

## GENOMICS RESEARCH CORE

**Sample submission for plasmid or BAC Sanger sequencing****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, outside room A916 (A-stem)
- 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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**Universal Primer Addition and Sequencing Reaction**

1. We provide addition of the following universal primers free of charge: M13F, M13R, SP6, T3 or T7. On the sample drop off form, in addition to other services requested, select "Addition of GENOMICS RESEARCH CORE Provided Universal Primers" and indicate clearly in the space provided which samples should be treated with each primer. Failure to mark this option may result in sample processing delay.
2. If submitting less than 48 samples, please submit 15 µl of template in 1.5 ml tubes to allow for accurate transfer and timely repeat in the event of technical failure. If you are requesting that samples be run with 2 different primers, please provide 2 separate tubes of template.

3. If submitting 48 or more samples, please submit 5  $\mu\text{l}$  of template in a 200-300  $\mu\text{l}$  96 well semi-skirted PCR compatible (conical) plate for addition of universal primers prior to sequencing reaction. If you are requesting that samples be run with 2 different primers, please provide 2 separate wells of template.
4. See table below for recommended template mass to be submitted.

### 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  $\mu\text{L}$  of DNA template (determined by the concentration of the template and the table below)

8.0 picomoles of primer (absolute mass not concentration)

Y  $\mu\text{L}$  of H<sub>2</sub>O (to bring total volume to 15  $\mu\text{L}$ )

15  $\mu\text{L}$  total volume

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

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

3.2 picomoles of primer

Y  $\mu\text{L}$  of H<sub>2</sub>O (to bring total volume to 6  $\mu\text{L}$ )

6  $\mu\text{L}$  total volume

The table below shows the recommended template quantity:

Sequencing Template	Tube submission (final volume 15 $\mu\text{L}$ )	Plate submissions (final volume 6 $\mu\text{L}$ )
Plasmid DNA	1 $\mu\text{g}$	500 ng
BAC	6 $\mu\text{g}$	3 $\mu\text{g}$