ILLUMINA SEQUENCING LIBRARY PREPARATION (LAST UPDATED 10/07/2015)

This protocol is modified from Baym et al. PLoS One. 2015 May 22;10(5).

For *n* samples (up to 96)

Number of rows (r) =
Number of columns (c) =
Total number of samples (n) =

		N7 barcodes											
N5 barcodes													

1. TAGMENTATION

It is critical that tagmentation steps be done cold (use freezer block) and as quickly as possible to avoid production of small fragments.

Transfer 1λ of gDNA (5 ng/ul) into PCR strips

Tagmentation Master Mix (TMM):

- Add *n* (____) x 1.2 x 1.25λ = ____ of **TD Buffer**
- Add *n* (____) x 1.2 x 0.25λ = ____ of **TDE1**
- Vortex briefly
- Distribute **1.5λ TMM per well**

Vortex briefly. Quick spin to get liquid to bottom.

Thermocycler: (“Nextera 1”)

- 55°C for 10 mins
- Hold at 4°C

2. INDEX PCR

Thaw KAPA polymerase mix and indices at room temp and vortex to mix.

Make *r* (____) Row Master Mixes (RMMs) in PCR tubes or strips:

- *c* (____) x 1.2 x 1.88λ = ____ **2x KAPA master mix**
- *c* (____) x 1.2 x 0.625λ = ____ **of each of the N5xx indices** in each of the *r* mixes
  **Note which tube gets which index
- Vortex briefly

Make *c* (____) Column Master Mixes (CMMs) in PCR tubes or strips:

- *r* (____) x 1.2 x 1.88λ = ____ **2x KAPA master mix**
- *r* (____) x 1.2 x 0.625λ = ____ **of each of the N7xx indices** in each of the *c* mixes
  **Note which tube gets which index
- Vortex briefly

Transfer **2.5λ of CMMs in each well** of the plate so that each column receives the same N7xx index

Transfer **2.5λ of RMMs in each well** of the plate so that each row receives the same N5xx index
**** Total volume = 7.5λ**

Put new caps on the strips. Vortex briefly. Quick spin to get liquid to bottom.

Thermocycler: ("Nextera 2")
- 72°C for 3 mins
- 98°C for 5 mins
- 98°C for 10 sec
- 62°C for 30 sec
- 72°C for 30 sec
- Repeat steps (3-5) 8 times
- 72°C for 5 mins
- Hold at 4°C

3. RECONDITIONING PCR

Make Reconditioning PCR Master Mix (RPMM)
- *n* (____) x 1.2 x 8.5λ = ____ **2x KAPA master mix**
- *n* (____) x 1.2 x 0.5λ = ____ **primer P1 (10 uM)****
- *n* (____) x 1.2 x 0.5λ = ____ **primer P2 (10 uM)****
  **1:10 dilution of stock primers P1 and P2 before use
- Vortex briefly

Distribute RPMM into *r* (____) tubes of a PCR strip with *c* (____) x 1.2 x 9.5λ = ____ **in each tube**

Use a multichannel to transfer **9.5λ of RPMM into each** well of the plate.
****Final PCR volume: 17λ**

Put new caps on the strips. Vortex briefly. Quick spin to get liquid to bottom.

Thermocycler: ("Nextera 3")
- 95°C for 5 mins
- 98°C for 20 secs
- 62°C for 20 secs
- 72°C for 30 secs
- Repeat steps (2-4) 5 times
- 72°C for 30 secs
- Hold at 4°C

4. PCR CLEAN UP & SIZE SELECTION

Prepare **100 mL** of **70% EtOH**
Prepare **50 ml** of **10 mM Tris-HCl (pH8)**

Prepare **Bead Plate**:
- Bring AMPure XP beads to room temp.
- Vortex the beads for 30 sec to ensure they are evenly dispersed
- Transfer **17λ of beads** into new 96-well plate

Spin PCR strips to make sure all liquid is at the bottom.

Add all 17 ul of PCR product to corresponding wells of **Bead Plate** and mix thoroughly by gently pipetting 20 times

**Use a P20 with a 1-2λ on a 20-200λ tip in order to get as much of the PCR product as possible

Incubate at room temp for 5 minutes. DNA is now on the beads

START BIOMEK (the following steps will be completed by running the program "Nextera PCR Cleanup"):

Place the plate on the magnetic stand and incubate for about 1 minute to separate beads from solution

Aspirate the clear solution from the plate and discard

** Do not disturb the beads! If beads are accidentally pipetted, resuspend them back and wait for solution to clear up and repeat.

While the plate is still on the magnetic stand, dispense 200λ **70% EtOH (100mL in reservoir)** into each well and incubate for 30 seconds at room temp

Aspirate out EtOH with out disturbing the beads and discard

Repeat for a total of 2 washes

Remove any remaining EtOH with a P10 pipette

Let the plate air dry for approximately 5 mins, do not over dry the beads

Take the plate off the magnetic stand and add 33λ **10 mM Tris-HCl (pH8) (50mL in reservoir)** to each well of the plate

Carefully resuspend the beads by mixing 20 times and incubate at room temp for 1 min, the DNA is now in solution

Place the plate back on the magnetic stand and incubate for about 1 min to separate beads from solution, wait for solution to become clear

While the plate is on the magnetic stand, aspirate the clear solution from the plate and transfer to a fresh plate

** Do not disturb the beads! If beads are accidentally pipetted, resuspend them back and wait for solution to clear up and repeat.