# Sample submission for PCR fragment Sanger sequencing

(Assumes PCR clean up has been performed in your own laboratory.)

## Sample Preparation Guidelines

General Sample submission guidelines:

1. Samples may be left at any of our drop off locations:
   - 3343 Forbes Avenue, Room 303
   - Scaife Hall, room S534A
   - Hillman Cancer Center Research Pavilion, room 2.15
   - Children's Hospital loading dock

   Please leave samples grouped together in the marked Sequencing box.
   **At Children's**, samples must be placed in the insulated container. Samples loose in the fridge will not be collected by the courier.
   **At Hillman,** please use the individual boxes supplied by Genomics Research Core. Please do NOT leave samples in bags, racks without lids or attached to the drop off form. Please note that courier service from the Hillman Center to the facility at 3343 Forbes Avenue is provided by, and at the convenience of, the Hillman Center and is not in any way coordinated by the Genomics Research Core or its staff.
2. Label sample tubes with initials and a single number (e.g. JD1, JD2, ...JD12, JD13....) on the top of the tube. There can be **NO periods, spaces, \ / : * ? " < > | or _** in sample names. PI name should be written on side. Keep an internal record of sample names; please do not send this key to the Genomics Research Core. Label plates on the side with PI name, date of submission and plate number if more than one is submitted.
3. Partial plates must be filled contiguously in column order, i.e. A1 through H1 then A2 through H2. Empty wells in the middle of a group will not be skipped and you will be charged for a reaction. Plates filled in row order will be returned for rearrangement prior to processing.
4. Please do not use tape anywhere.
5. Enter your service request in iLab. Sample ID's entered in iLab must match those written on the tube. There can be **NO periods, spaces, \ / : * ? " < > | or _** in sample names
6. If sample submission guidelines are not followed there may be a delay in processing your samples or additional charges.
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## Sequencing Reaction

1. For each reaction the following should be mixed prior to submission:

   - If submitting less than 48 samples in 1.5 ml tubes:

     X $\mu$L of DNA template (determined by the concentration of the template and the table below)

8.0 picomoles of primer (absolute mass not concentration)
Y $\mu$L of H20 (to bring total volume to 15 $\mu$L)
15 $\mu$L total volume

- If submitting 48 or more samples in a semi-skirted 200-300 $\mu$l 96-well PCR plate:

  X $\mu$L of DNA template (determined by the concentration of the template and the table below)
  3.2 picomoles of primer
  Y $\mu$L of H20 (to bring total volume to 6 $\mu$lL)
  6 $\mu$L total volume

The table below shows the recommended template quantity based on template size:

| PCR Product Size (bp) | Tube submission (final volume 15uL) | Plate submissions (final volume 6uL) |
|---|---|---|
| 100-200 bp | 2-6 ng | 1-3 ng |
| 200-500 bp | 6-20 ng | 3-10 ng |
| 500-1000 bp | 10-40 ng | 5-20 ng |
| 1000-2000 bp | 20-80 ng | 10-40 ng |
| >2000 bp | 80-200 ng | 40-100 ng |