**Plasmid Preparation Cassette**

Prepare a stock of 100uM primers (according to the manufacturer’s protocol), in Sigma Water

Dilute to get 10uM:

If the primer stock is 100uM- dilute 1:10 (1 ul primer in 9 ul sigma water)

If the primer stock is 1mM- dilute 1:100 (2 ul primer in 198 sigma water)

Prepare PCR samples:

|  |  |
| --- | --- |
| X2 Kapa ready mix | 12.5 ul |
| F Primer, 10uM | 1.25 ul |
| R Primer, 10uM | 1.25 ul |
| Plasmid (5-40 ng) | 1 ul |
| Sigma Water | 9 ul (to get total of 25ul per reaction) |
| Total | 25 ul/reaction |

Tip: While preparing the plasmid, it is preferable to dilute a few times and not take a small volume. If the plasmid concentration from the miniprep is ~100-500, then dilute 1:10 to get ~10-50 then take 1 ul for the PCR.

In the PCR machine: Run🡪Start🡪Kapa 120🡪 Start now

Run in gel: small gel (0.4gr in 40 ml TAE, 3 ul gel red) or big gel (0.8gr in 80 ml, ~5ul gel red). 100V, for approximately 40 min.

Check for bands and keep PCR products in -20.