Reactions – PCR – first amplification

1. 12.8 µl ddH_2O
2. 1.8 µl 10mM $MgCl_2$
3. 2.0 µl 10mM dNTPs
4. 2.5 µl 10x PCR Buffer (*Pfu* cloned includes 15 mM $MgCl_2$)
5. 2.5 µl 2µM primer1
6. 2.5 µl 2µM primer2
7. 0.3 µl of 10u KlenTaqLA

Mix and add 24 µl of master mix to each reaction. Add 1 µl DNA (5-100 ng/µl).

1. 68°C for 60 sec. (prewarm)
2. 93°C for 10 sec. (denature)
3. 52°C for 35 sec. (anneal)
4. 68°C for 90 sec. (extend) – time depends product length (at least 30 sec / 1 kb)
5. 68°C for 7 min. (final extend)

Repeat the parameters 2 – 4 30 times. I have found that a 2 or 5 minute "hot start" at 94°C does not work that well, but the 68°C preheating ensures that the first 93°C denaturing step will denature completely.

There is no need to test the quality of the PCR product after the first reaction.

Reactions – PCR – second (nested) amplification

1. 12.8 µl ddH_2O
2. 1.8 µl 10mM $MgCl_2$
3. 2.0 µl 10mM dNTPs
4. 2.5 µl 10x PCR Buffer (*Pfu* cloned)
5. 2.5 µl 2µM nested primer1
6. 2.5 µl 2µM nested primer2
7. 0.3 µl of 10u KlenTaqLA

Mix and add 24 µl of master mix to each reaction. Add 1 µl PCR product from previous reaction.

1. 68°C for 60 sec. (prewarm)
2. 93°C for 10 sec. (denature)
3. 55°C for 35 sec. (anneal)
4. 68°C for 90 sec. (extend) – time depends product length (at least 30 sec / 1 kb)
5. 68°C for 7 min. (final extend)

The nested design can be modified to a half-nested approach. The half-nested approach uses only one nested primer while the other primer is the same as in the original amplification reaction. Unless the primers are very degenerate or have too many multiple binding sites, this approach also works well.