**Nanopore Sequencing Workshop APS Plant Health 2024**

**SQK-RBK114.24 Rapid barcoding 24 V14 library preparation kit protocol**

1. Prepare 200ng of HMW DNA for each sample (each sample tube contains 200 ng DNA in 10 uL).
2. For each sample, mix the following:
   1. 10 uL template DNA (200ng)
   2. 1.5 uL rapid barcode (1 per sample)
3. Mix thoroughly by pipetting and spin down briefly.
4. Incubate the tubes at 30C for 2 minutes, then at 80C for 2 minutes. For this workshop, hold the tube in your hands for the 30C incubation, and place in the heat block at the front of the room for the 80C incubation. Put the tubes on ice to cool.
5. Spin down the tubes.
6. Pool all barcoded samples for your group in a clean 1.5 mL tube, noting the total volume. Each group has 4 samples, so the tube should contain 46 uL.
7. Resuspend the ampure XP beads (AXP) by vortexing.
8. Add an equal volume of ampure beads (46 uL) to the pooled sample and mix by flicking the tube.
9. Incubate for 10 minutes at room temperature, occasionally flipping the tubes in your hands.
10. Prepare at least 2 mL of fresh 80% ethanol in water (tube is provided).
11. Spin down the sample and pellet on a magnetic rack.
12. We will now wash the beads to clean the DNA.
    1. Keep the tube on the magnet and pipette off the supernatant.
    2. Keep the tube on the magnet and add 1 mL of 80% ethanol to wash the beads, without disturbing the pellet. Remove the ethanol with a pipette and discard
13. Repeat step 12 to wash the beads again.
14. Briefly spin down the tube and place it back on the magnet. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not overdry to the point of cracking.
15. Remove the tube from the rack and resuspend the pellet in 15 uL Elution Buffer (EB).
16. Incubate for 10 minutes at room temperature (alt: 37C improves HMW yield).
17. Pellet the beads on a magnet until the eluate is clear, then remove the full volume of eluate and store in a clean 1.5 mL tube.
18. Add 1 uL of diluted Rapid Adapter (dRA, prepared by the workshop TAs) to the tube and mix by flicking the tube.
19. Incubate the reaction for 5 minutes at room temperature.

The next steps to finalize and load the library will be prepared by the workshop instructors.