Nano pore 원리

PCR product

End-prep

이 과정을 통해 polyA 를 붙이고
인산기를 붙여줌

Ligation of barcodes

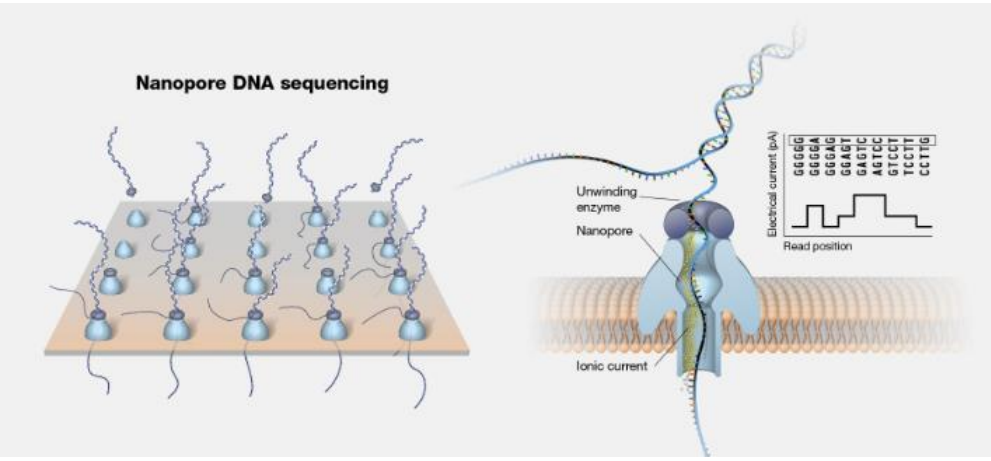
바코드와 TA ligation

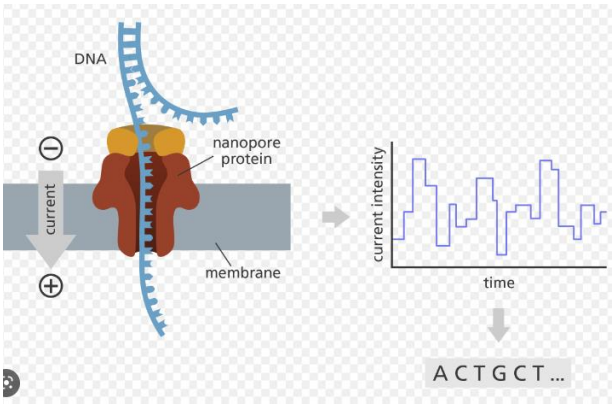
Ligation of sequencing adapters

바코드를 붙인 샘플을 pooling 후
(이때 농도 동일) Adapter ligation

10-20 fmol을 flowcell 에 로딩

Loading





Polymerase를 이용한 시퀀싱방법이 아닌 ATGC의 base에 따른 전위차에 의한 시퀀싱이 일어나는 것이기 때문에 샘플의 농도가 매우 중요함.

농도 측정시 nano drop을 사용.

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.

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.

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.	

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.	

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

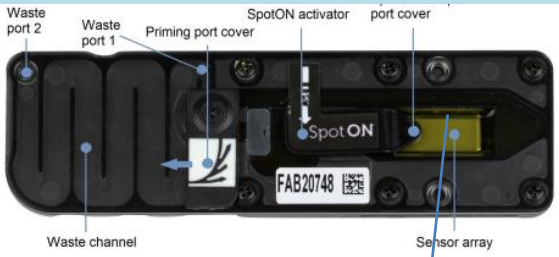
INSTRUCTIONS	NOTES/OBSERVATIONS
In a 1.5 ml Eppendorf LoBind tube, mix in the following order: total(50ul) ☐ 30 µl Pooled barcoded sample ☐ 5 µl Native Adapter (NA) ☐ 10 µl NEBNext Quick Ligation Reaction Buffer (5X) ☐ 5 µl Quick T4 DNA Ligase	
☐ Ensure the reaction is thoroughly mixed by gently pipetting 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℃)

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

INSTRUCTIONS

10min, Flow cell loading 전 QC 체크 후 (20min 소요) 포어가 800개 이상일때 진행하기

[4] Priming and loading the SpotON flow cell



IMPORTANT

☐ Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).

Using the Library Solution

☐ Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and one tube of Flow Cell Flush (FCF) at RT. Mix by vortexing and spin down.

IMPORTANT

☐ For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.

IMPORTANT

☐ We do not recommend using recombinant BSA.

매우 민감하며, 기포가 생기지 않도록 주의 하기, 열에 약하므로 샘플 로딩 후 티슈를 위에 깔고 아이스 팩 위에서 진행하기

To prepare the flow cell priming mix with BSA, add the following reagents directly to the tube of Flow Cell Flush (FCF), and mix by inverting the tube and pipette mix at RT:

- ☐ 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml
- ☐ 30 µl Flow Cell Tether (FCT)
- ☐ 1,205 µl Final total volume in Flow Cell Flush (FCF) tube

☐ Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.

☐ Slide the priming port cover clockwise to open the priming port.

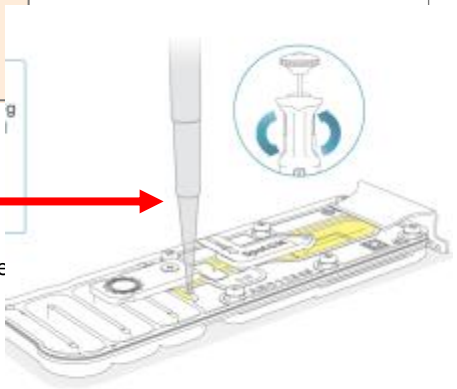
IMPORTANT

☐ Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles (a few µl):

- ☐ Set a P1000 pipette to ~~200 µl~~ 800ul
- ☐ Insert the tip into the priming port
- ☐ Turn the wheel until the dial shows ~~220-230 ul~~, to draw back 20-30 ul, or until you can see a small volume of buffer entering the pipette tip 820-830ul

액체가 조금 나왔다 싶으면 stop !!! 기포가 들어가면 안됩니다.



Note: Visually check that there is continuous buffer from the priming port across the sensor array.

☐ Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below.

☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

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

INSTRUCTIONS	NOTES/OBSERVATIONS
IMPORTANT ☐ The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	
In a new tube, prepare the library for loading as follows: total(75ul) ☐ 37.5 µl Sequencing Buffer (SB) ☐ 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using ☐ 12 µl DNA library	바로 flowcell에 로딩하기, buffer가 adapter 와 섞이는 순간 소모됨.
Complete the flow cell priming: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles.	기포 생기지 않도록 주의
☐ Mix the prepared library gently by pipetting up and down just prior to loading.	
☐ Add 75 µl of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.	한번에 다 넣지않고 방울방울 떨어뜨리기
☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION or GridION device lid.	
[5] Ending the experiment	
☐ After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Wash Kit instructions and store the washed flow cell at 2-8°C, OR ☐ Follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore.	Flowcell은 고가의 장비이므로 최대 4번까지 재사용할 수 있으므로 워시 후 4도씨 보관. Washing 프로토콜 참고 보관시 뒤집어두거나 세워주면 안됨.
IMPORTANT ☐ If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in the online version of this protocol.	

Flowcell loading 참고 영상

https://www.google.com/search?q=flow+cell+loading&rlz=1C1GCEU_koKR1050KR1050&sxsrf=APwXEdcOGDGoHVJYKuGDL0Jzz85DroBrig%3A1682920595061&ei=k1RPZKWhA_zO2roPzvmnoAI&oq=flowcell+loa&gs_lcp=Cgxnd3Mtd2l6LXNlcnAQARgAMgYIABAeEA0yCAgAEAUQHhANMggIABAFEB4QDTIICAAQBRAeEA0yCAgAEAUQHhANMggIABAIEB4QDTIKCAAQCBAeEA0QDzIICAAQCBAeEA06BwgjEOoCECc6BAgjECc6BwgjEIoFECc6EQguEIAEELEDEIMBEMcBENEDOggIABCABCxAzoLCC4QgAQQsQMQgwE6DgguEIAEELEDEMcBENEDOgcIABCKBRBDOgsILhCABBCxAxDUAjoFCAAQgAQ6CAguEIAEELEDOgUILhCABDoHCAAQgAQQCjoICAAQgAQQywE6DgguEIAEEMcBEK8BEMsBOgoIABCABBAKEMsBOgYIABAeEAo6CAgAEB4QDxAKOggIABAFEB4QCjoGCAAQCBAeSgQIQRgAULkEWMAiYL0waAJwAXgAgAGCAYgBhwySAQQwLjEzmAEAoAEBsAEKwAEB&sclient=gws-wiz-serp#fpstate=ive&vld=cid:58e0f0df,vid:Pt-iaemrM88

어느정도 읽혔는지 실시간으로 보이기 때문에 목표치 까지 시퀀싱이 일어났을경우 stop.
나노포어의 경우 지정한 시간동안 돌아가기 때문에 stop하지 않고 계속 돌아갈경우 pore 손상이 일어나 flowcell 재사용이 어려움.
Washing을 하더라도 0.1%로 제거되지 않은 DNA가 flowcell에 남아있기 때문에
한 flowcell을 여러 번 사용할 때는 바코드 번호가 겹치지 않게 하는 것이 중요함.
사용하지 않은 바코드 넘버 사용하기

Flow Cell Wash Kit (EXP-WSH004)

Version: WFC_9120_v1_revI_08Dec2020
Last update: 12/04/2023

Flow Cell Number: .....

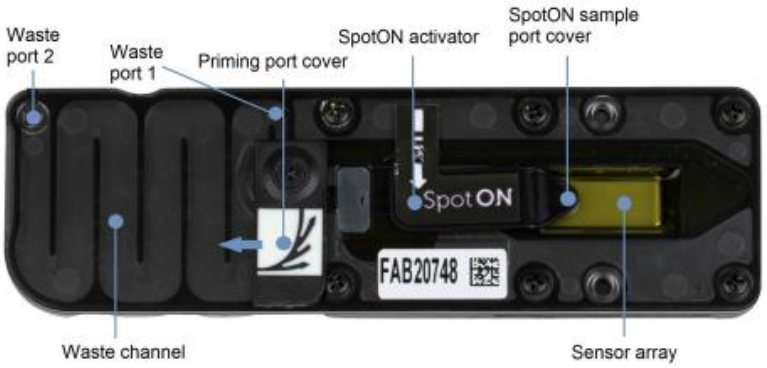
Before start checklist

Materials

Consumables

Equipment

- [ ] Flow Cell Wash Kit (EXP-WSH004)
- [ ] Flow cell priming reagents available in your sequencing kit or in the following kits:
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003) or Sequencing Auxiliary Vials (EXP-AUX002 or EXP-AUX001)
- [ ] Flow Cell Priming Kit (EXP-FLP004) or Flow Cell Priming Kit (EXP-FLP002)
- [ ] Ice bucket with ice
- [ ] Pipettes and pipette tips P20, P1000



INSTRUCTIONS

Flushing a MinION/GridION Flow Cell

Preparation to run the washing procedure

- [ ] Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.

Dnase 1 포함. 따라서 1시간 엄수

- [ ] Thaw one tube of Wash Diluent (DIL) at RT.
- [ ] Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, spin down briefly and place on ice.

In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:

- [ ] 2 µl Wash Mix (WMX)
- [ ] 398 µl Wash Diluent (DIL)
- [ ] Mix well by pipetting, and place on ice. Do not vortex the tube.
- [ ] Stop or pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.
- [ ] Before removing the waste fluid, ensure that the flow cell priming port cover and SpotON sample port cover are closed, as indicated in the figure below.

Waste port 앞까지만 제거하고 버블 안생기도록 주의

- [ ] Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.

IMPORTANT

- [ ] It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

- [ ] Rotate the flow cell priming port cover clockwise so that the priming port is visible.

After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

액체가 조금 나왔다 싶으면 stop !!! 기포가 들어가면 안됩니다.

- [ ] Set a P1000 pipette to 200 µl.
- [ ] Insert the tip into the flow cell priming port.
- [ ] Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip.
- [ ] Visually check that there is continuous buffer from the flow cell priming port across the sensor array.

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

INSTRUCTIONS	NOTES/OBSERVATIONS
IMPORTANT ☐ Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.	
☐ Load 400 µl of the prepared Flow Cell Wash Mix into the flow cell priming port, avoiding the introduction of air.	
☐ Close the flow cell priming port and wait for 60 minutes.	
☐ Before removing the waste fluid a second time, ensure that the flow cell priming port cover and SpotON sample port cover are closed, as indicated in the figure below.	
☐ Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
Follow one of the two options described in the next steps of the prot	바로 사용할 경우 QC 체크 필요. 하지만 4도에 어느정도 보관 후 사용하는 것을 추천 (온도가 올라가서 pore에 무리가 올 수 있으므로)
To run a second library on a MinION/GridION flow cell straight away	
IMPORTANT ☐ The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading a subsequent library. However, the first pore scan after a sequencing run has started will report the number of nanopores available.	
☐ To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of your library preparation protocol.	
IMPORTANT ☐ When priming a flow cell after a nuclease wash with the Flow Cell Wash Kit, it is vital to wait five minutes between the priming mix flushes to ensure effective removal of the nuclease.	
To store the MinION/GridION flow cell for later	4도에 보관할시 바로 이 step
Storage Buffer (S) can be used to flush flow cells for storage for later use or to check number of available nanopores before loading another library.	
☐ Thaw one tube of Storage Buffer (S) at RT.	
☐ Mix contents thoroughly by pipetting and spin down briefly.	
☐ Rotate the flow cell priming port cover clockwise so that the priming port is visible.	
After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles: ☐ Set a P1000 pipette to 200 µl. ☐ Insert the tip into the flow cell priming port. ☐ Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip. ☐ Visually check that there is continuous buffer from the flow cell priming port across the sensor array.	액체가 조금 나왔다 싶으면 stop !!! 기포가 들어가면 안됩니다.

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Slowly add 500 µl of Storage Buffer (S) through the flow cell priming port. ☐ Close the priming port. ☐ Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area. IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels. ☐ The flow cell can now be stored at 4-8°C.	
When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to RT for ~5 minutes.	
IMPORTANT After performing a flow cell wash, we recommend using the first pore scan to check number of available nanopores. ☐ stop your sequencing run, prime your flow cell and load the library and start a new sequencing run ☐ OR pause your sequencing run, prime your flow cell and load the library and restart the sequencing run	
Wahing 참고 영상 https://www.google.com/search?q=flow+cell+washing&rlz=1C1GCEU_koKR1050KR1050&sxsrf=APwXEddgjBmjF_J5jcayQmQG6w7AfdB-EQ%3A1682921801521&ei=SVlPZOK4H8_d2roPv8iC-AU&oq=flow+cell+lwash&gs_lcp=Cgxnd3Mtd2l6LXNlcnAQARgBMgcIABANEIAEMgcIABANEIAEMgcIABANEIAEMgcIABANEIAEMgcIABANEIAEMgYIABAeEA0yBggAEB4QDTIGCAAQHhANMgYIABAeEA06CggAEEcQ1gQQsAM6BAgjECc6CAgAEIAEEMsBOgQIABAeOgYIABAeEA86BggAEAUQHjoGCAAQCBAeOgUIIRCgAUoECEEYAFCEBFjdE2CMKmgBcAF4AIABiAGIAdsEkgEDMC41mAEAoAEByAEJwAEB&sclient=gws-wiz-serp#fpstate=ive&vld=cid:db69ba0a,vid:KQBOypLcnOE	