**Rapid Sequencing Protocol (SQK-RAD004)** Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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

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

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

**Materials**

* 1ug gDNA
* Flow Cell Priming Kit
* 1.5mL Eppendorf DNA LoBind tubes
* Nuclease-free water
* Thermal cycler at 30C and 80C

**Protocol**

1. Transfer gDNA into 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 (DNASample\_date) and FlowcellID.
   3. Perform Platorm QC and record active pores.
3. Library Prep
   1. Thaw SQB, LB, FLB and FLT at RT. Mix thawed reagents, FRA, and RAP. Spin down and keep on ice.
   2. **Add 2.5uL FRA** to 400ng gDNA. Mix by flicking and spin down. Incubate at 30C for 1 minute and then 80C for 1 minute. Put tube on ice.
4. Adapter Attachment
   1. **Add 1uL of RAP** to DNA tube. Mix by flicking and spin down. Incubate for 5 min at RT.
5. 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. **Add 30uL of FLT to FLB** tube to make priming mix. Mix by pipetting up and down. Load 800uL of priming mix into flow cell using the priming port. Wait 5 min.
   3. In a new LoBind tube **add 34uL SQB, 25.5uL LB, 4.5uL nuclease-free water, and 11uL DNA library**.
   4. Open the SpotON sample port and **load 200uL of priming mix** slowly.
   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.
6. Start MinION run
7. Analyze with EPI2ME while running
8. 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.