Prepping libraries for sequencing submission

```
source("../genomics/scripts/lab_helpers.R")
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
lab <- write_db("Laboratory")
```

```
## Loading required package: DBI
```

```
print(params)
```

```
## $date
## [1] "2018-09-13"
##
## $facility
## [1] "PrincetonLSI"
##
## $first_baits
## [1] "B001"
##
## $frag_length
## [1] 430
##
## $last_baits
## [1] "B003"
##
## $library_type
## [1] "ddRADSeq"
##
## $num_lanes
## [1] 2
##
## $pe_or_se
## [1] "se"
##
## $platform
## [1] "hiseq"
##
## $seq
## [1] "SEQ18"
```

```
# pull in the baits that are going to be pooled for sequencing
baits <- lab %>%
  tbl("baits") %>%
  collect()
```

```r
# update the db to reflect pooling
change <- baits %>%
  filter(baits_id >= params$first_baits & baits_id <= params$last_baits) %>%
  mutate(seq = params$seq)

baits <- change_rows(baits, change, "baits_id")
# dbWriteTable(write_lab, "baits", dat_db, row.names=F, overwrite = T)


# calculate the percent concentration of DNA in each bait
change <- change %>%
  mutate(vol = 20,
    perc = as.numeric(ng_ul)/as.numeric(vol))

# calculate the percent concentration of the pooled baits
tot <- change %>%
   # sum of percents
  summarise(tot_perc = sum(perc)) %>%
  # sum of volumes
  mutate(vol = change$vol[1] * 3,
    # calculate the ng_ul of the combined DNA
    ng_ul = tot_perc * vol,
    # calculate the nM of the combined DNA (430bp fragment length)
    nM = (ng_ul/(660*params$frag_length))*10^6,
    # calculate the volume of library to produce 10nM
    seq_vol = (30*10)/nM) # 30 because that is our desired final volume to send to seq, 10 because we w

knitr::kable(tot) %>%
  kableExtra::kable_styling()
```

```
## Warning in kableExtra::kable_styling(.): Please specify format in kable.
## kableExtra can customize either HTML or LaTeX outputs. See https://
## haozhu233.github.io/kableExtra/ for details.
```

tot_perc	vol	ng_ul	nM	seq_vol
0.1074	60	6.444	22.70613	13.21229

```r
# add to seq table in the db
dat_db <- lab %>%
  tbl("sequencing") %>%
  collect()

# create a row to add to the seq table in the db
change <- tibble(seq_id = params$seq, date = params$date, library_type = params$library_type, facility =

#_____________________________________________________________
# add columns to seq db (only have to do this once today)
dat_db <- dat_db %>%
  mutate(vol_pooled_libs = NA, vol_TE = NA)
dat_db <- dat_db %>%
  select(seq_id:contents, vol_pooled_libs, vol_TE, library_type:notes)
#_____________________________________________________________
```

```
dat_db <- rbind(dat_db, change) %>%
  mutate(vol_pooled_libs = formatC(vol_pooled_libs, width = 1),
    vol_TE = formatC(vol_TE, width = 1))

# dbWriteTable(lab, "sequencing", dat_db, row.names=F, overwrite = T)
```

Paste the qubit results into the PCR page of the Sample_Data file as directed above in the qubit protocol.

On the PCR page, in the µL PCR product to make 30µL 10nM column, pipet that number into the sequencing vial. Then pipet the number from the water column.

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):

Pinsky SEQ 04 12-10-2014 10 nM Indices 4,5,6,7

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 to freeze it. If it isn't ready to depart immediately, keep the 50mL falcon with the sequencing tube in it in the fridge.

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

Update 4/13/2015 - MRS

It is possible to calculate the concentration of each pool and then add 100ng of each:

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

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

Calculate the 4 pools independently on the PCR sheet in the Sample_Data file

Sum the volume on the Sequencing sheet in the Sample_Data file

Combine the 4 pools based on the volume calculated

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 to freeze it. If it isn't ready to depart immediately, keep the 50mL falcon with the sequencing tube in it in the fridge.

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If sending to Princeton, don't generate the PO until the seq is complete (particularly if you are submitting more than one seq within a month). Ask Princeton if they can add some description to the quote to make it look different than the rest of the quotes they have sent (like add the seq number). We have had problems in the past with multiple quotes from similar dates with no other difference on the page. Had to add a line of text saying "This invoice is for sequencing done to APCL12 DNA on February 6th, 2016".

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.