Flow Cell Number: ..... DNA Samples: .....

| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|

IMPORTANT

- [ ] Do not vortex the NEBNext Ultra II End Prep Enzyme Mix.

IMPORTANT

- [ ] It is important that the NEBNext Ultra II End Prep Reaction Buffer is mixed well by vortexing.

- [ ] Dilute your DNA Control Sample (DCS) by adding 105 µl Elution Buffer (EB) directly to one DCS tube. Mix gently by pipetting and spin down.

처음 키트 사용시에만 만들고 그 뒤부터는 만들어 진 것 사용 35 ul + 105 EB buffer (140회 분량)

- [ ] In clean 0.2 ml thin-walled PCR tubes (or a clean 96-well plate), aliquot 200 fmol (130 ng for 1 kb amplicons) of DNA per sample.

DNA 길이 별 fmol 농도 계산해서 만들기
예) 1.5 Kb 200 fmol 194.7 ng 이 11.5ul 필요하므로 17 ng/ul 농도로 넣어주기 (이때 농도 계산은 **나노드롭** 사용)

- [ ] Make up each sample to 11.5 µl using Nuclease-free water. Mix gently by pipetting and spin down.

Combine the following components per tube/well: total(15ul) PCR tube에 넣기

- [ ] 11.5 µl 200 fmol (130 ng for 1 kb amplicons) amplicon DNA
- [ ] 1 µl Diluted DNA Control Sample (DCS)
- [ ] 1.75 µl Ultra II End-prep Reaction Buffer
- [ ] 0.75 µl Ultra II End-prep Enzyme Mix

- [ ] Ensure the components are thoroughly mixed by pipetting. Close the tubes (or seal the plate) and spin down in a centrifuge.
- [ ] Using a thermal cycler, incubate at 20°C for 5 minutes and 65°C for 5 minutes. PCR 기계에서 바로 맞춰하기
- [ ] Transfer each sample into a clean 1.5 ml Eppendorf DNA LoBind tube. AXP과정 모두 PCR tube 사용
- [ ] Resuspend the AMPure XP beads (AXP) by vortexing.
- [ ] Add 15 µl of resuspended AMPure XP Beads (AXP) to each end-prep reaction and mix by flicking the tube. 1st(1X)
- [ ] Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.
- [ ] Prepare 500 µl of fresh 80% ethanol in Nuclease-free water.
- [ ] Spin down the samples and pellet the beads on a magnet until the eluate is clear and colourless. Keep the tubes on the magnet and pipette off the *supernatant.

*For trouble shooting
1) 1st 상층액 보관
2) bead 버리지 않고 보관
-> 37도 incubation
-> supernatant + bead reaction 다시 한번 진행

- [ ] Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.
- [ ] Repeat the previous step. 크랙이 생기지 않도록 주의!! 긴 단편의 DNA는 손상을 받을 수 있음.
- [ ] Briefly spin down and place the tubes back on the magnet for the beads to pellet. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellets to the point of cracking.

pellet 마르지 않도록(무광상태의 경우, DNA 깨질 수 있음, long read 끊어지지 않도록 주의)

- [ ] Remove the tubes from the magnetic rack and resuspend the pellet in 10 µl Nuclease-free water. Spin down and incubate for 2 minutes at RT.
- [ ] Pellet the beads on a magnet until the eluate is clear and colourless.