**Rapid Barcoding Protocol (SQK-RBK001)** Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

DNA Sample: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Flow Cell: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Active Pores: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Materials**

* 1ug gDNA per sample
* Library Loading Bead Kit (EXP-LLB001)
* NEB Blunt/TA Ligase Master Mix
* Agencourt AMPure XP beads
* Fresh 70% ethanol in nuclease free water
* 1.5mL Eppendorf DNA LoBind tubes
* 10mM Tris-HCl pH 8.0 with 50mM NaCl
* Nuclease-free water
* Gentle rotator mixer
* Magnetic rack
* Thermal cycler at 30C and 75C

**Protocol**

1. Transfer gDNA for each sample into a separate LoBind tube. Adjust final volume to 7.5uL with nuclease-free water, mix by flicking tube, and spin down.
2. Set up MinION
   1. Establish local connection.
   2. Input SampleID (ARES000X) and FlowcellID.
   3. Perform Platorm QC.
3. Library Prep
   1. **Add 2.5uL of Fragmentation Mix Barcode** (one for each sample). Mix by flicking tube and spin down.
   2. Incubate tubes at 30C for 1 minute then 75C for 1 min. Put tubes on ice.
   3. Pool all samples together into one 1.5mL LoBind tube.
   4. Vortex **AMPure XP beads** for use, and add an equal volume to pooled samples. Mix by flicking tube.
   5. Incubate on rotator mixer for 5 minutes at room temp.
   6. Spin down sample and pellet on magnet. Keep tube on magnet and pipette off the supernatant.
   7. Keep the tube on the magnet, and **wash with 200uL of 70% ethanol** without disturbing the pellet. Remove ethanol with pipette and discard.
   8. Spin down tube and place back on magnet. Remove any leftover ethanol and briefly dry.
   9. Resuspend the pellet in 10uL of **10mM Tris-HCl with 50mM NaCl**. Incubate at room temp for 2 minutes.
   10. Pellet beads on magnet until the eluate is clear and colorless.
   11. Remove the 10uL of eluate and place into new 1.5mL LoBind tube.
   12. **Add 2uL of RAD** to the barcoded DNA. Mix gently by flicking and spin down.
   13. **Add 0.2uL of Blunt/TA Ligase Master Mix** to the tube. Mix by flicking and spin down.
   14. Incubate at room temp for 5 minutes. Place library on ice.
4. Prime & Load Flow Cell
   1. Open priming port and check for bubbles. Draw back a small volume of buffer using a P1000 pipette. Set to 200uL, insert into priming port, and turn wheel to 230uL.
   2. **Mix 576uL of RBF and 624uL nuclease free water** in a LoBindtube to make priming mix. Load 800uL of priming mix into flow cell using the priming port. Wait 5 min.
   3. In a new LoBind tube, prep the library by adding **35uL RBF, 25.5uL LLB, 2.5uL nuclease-free water, and 12uL DNA library**.
   4. Open the SpotON sample port and **load 200uL of priming mix** slowly to the priming port.
   5. **Mix library** by gently pipetting up and down, then slowly **add 75uL dropwise** to SpotON sample port.
   6. Close SpotON sample port, then priming port and MinION lid.
5. Start MinION run – record active pores after start
   1. Note: turn off local base calling before start
6. Prepare flow cell for re-use with Wash Kit
   1. Open priming port and check that buffer is continuous. Some bubbles are normal are a run.
   2. **Add 150uL Solution A** through priming port. Wait 10 minutes.
   3. If adding next library, **add 150uL Solution B** through priming port. Load new library without priming flow cell. Start at step 5e; a Platform QC cannot be run if loading new library immediately.
   4. If storing for later use, slowly **add 500uL Storage Buffer** through priming port. Close priming port and remove buffer from waste section using waste ports. Store at 4-8C.