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Guidelines for gDNA samples submission for PCR and sequencing - PLEASE READ CAREFULLY -

Preparations of gDNA are submitted in 96-well PCR plates. Often, the cells from a particular arm of an experiment will exceed the capacity of a single well (10 µg) and thus must be split across multiple wells. Or, a particular sample of gDNA will need to be split across multiple wells because it is in a large volume (maximum of 50 µL of gDNA per well). We can use up to 96 barcodes; if you have more than 96 wells of gDNA, you will need multiple plates and they will be sequenced on different Illumina lanes. The PCR plate will be stored for 3 months after which it will be discarded, if you wish to retrieve the plate, please notify us.

Billing

- We bill per plate rather than number of samples or wells.
- You need a quote to submit your samples:
 - If you already have one, please indicate the quote ID (RNAI...) on the conditions file.
 - If you require a new quote, email gpp-gdnasubmission@broadinstitute.org with the name of your PI and the number of plates.

Samples requirements and plate layout

- Submit samples in a **skirted, 96-well PCR plate** (for example: Eppendorf Cat.# 951020401). PCR will be performed directly in this plate. **Clearly label the plate with date in YYYY-MM-DD_Last Name_PerturbationType** (for example: [2010-11-20_Belichick_sgRNA](#)). Do not submit samples in tubes.



Skirted



Non-skirted

- Each sample should be submitted in a **final volume of 50 μ L** (gDNA-only or gDNA plus water), with a maximum amount of **10ug gDNA per well**. There is no minimum per se, but particularly low input amounts (< 1 ug per well) may require additional PCR cycles.
- **Double-check with your screening scientist how much gDNA you should submit!!** To give you an idea of the scale:
 - o Positive selection: submit gDNA from all the cells that survived selection.
 - o Negative selection (standard viability screen): # of perturbations x 500 cells x 6.6 pg DNA/cell
- Preferably, each sample will contain similar amounts of gDNA. Keep in mind that we will pool PCR product equally by volume from all wells before sequencing.
- If using less than a whole plate, please load samples across rows rather than columns.
- If submitting multiple plates, please **distribute samples evenly across plates**. For example, load 2 plates with 50 samples each as opposed to 1 plate with 96 samples and 1 plate with 4 samples. Likewise, if loading multiple wells for a given sample, we recommend that you distribute those wells evenly across plates.
- If submitting multiple plates that are not full, it is helpful to vary the locations of empty wells, to ensure that plate swaps can be easily detected.
- All samples on the plate must have the same insert type (sgRNA, shRNA, ORF) and be vectors that use the same primers, if you're unsure email gpp-gdnasubmission@broadinstitute.org

Conditions file guidelines

- Complete the conditions file Excel spreadsheet with the contents of each well and email to gpp-gdnasubmission@broadinstitute.org before dropping off the plate.
- Indicate the quote ID (RNAi...) on the conditions file. **Sample submissions without a valid quote ID will not be processed.**
- Indicate which Screening Scientist is working on your project.
- **Note which library should be used for deconvolution (CP#####). CPs can be found on the [GPP website](#). Selecting the wrong CP may result in sample loss, if you are unsure or have a custom pool, do not guess, contact gpp-gdnasubmission@broadinstitute.org**
- If multiple CPs were used on the same plate, indicate which wells uses which CP.
- The Meta Info columns are for your samples descriptions (treatment, time point, etc.), do not use commas in these columns.
- Use one sheet per plate. If you have multiple plates, submit multiple worksheets.
- Please name the file as you named the plate: YYYY-MM-DD_Last Name_PerturbationType.

Samples dropoff

If you have access to Broad:

- pack the plates in a box on dry ice
- include your name and quote ID
- email gpp-gdnasubmission@broadinstitute.org to indicate when you will come by during Mon-Fri 9am-4pm
- place the box on the shelf clearly indicated for gDNA drop-off in the hallway of 75A - 5th floor
- DO NOT leave the box on the floor as it might be mistaken for trash.**

If you don't have access to Broad:

- ship the plates on dry ice to:
The Broad Institute
GPP-gDNA submission
75 Ames Street, room 2051
Cambridge, MA 02142
Tel: 617-714-7257/617-714-8082
- secure the plate with bubble wrap and in a plastic bag
- include at least two layers (styrofoam box + cardboard box)
- for domestic shipments, use Fedex priority overnight, DO NOT USE A COURIER
- for international shipments, use a company that replenishes dry ice (ex: World Courier, NOT FEDEX)
- ship at the beginning of the week to ensure that delays do not result in the shipment arriving on a weekend.
- email gpp-gdnasubmission@broadinstitute.org to indicate when you have shipped.