctgaggtggtatcggcaatgaccgatgccgcgacagccccagtagctcctgtg 7350

>>>
TetA

7351 atccctgaaagcaaacggcctaaatacagcatccaaagcgcacttgaaaaagccagcaataagtaatccagcgat 7425

>>>
TetA

7426 gcgcctattaatgacaacaacagcactgggcgccgaccaaatcggtcagacatttttccaagccaaggagcaaag 7500

>>>
TetA

7501 ataacctgcattaacgcataaagtgcaagcaatacgccaaagtggttagcgatatcttccgaagcaataaattca 7575

>>>
TetA

7576 cgtaataacgttggcaagactggcatgataaggccaatccccatggcatcgagtaacgtaattaccaatgcgatc 7650

>>>
TetA

7651 tttgtcgaactattcatttcacttttctctatcactgatagggagtggtaaaataactctatcaatgatagagtg 7725

>>>>>>>>>>>>>>>>>
TetA

7726 tcaacaaaaattaggaattaatgatgtctagattagataaaagtaaagtgattaacagcgcattagagctgctta 7800

>>
TetR

7801 atgaggtcggaatcgaaggtttaacaacccgtaaactcgcccagaagctaggtgtagagcagcctacattgtatt 7875

>>>
TetR

7876 ggcatgtaaaaaataagcgggctttgctcgacgccttagccattgagatgttagataggcaccatactcactttt 7950

>>>
TetR

7951 gccctttagaaggggaaagctggcaagatttttacgtaataacgctaaaagttttagatgtgctttactaagtc 8025

>>>
TetR

8026 atcgcgatggagcaaaagtacatttaggtacacggcctacagaaaaacagtatgaaactctcgaaaatcaattag 8100

>>>
TetR

8101 ccttttatgccaacaaggtttttcactagagaatgcattatatgcactcagcgctgtggggcattttactttag 8175

>>>
TetR

8176 gttgcgtattggaagatcaagagcatcaagtcgctaaagaagaaagggaaacacctactactgatagtatgccgc 8250

>>>
TetR

8251 cattattacgacaagctatcgaattatttgatcaccaaggtgcagagccagccttcttattcggccttgaattga 8325

>>>
TetR

8326 tcatatgcggattagaaaaacaacttaaatgtgaaagtgggtcttaaaagcagcataacctttttccgtgatggt 8400

>>>
TetR

8401 aacttcacggtaaccaagatgtcgagttaaccacccttagattcataaagcgaaaataatgcggctccaacgta 8475

8476 cccacctaaatggaaacggcgttcactccaatctaaacacgcacaacagattttacgtgaatgtttggaaggaac 8550

8551 gtcaattcccatttcatgaaaatattgaataccacttaatgtgatcattgaaccattttcagtgatccattgctg 8625

8626 ttgacaaagggaatcatagatccctttagggttccgatttagtgctttacggcacctcgacctccaaaaacttga 8700

8701 tttgggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgtt 8775

8776 ctttaatagtggactcttgttccaaactggaacaacactcacaactaactcggcctattcttttgatttataagg 8850

8851 attttgtcattttctgcttactggttaaaaaataagctgatttaacaaatatttaacgcgaaatttaacaaaac 8925

8926 attaacgtttacaatttaaatatttgcttatacaatcatcctgtttttggggcttttctgattatcaatcggggt 9000

9001 acatatgattgacatgctagttttacgattaccgttcatcgattctcttgtttgctccagactttcaggtaatga 9075

9076 cctgatagcctttgtagacctctcaaaaatagctaccctctccggcatgaatttatcagctagaacggttgaata 9150

9151 tcatattgacggtgatttgactgtctccggcctttctcacccgtttgaatctttgcctactcattactccggcat 9225

9226 tgcatttaaaatatatgagggttctaaaaatttttatccctgcgttgaaattaaggcttcaccagcaaaagtatt 9300

9301 acagggtcataatgtttttggtacaaccgatttagctttatgctctgaggctttattgcttaattttgctaactc 9375

9376 tctgccttgcttgtacgatttattggatgtt 9406