

Template and Primer Requirements

Updated February 2021

Template Requirements

All Samples

Clean up your samples prior to submission.

Suggested kits: Zymo DNA Clean and Concentrator, Qiagen PCR Purification Kit, etc.

QC your samples using the NanoDrop One.

Quantification

Provide the requested amount of sample in the requested volume.

Too much or too little template will negatively impact your results.

Too much template will also shorten the life of the capillary.

Quality

Your sample 260/230 and 260/280 should be > 1.8.

A 260/230 < 1.8 indicates a contaminating reagent in the sample that could be inhibitory to the sequencing reaction.

Contaminant that absorbs at 230 or 280 will often impact the 260 reading, making the quantification unreliable.

Evaluate your PCR products on an agarose gel.

Do you have a single band, artifacts, primer dimer?

This is critical if your sequencing primer is one of the PCR primers.

If the PCR fragment of interest is not pure, we recommend using a nested primer.

What You Should Provide Us

All Samples

Fill out the Sample Submission Form and send to ccg@uiowa.edu.

Note: the Sample Name starts with your initials, the line number on the form for that sample, and, if you choose, a sample name.

Provide your sample or sample/primer mix in a 0.5 ml tube.

Label the tube cap with your initials and the sample number (the line number on the sample form).

PCR Templates (10 ul total volume without primer, or 12 ul total volume with primer added)

| PCR product length | concentration per 1 μ l | concentration in 10 μ l of sample |
|--------------------|-----------------------------|---------------------------------------|
| 100-200 bp | 0.5-1.5 ng | 5-15 ng |
| 200-500 bp | 1.5-5 ng | 15-50 ng |
| 500-1000 bp | 2.5-10 ng | 25-100 ng |
| 1000-2000 bp | 5-20 ng | 50-200 ng |
| >2000 bp | 10-25 ng | 100-250 ng |

If adding your own primer, the final total quantity should be ~8-10 pmol.

10 pmol is equal to 2 ul of 5uM primer.

Plasmid Templates

| Plasmid Size (vector + insert) | concentration per 1µl | concentration in 10µl of sample |
|--------------------------------|-----------------------|---------------------------------|
| <6 kb | ~50 ng | ~500 ng |
| 6-10 kb | ~80 ng | ~800 ng |
| >10 kb | ~100 ng | ~1000 ng |

If adding your own primer, the final total quantity should be ~8-10 pmol.

10 pmol is equal to 2 ul of 5uM primer.

For plasmids, please specify the length of your insert on your sequencing request.

Primers:

CCG Sequencing Primers:

GW1 GTT GCA ACA AAT TGA TGA GCA ATG C

GW2 GTT GCA ACA AAT TGA TGA GCA ATT A

M13F (-20) GTA AAA CGA CGG CCA GT

M13R (-27) CAG GAA ACA GCT ATG AC

SP6 TAC GAT TTA GGT GAC ACT ATA G

T3 AAT TAA CCC TCA CTA AAG GG

T7 TAA TAC GAC TCA CTA TAG GG

T7 – terminator GCT AGT TAT TGC TCA GCG G