Protocols for PCR Amplification of Killer Red ssrA tag Insert
iGEM Grenoble-EMSE-LSU 2013

Stewart Humble (stewhum1@me.com)

June 2013

1. Amplification of KRssrA using PCR

KillerRed sequence:

ATGGGTTCAGAGGGCGGCCCCGCCCTGTTCCAGAGCGACATGACCTTCAAAATCTTCATCGACGGCGAGGTGAACGGCCAGAAGTTCACCATCGTGGCCGACGGCAGCAGCAAGTTCCCCCACGGCGA
CTTCAACGTGCACGCCGTGTGCGAGACCGGCAAGCTGCCCATGAGCTGGAAGCCCATCTGCCACCTGATCCAGTACGGCGAGCCCTTCTTCGCCCGCTACCCCGACGGCATCAGCCATTTCGCCCAGGA
GTGCTTCCCCGAGGGCCTGAGCATCGACCGCACCGTGCGCTTCGAGAACGACGGCACCATGACCAGCCACCACACCTACGAGCTGGACGACACCTGCGTGGTGAGCCGCATCACCGTGAACTGCGACG
GCTTCCAGCCCGACGGCCCCATCATGCGCGACCAGCTGGTGGACATCCTGCCCAACGAGACCCACATGTTCCCCCACGGCCCCAACGCCGTGCGCCAGCTGGCCTTCATCGGCTTCACCACCGCCGAC
GGCGGCCTGATGATGGGCCACTTCGACAGCAAGATGACCTTCAACGGCAGCCGCGCCATCGAGATCCCCGGCCCACACTTCGTGACCATCATCACCAAGCAGATGAGGGACACCAGCGACAAGCGCGA
CCACGTGTGCCAGCGCGAGGTGGCCTACGCCCACAGCGTGCCCCGCATCACCAGCGCCATCGGTAGCGACGAGGAT

Number of primers: 2

Name: KR_left_pQE

TAATTCCGGATCCATGGGTTCAGAGGGCGGC (Tm=67∞C for 31 bases)

Restriction Site: (BamH1)

Name: KR_right_pQE_ssrA_downstream

CAGGTACCttaCGAAGCGTCGGCATAGTTTTCTGAATAGTTTTCATCGTTGGCCGCATCCTCGTCGCT
ACCGATGG

(Tm=55,4∞C for 76 bases)

Restriction Site: (Kpn1)

1. **Prepare 5x buffer, dNTPs, primers**
2. **Prepare a premix of 100 µL:**

	Volumes (µL)	Final Concentration
H_2O	60	
Buffer 5x with Mg^{2+} and loading dye	20	1x
dNTPs 2 mM	10	0.2 mM
primer KR-5'	5 of stock* diluted 10 x	50 pmoles
primer KR-3'	5 of stock* diluted 10 x	50 pmoles

* primers are at 100 nmoles/mL

3. **Prepare 4 samples of 18 µL each. Add 1µL of pBabe-KR (7ng/µL) in 3 of them and 1 µL H_20 in the last tube. Add 1 µL of GoTaq polymerase (2 U). The final volume is 20 µL.**

4. **Program the PCR thermocycler as follows:**

94°C, 2 min	*Initial Denaturation of Matrix DNA*
94°C, 30 sec	*Denaturation*
50°C, 30 sec	*Annealing*
72°C, 1 min	*Extension*
20 cycles	
68°C, 10 min	*Extension of All Generated Fragments*
4°C, no time limit	*Sample Conservation*

5. **Meanwhile, pour a 1.2 % agarose gel**
6. **Perform the migration (30 min, 135V)**
7. **Let the gel stand 15 min in BET, and then 2 min in Wash buffer**
8. **Take a picture, and cut the stripes of interest**
9. **Perform the Gel extraction, following the Qiagen gel extraction kit**
10. **Nanodrop the DNA samples**

Sample volume :

Sample concentration :

µg of DNA :