Polymerase Chain Reaction (PCR): Amplification of insert

1) Resuspend primers in buffer EB (elution buffer from Qiagen kit) to a final concentration of 60 μM. For 25 nmol of primers, 400 μl EB.
2) Taq Readymix + MgCl2 from Sigma contains dNTPs, reaction buffer, MgCl2, and Taq enzyme. Please use aerosol plugged tips to minimize accidental DNA transfer. Be ready to PCR immediately after aliquoting to minimize primer dimers.

• Template (100 ng miniprep DNA):	1 μl
• 5'-primer:	1 μl (1 μM)
• 3'-primer:	1 μl
• Taq mix (Sigma):	25 μl
• MilliQ Water:	22 μl
TOTAL :	**50** μl

3) Use PCR program 'PCR RRM'. Adjust extension time for the length of your amplified fragment: 1 min. per 1 kb.

i. Heated lid: 105ºC
ii. Preheat lid: on
iii. Pause: off
iv. Initial denaturation: 94 ºC 1 min.
v. Hot start: off
vi. ====Loop 1====
vii. Number of cycles 4
viii. Segment 94 ºC 1 min.
ix. Segment 68 ºC 3 min. 30 sec.
x. =====End loop=====
xi. =====Loop 2=====
xii. Number of cycles 26
xiii. Segment 94 ºC 50 sec.
xiv. Segment 62 ºC 1 min.
xv. Segment 70 ºC 1 min. (adjust)
xvi. =====End loop======
xvii. Final extension 72ºC 5 min.
xviii. Final Hold 4 ºC