

# PETRI-seq Detailed Protocol

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## Some Notes Before Starting

- PETRI-seq cell preparation and split-pool barcoding can be done in 2 days, as detailed below. Please be aware that day 2 takes about 12 hours and does not include any stopping points prior to lysis. We will update this protocol if we later find any feasible stopping points.
- Please go through the materials listed below, and prepare reagents as needed. Specifically, we recommend diluting and annealing the barcoding oligos in the first half of day 2. We usually do this during the many centrifugations.

## Materials Needed

*click name of item for order information*

- 4% [formaldehyde](#) diluted in [1x PBS](#) (ice-cold for use on Day 1)
- PBS-RI (ice-cold for use on Day 2): [1x PBS](#) supplemented with 0.01 U/ $\mu$ L [SUPERase In RNase Inhibitor](#)
- [Lysozyme](#) and/or [lysostaphin](#) (depending on what kind of cells are being sequenced)
- 100% Ethanol (ice-cold for use on Day 2)
- TEL-RI (ice-cold for use on Day 2): 100 mM [Tris pH 8.0](#), 50 mM [EDTA](#), 0.1 U/ $\mu$ L [SUPERase In RNase Inhibitor](#) (10x more RNase inhibitor than PBS-RI)
- DNase-RI buffer (ice-cold for use on Day 2): 1.1x [DNase I reaction buffer](#), 0.1 U/ $\mu$ L [SUPERase In RNase Inhibitor](#)
- [DNase I](#)
- [Semen test hemocytometers](#)
- [Maxima H Minus Reverse Transcriptase](#)
- [dNTPs](#) (10 mM each)
- [96-well PCR plates](#)
- [Foil seals for PCR plates](#)
- 5% Tween-20
- [T4 Ligase](#)
- [BSA](#)
- 0.5 M [EDTA](#)
- 2x Lysis-T: 50 mM [EDTA](#), 400 mM [NaCl](#), 1% Triton X-100
- [Proteinase K](#)
- [AMPure XP beads](#)
- 80% ethanol (room temperature for AMPure washes)
- Magnetic rack for eppendorf or PCR tubes (we use eppendorf)
- [NEBNext Second Strand Synthesis Module](#)

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- [Nextera XT DNA Library Preparation Kit](#)
  - [Nextera Index Kit v2 Set A](#)
  - If using [EZ-Tn5 Transposase](#) instead of Nextera, then the transposase must be loaded as follows:
    1. Anneal 5 $\mu$ L of 100 $\mu$ M SB117 (5Phos/CTGTCTCTTATACACATCT) and 5 $\mu$ L of 100 $\mu$ M SB118 (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG).
    2. Dilute annealed primers 5x (to 10 $\mu$ M each).
    3. Take 10 $\mu$ L of annealed primers and add 10 $\mu$ L 100% glycerol.
    4. Combine 8 $\mu$ L of annealed primers in 50% glycerol with 2 $\mu$ L of EZ-Tn5.
    5. Incubate 30 minutes at room temperature to load the transposase. Store at -20°C until ready to use.
  - [NEBNext Multiplex Oligos for Illumina](#)
  - [Agilent Bioanalyzer HS DNA Kit](#)
  - [Qubit HS dsDNA Kit](#)
  - Single-tube oligonucleotides (see Oligonucleotide table)
    - Resuspended and annealed oligos, 400 $\mu$ M: SB84 (~40 $\mu$ L), SB81 (~80 $\mu$ L)
      - \* To anneal each oligo (to form an intramolecular hairpin), heat to 95 °C for 3 minutes then decrease the temperature to 20°C at a ramp speed of -0.1°C/second.
    - Resuspended oligos, 400 $\mu$ M: SB85 (~40 $\mu$ L), SB82 (~80 $\mu$ L)
    - Resuspended oligos, 100 $\mu$ M: SB83, 70 $\mu$ M SB80
  - 96 Round 1 RT oligonucleotides (see Oligonucleotide table)
    - Oligos can be ordered from IDT, resuspended in IDTE Buffer pH 8.0 at a concentration of 100 $\mu$ M.
    - Before use on Day 2, dilute the oligos to 10 $\mu$ M.
  - 96 Round 2 ligation oligonucleotides, annealed (see Oligonucleotide table)
    - Oligos can be ordered from IDT, resuspended in IDTE Buffer pH 7.5 at a concentration of 100 $\mu$ M
    - To anneal the oligos, add the following to each well of a 96-well plate (scaled up or down as needed - only 2.24 $\mu$ L will be used per barcoding plate):
      - \* 3.52 $\mu$ L of 100 $\mu$ M linker SB83
      - \* 2.64 $\mu$ L RNase-free water
      - \* 3.84 $\mu$ L of 100 $\mu$ M barcode oligo
    - After combining the oligos, briefly spin down the plate then anneal them by heating the plate to 95°C for 3 minutes then decreasing the temperature to 20°C at a ramp speed of -0.1°C/second.
  - 96 Round 3 ligation oligonucleotides, annealed (see Oligonucleotide table)
    - Oligos can be ordered from IDT, resuspended in IDTE Buffer pH 7.5 at a concentration of 70 $\mu$ M
    - To anneal the oligos, add the following to each well of a 96-well plate (scaled up or down as needed - only 3.49 $\mu$ L will be used per barcoding plate):
      - \* 6.6 $\mu$  of 70 $\mu$ M linker SB80
      - \* 7.2 $\mu$ L of 70 $\mu$ M barcode oligo
    - After combining the oligos, briefly spin down the plate then anneal them by heating the plate to 95°C for 3 minutes then decreasing the temperature to 20°C at a ramp speed of -0.1°C/second.

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# Cell Preparation

## Day 1

*Note:* Cells should be grown on Day 1 to the desired growth phase, which may require advanced preparation on preceding days depending on the experiment. For example, in our publication, we started *E. coli* and *S. aureus* overnights on the day before, then diluted cells into fresh media on "Day 1", and grew the cells to exponential or stationary phase. The following steps begin with cells that are in the growth phase and condition of interest.

1. Transfer  $\sim 9.6 \times 10^8$  cells (calculated roughly based on  $1 \text{ OD}_{600} = 8 \times 10^8$  cells/mL) to a centrifuge tube (room temperature) and spin at  $5,525g$  for 2 minutes at  $4^\circ\text{C}$ .
  - The number of cells can be varied, but we recommend a corresponding adjustment of volumes used throughout the cell preparation protocol. For example, if double the number of cells are taken, then fix cells in 6 mL formaldehyde instead of 3 mL (and so on for the remaining steps). In our [paper](#), we used  $\sim 2.2 \times 10^9$  cells fixed in 7 mL, which results in many more cells than needed for split-pool barcoding.
  - If combining multiple cell types for fixation (as we did with stationary-phase RFP *E. coli* and exponential-phase GFP *E. coli*), measure the OD600 of these cultures, then combine them to reach  $\sim 9.6 \times 10^8$  cells total (in the desired ratio of cell types).
2. Take out tubes immediately after spin, and keep cold on ice. Remove supernatant.
3. Resuspend cells in 3 mL of ice-cold 4% formaldehyde.
4. Rotate cell suspension for  $\sim 16$  hours at  $4^\circ\text{C}$ .
  - Using something like a [Thermo Scientific Lab Quake Rotator](#), for example.

## Day 2

\*\* Keep cells on ice as much as possible (unless otherwise indicated) throughout Day 2 and chill all reagents on ice before use \*\*

1. Centrifuge cells at  $5,525g$  for 10 minutes at  $4^\circ\text{C}$ .
2. Remove supernatant, and resuspend cells in 3 mL PBS-RI.
3. Centrifuge cells at  $5,525g$  for 10 minutes at  $4^\circ\text{C}$ .
4. Remove supernatant and resuspend cells in  $300\mu\text{L}$  PBS-RI.
5. Centrifuge cells at  $7,000g$  for 8 minutes at  $4^\circ\text{C}$ .
6. Remove supernatant and resuspend cells in  $150\mu\text{L}$  PBS-RI. Add  $150\mu\text{L}$  100% ethanol and invert tube to mix.
7. Centrifuge cells at  $7,000g$  for 8 minutes at  $4^\circ\text{C}$ .
8. Remove supernatant and resuspend cells in  $300\mu\text{L}$  PBS-RI (wash #1).
9. Centrifuge cells at  $7,000g$  for 8 minutes at  $4^\circ\text{C}$ .
10. Remove supernatant and resuspend cells in  $300\mu\text{L}$  PBS-RI (wash #2).
11. Resuspend cells in the appropriate cell wall degrading enzyme.
  - Lysozyme:  $45\mu\text{L}$  of  $100\mu\text{g}/\text{mL}$  lysozyme in TEL-RI
  - Lysostaphin:  $45\mu\text{L}$  of  $40\mu\text{g}/\text{mL}$  lysostaphin in TEL-RI
  - *Notes:* We have validated lysozyme for *E. coli* permeabilization and lysostaphin for *S. aureus* permeabilization. We have only used the enzymes separately (i.e. *E. coli* prepared alone or *S. aureus* prepared alone). The lysozyme concentration was set after titrating from 4 to  $500\mu\text{g}/\text{mL}$

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with little change in cDNA yield (measured by qPCR).

12. Permeabilize cells for 15 minutes at room temperature.
13. Centrifuge cells at 7,000g for 8 minutes at 4°C.
14. Remove supernatant and resuspend cells in 75 $\mu$ L PBS-RI (wash #1).
15. Centrifuge cells at 7,000g for 8 minutes at 4°C.
16. Remove supernatant and resuspend cells in 75 $\mu$ L PBS-RI (wash #2).
17. Centrifuge cells at 7,000g for 8 minutes at 4°C.
18. Resuspend cells in 30 $\mu$ L DNase-RI buffer.
19. Add 3 $\mu$ L DNase I
20. Incubate cells at room temperature for 30 minutes
21. Add 3 $\mu$ L DNase I stop solution
22. Heat cells to 50°C for 10 minutes with shaking at 500 rpm
  - We use a [multi-therm shaker](#) for this
  - DNase I is typically inactivated at 70°C, but we inactivate at 50°C to preserve cell integrity
23. Centrifuge cells at 7,000g for 8 minutes at 4°C.
  - The cell pellet usually looks different after DNase activation, specifically more pale and smaller than before DNase. While the pellet should remain visible, this difference in appearance is to be expected.
24. Remove supernatant and resuspend cells in 75 $\mu$ L PBS-RI (wash #1).
25. Centrifuge cells at 7,000g for 8 minutes at 4°C.
26. Remove supernatant and resuspend cells in 75 $\mu$ L PBS-RI (wash #2).
27. Centrifuge cells at 7,000g for 8 minutes at 4°C.
28. Remove supernatant and resuspend cells in 75 $\mu$ L 0.5x PBS-RI.
29. Count cells using a [semen test hemocytometer](#).
  - Determine volume,  $v_c$ , of  $3 \times 10^7$  cells

## Split-Pool Barcoding

### Day 2 (contd.)

1. Using a multi-channel pipette, aliquot 2 $\mu$ L of each Round 1 RT oligo (10 $\mu$ M) across a 96-well plate.
  - We typically seal the plate with a foil seal at this point and centrifuge the plate to ensure oligos go to bottom of tubes.
2. Prepare an RT reaction mix (total volume 960 $\mu$ L) by combining the following:
  - 240 $\mu$ L 5x Maxima H Minus RT buffer
  - 24 $\mu$ L dNTPs
  - 12 $\mu$ L SUPERase In RNase Inhibitor
  - 24 $\mu$ L Maxima H Minus Reverse Transcriptase
  - (660 -  $v_c$ )  $\mu$ L RNase-free water
  - $v_c$   $\mu$ L cells ( $3 \times 10^7$  cells)
    - If multiple cell types are to be combined at this stage, add a total of  $3 \times 10^7$  cells (e.g.  $1.5 \times 10^7$  *S. aureus* and  $1.5 \times 10^7$  *E. coli*)
3. Add 8 $\mu$ L of the RT reaction mix to each well of the 96-well plate containing the Round 1 RT oligos

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(already containing 2 $\mu$ L oligos)

- We typically aliquot 106 $\mu$ L of the RT reaction mix across an 8-strip PCR tube then use a multi-channel pipette to aliquot 8 $\mu$ L into each well of the 96-well plate. To avoid cells pelleting in the PCR plate, we do not centrifuge the plate after adding cells. Instead, we try to add cells directly to the bottom of the plate and pipette up and down a few times.
4. Incubate the PCR plate for RT as follows:
    - 50°C 10 minutes
    - 8°C 12 seconds
    - 15°C 45 seconds
    - 20°C 45 seconds
    - 30°C 30 seconds
    - 42°C 6 minutes
    - 50°C 16 minutes
    - 4°C hold
  5. Immediately after RT finishes and reaches 4°C, pool reactions into one eppendorf tube.
  6. Add 5% Tween-20 to the pooled cells, reaching a final concentration of 0.04% (125x dilution)
    - The volume of 5% Tween-20 to add should be calculated after measuring the volume of recovered pooled cells. For example, if the volume of pooled cells after RT is 830 $\mu$ L, add 6.6 $\mu$ L 5% Tween-20. We split the pooled cells (before adding Tween-20) into a few tubes so the volume in each is less than ~350 $\mu$ L. This is important for the dilution in the next step.
  7. Incubate cells in 0.04% Tween-20 on ice for 3 minutes.
  8. Add PBS-RI to bring the final concentration of Tween-20 to 0.01%.
    - For example, add a total of 2510 $\mu$ L PBS-RI to 836.6 $\mu$ L of 0.04% PBS-RI. If 836.6 $\mu$ L is split into 3 tubes of 279 $\mu$ L, then add 836.6 $\mu$ L PBS-RI to each of these tubes.
  9. Centrifuge cells at 7,000g for 10-20 minutes at 4°C. A small pellet should be visible.
    - We were routinely centrifuging for 20 minutes (and sometimes at 10,000g), but we have noticed that cells pellet sooner than 20 minutes, so it should be fine to centrifuge for less time if a pellet is visible. We also often remove most of the supernatant and then spin an additional few minutes before removing the rest of it.
  10. *During centrifugation*, aliquot 2.24 $\mu$ L of annealed Round 2 Ligation Oligos into a new 96-well plate. Briefly centrifuge the plate to collect volume at the bottom.
  11. *After centrifugation*, remove supernatant and resuspend cells in 600 $\mu$ L 1x T4 ligase buffer supplemented with 0.1 U/ $\mu$ L SUPERase In RNase Inhibitor.
  12. Prepare a ligation master mix as follows:
    - 7.5 $\mu$ L RNase-free water
    - 37.5 $\mu$ L 10x T4 ligase buffer
    - 16.7 $\mu$ L SUPERase In RNase Inhibitor
    - 5.6 $\mu$ L BSA
    - 27.9 $\mu$ L T4 ligase
    - 600 $\mu$ L resuspended cells
  13. Add 5.76 $\mu$ L of the RT reaction mix to each well of the 96-well plate containing the Round 2 ligation oligos (already containing 2.24 $\mu$ L oligos)
    - We typically aliquot 76 $\mu$ L of the ligation reaction mix across an 8-strip of PCR tubes then use a multi-channel pipette to aliquot 5.76 $\mu$ L into each well of the 96-well plate. To avoid cells pelleting in the PCR plate, we do not centrifuge the plate after adding cells. Instead, we try to add cells

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- directly to the bottom of the plate and pipette up and down a few times.
14. Incubate ligations for 30 minutes at 37°C.
  15. After the ligation, add 2 $\mu$ L Round 2 blocking mix to each well.
    - Round 2 blocking mix includes:
      - 37.5 $\mu$ L 400 $\mu$ M SB84 blocking oligo (annealed to self, see explanation in materials)
      - 37.5 $\mu$ L 400 $\mu$ M SB85 blocking oligo
      - 25 $\mu$ L 10x T4 ligase buffer
      - 150 $\mu$ L RNase-free water
    - We typically aliquot 27 $\mu$ L of blocking mix across an 8-strip of PCR tubes then use a multi-channel to aliquot 2 $\mu$ L into each well
  16. Incubate the blocking reaction for 30 minutes at 37°C.
  17. *During blocking*, aliquot 3.49 $\mu$ L of annealed Round 3 Ligation Oligos into a new 96-well plate. Briefly centrifuge the plate to collect volume at the bottom.
  18. Pool cells after blocking into a single eppendorf tube.
  19. Add the following to the pooled cells for the Round 3 ligation:
    - 46 $\mu$ L 10x T4 ligase buffer
    - 12.65 $\mu$ L T4 ligase
    - 115 $\mu$ L RNase-free water
      - In earlier iterations of PETRI-seq, we did not add a substantial amount of water to the round 3 mix. The 115 $\mu$ L recommended here amounts to only 1 $\mu$ L per 12 $\mu$ L in each well. We decided to add it, effectively diluting the reactions a bit, in order to facilitate further optimizations to the protocol. Specifically, we were able to test supplementing the reactions with PEG, which increased capture by 30% in preliminary experiments.
  20. Add 8.51 $\mu$ L of the RT reaction mix to each well of the 96-well plate containing the Round 3 ligation oligos (already containing 3.49 $\mu$ L oligos)
    - We typically aliquot 110 $\mu$ L of the ligation reaction mix across an 8-strip of PCR tubes then use a multi-channel pipette to aliquot 8.51 $\mu$ L into each well of the 96-well plate. To avoid cells pelleting in the PCR plate, we do not centrifuge the plate after adding cells. Instead, we try to add cells directly to the bottom of the plate and pipette up and down a few times.
  21. Incubate ligations for 30 minutes at 37°C.
  22. After the ligation, add 10 $\mu$ L Round 3 blocking mix to each well.
    - Round 3 blocking mix includes:
      - 72 $\mu$ L 400 $\mu$ M SB81 blocking oligo (annealed to self, see explanation in materials)
      - 72 $\mu$ L 400 $\mu$ M SB82 blocking oligo
      - 120 $\mu$ L 10x T4 ligase buffer
      - 336 $\mu$ L RNase-free water
      - 600 $\mu$ L 0.5 M EDTA
    - We typically aliquot 133 $\mu$ L of blocking mix across an 8-strip of PCR tubes then use a multi-channel to aliquot 10 $\mu$ L into each well
  23. After adding the blocking mix, pool cells (total volume ~2.1 mL) and split into 2 eppendorf tubes.
  24. Measure the volume of pooled cells, and add Tween-20 to a final concentration of 0.01%.

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25. Centrifuge cells at 7,000g for 10 minutes at 4°C.
  26. Remove supernatant and resuspend cells in 500 $\mu$ L TEL-RI + 0.01% Tween-20.
    - The cells in the two tubes can be combined here in a total of 500 $\mu$ L.
  27. Centrifuge cells at 7,000g for 10 minutes at 4°C.
  28. Remove supernatant and resuspend cells in 30 $\mu$ L TEL-RI.
  29. Count cells using a [semen test hemocytometer](#).
  30. Transfer ~10,000 cells into eppendorf tubes for lysis.
    - Up to 40,000 cells can be taken for each lysate, but we typically take 10,000 cells in case cell counts are underestimated. We usually prepare 10-20 lysates from the pooled cells at this stage to be safe in case anything goes wrong in downstream steps or to be able to sequence more cells as needed.
    - We recommend counting cells and aliquoting lysates *quickly*, as cell lysis may occur prior to making lysates and result in higher levels of cross-contamination in the final library. We also may later recommend more washes, but at this point we have only tested a single wash before lysis.
  31. Add TEL-RI to the 10,000 cells to bring the volume to 25 $\mu$ L.
  32. Add 25 $\mu$ L 2x lysis-T and 5 $\mu$ L proteinase K (20 mg/mL) to the cells in TEL-RI.
  33. Lyse cells for 1 hour at 55°C with shaking at 750 rpm.
    - We use a [multi-therm shaker](#) for this.
  34. Save lysates at -80°C.
    - We typically proceed to library preparation the following day.

## Library Preparation

### Day 3

Note that during library preparation, it is critical to carry over the entire library at each step before amplification. Any lost cDNA prior to amplification will correspond to a reduction in UMIs captured per cell.

1. Thaw lysates on ice
2. Purify lysates by adding AMPure XP beads at a 1.8x ratio (approximately 99 $\mu$ L of beads, but measure the volume of lysate to be precise). Elute in 20 $\mu$ L RNase-free water after following the purifications steps below.
3. We use the following AMPure purification protocol:
  - (a) Incubate the lysate with beads for at least 5 minutes
  - (b) Place tubes on a magnetic stand.
  - (c) Let sit for about 2 minutes to allow beads to separate.
  - (d) Once the solution is clear, remove the liquid, leaving behind the beads and being *very* careful not to remove any beads
  - (e) Add 200 $\mu$ L 80% ethanol and let sit for at least 30 seconds, keeping tubes on magnetic rack (wash #1).
  - (f) Remove ethanol.
  - (g) Add 200 $\mu$ L 80% ethanol and let sit for at least 30 seconds, keeping tubes on magnetic rack (wash #2).
  - (h) Remove ethanol.

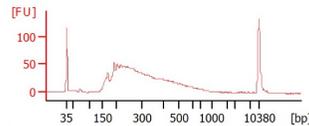
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- (i) Keeping tubes on magnetic rack with lids open, allow to dry for ~10 minutes, watching to be sure beads are not over-dried.
  - (j) Take tubes off magnetic rack, and resuspend beads in RNase-free water.
  - (k) Incubate beads in water for ~10 minutes to elute cDNA.
  - (l) Place tubes on the magnetic stand.
  - (m) Let sit for ~1 minutes, until solution is clear.
  - (n) Remove the entire volume, leaving behind all of the beads, and transfer the volume to a PCR tube. We find that it is possible to recover the entire volume while excluding beads if pipetting carefully.
4. Add the following mix to each purified lysate:
    - 14 $\mu$ L RNase-free water
    - 4 $\mu$ L NEBNext Second Strand Synthesis Reaction Buffer
    - 2 $\mu$ L NEBNext Second Strand Synthesis Enzyme Mix
  5. Incubate reaction for 2.5 hours at 16°C with heated lid off.
  6. Purify the reaction by adding 72 $\mu$ L AMPure XP beads and following the purification protocol above. Elute in 20 $\mu$ L water.
  7. Proceed to tagmentation or store the double-stranded cDNA at -20°C.
  8. For tagmentation, we use a slightly modified version of the [Nextera XT protocol](#).
  9. Add 25 $\mu$ L TD buffer and 5 $\mu$ L ATM to the purified lysate.
    - We have preliminary results using [EZ-Tn5 Transposase](#) instead of the Nextera kit. EZ-Tn5 gives a higher yield (theoretically 2x) and is less expensive. To use EZ-Tn5, replace ATM with 0.125 $\mu$ L of loaded EZ-Tn5 (see materials) and 4.875 $\mu$ L water.
  10. Briefly spin down the reaction contents.
  11. Incubate the reaction on a preprogrammed thermocycler: 5 minutes at 55°C, hold at 10°C.
  12. Once the reaction reaches 55°C, immediately add 12.5 $\mu$ L NT.
  13. Briefly spin down the reaction contents.
  14. Incubate at room temperature for 5 minutes.
  15. Add the following to the reaction:
    - 2.5 $\mu$ L i50x primer (NEB kit E7600S)
    - 2.5 $\mu$ L N70x primer (Nextera Index Kit v2)
    - 20 $\mu$ L water
    - 37.5 $\mu$ L NPM
      - In this reaction, NPM is used at a much higher rate than the other Nextera reagents. We recognize that this will lead to extra expense that we think can be avoided by using another PCR mix. We have had success using [Q5](#) (with GC enhancer) and [phusion](#). With Q5, we first diluted the tagmentation reaction (after NT) 4x and set up the PCR in a total volume of 375 $\mu$ L. With Phusion, we first diluted the tagmentation reaction (after NT) 2x and set up the PCR in a total volume of 187.5 $\mu$ L.
  16. Thermocycle until early-exponential phase of amplification:
    - 72°C 3 min
    - 95°C 30 sec
    - ~16x [until early-exponential]:

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- 95°C 10 sec
  - 55°C 30 sec
  - 72°C 30 sec
  - 72°C 5 min
  - 4°C hold
    - To determine the number of cycles to amplify, we first run 8 PCR cycles and then remove 5 $\mu$ L from the reaction. We add SYBR and rox to the 5 $\mu$ L and then amplify on our qPCR. Alternatively, SYBR could be added to the initial reaction, and the entire reaction could be run on a qPCR.

17. Purify the products with AMPure beads at a 1x ratio. Elute in 20-40 $\mu$ L.

18. Quantify the library concentration using the Qubit dsDNA HS kit and Agilent Bioanalyzer HS DNA assay.

- We usually see a concentration ~1-5 ng/ $\mu$ L on the Qubit.
- Purify again if there is a spike in the bioanalyzer trace below ~200 bp. The empty construct is 185 bp.
- The Bioanalyzer size distribution does usually skew towards short fragments. Here is an example of a library that gave good sequencing results:



- We use the molarity given by the bioanalyzer to determine the amount of sample to load for sequencing.
19. Sequence libraries using at least 75 cycles paired-end (58 cycles read 1,  $\geq$ 17 cycles read 2). We recommend about 50 million reads per PETRI-seq library.