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

INSTRUCTIONS	NOTES/OBSERVATIONS
In a 1.5 ml Eppendorf LoBind tube, mix in the following order: total(50ul) ☐ 30 µl Pooled barcoded sample ☐ 5 µl Native Adapter (NA) ☐ 10 µl NEBNext Quick Ligation Reaction Buffer (5X) ☐ 5 µl Quick T4 DNA Ligase	
☐ Ensure the reaction is thoroughly mixed by gently pipetting and spin down briefly.	
☐ Incubate the reaction for 20 minutes at RT.	
IMPORTANT ☐ The next clean-up step uses Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) rather than 80% ethanol to wash the beads. The use of ethanol will be detrimental to the sequencing reaction.	80% EtOH 사용하지 않음(adapter ligation을 EtOH 방해할 수 있음, motor protein 씻겨나감)
☐ Resuspend the AMPure XP Beads (AXP) by vortexing.	
☐ Add 20 µl of resuspended AMPure XP Beads (AXP) to the reaction and mix by pipetting. 3rd (0.4X)	
☐ Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT. 훌라믹서가 없을 시 손으로 inverting	
☐ Spin down the sample and pellet on the magnetic rack. Keep the tube on the magnet and pipette off the supernatant.	
Metagenome의 경우 SFB 사용함.	
☐ Wash the beads by adding either 125 µl Long Fragment Buffer (LFB) or Short Fragment Buffer (**SFB**). Flick the beads to resuspend, spin down, then return the tube to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard.	
☐ Repeat the previous step.	
☐ Spin down and place the tube back on the magnet. Pipette off any residual supernatant.	
☐ Remove the tube from the magnetic rack and resuspend pellet in 15 µl Elution Buffer (EB).	
☐ Spin down and incubate for 10 minutes at 37°C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution.	
☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.	
☐ Remove and retain 15 µl of eluate containing the DNA library into a clean 1.5 ml Eppendorf DNA LoBind tube.	
3rd ☐ Quantify 1 µl of eluted sample using a Qubit fluorometer. 농도 측정 10-20 fmol 이상되어야 함. 더 큰경우 희석이 가능하지만 낮은 경우 이전 step으로 돌아가 다시 진행함.	
☐ Make up the library to 12 µl at **10-20 fmol.**	
IMPORTANT 8kb 기준, 약 50~100ng) ☐ We recommend loading 10 - 20 fmol of this final prepared library onto the R10.4.1 flow cell.	
The prepared library is used for loading onto the flow cell. Store the library on ice until ready to load.	실험 Stop point (3) 4℃ for 4 weeks, 3개월 이상의 long term storage (-80℃)