Primers to introduce a mTEV protease site in PAX

For: GST-ENLYFQ-Cys-Pax(38-557)-FLAG

4 flanking bases

```
                                    E   N   L   Y   F   Q
5'  GCC  GGA  ATT  CGT  GAA  AAC  CTG  TAT  TTT  CAG  *
                                    A
```

EcoRI site

mTEV cut site

```
                C   H   T   Y   Q   E   I
5'  TGC  CAC  ACA  TAC  CAG  GAG  ATT
```

residue 38-43 of paxillin

For cloning into pGEX-4T-2 vector of the pcr product.
The reverse primer used will be BACGSTPA (p. 3-123.)

FOR: 107.1 µg in 214.2 µL 0.5 µg/µL

FOR1104 (030219-1) **11/05/2003**

51 mer
5'-GCC GGA ATT CGT GAA AAC CTG TAT TTT
CAG TGC CAC ACA TAC CAG GAG ATT

1 OD = 31.5 µg = 2015 pmol
MW = 15631.28 E260 = 496.4 L/mmol-cm
Tm = 65.2 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 3.4 OD; Purified (RPC)

yield: 107.1 µg

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Thursday, November 6, 2003

PCR for ligation into pGEX-4T-2

- 50 µL of the following reaction mixture was added to each of 10 sterile pcr tubes after gentle mixing by flicking:
  - a. 65 µL 10 × HIFI buffer
  - b. 3.25 µL 100 mM dNTP's
  - c. 19.5 µL 50 mM MgCl2
  - d. 6.5 µL FOR1104 (0.5 µg/µL)
  - e. 6.5 µL BACGSTPA (0.5 µg/µL)
  - f. 5.2 µL DNA template
  - g. 404.3 µL sterile water (should be 410.8)
  - h. 6.5 µL Taq HIFI (5 U/µL)

- The thermal cycle was run as shown on p. 4-67, except each 72°C extension was carried out for 1 min. (not 30 s) and the final 72°C hold was for 10 min.