

General PCR set up for genomic DNA, Plasmid DNA and/or RT-PCR

Make up PCR mix for your desired product and number of reactions

- a. PCR mix per reaction
 - i. 2.5ul of 10X Hotstart Taq Buffer (Qiagen)
 - ii. 0.5ul 10mM dNTP mix
 - iii. Forward Primer of 0.15ul 20uM
 - iv. Reverse Primer of 0.15ul 20uM
 - v. 1ul 25mM MgCl₂
 - vi. H₂O up to 25 ul total
 - vii. Taq Polymerase (Qiagen) 0.125ul per reaction
- add 24 ul mix to each PCR reaction tube

-FOR GENOMIC DNA = add 1 ul of DNA sample to each tube (this amount may vary depending on the type of DNA you are using for PCR)

-FOR PLASMID DNA = add 10-20 ng per of DNA per PCR reaction

-FOR RT-PCR start with 2 ul of RT reaction per PCR reaction

Set PCR machine for the desired settings according to your primers and product size.

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|--------|------------|---|
| Step 1 | 95 degrees | 15 min |
| Step 2 | 95 degrees | 1 min |
| Step 3 | X degrees | 1 min (annealing temp should be about 5 degrees lower than primer T _m) |
| Step 4 | 72 degrees | 1 min per kb product size
Decide the cycle number desired and repeat step 2-4 that number of times |
| Step 5 | 72 degrees | 10 min |
| Step 6 | 4 degrees | until you can take it out. |

Run on an agarose gel and look for the appropriately sized bands.