Protocol – NEBNext Ultra II FS

# Step 1

1. Vortex and mix reagents

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| **Component** | **Volume** |
| DNA (500ng) diluted in a total volume of 26ul | 26ul |
| (yellow) Reaction Buffer | 7ul |
| (yellow) Enzyme Mix | 2ul |

1. Vortex (5 sec) and spin down
2. Incubate (program **Step 1**), set lid @75°C

* 15 min @37°C
* 30 min @65°C
* Hold @4°C

# Step 2

1. Vortex and mix reagents directly on the mix from step 1

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| **Component** | **Volume** |
| (red) Ligation Master Mix | 30ul |
| (red) Ligation Enhancer | 1ul |
| (red) Adaptor for Illumina | 2.5ul |

1. Vortex (5 sec) and spin down
2. Incubate in a thermocycler (program **Step 2**) @20°C for 15 min
3. Add 3ul (red) USER Enzyme to the mix
4. Vortex (5 sec) and spin down
5. Incubate (program **Step 3**) @37°C for 15 min (lid 47°C)

# Step 3

1. Add 28.5ul of 0.1X TE to the reaction mix
2. Resuspend beads
3. Add 30ul of beads to the reaction mix, vortex, and incubate @RT for 5 min
4. Place on magnet of 3 min
5. Transfer supernatant to a new tube (discard beads)
6. Add 15ul of beads to the reaction mix, vortex, and incubate @RT for 5 min
7. Place on magnet for 3 min
8. Discard supernatant (keep beads)
9. Add 200ul of EtOH 80% and incubate 30 sec
10. Discard supernatant
11. Add 200ul of EtOH 80% and incubate 30 sec
12. Discard supernatant
13. Air dry for 5 min
14. Add 17ul of 0.1X TE, vortex, and incubate @RT for 5 min
15. Place on magnet of 3 min
16. Transfer supernatant (15ul) to a new tube (tip: extract 7.5ul twice with a small tip)

# Step 4

1. Vortex and mix reagents directly on the mix from step 2

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| **Component** | **Volume** |
| (blue) Q5 Master Mix | 25ul |
| (blue) Primer i7 | 5ul |
| (blue) Universal PCR primer | 5ul |

1. Vortex (5 sec) and spin down
2. Incubate (**program Step 4**)

* 98°C for 30sec
* 98°C for 10sec + 65°C for 75sec (5 times)
* 65°C for 5 min
* Hold @4°C

# Step 5

1. Add 45ul of beads to the reaction mix, vortex, and incubate @RT for 5 min
2. Place on magnet for 3 min
3. Discard supernatant (keep beads)
4. Add 200ul of EtOH 80% and incubate 30 sec
5. Discard supernatant
6. Add 200ul of EtOH 80% and incubate 30 sec
7. Discard supernatant
8. Air dry for 5 min
9. Add 33ul of 0.1X TE, vortex, and incubate @RT for 5 min
10. Place on magnet of 3 min
11. Transfer supernatant (30ul) to a new tube (tip: extract 10ul three times with a small tip)