



The Centre for Applied Genomics DNA Sequencing Facility

Sample preparation

- If you are planning to submit 20 or more samples/reactions for sequencing, please consider sending them in PCR strips or a PCR plate. You can label the tubes as the sample number as well. This helps move things through the pipeline faster for all users. Thanks for your consideration.
- Submitted samples must contain 7.0 ul purified template DNA (at the appropriate concentration - see guidelines below)
- The entire sample volume will be used for the sequencing reaction. The facility will not store leftover DNA.
- If you would like the facility to add a standard vector primer (www.tcag.ca/primerList.html), this can be done at no extra charge. You can request this service by selecting the primer from the dropdown menu in the “Primer Type” column of the order form.
- If you are using your own primer, please select “user added” from the dropdown menu in the “Primer Type” column of the order form. DNA template must be premixed with the primer in one tube in order to be processed. The primer must be added at a concentration of ~50 mg (~5 pmol) in 0.7 ul volume, for a total submitted volume of 7.7 ul. Be sure to only add ONE primer to each tube.
- All submitted samples will be billed, unless the DNA sequencing facility experiences a system failure. We run a control reaction on each plate as an internal quality control measure.

DNA Template Concentration

The quantity of DNA template depends on the size of the template you are sequencing.

- Plasmids – 200 – 300ng in 7 ul
- PCR product (>4kb) – 100 – 200ng in 7 ul
- PCR products (2-4kb) – 100 – 150ng in 7 ul
- PCR products (1-2kb) – 50 – 100ng in 7 ul
- PCR products (<1kb) – 50ng in 7 ul
- PCR products (<500bp) – 20ng in 7 ul
- PCR products (<200bp) – 10ng in 7 ul
- BAC/Cosmid DNA – 600 – 800ng in 7 ul

Please contact Tara Paton (tpaton@sickkids.ca) or Beverly Apresto (bapresto@sickkids.ca) with questions (416) 813-7654 x308643