Tagmentation, Post Tagmentation Clean Up & Amplification

1. Label PCR plate (or equivalent).
2. Add molecular grade water to wells.
3. Add sample DNA to wells.
4. Vortex pre-warmed BLT for 10s.
5. Prepare tagmentation master mix and vortex well.
6. Add 20 µl of master mix (BLT + TB1) to each sample well.
7. Pipet well to mix.
8. Seal the plate and incubate on thermal cycler (55°C for 15 min, then held at 10°C)
9. Add 10 µl of TSB to sample wells.
10. Pipet gently to mix.
11. Seal the plate and incubate on thermal cycler (37°C for 15 minutes, held at 10°C).
12. Place the plate on the magnet for 3 minutes.
13. Remove and discard supernatant.
14. Remove from magnet and add 100 µl TWB.
15. Pipet gently to resuspend the pellets.
16. Place back on the magnet for 3 minutes.
17. Remove and discard supernatant.
18. Remove the plate from the magnet and add 100 µl of TWB.
19. Pipet gently to resuspend the pellets.
20. Place back on the magnet for at least 3 minutes.
21. Remove and discard supernatant.
22. Remove from the magnet and add 100 µl of TWB.
23. Pipet gently to resuspend the pellets.
24. Place back on the magnet for at least 3 minutes.
25. While incubating on the magnet, prepare PCR master mix (EPM + molecular grade water)
26. Vortex and quick spin master mix.
27. Remove the TWB from the bead pellets.
28. Remove all excess TWB with smaller pipet tip.
29. Remove from magnet and add 40 µl of PCR master mix, pipet to mix.
30. Add 10 µl of indices from the appropriate well of the index plate to appropriate sample well.
31. Mix by pipetting (10x minimum).
32. Seal the plate and amplify on the thermal cycler.

Post-PCR Clean Up & Pooling

1. Centrifuge the plate at 280 x g for 1 minute
2. Place on the magnet for 5 minutes.
3. Transfer 45 µl of supernatant to new wells on the PCR plate (or a MIDI plate).
4. Vortex IPB stock solution.
5. Prepare IPB master mix (IPB + molecular grade water).
6. Vortex master mix well.
7. Remove plate from the magnet and add 85 µl IPB master mix to each sample.
8. Pipet a minimum of 10x to mix.
9. Incubate for 5 minutes.
10. Place on magnet for 3 minutes.
11. During incubation, vortex stock IPB thoroughly.
12. Transfer 125 µl of supernatant to new wells.
13. Remove from magnet and add 15 µl of IPB stock solution to each sample.
14. Pipet a minimum of 10x to mix.
15. Incubate for 5 minutes.
16. Make fresh 80% EtOH
17. Place plate back on the magnet for 3 minutes.
18. Discard the supernatant.
19. While on the magnet, add 170 µl of 80% EtOH and incubate for 30 seconds.
20. Discard the supernatant.
21. While on the magnet, add 170 µl of 80% EtOH and incubate for 30 seconds.
22. Discard the supernatant, and remove the excess with a smaller volume pipet tip.
23. Allow pellets to air dry 3—5 min.
24. Remove from the magnet and add 32 µl of room temperature RSB.
25. Pipet to mix thoroughly and incubate at room temperature 2-5 min.
26. Place back on the magnet for 3 minutes.
27. Transfer 30 µl of the supernatant to new wells or a new plate (**final product**).
28. Pooling for MiSeq and MiniSeq: 2.5 µl *Campylobacter* and 5 µl other organisms. Pooling for iSeq: 2.0 µl *Campylobacter* and *Listeria*, 5 µl others. Pool may now be quantified, denatured and diluted for loading onto a sequencing instrument