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

INSTRUCTIONS	NOTES/OBSERVATIONS
Remove and retain 10 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Dispose of the pelleted beads	
1st Quantify 1 µl of each eluted sample using a Qubit fluorometer.	
Take forward an equimolar mass of samples to be barcoded and pooled forward into the native barcode ligation step. However, at this point it is also possible to store the sample at **4°C overnight.**	실험 Stop point (1)
[2] Native barcode ligation ← 바코드를 붙여주는 과정 60min (NB01-24까지 있음. 한 flowcell에 중복되게 사용하지 않기)	
Prepare the NEB Blunt/TA Ligase Master Mix 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.	
☐ Thaw the EDTA at RT, mix by vortexing, spin down and place on ice.	
☐ Thaw the Native Barcodes (NB01-24) required for your number of samples at RT. Individually mix the barcodes by pipetting, spin down, and place them on ice.	
☐ Select a unique barcode for each sample to be run together on the same flow cell. Up to 24 samples can be barcoded and combined in one experiment.	
In clean 0.2 ml PCR-tubes or a 96-well plate, add the reagents in the following order per well: total(20ul) ☐ 7.5 µl End-prepped DNA ☐ 2.5 µl Native Barcode (NB01-24) 바코드 하나에 F/R 동시에 들어가 있음. 한 샘플당 바코드 하나 ☐ 10 µl Blunt/TA Ligase Master Mix	
☐ Ensure the reaction is thoroughly mixed by gently pipetting and spin down briefly.	
☐ Incubate for 20 minutes at RT.	
☐ Add 2 µl of EDTA to each well and mix thoroughly by pipetting and spin down briefly.	adding EDTA to stop the reaction
☐ Pool the barcoded samples in a 1.5 ml Eppendorf DNA LoBind tube.	
☐ Resuspend the AMPure XP Beads (AXP) by vortexing.	
☐ Add AMPure XP Beads (AXP) to the pooled reaction, and mix by pipetting for a 0.4X clean. 바코딩할 샘플당 8ul AXP 첨가	
☐ Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT. 2nd 훌라믹서가 없을 시 손으로 inverting	
☐ Prepare 2 ml of fresh 80% ethanol in Nuclease-free water.	
Spin down the sample and pellet on a magnet for 5 minutes. Keep the plate on the magnetic rack until the eluate is clear and colourless, and pipette off the supernatant.	