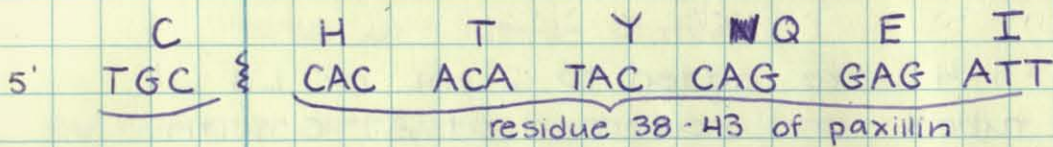
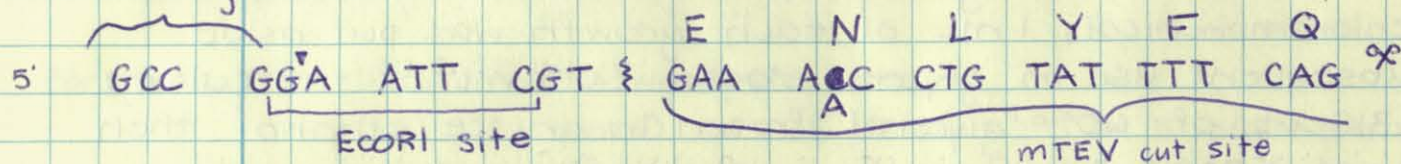


Primers to introduce a mTEV protease site in PAX

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

4 flanking bases



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

FOR: 107.1 μ g in 214.2 μ L

0.5 μ g/ μ L

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 μ g = 2015 pmol
MW = 15631.28 E260 = 496.4 L/mmol-cm
Tm = 65.2 $^{\circ}$ C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 3.4 OD; Purified (RPC)

yield: 107.1 μ g

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Thursday, November 6, 2003

PCR for ligation into pGEX-4T-2

50 μ L of the following reaction mixture was added to each of 10 sterile PCR tubes after gentle mixing by flicking:

- a. 65 μ L 10 \times HIFI buffer
- b. 3.25 μ L 100 mM dNTP's
- c. 19.5 μ L 50 mM MgCl₂
- d. 6.5 μ L FOR1104 (0.5 μ g/ μ L)
- e. 6.5 μ L BACGSTPA (0.5 μ g/ μ L)
- f. 5.2 μ L DNA template
- g. 404.3 μ L sterile water (should be 410.8)
- h. 6.5 μ L Taq HIFI (5 U/ μ L)

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