Flow Cell Number: .....

Metagenome의 경우 PCR 후 PCR product로 진행, 바코드 없는 프라이머 사용

Before start checklist

Materials

- [ ] Native Barcoding Kit 24 V14 (SQK-NBD114.24)
- [ ] 200 fmol (130 ng for 1 kb amplicons) DNA per sample to be barcoded

http://molbiol.ru/eng/scripts/01_07.html
DNA 길이 별 fmol 농도 계산
DNA 농도는 나노드롭 사용

- 200 fmol (194.7 ng for 1.5 Kb)
- 200 fmol (64.9 ng for 0.5 Kb)
- 200 fmoll (32. 45 ng for 0.25 Kb)

Consumables

- [ ] NEB Blunt/TA Ligase Master Mix (NEB, Cat # M0367)
- [ ] NEBNext Ultra II End repair/dA-tailing Module (E7546)
- [ ] NEBNext Quick Ligation Module (E6056)
- [ ] Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Cat # 0030129504) with heat seals
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 2 ml Eppendorf DNA LoBind tubes
- [ ] Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- [ ] Freshly prepared 80% ethanol in nuclease-free water
- [ ] Qubit™ Assay Tubes (ThermoFisher, Q32856)
- [ ] Qubit dsDNA HS Assay Kit (ThermoFisher Q32851)
- [ ] (Optional) Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, Cat # AM2616)

Equipment

- [ ] Hula mixer (gentle rotator mixer)
- [ ] Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, # 11766427)
- [ ] Magnetic rack suitable for 0.2 ml thin-walled PCR tubes or 96-well plates
- [ ] Microfuge
- [ ] Vortex mixer
- [ ] Thermal cycler
- [ ] Ice bucket with ice
- [ ] Timer
- [ ] Eppendorf 5424 centrifuge (or equivalent)
- [ ] Qubit fluorometer (or equivalent for QC check)
- [ ] Pipettes and pipette tips P2, P10, P20, P100, P200, P1000

PCR 과정 중 생긴 nick과 polyA를 붙여주는 과정 20min

INSTRUCTIONS	NOTES/OBSERVATIONS
[1] End-prep	Base colling 과정에서 성공적으로 일어났는지 확인하기 위한 샘플 (λ phage genome)

- [ ] Thaw the AMPure XP Beads (AXP) and DNA Control Sample (DCS) at RT and mix by vortexing. Keep the beads at RT and store the DNA Control Sample (DCS) on ice.

Prepare the NEBNext Ultra II End Repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice:

- [ ] Thaw all reagents on ice.
- [ ] Flick and/or invert the reagent tubes to ensure they are well mixed.
  Note: Do not vortex the Ultra II End Prep Enzyme Mix.
- [ ] Always spin down tubes before opening for the first time each day.
- [ ] The Ultra II End Prep Buffer may have a little precipitate. Allow the mixture to come to RT and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for 30 seconds to solubilise any precipitate.