Samples have been:

Extracted - Extraction Qiagen DNeasy Protocol

Quantified - quant-it pico green

Maybe Cleaned - Ampure clean up protocol

Digested - Digestion Reactions

Cleaned - Ampure clean up protocol

Quantified - quant-it pico green

Ligated - Ligation

Pooled -

Cleaned 2X - Ampure clean up protocol

Pippin - Pippin Prep

PCR’d - PCR

Cleaned - Ampure clean up protocol

Quantified - Qubit Protocol

And are ready to prepare for the sequencing facility

Based on the qubit quantification (X), determine the volume of sample (Y) that will supply 100ng to the sequencing pool:

100ng ÷ X ng/µL = Y µL

Calculate the 4 pools independently on the PCR sheet in the [Sample\_Data file](https://docs.google.com/a/scarletmail.rutgers.edu/spreadsheets/d/1Rf_dFJ5WK-vTTsIT_kHHOcFrKzQtMFtKiuXiFw1lh9Y/edit#gid=637667576)

Sum the volume on the Sequencing sheet in the [Sample\_Data file](https://docs.google.com/a/scarletmail.rutgers.edu/spreadsheets/d/1Rf_dFJ5WK-vTTsIT_kHHOcFrKzQtMFtKiuXiFw1lh9Y/edit#gid=637667576)

The concentration of this pooled sequencing sample is calculated in the next cell by dividing 400 by the sum volume (400 because you added 100ng of 4 pools) - this value should calculate automatically in the spreadsheet

We are using 430bp fragments (375 from pippin plus 55 added by illumina primer for index and flowcell attachment) - enter this value into the spreadsheet

The spreadsheet will calculate nmol/µL by dividing the concentration by the product of 660 and the number of basepairs (430) …660\*430=283,800

The spreadsheet will calculate nM (aka nmol/L) by multiplying nmol/µL by one million

Label a vial with the SEQ number on top (SEQ 04) and SEQ # date nM ng/µL indices on the side for example (this really does fit):

SEQ 04

12-10-2014

65.05 nM 18.46ng/µL

Indices 4,5,6,7

Combine the 4 pools based on the volume calculated on the PCR page

We would like to send 10nM to the sequencing facility. Most likely the sequencing pool will need to be diluted in order to bring it down to 10nM. In a new tube with all of the above labeling but also "Pinsky" so it reads

Pinsky

SEQ 04

12-10-2014

10 nM 2.83ng/µL

Indices 4,5,6,7

The next two cells in the Sequencing spreadsheet calculate the volume of sample and the volume of water to be combined in this new tube - the volume of pooled sequencing sample is calculated by dividing 300 by the nM and the volume of water is calculated by subtracting the volume of sequencing sample from 30.

Combine the sequencing sample and Pure water in the new tube.

Stuff a kim wipe into a 50mL falcon tube, place the diluted 10nM sequencing tube inside, stuff in another kim wipe, screw on the cap and place in a styrofoam cooler with an icepack. We want to keep the sample cool but we do not want it to freeze. If it isn’t ready to depart immediately, keep the 50mL falcon containing the sequencing tube n the fridge (4˚C).

Place the original pooled sequencing sample vial in the freezer box in the -20

If sending the sample to Princeton, Malin will fill out the submission form on this webpage:

<https://htseq.princeton.edu>

Princeton also has guidelines on this webpage:

<http://www.princeton.edu/genomics/sequencing/instructions/sample-preparation/>

Their guidelines differ from our personal contact’s recommendations at Princeton and we will follow our own set of guidelines unless instructed otherwise by Malin.