

PCR Sample Submission Requirements

Reaction Submissions: Only if we are running your cycle sequencing reactions!

In order to keep our sequencing prices at current levels we are now requesting that you submit reactions to be run by following the guidelines below (for each reaction):

| Template | Quantity |
|---|---|
| PCR Product | 1-3ng for each 100 base pairs template + 3.2 pmoles primer in 12ul sterile H ₂ O |
| Plasmid (<9kb) | 50-100ng per kb template (total length) + 3.2 pmoles primer in 12ul sterile H ₂ O |
| Plasmid (>9kb) | 1.0ug template + 15 pmoles primer in 12ul sterile H ₂ O |
| Single-Stranded | 200ng template + 3.2 pmoles primer in 12ul sterile H ₂ O |
| Template only using one of our standard primers | If you are submitting your template and we are adding standard primers then: Template in 8ul sterile H ₂ O (call for a list of our standard primers) |

Since we will be setting up 20ul reactions, **all DNA + primer + H₂O should be in total volume of 12ul.** Never use TE; excess EDTA will result in poor sequencing results.

Standard Primers and Vector Primers Available at PNAACL

| Primer Name | Primer Sequence | Tm |
|-------------------|--------------------------------|-------|
| BGH Reverse | TAG AAG GCA CAG TCG AGG | 54.03 |
| GL1 | TGT ATC TTA TGG TAC TGT AAC TG | 50.75 |
| GL2 | CTT TAT GTT TTT GGC GTC TTC CA | 55.73 |
| M13 FORWARD (-20) | GTA AAA CGA CGG CCA GTG | 54.37 |
| M13 FORWARD (-41) | GGT TTT CCC AGT CAC GAC | 53.77 |
| M13 REVERSE (-27) | GGA AAC AGC TAT GAC CAT G | 51.47 |
| M13 REVERSE (-48) | AGC GGA TAA CAA TTT CAC AC | 52.2 |
| pCMV FORWARD | CGC AAA TGG GCG GTA GGC GTG | 64.76 |
| pGEX 3' | CCG GGA GCT GCA TGT GTC AGA GG | 65.18 |
| pGEX 5' | GGG CTG GCA AGC CAC GTT TGG TG | 67.02 |
| RV PRIMER 3 | CTA GCA AAA TAG GCT GTC CC | 53.9 |
| RV PRIMER 4 | GAC GAT AGT CAT GCC CCG CG | 61.15 |
| SP6 PROMOTER | TAC GAT TTA GGT GAC ACT ATA G | 50 |
| T3 PROMOTER | CAA TTA ACC CTC ACT AAA GG | 49.05 |
| T5 Promoter | CCC GAA AAG TGC CAC CTG | 57 |
| T5 Terminator | GTT CTG AGG TCA TTA CTG G | 50.4 |
| T7 PROMOTER | GTA ATA CGA CTC ACT ATA GGG | 49.88 |
| T7 TERMINATOR | GCT AGT TAT TGC TCA GCG G | 54.09 |

If you have any questions about your sample submission please call 314-362-0282. Thank you in advance for your cooperation.