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## HCASMC CRISPRi TAPseq

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We are still developing and optimizing this protocol

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## Abstract

This protocol outlines the steps for conducting a CRISPR interference (CRISPRi) TAP-seq experiment in human coronary artery smooth muscle cells (HCASMCs). The procedure includes virus transduction, MOI titration using digital PCR (dgPCR), and detailed instructions for setting up and running the experiment. Key steps involve configuring the experiment in the software, setting fluorescence thresholds for droplet analysis, and calculating target concentrations using the Poisson equation. The protocol ensures accurate quantification of target sequences and reference controls. This method provides a robust framework for analyzing gene expression and regulatory elements in HCASMCs using CRISPRi and TAP-seq technologies.



## Library cloning for guides

- 1
  - Vector Preparation – pBA904 (direct capture for TAPseq); CROP-seq-opti for TSS perturbseq

### 1.1 TAPseq:

Digest 1ug\* vector x8 reactions overnight at 37C in thermocycler (max vol is 50ul per PCR tube)

#### 20ul Reaction

|                      |      |
|----------------------|------|
| Water                | 15ul |
| NEB Buffer 2.1 10x** | 2ul  |
| pBA904               | 1ul  |
| BspI                 | 1ul  |
| BstXI                | 1ul  |

\*Can increase this to 3-5ug vector x2-3 reactions

\*\*BstXI only has 50% activity in NEB Buffer 2.1, but this is the best buffer for the combination of these two enzymes

Negative controls to include: single digests and no enzyme

### 1.2 TSS perurbseq:

#### **Digest Crop-Opti with BsmBI**

|                            |                 |
|----------------------------|-----------------|
|                            |                 |
|                            | µL per reaction |
| NEB Buffer 3.1 (10X)       | 10 µL           |
| 20 µg plasmid (1271 ng/µL) | 15.7 µL         |
| BsmBI-v2                   | 4 µL            |
| Water                      | 70.3 µL         |
| Total                      | 100 µL          |

1. Incubate at 55°C overnight (16 h) -- started at 4 pm on 8/4/21.

2. Next morning, spike in 4 µL BsmBI-v2 to the tube and continue incubating at 55°C for several more hours -- from 9:30 am to 5:00 pm on 8/5/21 (total 25 h).

#### **0.7X SPRI (Ampure XP) clean up**

1. Pre-warm beads from 4°C to room temperature.
2. Add 70 µL SPRI to the tube, pipette to mix, and incubate for 10 min.
3. Wash with 150 µL 70% ethanol twice.
4. Remove ethanol and air dry for 10 min.
5. Add 89 µL water and heat 37°C for 10 min.



6. Put on magnet and transfer eluate (89  $\mu\text{L}$ ) to a new tube.

7. (Store at  $-20^{\circ}\text{C}$ )

#### **Digest Crop-Opti with Clal**

1. Add enzyme last

|  |          |                            |
|--|----------|----------------------------|
|  |          |                            |
|  |          | $\mu\text{L}$ per reaction |
|  | Cutsmart | 10 $\mu\text{L}$           |
|  | Eluate   | 89 $\mu\text{L}$           |
|  | Clal     | 1 $\mu\text{L}$            |
|  | Total    | 100 $\mu\text{L}$          |

1. Incubate at  $37^{\circ}\text{C}$  overnight (16 h) -- start at 5:30 pm on 8/5/21.

2. Next morning, spike in 1  $\mu\text{L}$  Clal to the tube and continue incubating at  $37^{\circ}\text{C}$  for several more hours. -- from 9:30 am to 4:00 pm on 8/6/21 (total 24 h).

#### **0.7X SPRI (Ampure XP) clean up**

1. Pre-warm beads from  $4^{\circ}\text{C}$  to room temperature.

2. Add 70  $\mu\text{L}$  SPRI to the tube, pipette to mix, and incubate for 10 min.

3. Wash with 150  $\mu\text{L}$  70% ethanol twice.

4. Remove ethanol and air dry for 10 min.

5. Add 80  $\mu\text{L}$  water and heat  $37^{\circ}\text{C}$  for 10 min.

6. Put on magnet and transfer eluate (80  $\mu\text{L}$ ) to a new tube.

7. Nanodrop the sample.

8. Run 20 ng cleaned product on a gel. Expected band sizes as below.

9. Store at  $-20^{\circ}\text{C}$ .

## **2 Insert Preparation: PCR**

### **2.1 Set up 3 $\times$ 50ul reactions and a "NO template" negative control.**

#### 50ul reaction

|  |                      |                    |
|--|----------------------|--------------------|
|  | Reagent              | Volume             |
|  | Water                | 30.5 $\mu\text{L}$ |
|  | 5x Phusion HF Buffer | 10 $\mu\text{L}$   |
|  | DMSO (100%)          | 1.5 $\mu\text{L}$  |

| Reagent               | Volume |
|-----------------------|--------|
| dNTPs (10 mM)         | 1 µL   |
| Primer For (10 µM)    | 2.5 µL |
| Primer Rev (10 µM)    | 2.5 µL |
| Template (0.05 pM/µL) | 1 µL   |
| HF Phusion            | 1 µL   |

- 2.2 \*Sequencing of single colonies (after ligation) gave 2/10 colonies with the same guide. Retried PCR with 1/10 and 1/100 dilution of template to see if we could get rid of this duplication. 1/10 seem to work well

PCR conditions

98C, 30s

15 cycles

98C, 15s

56C, 15s

72C, 15s

72C, 10m

7C, hold

- 2.3 Combine reactions. Run 5ul of combined reactions on a 20% acrylamide gel (Thermo Fisher Novex TBE 10% gel, cat # EC6315BOX). Run until the dye reaches the last third of the gel. Watch carefully so that it doesn't run off the gel!
- Optional: save 5ul reaction to run with the digested PCR product later, to not waste a gel with just one sample
  - Use purple gel loading dye without SDS (NEB cat # B7025S) and Low Molecular Weight DNA Ladder (NEB cat # N3233S)
  - Expected insert size is ~86bp

- 2.4 Purify remaining PCR product on one Nucleospin Gel and PCR Clean Up column
- Add 4x sample volume of PB to combined PCR product. Add 1/100th volume 3M NaOAc.
  - Bind to column
  - Wash 2x with 750ul PE
  - Dry spin
  - Elute 15ul of EB, wait 5 min, spin (use QIAGEN PB)
  - Your recovery should be about ~0.75ug to 1.5ug (if using 3× 100ul reactions). If your recovery is low, you will need to optimize your PCR before proceeding to digest

3 Insert Digest



- 3.1 Digest 1ug of insert. Incubate digest at 37C overnight  
30ul reaction  
Insert 1ug  
NEB Buffer 2.1 10x  
BlnI  
BstXI  
Water
- 3.2 Gel purification
  - i. Run digest on 20% acrylamide gel until dye is about 2/3 through the gel (about 200 V, 20-30min)
  - ii. Use purple gel loading dye without SDS (NEB cat # B7025S) and Low Molecular Weight DNA Ladder (NEB cat # N3233S)
  - iii. While gel is running prepare tubes and tips for rapid extraction
- 3.3 Pierce the bottom of a 0.5ml low binding tube (Qubit tube) with an 18.5 gauge needle and place inside a 1.5ml low binding tube. Use a clean blade or scissors to cut the tips off a 1000ul pipette tips.
  - iv. Stain gel with SYBRsafe for 30m
  - v. Excise insert (33bp)
  - vi. Place gel piece inside 0.5ml nonstick tube and proceed with rapid extraction
- 3.4 Rapid Extraction
  - a. Spin tube with gel piece at 20,000 x g for 3 min. This will force the gel through the hole and crush it into tiny pieces. Check for gel pieces in the small tube. Residual pieces should be transferred to large tube either by tapping to get the pieces to fall through the hole or by pipetting.
  - b. Add 200ul of water to gel pieces and incubate at 70C for 10 min.
  - c. Vortex gel slurry for 30 sec and use cut 1000ul tip to transfer gel mixture to Costar Spin-X column (Thermo Fisher cat # 07-200-388)
  - d. Spin tubes at 20,000 x g for 3 min to recover the elution mixture free of gel debris
  - e. Transfer eluate to new 1.5ml low binding tube
  - f. Isopropanol Precipitation: mix the following (invert 10-15x)

|                                                      |         |
|------------------------------------------------------|---------|
| Gel eluate                                           | 200ul   |
| Glycobblue (this will help you visualize the pellet) | 1.5-2ul |
| 3M NaOAc or 3M NaCl                                  | 25ul    |
  - i. Add 750ul isopropanol, mix well
  - ii. Incubate -20C 30 min up to 18h or -80C for 30 min
  - iii. Spin at 20,000 x g for 30 min at 4C ((if no precipitate, leave overnight at -20C, and continue from then)
  - iv. Remove supernatant
  - v. Wash pellet 3x with ice cold 80% EtOH

- vi. Air dry
- vii. Resuspend pellet in 15ul water or Qiagen EB
- viii. Nanodrop sample. You may observe a peak at 230. This is from the glycobblue. Concentration should be about 20-25 ng/ul with glycobblue, and 10-15 ng/ul without glycobblue. Qubit HS assay will give ~0.5-2 ng/ul (use this concentration to proceed).

#### 4 Ligation

- 4.1 Set up three reactions at different molar ratios of (vector:insert) 2:1, 1:1, 1:2. Adding too much insert can cause sgRNA concatemers (which was seen at 1:3) (2:1 ratio was the best with the 9p21 library)

- 4.2 Also set up negative control without insert  
50ul reaction (can be done in 20ul reaction).

Vector 500ng

Insert – must be calculated

Ligase buffer 10x

T5 Ligase

Water

Incubate in thermocycler at 16C over night

- 4.3 Ligation reaction clean up: EtOH precipitation

- i. Removing excess salt in the ligation reaction will help improve electroporation conditions
- ii. Bring ligation reaction volume up to 200ul with water
- iii. Add 1.5-2ul glycobblue (to help with visualizing pellet)
- iv. Add 1/10 reaction volume of 3M NaOAc. Mix well.
- v. Add 3x reaction volume of 100% EtOH. Invert 10X to mix.
- vi. Place at -20C for 30 min up to overnight or -80C for 30 min
- vii. Spin at 20,000 x g for 30 min at 4C
- viii. Remove supernatant
- ix. Wash pellet 2x with ice cold 80% EtOH
- x. Air dry
- xi. Resuspend pellet in 20ul water

#### 5 Test Transformation

- 5.1
  - add 0.5ul cleaned ligation reaction to 50ul DH5a, gently mix.
  - Incubate on ice 15-30 min
  - Heat shock at 42C for 30 sec
  - Incubate on ice for 2 min

- Add 180ul SOC and recover at 37C for 30 min
- Plate 160ul on warmed plates
- Dilute 16ul with 160ul SOC and plate (1:10 dilution)
- Incubate at 37C overnight
- Compare the plates with different molar ratios
- Pick plate with the greatest number of colonies over the control. This is the ratio you should move forward with.

- 5.2
- i. If the colonies are mixed sizes, scrape all colonies off with 1.5ml LB, miniprep and transform again with 10-50 pg DNA + 50ul cells and plate again.
  - ii. If colonies are uniform in size, go ahead and pick 10-20 colonies for minipreps
  - iii. Send to sequence with mU6F
  - iv. Make sure sequences of guides are correct and that one guide does not appear more than once
  - v. If 60-80% of sequences are perfect and match the library it is safe to proceed with the large scale transformation.

## 6 Large Scale Transformation

- 6.1 Before starting, plan out the number of plates you will need. You want 1 plate per 3666.6 oligos for roughly 30 fold coverage at 100,000 colonies per plate.

- i. For example, if we would like 100 fold coverage and have 220 guides:  
 $100 \times 220 = 44,000$  = this is the number of colonies that we want to get total  
 $44,000 / 3666.6 = 6$  = number of plates we needed for the large scale transformation

- 6.2 Pour LB carbenicillin 75ug/ml (or amp) plates

- i. Use Thermo Fisher cat # NC9372402 square plates
- ii. Each plate needs 150-200ml LB agar

- 6.3
- Thaw Mega-X cells on ice. Warm carbenicillin plates. Chill 10% glycerol on ice. Chill cuvettes (Bio-Rad cat # 165-2089) on ice.
  - In 1.5ml tubes, gently mix 20ul Mega-X cells with 100 ng ligation per plate.
  - Incubate on ice 30 min
  - Take everything to Beckman B261 for electroporation.
  - Add equal volume chilled 10% glycerol to Mega-X ligation mixture and transfer to chilled cuvette. Immediately proceed to electroporation.
  - Electroporate at 2.0 kV, 200 ohms, 25 uF in 0.1 cm cuvette (Bio-rad Gene Pulser Xcell)
  - Add 300ul SOC to the cuvette and transfer to round bottom 15ml tube.
  - Add another 300ul SOC to the cuvette and add to the first tube, for a total of 600ul for each electroporation
  - Shake at 250 rpm at 37C for 1.5 hours
  - Plate 600ul per plate
  - Incubate at 37C overnight

## 7 Scrape Plates

### 7.1 Make glass scraper with 9" glass Pasteur pipettes

- i. Sterilize narrow end of the pipette by passing it over a flame a few times slowly
- ii. To make an L shaped scraper, hold one point of the narrow end over the flame. The glass will soften and bend. Remove from heat as soon as it bends to a 90 degree angle

### 7.2 Add 15-25ml LB to each plate. Using the newly made glass scraper, scrape colonies and transfer to collection tube. Add another 5-15ml LB, scrape again and transfer to same collection tube.

- i. If you're scraping more than one plate, place collection tube on ice while collecting other plates.
- ii. Once you are done with scraping, spin culture at 4000 rpm for 20 min.
- iii. The pellet should be tight.
- iv. Pour off media and free pellet or proceed with plasmid preparation

## 8 Plasmid Preparation

### 8.1 Use Qiagen or Sigma midi, maxi, mega or giga plasmid prep kits. Make sure columns are dry prior to final elution. Wet columns will give a low recovery

### 8.2 Weigh pellet and divide between columns depending on the capacity per column, eg each column of Maxi kit can handle approximately 0.45 g of bacterial pellet.

## 9 Prepare Samples for Illumina Sequencing

### 9.1 Crispri TSS (Index) paired end 5'

- i.  
aatgatacggcgaccaccgaGATCTACACGATCGGAAGAGCACACGTCTGAACTCCAGTCACG  
CCAATgcacaaaaggaaactcacct

### 9.2 Crispri TSS Common paired end 3'

- i. CAAGCAGAAGACGGCATACGAGATaatggatcctagtactcgagaaaa

### 9.3 Index PCRs (making NGS library for sequencing)

- i. Run 3× 100ul reactions for column purification or 1× 100ul reaction if using SPRI for clean up

100ul reaction



Library 100ng  
5x Phusion HF Buffer 20ul  
DMSO (100%) 3ul  
dNTPS (10mM) 1ul  
Primer (Index) 10uM 2.5ul  
Primer (Common) 10uM 2.5ul  
HF Phusion 1ul  
Bring reaction volume up to 100ul with water

9.4 Divide reaction into 2 PCR tubes (thermocycler max volume is 50ul)

PCR conditions

98C, 30 s  
15 cycles  
98C, 15 s  
56C, 15s  
72C, 15s  
72C, 10 min  
7C, hold

9.5 Combine reactions into a single 1.5ml low binding tube and proceed with SPRI bead purification

- i. Index PCR library is ~337 bp
- ii. Add 0.6X reaction volume SPRI beads (60ul) to PCR product
- iii. Pipette mix 15x
- iv. Incubate at RT for 5 min
- v. Place on magnet stand for 5 min or until supernatant becomes clear
- vi. Transfer supernatant to a new low binding tube. Do not discard the supernatant.
- vii. Rebind your library to the beads by adding 0.9x your initial reaction volume SPRI beads (90ul)
- viii. Pipette mix 15x
- ix. Incubate at RT for 5 min
- x. Place on magnet stand for 5 min or until supernatant becomes clear
- xi. Keep beads. Remove supernatant
- xii. Wash beads by adding 1ml 80% EtOH. Incubate at RT 2 min. Remove EtOH. Repeat for a total of two washes
- xiii. Air dry. Beads will change from a glossy texture to a matte and will look dry. This can take 5-15 min.
- xiv. Elute with 50ul Qiagen EB

9.6 - Add 50ul EB to beads  
- Pipette mix 15x  
- Place on magnet until solution is clear  
- Transfer supernatant to fresh low binding tube – this is your library



## 10 Sample Validation

### 10.1 Nanodrop

- i. Expect: 30-300 ng/ul
- ii. Useful for making dilutions for running bioanalyzer

Qubit

- i. Useful for making dilutions for bioanalyzer
- ii. Useful when pooling libraries

Agilent Bioanalyzer

- i. High sensitivity kit
- ii. Load 400 ng
- iii. You should see a nice peak at 337 bp

### 10.2 Pool samples for submission

## 11 Sequencing

Provide sequencing vendor with custom sequencing primer:

oCrispri TSS seq

GTGTGTTTTGAGACTATAAGTATCCCTTGGAGAACCACCTTGTTG

## HCASM virus transduction and MOI titration with ddPCR

### 12 Goal

- Confirm MOI
- Using volumes of 2, 5, 30 and 60 ul and negative control

### 13 gDNA purification/quantification

1. Prepare 1M K562 cells in 200 µl PBS in 1.5 mL tubes. Use 2 samples of uninfected K562 Ci cells for control.
2. Total samples from large library: 8
3. Total samples from CS96: 8
4. Total uninfected samples: 2
5. Add 25 µl Proteinase K.
6. Add 200 µl Buffer B3 to the samples and vortex vigorously (10-20s).
7. Note: Vigorous mixing is important to obtain high yield and purity of DNA
8. Incubate samples at 70°C for 10-15 min.
9. Add 210 µL ethanol (96 – 100 %) to each sample and vortex again.
10. For each preparation, take one NucleoSpin Blood Column placed in a Collection Tube and load the sample.
11. Bind DNA: Centrifuge 1 min at 11,000g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000g).

12. Discard Collection Tube with flow-through.
13. 1st wash: Place the NucleoSpin Blood Column into a new Collection Tube (2 mL) and add 500 µl Buffer BW. Centrifuge 1 min at 11,000g and discard Collection Tube with the flow-through.
14. 2nd wash: Place the NucleoSpin Blood Column into a new Collection Tube (2 mL) and add 600 µl Buffer B5. Centrifuge 1 min at 11,000g. Discard flow-through and reuse Collection Tube.
15. Place the NucleoSpin Blood Column back into the Collection Tube and centrifuge 1 min at 11,000g. Residual ethanol is removed during this step.
16. Place the NucleoSpin Blood Column in a 1.5 mL microcentrifuge tube and add 100 µL preheated Buffer BE(70°C). Dispense buffer directly onto the silica membrane.
17. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000g.
18. Quantify samples using Qubit dsDNA HS assay (Quantitation Range 0.1 - 120 ng).
19. Diluted sample 1:5 to fit Qubit range
20. Dilute gDNA to 500 ng / 44 µl = 11.4ng/µl concentration.

#### 14 Nucleic acid digestion

Digest purified gDNA (500 ng) with HindIIIHF (NEB) enzyme with 10–20 units in a total reaction mixture of 50 µl at 37°C for 1 hr. HindIIIHF stock is 20,000 units/ml.

1. Make a master mix on ice.

| 1 | Reagents             | Final conc | 1X (µl)   | 17.6X (µl) |
|---|----------------------|------------|-----------|------------|
| 2 | gDNA ( ng/µl)        | 500 ng     | 44        | -          |
| 3 | Water                | -          | -         | -          |
| 4 | 10X rCutSmart Buffer | 1X         | 5         | 88         |
| 5 | HindIIIHF            | 0.4 U/µl   | 1         | 17.6       |
| 6 | <b>Total</b>         |            | <b>50</b> |            |

Aliquot 6 µl master mix into strip tubes and 44 µl of each gDNA.

1. Incubate at 37°C for 1 hr.
2. Heat kill at 80°C for 20 min.
3. Add 150 µl water to dilute gDNA samples 1:20 to obtain concentrations of 2.5 ng/µl.

#### 15 Multiplex ddPCR

Preparation of PCR reaction

- Make 50 µl primer mix at a final concentration of 22.5 µM. Combine 11.2 µl of each primer (100 µM stock) + 5.2 µl H<sub>2</sub>O.

1. hALB\_F: 5' -GCTGTCATCTCTTGTGGGCTGT -3'
2. hALB\_R: 5' -ACTCATGGGAGCTGCTGGTTC -3'
3. gRNA\_scaffold\_F: 5'- AGAGCTAAGCTGGAAACAGC -3'
4. gRNA\_scaffold\_R: 5'- GTGCCACTTGGCCTTGCTAG -3'



- Make 32  $\mu\text{l}$  TaqMan probe mix at a final concentration of 6.25  $\mu\text{M}$ . Combine 2  $\mu\text{l}$  of both probes (100  $\mu\text{M}$  stock) + 28  $\mu\text{l}$  H<sub>2</sub>O. Wrap the tube in aluminum foil.  
hALB (M12523.1) Probe: 5' -  
CCTGTCATGCCCACACAAATCTCTCC-3' (5'VIC/3'QSY)  
gRNA scaffold Probe: 5'-AGGCTAGTCCGTTATCAACTTGGCCG-3' (5'FAM/3'QSY)
- Thaw and equilibrate reaction components to RT
- Vortex ddPCR Supermix thoroughly to ensure homogeneity, as a concentration gradient may form during -20°C storage. Centrifuge briefly.

Prepare ddPCR master mix:

| 1 | Reagents                      | Final concentration | 1x volume ( $\mu\text{l}$ ) | 17.6x volume ( $\mu\text{l}$ ) |
|---|-------------------------------|---------------------|-----------------------------|--------------------------------|
| 2 | gDNA (2.5 ng/ $\mu\text{l}$ ) | 25 ng               | 10                          | -                              |
| 3 | Primers                       | 0.9 $\mu\text{M}$   | 1                           | 17.6                           |
| 4 | TaqMan Probes                 | 0.25 $\mu\text{M}$  | 1                           | 17.6                           |
| 5 | 2X ddPCR Supermix             | 1X                  | 12.5                        | 220                            |
| 6 | Water                         |                     | 0.5                         | 8.8                            |
| 7 | Total                         |                     | 25                          |                                |

Aliquot 15  $\mu\text{l}$  master mix in strip tubes and add 10  $\mu\text{l}$  gDNA. Mix by vortexing in short pulses, and centrifuge briefly.

## 16 Droplet generation on QX100

1. Bring the samples, a 96-well PCR plate, foil seal, 2 cartridges, 2 gaskets, 1 holder, p20 & p200 multichannels, tips, and reservoir to SFGF.
2. Insert the cartridge into the holder with the notch in the cartridge at the upper left of the holder. Avoid contacting the bottom surface of the cartridge with gloved hands.
3. Open the cartridge holder by pressing the latches in the middle.
4. Slide the cartridge into the right half of the holder, then drop it down.
5. Press the halves of the holder together to snap it closed.
6. Transfer 20  $\mu\text{l}$  of samples into the middle row (sample wells) using multi-channel.
7. Put 700  $\mu\text{l}$  Droplet Generation Oil for Probes (critical to not use HYPR oil) into a reservoir. Using a multichannel, dispense 70  $\mu\text{l}$  oil in the bottom row of the cartridge. Use 50% glycerol for empty wells as the chip will not run dry.
8. Hook the gasket over the cartridge holder using the holes on both sides.
9. Place the cartridge holder into the QX100 droplet generator and initiate droplet generation:
10. Open the instrument by pressing the button on the green top.



11. Place the cartridge holder into the instrument. When the holder is in the correct position, both the power (left light) and holder (middle light) indicator lights are green.
12. Press the button on the top again to close the door. This initiates droplet generation. The droplet indicator light (at right) flashes green after 10s to indicate droplet generation is in progress.
13. When droplet generation is complete, all three indicator lights are solid green. Open the door and remove the holder from the unit. Remove the disposable gasket from the holder and discard. The top wells now contain droplets, and the middle and lower wells are nearly empty with a small amount of residual oil. Keep the cartridge in the holder.

## 17 PCR amplification

### 17.1

1. Pipet 40  $\mu$ l of the contents of the top wells (the droplets) into a 96-well plate as shown below. Slowly! Avoiding the gray area since they are prone to evaporation.
  2. Important: See page 16 of their [manual](#) (or [appendix](#)) for pipetting techniques
- 
1. Cover the 96-well plate with a tip box lid and repeat droplet generation with the other two cartridges.
  2. Immediately place one sheet of pierceable foil seal (with red band facing up) over the plate.
  3. Touch the eject button on the [PX1 PCR Plate Sealer](#) instrument. Place the Plate Support Block and Sealing Frame in the drawer.
  4. Place the PCR plate on the Plate Support Block. Adjust foil if needed.
  5. Click Seal when the set temperature has been reached and the seal button is green. The drawer will close and the plate will be sealed.
  6. Seal the plate at 180°C for 5 s. Check that the wells are sealed - depressions of the wells should be visible on the foil.
  7. When the sealing is complete, the drawer will automatically open and the plate can be removed. Take the Plate Support Block outside as well.
  8. Transfer the sealed 96-well plate to a thermal cycler.
  9. Perform PCR within 30 min after completing droplet generation using a UNO96 thermal cycler with the following cycling conditions (ramp rate should be 2°C at every step).

|  |   |      |       |  |
|--|---|------|-------|--|
|  |   |      |       |  |
|  | 1 | 95°C |       |  |
|  | 2 | 95°C | 10:00 |  |

|  |   |      |       |     |
|--|---|------|-------|-----|
|  |   |      |       |     |
|  | 3 | 94°C | 0:30  | x40 |
|  | 4 | 60°C | 1:00  |     |
|  | 5 | 98°C | 10:00 |     |
|  | 6 | 4°C  | ∞     |     |

- 17.2 After thermal cycling, store the plate at 4°C overnight in the dark.
1. Next day, secure the PCR plate containing the droplets in the plate reader holder of QX200 droplet reader (Bio-Rad).
  2. Start **QuantaSoft** software.
  3. Click Setup > Template > New.
  4. Double-click on the well(s) you wish to edit. Selected wells are highlighted in gray, and the well editor appears across the top of the interface.
  5. Sample:
  6. Name: e.g. virus volume. The sample name is case sensitive; only wells with identical names can be treated as merged wells during analysis.
  7. Experiment: NAME
  8. Supermix: ddPCR Supermix for Probes (no dUTP)
  9. Target 1 (channel 1, the FAM channel):
  10. Name: gRNA
  11. Type: Ch1 Unknown
  12. Target 2 (channel 2, the VIC channel):
  13. Name: hALB
  14. Type: Ch1 Reference
  15. Click OK to save changes and close well editor.
  16. To open Experiment Editor, select Experiment > add experiment... in the well editor or select New or Edit (then double-click on an experiment name) in the Experiments window under Setup.
  17. Reference Copies = 3 (three copies for a triploid genome of K562)
  18. Click Apply or OK to save the experiment information.
  19. Save the template.
  20. Click Run in the left navigation bar to start the run. It takes 1-2 min/well.
  21. In the Run Options window, select:
  22. the dye pair in use (FAM/VIC)
  23. the direction for the wells to be read (by column or by row)
  24. Up to 1 minute later, a green circle appears next to the abort button and flashes to indicate the run is in progress. Active and analyzed wells are also highlighted in green in the plate map.
  25. Analysis



26. In the Setup window, load a plate (filename.qlps), then click Analyze to open and analyze the data.
27. The QuantaSoft software will automatically set a fluorescence threshold for each well to differentiate positive and negative droplets. Once the threshold has been set, all of the droplets with fluorescence above the threshold are considered positive, and all droplets below the threshold are negative.
28. Run analysis of results to convert positive and negative droplets to concentration.
29. Manually inspect the results of each well. Each dot on the graph represents the fluorescence detected from a single droplet. If necessary, set or reset the threshold to differentiate positive and negative droplets.
30. Open Table results to find the concentration of each target in copies/ $\mu$ l, automatically calculated using the Poisson equation.
31. CNV for each sample is reported in this table and is automatically calculated by multiplying by 3 (for triploid genome), the ratio of the target sequence to the REF concentration.
32. Take your data
33. Each run is saved as a folder
34. C drive > Quantalite > Data > Copy the entire run folder to your USB drive

## Harvesting and 10X loading of hTerT-HSCASMs w/Cas9 cells for the enhancer screens

- 18    Materials-    Chromium Next GEM Single Cell 3' GEM Kits
  - Chromium Next GEM Single Cell 3' Gel Bead Kit v3.1 PN-1000122
  - 3' Feature Barcode Kit PN-1000262
  - Chromium Next GEM Chip G Single Cell Kit PN-1000120
  - Dual Index Kit TT Set A PN-1000215
  - Dual Index Kit NT Set A PN-1000242
  - Htert 2105 HCASCMs dCas9-KRAB cells transduced with sgRNA library
  - Library construction primers
- 19    Check cell  
BFP expression for CRISPRi expression and scatter plot distribution for cell viability using flow cytometry. Take pictures of cells with and without BFP and TdTomato
- 20    Harvest cells
- 20.1
  - Refer to 10X Cell Preparation Guide
  - Ideally, input cell suspensions should contain more than 90% viable cells.
  - Cell suspensions should be loaded as soon as possible after preparation, ideally within 30 min.

- When washing and resuspending cells, always use sufficient volumes to maintain concentrations at less than 5000 cells/ $\mu$ l (5-9M/ml).
- Expect 25% cell loss during wash steps, and 30% cell loss during Flowmi.
- Make PBS with 0.04% BSA

|  | 1 | Reagent | Stock Conc | Final Conc | 6,000 $\mu$ l | 10,000 $\mu$ l |
|--|---|---------|------------|------------|---------------|----------------|
|  | 2 | PBS     | 100%       | 99.96%     | 5,976         | 9,960          |
|  | 3 | BSA     | 10%        | 0.04%      | 24            | 40             |

## 20.2 Prepare cells in a 15 ml tube.

Centrifuge cells at 200g for 3 min at RT.

Remove supernatant without disrupting the cell pellet. Count cells!

Using a wide-bore pipette tip, add 1 ml PBS with 0.04% BSA to the tube. Gently pipette mix 5 times to resuspend the cell pellet.

Centrifuge cells at 200g for 3 min.

Remove supernatant without disrupting the cell pellet.

Add 400-500  $\mu$ l (target 7-8million/ml) of PBS with 0.04% BSA to achieve the target cell concentration. Gently pipette mix 10 – 15 times or until the cells are completely suspended.

Wet the FACS tube Flowmi with PBS

Use a 40  $\mu$ m Flowmi<sup>®</sup> Tip Strainer (FACS tube strainer) to remove any remaining cell debris and large clumps. FACS sorting at FACS core, Sort into 2ml low bind tubes.

After coming from FACS, spin down 500g x5 mins, resuspend in 1000  $\mu$ l, check viability with Countess and manually count cells (countess 10 $\mu$ l of cell suspension, 10  $\mu$ l of trypan blue)

Spin down 500g x 5 mins

Make cell suspension (based on manual cell counting) of the sample such that the cell concentration is (1000) 1100-1300 cells/ $\mu$ l.

Place the cells on ice and immediately proceed to 10x Genomics Single Cell Protocol.

## 21 10X loading

### 21.1 GEM Generation & Barcoding- Follow all the steps according to the 10X protocol.

- Load XX x 10X lanes (Numbers here calculated based on a previous exp)
- Use the volumes in the red box (corresponding to ~10,000 cells loaded) as shown below to load onto lanes.
- When transferring GEMs in step 1.4, slowly aspirate 100  $\mu$ l GEM as written in the protocol. Then go back and try to recover 10-20  $\mu$ l additional volumes from ALL wells (be consistent across wells)

## 21.2 EM production, RT reaction

- Make the master mix containing RT reagent , TSO and other reagents as described in Step 1.1 of the **10x genomics protocol**.
- Use the TSO already resuspended in 80 µl 1x low TE (pure 10 mM Tris pH 8, 0.1 mM EDTA, made by SQM).
- Should be done with sorting around 10.00-10.15 and then walking plus cell suspension, so thaw reagents around at 10 am, will slack message when I know how the sorting goes.
- Add 31.9 µl into 8 tubes of a PCR 8-tube strip on ice.
- To 31.9 µl of the RT master mix directly add the following volumes of water and the cell suspension to achieve a final volume of 75 µl.
- Mix the sample by pipetting.
- Use 70 µl of the final mixture to load one well in row 1 of the chip (avoid bubbles).
- Snap the tube strip holder with the gel bead strip into a 10x vortex adapter. Vortex 30 s. Spin strip for 5 s. Place the strip back in the holder. Puncture the foil seal.
- Load 50 µl of the gel beads to row 2 (avoid bubbles).
- Load 45 µl partitioning oil to row 3.
- Finally, we have used full 2 chips and 6 wells of the 3rd chip and for the unused wells add 50% glycerol in the three rows with the same volumes as above.
- Follow the instructions of the protocol to set up the GEM reaction (~18 min).
- Place a tube strip on ice and transfer the GEMs carefully (100 µl) by releasing slowly on the tube wall.
- TRIEU+MARKUS:Setup the RT reaction in the thermal cycler for ~55 min (once 1 ones comes from chromium start RT-PCR).
- Store the reactions at -20°C.
- STOP HERE POSSIBLE, Freeze down samples at -20 for max 1 week.
- Cleanup the reactions as instructed in the 10x protocol. Final volume of the purified cDNA is 35 µl.

## 22 TAP-seq PCRs

### 22.1 All-in-one PCR reaction

- a. Use the outer primer mix from the **original TAP-seq** experiment without the BFP primer but with MYC primer
- b. Setup the PCR reaction with the following reagents (keep on ice)

|  | 1 | <b>Reagents</b>      | <b>Volume (µl)</b> | <b>Volume 6.5x (µl)</b> |
|--|---|----------------------|--------------------|-------------------------|
|  | 2 | <b>cDNA</b>          | 35                 |                         |
|  | 3 | 10 uM Partial Read 1 | 4                  | 26                      |



|  |   |                         |            |       |
|--|---|-------------------------|------------|-------|
|  |   |                         |            |       |
|  | 4 | 100 uM outer primer mix | 2.5        | 16.25 |
|  | 5 | 40 uM Partial Read 1N   | 1          | 6.5   |
|  | 6 | 100 uM Partial TSO      | 1.2        | 7.8   |
|  | 7 | KAPA HiFi               | 50         | 325   |
|  | 8 | Water                   | 6.3        | 40.95 |
|  | 9 | <b>Total</b>            | <b>100</b> |       |

- 22.2
- c. Divide 65  $\mu$ l in 6 tubes. Add 35  $\mu$ l of respective cDNA.
  - d. Run PCR1 with the following program
  - 
  - e. For each tube add 65  $\mu$ l SPRI beads (0.65x) to the reactions and incubate at room temperature for 5 min. Place the tube on a 10x magnet (high) until the solution clears.
  - f. Transfer the supernatant (gRNA) into new PCR strip tubes without disturbing the pellet (mRNA, 165  $\mu$ l). Set aside this supernatant at room temperature for gRNA amplicon cleanup for step i. below.
  - g. mRNA amplicon
  - Add 200  $\mu$ l 80% ethanol to the pellet. Wait 30 sec.
  - Remove the ethanol and repeat the wash step one more time.
  - Centrifuge briefly and place on magnet (low). Remove trace ethanol.
  - Air dry for 2 min (do not exceed 2 min as this will decrease elution efficiency).
  - Remove from the magnet. Add 41  $\mu$ l Buffer EB. Pipette mix 15x.
  - Incubate 2 min at room temperature. Place the tube on magnet (high) until the solution clears.
  - Transfer 40  $\mu$ l to a fresh tube.
  - Measure concentration by Qubit dsDNA HS assay (Typical yields are 10-100 ng).
  - Qubit concentration in 30  $\mu$ l final volume

|  |   |               |                                                        |             |                                |
|--|---|---------------|--------------------------------------------------------|-------------|--------------------------------|
|  |   |               |                                                        |             |                                |
|  | 1 | <b>Sample</b> | <b>Concentration after PCR1 (ng/<math>\mu</math>l)</b> | <b>Code</b> | <b>Dilute 2ul with x water</b> |
|  | 2 | T1            | 14.2                                                   | m1          | 12.2                           |
|  | 3 | T2            | 69.2                                                   | m2          | 67.2                           |
|  | 4 | T3            | 31.6                                                   | m3          | 29.6                           |
|  | 5 | T4            | 77.2                                                   | m4          | 75.2                           |
|  | 6 | T5            | 73.6                                                   | m5          | 71.6                           |



|  |   |    |    |    |    |
|--|---|----|----|----|----|
|  |   |    |    |    |    |
|  | 7 | T6 | 76 | m6 | 74 |

Store samples at -20°C overnight or proceed with PCR2.

## 23 gRNA amplicon cleanup

- 23.1
- Add 55 µl of SPRI beads (1.2x) to the supernatant from step f. and pipette mix 15x (pipette set to 200 µl).
  - Incubate at room temperature for 5 min.
  - Place the tube on magnet (high) until solution clears.
  - Discard supernatant.
  - Add 200 µl 80% ethanol to the pellet. Wait 30 sec.
  - Remove the ethanol and repeat the wash step one more time.
  - Centrifuge briefly and place on magnet. Remove trace ethanol.
  - Air dry for 2 min (do not exceed 2 min as this will decrease elution efficiency).
  - Remove from the magnet. Add 41 µl Buffer EB. Pipette mix 15x.
  - Incubate 2 min at room temperature. Place the tube on magnet (high) until the solution clears.
  - Transfer 40 µl to a fresh tube.
  - Make sure to use the code for the samples

|  |   |               |             |
|--|---|---------------|-------------|
|  |   |               |             |
|  | 1 | <b>Sample</b> | <b>Code</b> |
|  | 2 | T1            | g1          |
|  | 3 | T2            | g2          |
|  | 4 | T3            | g3          |
|  | 5 | T4            | g4          |
|  | 6 | T5            | g5          |
|  | 7 | T6            | g6          |

- 23.2
- Store at 4°C for up to 72 h or at -20°C for up to 4 weeks, or proceed to **Step 6. CRISPR Screening Library Construction.** (Try to take through step 6, first cleanup and then store) PCR2 (mRNA)
- Thaw inner primers, which have already been pooled here (without BFP primer).
  - Dilute all PCR1 products to 2 ng/ul (Or dilute all to lowest conc if under 2ng/ul)
  - Make the mix for PCR2 in the following way (on ice):



| 1 | Reagents                | Volume (μl)  | Volume 6.5x (μl) |
|---|-------------------------|--------------|------------------|
| 2 | PCR1 cleanup            | 10 ng (5 μl) |                  |
| 3 | 10 uM Partial Read 1    | 4            | 26               |
| 4 | 100 uM inner primer mix | 2.5          | 16.25            |
| 5 | KAPA HiFi               | 50           | 325              |
| 6 | Water                   | 38.5         | 250.25           |
| 7 | <b>Total</b>            | <b>100</b>   |                  |

- 23.3
- Divide 95 μl in 6 tubes. Add 5 μl of respective DNA (at 10ng).
  - Run PCR2 with the following program

|   |      |       |                                                    |
|---|------|-------|----------------------------------------------------|
| 1 | 95°C | 3 min |                                                    |
| 2 | 98°C | 20 s  | x 8 cycles <i>Ran 11 cycles as yields were low</i> |
| 3 | 67°C | 60 s  |                                                    |
| 4 | 72°C | 60 s  |                                                    |
| 5 | 72°C | 5 min |                                                    |
| 6 | 4°C  | hold  |                                                    |

- 23.4
- Cleanup the reactions with 150 μl (1.5x) SPRI beads. Add beads and incubate at room temperature for 5 min. Place the tube on a magnet (high) for 2 min and remove the supernatant.
  - Wash once with 300 μl of 80% Ethanol without disturbing the beads.
  - Wash twice with 200 μl of 80% Ethanol without disturbing the beads.
  - Spin down the beads, place on magnet (low) and remove traces of ethanol. Dry for 2 min
  - Elute by adding 32 μl of Buffer EB and incubate at room temp for 5 min.
  - Place the tube on a magnet (low) and transfer 30ul the eluate to a fresh tube.
  - Measure concentration by Qubit dsDNA HS assay (Typical yields are 10-100 ng).
  - Concentrations

| 1 | Sample | Concentration after PCR2 (ng/ul) | Code |
|---|--------|----------------------------------|------|
| 2 | T1     | 1.01                             | m1   |
| 3 | T2     | 2.48                             | m2   |



|  |   |    |      |    |
|--|---|----|------|----|
|  |   |    |      |    |
|  | 4 | T3 | 1.47 | m3 |
|  | 5 | T4 | 1.83 | m4 |
|  | 6 | T5 | 3.56 | m5 |
|  | 7 | T6 | 1.82 | m6 |

23.5 - Store samples at -20°C overnight or proceed with PCR3.

PCR3 (mRNA)

- Dilute all PCR2 products to 1 ng/ul.
- Make the PCR3 mix for all the above samples in the following way (on ice)

|  |   |                        |                    |                         |
|--|---|------------------------|--------------------|-------------------------|
|  |   |                        |                    |                         |
|  | 1 | <b>Reagents</b>        | <b>Volume (µl)</b> | <b>Volume 6.5x (µl)</b> |
|  | 2 | PCR2 cleanup           | 10 ng (10 µl)      |                         |
|  | 3 | 10 uM Targeted_10X     | 4                  | 26                      |
|  | 4 | 10 uM JR19_N70n primer | 2.5                |                         |
|  | 5 | KAPA HiFi              | 50                 | 325                     |
|  | 6 | Water                  | 33.5               | 217.75                  |
|  | 7 | <b>Total</b>           | <b>100</b>         |                         |

23.6 Divide 87.5 µl in 6 tubes. Add 10 µl of respective DNA and 2.5 µl of the respective N70n primer. Note down the BCs (see below)

- Run PCR3 with the following program

|  |   |      |       |            |
|--|---|------|-------|------------|
|  |   |      |       |            |
|  | 1 | 95°C | 3 min |            |
|  | 2 | 98°C | 20 s  | x 8 cycles |
|  | 3 | 60°C | 15 s  |            |
|  | 4 | 72°C | 45 s  |            |
|  | 5 | 72°C | 5 min |            |
|  | 6 | 4°C  | hold  |            |



- 23.7 - Cleanup the reaction with 150  $\mu$ l (1.5x) SPRI beads. Add beads and incubate at room temperature for 5 min. Place the tube on a magnet (high) for 2 min and remove the supernatant.
- Wash once with 300  $\mu$ l of 80% Ethanol without disturbing the beads.
  - Wash twice with 200  $\mu$ l of 80% Ethanol without disturbing the beads.
  - Spin down the beads, place on magnet (low) and remove traces of ethanol. Dry for 2 min
  - Elute by adding 32  $\mu$ l of Buffer EB and incubate at room temp for 5 min.
  - Place the tube on a magnet and transfer 30  $\mu$ l of the eluate to a fresh tube.
  - Measure final library concentration by Qubit dsDNA HS assay (Typical yields are in the 100 ng range).

|  | 1 | S# | Code | Barcode | Concentration (ng/ $\mu$ l) |
|--|---|----|------|---------|-----------------------------|
|  | 2 | 1  | m1   | N701    | 3.54                        |
|  | 3 | 2  | m2   | N702    | 3.05                        |
|  | 4 | 3  | m3   | N703    | 3.54                        |
|  | 5 | 4  | m4   | N704    | 4.45                        |
|  | 6 | 5  | m5   | N705    | 6.42                        |
|  | 7 | 6  | m6   | N706    | 6.07                        |

- 23.8 - Store samples at -20°C.
- Sequence the library like standard 10x genomics 3' RNA libraries using Illumina Read 1(28 cycles), Read 2 (90 cycles) and Sample index i7 (8 cycles).

## 24 CRISPR Screening Library Construction for gRNA

- For this follow the entire **10X genomics protocol**.

### 24.1 Setup the Feature PCR according to this table

|  | 1 | Reagents                       | Volume ( $\mu$ l) | Master mix (6.5x) |
|--|---|--------------------------------|-------------------|-------------------|
|  | 2 | DNA                            | 5                 |                   |
|  | 3 | 10 $\mu$ M Read 1N_Feature     | 6                 | 39                |
|  | 4 | 10 $\mu$ M Read 2_pTSO_Feature | 6                 | 39                |
|  | 5 | KAPA HiFi                      | 50                | 325               |
|  | 6 | Water                          | 33                | 214.5             |



|   |              |            |  |
|---|--------------|------------|--|
|   |              |            |  |
| 7 | <b>Total</b> | <b>100</b> |  |

- 24.2
- Divide 95  $\mu$ l in 6 tubes. Add 5  $\mu$ l of respective cDNA to each tube.
  - Run the following program for Feature PCR

|   |      |       |             |  |
|---|------|-------|-------------|--|
|   |      |       |             |  |
| 1 | 95°C | 3 min |             |  |
| 2 | 98°C | 20 s  | x 11 cycles |  |
| 3 | 58°C | 5 s   |             |  |
| 4 | 72°C | 5 s   |             |  |
| 5 | 72°C | 1 min |             |  |
| 6 | 4°C  | hold  |             |  |

- 24.3
- Cleanup the reaction with 100  $\mu$ l (1x) SPRI beads.
  - Add beads and incubate at room temperature for 5 min. Place the tube on a magnet (high) for 2 min and remove the supernatant.
  - Wash once with 300  $\mu$ l of 80% Ethanol without disturbing the beads.
  - Repeat wash.
  - Spin down the beads, place on magnet (low) and remove traces of ethanol. Dry for 1 min.
  - Elute by adding 31  $\mu$ l of Buffer EB and incubate at room temp for 5 min.
  - Place the tube on a magnet (low) and transfer 30  $\mu$ l of the eluate to a fresh tube.
  - Qubit the samples (the WTA/amplicon libraries were between 5 and 34.4ng/ $\mu$ l, the first PCR before custom primers were between 2.36 and 5.52ng/ $\mu$ l and the PCR post custom primers were between 2.26 and 30.4ng/ $\mu$ l) and run them on a 2% gel.

Custom gRNA PCR2-

|   |               |                                                        |             |
|---|---------------|--------------------------------------------------------|-------------|
|   |               |                                                        |             |
| 1 | <b>Sample</b> | <b>Concentration after PCR2 (ng/<math>\mu</math>l)</b> | <b>Code</b> |
| 2 | T1            | 6.88                                                   | g1          |
| 3 | T2            | 6.10                                                   | g2          |
| 4 | T3            | 2.26                                                   | g3          |
| 5 | T4            | 7.44                                                   | g4          |



|  |   |    |      |    |
|--|---|----|------|----|
|  |   |    |      |    |
|  | 6 | T5 | 11.5 | g5 |
|  | 7 | T6 | 30.4 | g6 |

- 24.4 - Setup the Sample Index PCR with 5 µl of the eluate.

|  |   |                                                    |                    |                          |
|--|---|----------------------------------------------------|--------------------|--------------------------|
|  |   |                                                    |                    |                          |
|  | 1 | <b>Reagents</b>                                    | <b>Volume (µl)</b> | <b>Master mix (6.5x)</b> |
|  | 2 | DNA                                                | 5                  |                          |
|  | 3 | 10 uM P5_Feature                                   | 6                  | 39                       |
|  | 4 | 10 uM P7_Feature_An (See barcode assignment below) | 6                  |                          |
|  | 5 | KAPA HiFi                                          | 50                 | 325                      |
|  | 6 | Water                                              | 33                 | 214.5                    |
|  | 7 | <b>Total</b>                                       | <b>100</b>         |                          |

- 24.5 - Divide 89 µl in 6 tubes. Add 5 µl of the respective DNA and 6 µl of the respective P7 primer to each tube. Use P7\_Feature\_A6 – A11.
- Run the following program for Sample index PCR

|  |   |      |       |             |
|--|---|------|-------|-------------|
|  |   |      |       |             |
|  | 1 | 95°C | 3 min |             |
|  | 2 | 98°C | 20 s  | x 12 cycles |
|  | 3 | 54°C | 30 s  |             |
|  | 4 | 72°C | 20 s  |             |
|  | 5 | 72°C | 1 min |             |
|  | 6 | 4°C  | hold  |             |

- 24.6 - Cleanup the reaction with 80 µl (0.8x) SPRI beads.
- Vortex and add the beads to each sample. Incubate at room temperature for 5 min. Place the tube on a magnet (high) for 2 min and save the supernatant (170 µl).



- Add 20  $\mu$ l (1x) SPRI beads to the supernatant. Mix and incubate at room temperature for 5 min. Place the tube on a magnet (high) for 2 min. Remove the supernatant.
  - Wash once with 300  $\mu$ l of 80% Ethanol without disturbing the beads.
  - Wash once with 200  $\mu$ l of 80% Ethanol without disturbing the beads.
  - Spin down the beads, place on magnet (low) and remove traces of ethanol. Dry for 1 min.
  - Elute by adding 32  $\mu$ l of Buffer EB and incubate at room temp for 5 min.
  - Place the tube on a magnet (low) and transfer 30  $\mu$ l of the eluate to a fresh tube.
  - Measure concentration by Qubit dsDNA HS assay. Run a bioanalyzer– diluted all samples to 1ng/ $\mu$ l to load.
- Final gRNA library concentration (Qubit dsDNA)

|  | 1 | S# | Sample | Barcode        | Concentration (ng/ $\mu$ l) |
|--|---|----|--------|----------------|-----------------------------|
|  | 2 | 7  | g1     | P7_Feature_A6  | 27.4                        |
|  | 3 | 8  | g2     | P7_Feature_A7  | 32.0                        |
|  | 4 | 9  | g3     | P7_Feature_A8  | 26.6                        |
|  | 5 | 10 | g4     | P7_Feature_A9  | 35.4                        |
|  | 6 | 11 | g5     | P7_Feature_A10 | 51.8                        |
|  | 7 | 12 | g6     | P7_Feature_A11 | 49.0                        |

#### 24.7 Bioanalyzer

- Run bioA only for samples S1-S11. (add bioA traces here)
- Dilute all the samples to 1 ng/  $\mu$ l. Load 1  $\mu$ l on the DNA high sensitivity chip and run the bioanalyzer. Complete bioanalyzer results can be found here

## Sequencing

- 25 Pool all the libraries together (1x-gRNA, 2.5x-mRNA). Sequence the pool on a NextSeq (150 cycles)