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Nextera Flex DNA

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John E Gorzynski[1]

[1]Stanford University

John E Gorzynski
Stanford University

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Troubleshooting

NEXTERA FLEX PROTOCOL
1. In a 96 well plate add up to 10ug of DNA and bring the volume to 30ul for ALL samples
label the plate well
2. TAGMENT
A. Make a master mix (MM) with 11ul * #samples **BLT** + 11ul * #samples **TB1**
Make sure BLT is mixed well before making master mix*
-BLT in Deli Fridge TB1 in -20
B. divide the MM equally into an 8 tube PCR strip
C. use a multichannel pipette to add 20ul of MM to each sample.
D. Pipette up and down 10 times to mix well (be gentle)
F. Put adhesive foil over the 96 well plate
G. Put in the thermal cycler (preset program-- Folder= Covid, Program=1 Tagmentation)
(lid 100C, 55C 15 min, hold at 10C)
3. POST TAGMENTATION CLEANUP
A. Add 10ul **TSB** to each reaction (use a multichannel pipette and 8 tube pcr strip)
-TSB in deli fridge (use at RT)
B. Pipette up and down 10 times
C. Seal plate with new adhesive foil
D. Put in thermal cycler (preset program-- Folder=Covid, Program=2 Cleanup)
(lid 100, 37C 15 min, hold at 10C)
E. Remove plate from thermocycler and put on magnet ~3min until solution is clear
F. Remove supernatant while plate is on the magnet
G. Remove plate from magnet and add 100ul **TWB** to each well and pipette slowly up and down to resuspend the beads
- TWB in deli fridge (use at RT)
H. Place plate back on the magnet and wait ~3 until the solution is clear
REPEAT STEPS F, G, H
4 INDICES and AMPLIFICATION
A. Prepare PCR master mix: 22ul * #samples **EPM** + 22ul * #samples WATER
Mix Well -EPM in -20
B. Remove supernatant while plate is on the magnet
C. Take the plate off of the magnet and add **40ul** PCR master mix directly to the beads and pipette up and down to resuspend.
D. Seal plate and centrifuge at 280g for 3 seconds (TQ cell culture)
E. Add 10ul indices (using a 20ul multichannel pipette puncture the foil on the indices plate and transfer 10ul of each indices to sample plate)
Pipette up and down to resuspend
F. Seal with adhesive foil and spin at 280g for ~15-30sec
G. Put in thermal cycler and run (Folder=Covid, Program=3 Amplification)
When complete, put in 4 degrees for storage

5. LIBRARY CLEANUP
1. Centrifuge plate at 280g for 1 min
2. Place plate on magnet and wait until liquid is clear
3. Transfer supernatant to a new 96 well plate (keep samples in order)
4. Add 81ul **SPB** to each sample and pipette up and down 10 times to mix

Vortex and invert SPB to mix

1. Seal and incubate at RT for 5 minutes
2. Place plate on magnet and wait until liquid is clear
3. Remove the supernatant and discard (without disturbing the beads)
4. Wash two times:
5. With plate on magnet add 200ul 80% EtOH
6. Incubate for 30 seconds
7. Without disturbing the beads remove and discard the supernatant
8. Use 20ul pipette to remove and discard the residual ETOH
9. Air Dry the beads on the magnet for 5 minutes
10. Remove plate from magnet and add 32ul RSB and pipette to resuspend beads
11. Incubate for 2min at RT
12. Place plate on magnet and wait until liquid is clear
13. Transfer ~30ul supernatant to a new 96 well plate (keep samples in order)

6. QUBIT SAMPLES-- USE High Sensitivity Kit!!!
1. Tubes QuBit tubes for each sample and TWO standards (Standard 1 and Standard 2)
2. In a 15ml falcon tube add 8800ul **Qubit HS Buffer** and 44ul**HS qubit dye** and vortex
3. Add 190ul of Buffer+Dye solution to the TWO standard tubes
4. Add 199ul of Buffer+Dye to the sample tubes
5. Add 10ul of Standard 1 to the Standard 1 tube and Vortex
6. Add 10ul of Standard 2 to the Standard 2 tube and Vortex
7. Add 1ul of Samples to each Sample tube and Vortex
8. Read standards on QuBit to generate a standard curve
9. Read Samples

**Record samples values in ng/ul

**Make sure you set the dilution factor to 1ul!!!

***For samples >600ng/ML please nanodrop to get a concentration

7. Dilute to 4nanoMolar assuming 300bp average library size (calculations on spreadsheet)

STOP

8. Pool 1ul of each sample
9. Tapestation
10. Run MySeq