

Protocols for PCR Amplification of Killer Red Insert
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1. Amplification of KR using PCR

KillerRed sequence:

ATGGGTTTCAGAGGGCGCCCTGTTCCAGAGCGACATGACCTTCAAAATCTTCATCGACGGCGAGGTGAACGGCCAGAAAGTTACCATCGTGGCCGACGGCAGCAGCAAGTTCCCCACGGCGA
 CTTCAACGTGCACGCGTGTGCGAGACCGGCAAGCTGCCCATGAGCTGGAAGCCCATCTGCCACCTGATCCAGTACGGCGAGCCCTTCTTCGCCCCGCTACCCCGACGGCATCAGCCATTTCGCCAGGA
 GTGCTTCCCCGAGGGCTGAGCATCGACCGCACCCTGCGCTTCGAGAACGACGGCACCATGACCAGCCACACCTACGAGCTGGAGCAGACCTGCGTGGTGGAGCCGCATCACCCTGAAGTGCAGC
 GCTTCAGCCCCAGGGCCCATCATCGCGACCAAGCTGGTGGACATCTGCCCAACGAGACCCACATGTTCCCCACGGCCCAACGCCGTGCGCCAGCTGGCCTTCATCGGCTTCACCACCGCCGAC
 GCGGCGCTGATGATGGCCACTTCGACAGCAAGATGACCTTCAACGGCAGCCGCGCCATCGAGATCCCCGGCCACACTTCGTGACCATCATCACCAGCAGATGAGGGACACCAGCGACAAGCGCGA
 CCACGTGTGCCAGCGAGGTGGCTACGCCCACAGCGTCCCCGCATCACCAGCGCCATCGGTAGCGACGAGGAT

Number of primers: 2

Name: KR_left_pQE

TAATTCCGGATCCATGGGTTTCAGAGGGCGGC (Tm=67°C for 31 bases)

Restriction Site: (*Bam*H1)

Name: KR_right_pQE

CAGGTACCTtaATCCTCGTCGCTACCGATGG (Tm=66°C for 31 bases)

Restriction Site: (*Kpn*1)

1. Prepare 5x buffer, dNTPs, primers

2. Prepare a premix of 100 µL:

	Volumes (µL)	Final Concentration
H ₂ O	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 H₂O 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	<i>Initial Denaturation of Matrix DNA</i>
94°C, 30 sec	<i>Denaturation</i>
50°C, 30 sec	<i>Annealing</i>
72°C, 1 min	<i>Extension</i>
20 cycles	
68°C, 10 min	<i>Extension of All Generated Fragments</i>
4°C, no time limit	<i>Sample Conservation</i>

- 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 :