**How to insert the shRNA sequence into pT3-EF1 vector**

Example:

**Shraptor 97 Sequence:**

TGCTGTTGACAGTGAGCGCttgaaggacttgacgatgaggTAGTGAAGCCACAGATGTAcctcatcgtcaagtccttcaaATGCCTACTGCCTCGGA

ttgaaggacttgacgatgagg and cctcatcgtcaagtccttca are shRaptor sequences

**Then we add the restriction enzyme sequence to the shRaptor, so we get the template sequence as following:**

**Shraptor 128:** CAGAAGG**ctcgag**aaggtatatTGCTGTTGACAGTGAGCGCTTGAAGGACTTGACGATGAGGTAGTGAAGCCACAGATGTACCTCATCGTCAAGTCCTTCAAATGCCTACTGCCTCG**gaattc**AACTG

**ctcgag** and **gaattc** are added restriction enzyme sites.

**Design Primers:**

Shraptor-F1:CAGAAGG**ctcgag**aaggtatatTGCTGTTGACAGTGAGCGCTTGAAGGAC (1-50)

Shraptor-F2:TTGACGATGAGGTAGTGAAGCCACAGATGTACCTCATCGTCAAGTCCTTC(51-100)

Shraptor-R1:TGTGGCTTCACTACCTCATCGTCAAGTCCTTCAAGCGCTCACTGTCAACA(27-76)

Shraptor-R2:CAGTTgaattcCGAGGCAGTAGGCATTTGAAGGACTTGACGATGAGGTACATC(76-128)

F1 (1-50)

F2 (51-100)

1 128

R2(76-1328)

R1(27-76)

**PCR Program**:

First: Tube 1: F1+R1 --------------PCR -----5 Cycles

Tube2: F2+R2 --------------PCR -----5 Cycles

Second: get 12.5ul from Tube1 and 12.5ul from Tube 2 -----------mix into tube3-------

--------PCR -----5 Cycles

Third: get 2ul from Tube3 as template and add F1+R2--------- PCR -----35 Cycles