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Guidelines for gDNA samples submission for PCR and sequencing

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 in order 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 µL** (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:
    - Positive selection: submit gDNA from all the cells that survived selection.
    - 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 in the same vector (pXPR_003, pLKO.1/pLKO.5, etc.).

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 the library that should be used for deconvolution (CP####); CP numbers can be found on the second tab of the conditions file. If you are unsure or have a custom pool, please 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.). Please do not use commas in these columns.
- Indicate the vector your library constructs are in, (e.g. pXPR_003, pLKO.1/pLKO.5, pLX_317, etc.). **Incorrect vector designation will result in PCR failure and sample loss.**
- Use one sheet for each plate. If you have multiple plates, submit multiple worksheets.

- Please name the file according to the following convention: Date in YYYY-MM-DD_Last Name_PerturbationType. For example: 2017-11-20_Belichick_sgRNA

Samples dropoff

If you have access to Broad:

-pack the plates in a box on dry ice
-include a printout of the conditions file or 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

-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.