**DNA Sequencing Library Prep**

**Rapid Barcoding Prep (>400 ng DNA)**

**Materials:**

* Rapid barcoding kit (SQK-RB004)
* Magnetic tube rack
* Magnetic SPRI beads
* Fresh 70% EtOH (500 µL per sample)
* DNase-free H2O
* Thermal cycler (or two heat blocks, one at 30ºC and one at 80ºC)
* 1.5 mL microcentrifuge tubes
* DNA sample

**Procedure:**

1. Add 2.5 µL Fragmentation Mix (RB01-12)
2. Incubate for 1 minute at 30ºC (fragment and barcode DNA)
3. Incubate for 1 minute at 80ºC (inactivate fragmentase)
4. Add equal volume SPRI beads (50 µL)
5. Incubate for 5 min at room temp
6. Incubate in magnetic tube rack at 4ºC overnight
7. While sample is still on magnetic rack, pipet off supernatant
8. Add 200 µL fresh EtOH
9. Pipet off and discard supernatant
10. Repeat steps 8,9
11. Let pellet dry for ~30 seconds
12. Add 12 µL DNase-free H2O
13. Incubate at room temperature for 10 minutes
14. Pellet on magnetic rack (~10 minutes), pipet 10 µL of supernatant and add to library pool

**PCR Rapid Barcoding (<400 ng DNA):**

**Materials:**

* DNA sample(s)
* Rapid PCR Barcoding Kit (SQK-RPB004)
* PCR tubes
* 1.5 mL microcentrifuge tubes
* Thermal cycler
* Magnetic tube rack
* Magnetic SPRI beads
* Fresh 70% EtOH (500 µL per sample)
* DNase-free H2O

**Procedure:**

1. While on ice, add 5 ng DNA to PCR tube
2. Add 1 µL Fragmentation Mix
3. Incubate for 1 minute at 30ºC (fragment DNA)
4. Incubate for 1 minute at 80ºC (inactivate fragmentase)
5. Add 20 µL DNase-free H2O
6. Add 1 µL Rapid Barcode (RLB01-RLB12)
7. Add 25 µL LongAmp Taq
8. Amplify under the following conditions:

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1. Transfer to 1.5 mL microcentrifuge tube, add 50 µL SPRI beads
2. Incubate for 5 min at room temp
3. Place on magnetic tube rack, incubate at room temperature for 1 hr
4. After incubation, and while sample is still on magnetic rack, pipet off supernatant
5. Add 200 µL fresh EtOH
6. Pipet off and discard supernatant
7. Repeat steps 13,14
8. Let pellet dry for ~30 seconds
9. Add 12 µL DNase-free H2O
10. Incubate at room temperature for 10 minutes
11. Pellet on magnetic rack (~10 minutes), pipet 10 µL of supernatant and add to library pool