**PCR Protocol**

Written by S. Tsui on 8/9/2022; Updated 3/15/2024

**Supplies**

* (non-PCR) pipettes (P200, P2) and tips (200uL, 2uL)
* (non-PCR) blue ice bucket
* PCR tube tray
* PCR tubes and caps, autoclaved
* 1.5 mL autoclaved tubes
* Fine tip sharpie
* MasterMix, forward and reverse primers, nuclease-free H2O, and samples
* Ethanol and KimWipes

1. Turn off the PCR hood to disable the fan.
2. Wipe down the PCR bench with 10% bleach. Allow it to sit for a moment before wiping. Let it dry completely before proceeding. Wipe down the PCR bench, pipettes (non-PCR), tube tray, sharpie, and the outsides of autoclaved tube containers with 70% ethanol. **Clean your gloves with ethanol throughout.**
3. Fill the blue ice bucket (NOT labeled for PCR) to the near top.
4. Check the Google Sheet to see what reverse primers are available to be used. Record in your notebook what reverse primer is used for each sample.
5. Thaw MasterMix on the tube tray. After thawing, place on ice. This step can also be done with the primers in Step 8 if the MasterMix thaws faster.
6. Gather 4 PCR tubes (3 replicates, 1 blank). Place the tubes on ice and label them with the reverse primer ID across. The tubes should be laid vertically. This will be enough to PCR 8 samples at once.
7. Gather 1.5 uL autoclaved tubes for each individual cocktail. Label them with the reverse primer ID. **Each sample has its own cocktail.** If you are PCRing 8 samples, you will need 8 1.5 uL autoclaved tubes.
8. Gather the forward and reverse primers, nuclease-free H2O, and samples from the -20C freezer. Keep them on ice. Make sure the primers stay on ice as much as possible, but they also need to be fully thawed.
9. Make the cocktail for each sample in a 1.5 uL autoclaved tube. **The amount of water and MM might vary depending on the MasterMix.** Flick each reverse primer before adding to make sure it is mixed. Then, return the MasterMix and primers to the -20C freezer.

|  | **Amount per tube** | **Total amount needed (x4.5)** |
| --- | --- | --- |
| **Nuclease-free H2O** | 11 uL | 49.5 uL |
| **MasterMix (MM)** | 12.5 uL | 56.25 uL |
| **Forward primer (FPr)** | 0.25 uL | 1.125 uL |
| **Reverse primer** | 0.25 uL | 1.125 uL |

1. Add 24uL of cocktail to each tube on the strip from left to right. **Before each row, push the pipette up and down to mix the cocktail.** Change the pipette tip after each row. Keep the tubes covered as much as possible and open them one at a time.
2. Add 1 uL of your sample DNA to each strip in triplicate (left to right). Then, add H2O to the final strip. Repeat for each row and change the tip after each row. Keep the tubes covered as much as possible and open them one at a time.
3. Once all the samples are added, place the PCR tubes (in tray) on the centrifuge and pulse to bring the liquid down.
4. Load the strips in the Thermocycler. It will run for ~2.5 hours. In ~2 hours, prepare your gel (see below). Place the 1st strip in the leftmost block, the 2nd strip in the middle block, and the 3rd and 4th strips in the rightmost block.
   1. Set up run, open method, select MiSeq 5pr, press verify block, select all three blocks, and start the run.
   2. The thermocycler can be left to run overnight.
   3. Store completed PCR product in the PCR freezer.
5. Discard individual cocktail solutions. Clean the PCR hood and turn on the UV light for at least 15 minutes to sterilize the PCR hood. Dump the remaining ice into the sink.
6. Update the Google Sheet with the reverse primers you used.

**Gel Protocol**

Written by S. Tsui on 8/9/2022

**Supplies**

* Gel tray and 16-well comb (green-colored tape is best)
* Label tape
* Large weigh paper
* Gel flask (on drying rack)
* Graduated cylinder (on drying rack)
* P10 pipette
* 0.9g agarose (+/- 0.05g)
* 60mL SB buffer
* 6uL sybr safe
* Tupperware and ice pack
* 1x loading dye from PCR fridge (dilute the stock with DEPC water, store in a 1.5ml tube)

1. Prepare the gel tray with the 16-well comb. Use label tape to enclose each end so the gel will not spill out as it cools.
2. Make the gel.
   1. Weigh 0.9g (+/- 0.05g) of agarose on weigh paper.
   2. Combine agarose and 60mL of SB buffer to the gel flask (on drying rack).
   3. Microwave the mixture for 1 minute. Watch the microwave carefully and make sure the mixture does not bubble over. Check after 40 seconds and stop periodically to avoid overflow.
   4. Add 6uL of sybr safe.
   5. Pour the mixture into the gel tray. Avoid bubbles. Cover it with a tupperware container to avoid any dust from falling. Place an ice pack over the tupperware and allow the gel to cool (~20 minutes).
3. Run the gel.
   1. Add 5uL of 1x dye to each sample. Mix with the pipette.
   2. Remove the label tape around the gel. Submerge it in SB buffer. Remove the comb.
   3. Pipette 5uL of dyed sample in triplicate + blank from left to right.
   4. Connect the gel and run at 160 for 10 minutes. Run to red and back to black.
   5. Review the gel in the dark room (turn off lights and use the blackout curtain) over the lightbox. To take a photo of it with your phone, use the cardboard box.

**Sodium Borate (SB) Buffer Protocol**

Written by S. Tsui on 3/15/2024

**Supplies**

* 1-L 20X SB Buffer container, with magnetic stir bar
* 47g boric acid
* 8g NaOH
* MilliQ water
* Weigh paper
* 1-L graduated cylinder
* Hot plate
* Label tape

1. Using the original 1-L bottle for 20x SB buffer (do not need to clean), add 600mL of milliQ water. It does not need to be exact.
2. On weigh paper, weigh 47g of boric acid and 8g of NaOH. Add both to the 1-L bottle.
3. Place on a hot plate and stir on low heat. Make sure the magnetic bar is centered correctly. Make sure the bar does not over stir the solution and risk it from splashing. You can add more milliQ but the solution must be under 1L in total volume.
4. Once dissolved, pour the contents into a 1-L graduated cylinder. Add milliQ until the solution reaches 1L.
5. Pour it back into the bottle and relabel the bottle with the date and your initials. Store in the fridge door.

**Diluting 20X Sodium Borate (SB) Buffer to 1X for Gels**

Written by S. Tsui on 3/15/2024

Supplies

* 2-L 1X SB Buffer container
* 20X SB Buffer (in fridge)
* MilliQ water
* 1-L graduated cylinder

1. To make 1L of 1X SB buffer, measure 50mL of 20X SB into a 1-L graduated cylinder.
2. Fill the graduated cylinder with 950mL of milliQ water. It should reach 1-L.
3. Pour into 1X SB Buffer container. Label with the date and your initials.
4. To make 2L of 1X SB buffer, repeat steps 1-3.

SB Buffer can be disposed by diluting and pouring down the sink.