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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Keep the tube on the magnetic rack and wash the beads with 700 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.	
☐ Repeat the previous step.	
☐ Spin down and place the tube back on the magnetic rack. Pipette off any residual ethanol. Allow the pellet to dry for ~30 seconds, but do not dry the pellet to the point of cracking.	
☐ Remove the tube from the magnetic rack and resuspend the pellet in 35 µl Nuclease-free water by gently flicking.	
☐ Incubate for 10 minutes at 37°C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution. 37도 10분간 incubation, 2분마다 tapping	
☐ Pellet the beads on a magnetic rack until the eluate is clear and colourless. Remo	
☐ ve and retain 35 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. 다음 step에서 30ul 필요하므로 갈색 AXP가 딸려오지 않도록 주의	
2nd Quantify 1 µl of eluted sample using a Qubit fluorometer. DNA loss가 생기는 지 매 step마다 농도 측정하여 확인, loss가 심하면 sample을 버리지 않고 현재 step을 다시해야함.	
Take forward the barcoded DNA library to the adapter ligation and clean-up step. However, at this point it is also possible to store the sample at 4°C overnight.	실험 Stop point (2)
[3] Adapter ligation and clean-up ← 시퀀싱을 위한 어뎁터를 붙여주는 과정 50min. 바코드 붙인 샘플을 동일한 농도로 pooling 후 진행. Adaptor ligation 후 최종 10-20 fmol이 나와야 하므로 loss가 안생기도록 주의하기. 농도가 낮은경우 2step에서 다시 진행.	
IMPORTANT ☐ The Native Adapter (NA) used in this kit and protocol is not interchangeable with other sequencing adapters.	
Prepare the NEBNext Quick Ligation Reaction Module according to the manufacturer's instructions, and place on ice: ☐ Thaw the reagents at RT. ☐ Spin down the reagent tubes for 5 seconds. ☐ Ensure the reagents are fully mixed by performing 10 full volume pipette mixes.	
IMPORTANT ☐ Do not vortex the Quick T4 DNA Ligase.	
☐ Spin down the Native Adapter (NA) and Quick T4 DNA Ligase, pipette mix and place on ice.	
☐ Thaw the Elution Buffer (EB) at RT, mix by vortexing, spin down and place on ice.	
IMPORTANT Metagenome의 경우 SFB 사용함. Depending on the wash buffer (LFB or **SFB**) used, the clean-up step after adapter ligation is designed to either enrich for DNA fragments of >3 kb, or purify all fragments equally. ☐ To enrich for DNA fragments of 3 kb or longer, use Long Fragment Buffer (LFB) ☐ To retain DNA fragments of all sizes, use Short Fragment Buffer (SFB)	
☐ Thaw either Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) at RT, mix by vortexing, spin down and place on ice.	