

# The Open Food Repo DNA



04.05.19

Samples to be sequenced:

Food product	Brand
Pesto Basilico	Alnatura
Cantadou au raifort	Cantadou
Paprika original chips	Zweifel
Sandwich Thon	M Classic
Falafel	Cornatur -VEGAN
Super mix: millet, quinoa, riz, lentilles	Migros BIO - YOU
Amande moulues	Coop Qualité et prix
Viande hâchée	MClassic
Yogourt Baies des bois	Aha
Bâtonnets de cabillaud	Pelican
Germes d'onions	Coop Naturaplan
Salade melee avec du poulet	Migros Daily

### DNA EXTRACTION 45 minutes

Sol 1: NaOH 0.1 M lysis buffer

Sol 2: Tris HCl EDTA\*Na<sub>2</sub>\*2H<sub>2</sub>O neutralization buffer

- Weigh around 100 mg of food sample into a 2 ml tube.
- Re suspend in 150 ul Sol1 or 200 ul more according to the sample.
- Incubate 10 min @ 85°C.
- Add 750 ul of Sol2 or 1 ml.
- Centrifuge 5 min.
- Transfer the supernatant into a new 1.5 ml tube.
- Prepare 1:10 dilution.

### DNA QUANTIFICATION 10 minutes

- Prepare measuring solution with 199 ul of assay reagent and 1 ul of dye.
- Mix it well.
- Transfer 195 ul into a 0,5 ml tube.
- Add 5 ul of DNA extract.
- Measure by fluorimeter.

### BARCODES AMPLIFICATION 30 + 90 minutes

- Prepare the master mix:

Primers 16S F 0,75 ul

Primers 16S R 0,75 ul

Primers RBC F 0,75 ul

Primers RBC R 0,75 u

MyTaq mix 2X 12,5 ul

water to 25 ul

Mix gently by flicking the tube, and spin down.

- Aliquot 22,5 ul of master mix into 0,2 ml tubes, then add 2,5 ul of DNA (1:10 dilution).
- Amplify using the following cycling conditions:

Initial Denaturation	95°C	2 min
Denaturation	95°C	15 s
Annealing	56°C	15 s x 50
Extension	72°C	10 s
Final amplification	72°C	1 min

### Quality check 20 + 30 minutes

- Prepare the master mix:

Agarose 4 g

TAE 50X 4 ml

Water to 200 ml

Mix gently and warm it up until the agarose dissolves.

- Add 5 ul of Syber Safe, mix and pour into the gel support with the comb.
- Mix 5 ul of each sample with 1 ul loading dye and water to 10 ul.
- Load the gel with the samples and the marker
- Run it around 20 minutes and check it by the transilluminator.

### LIBRARY PREPARATION

#### Check your flow cell

- Set up the MinION, flow cell and host computer.
- Once successfully plugged in, you will see a light and hear the fan.
- Open the MinKNOW GUI from the desktop icon and establish a local or remote connection.
- If running a MinION on the same host computer, plug the MinION into the computer.
- Choose the flow cell type from the selector box. Then mark the flow cell as "Selected".
- Click "Check flow cells" at the bottom of the screen.
- R9.4.1 FLO-MIN106.
- Click "Start test".
- Check the number of active pores available for the experiment, reported in the System History panel when the check is complete.

### LIBRARY PREPARATION - IF USING THE PCR BARCODING KIT 15 + 90 + 30 minutes

- Prepare the master mix:

Primer tailed RBC F (10uM) 0,125 ul

Primertailed RBC R (10uM) 0,125 ul

Primertailed 16S F (10uM) 0,125 ul

Primertailed 16S R (10uM) 0,125 ul

LongAmp mix 2X 12,5 ul

water to 25 ul

Mix gently by flicking the tube.

- Aliquot 18,75 ul of master mix into 0,2 ml tubes, then add 5 ul of the PCR product and 0,75 ul of the specific adapter.

- Amplify using the following cycling conditions:

Initial Denaturation	95°C	2 min
Denaturation	95°C	15 s
Annealing	56°C	15 s x 5
Extension	72°C	10 s
Denaturation	95°C	15 s
Annealing	60°C	15 s x 30
Extension	72°C	10 s
Extension	72°C	1 min

- Re suspend by vortexing the AMPure XP beads.
- Transfer the sample to a 1.5 ml DNA LoBind Eppendorf tube.
- Add 40 µl of resuspended AMPure XP beads to the reaction and mix by pipetting.
- Incubate for 5 minutes at RT.
- Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.
- Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.
- Keep on magnet, wash beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the 70% ethanol using a pipette and discard.
- Repeat the previous step.
- Spin down and place the tube back on the magnet. Pipette off any residual 70% ethanol. Briefly allow to dry.

- Remove the tube from the magnetic rack and resuspend pellet in 10  $\mu$ l of 10 mM Tris-HCl pH 8.0 with 50 mM NaCl. Incubate for 2 minutes at RT.
- Pellet beads on magnet until the eluate is clear and colourless.
- Remove and retain 10  $\mu$ l of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.

### Quality check 20 + 30 minutes

- Prepare the master mix:

Agarose 4 g

TAE 50X 4 ml

Water to 200 ml

Mix gently and warm it up until the agarose dissolves.

- Add 5  $\mu$ l of Syber Safe, mix and pour into the gel support with the comb.
- Mix 1  $\mu$ l of each sample with 1  $\mu$ l loading dye and water to 10  $\mu$ l.
- Load the gel with the samples and the marker.
- Run it around 20 minutes and check it by the transilluminator.

- Quantify 1  $\mu$ l of eluted sample using a fluorimeter.
- Pool equally the libraries into 10  $\mu$ l.
- Add 1  $\mu$ l RAP to the 10  $\mu$ l amplified DNA library.
- Mix gently by flicking the tube, and spin down.
- Incubate the reaction for 5 minutes at RT.
- Store the library on ice or -20°C until ready to load.

### PRIMING AND LOADING THE SPOTON FLOW CELL 15 minutes

- Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FLB) at RT before placing the tubes on ice as soon as thawing is complete.
- Mix the Sequencing Buffer (SQB) and Flush Buffer (FLB) tubes by vortexing, spin down and return to ice.
- Spin down the Flush Tether (FLT) tube, mix by pipetting, and return to ice.
- **Important** open the lid of the nanopore sequencing device and slide the flow cell's priming port cover clockwise so that the priming port is visible.

After opening the priming port, check for small bubble under the cover. Draw back a small volume to remove any bubble (a few  $\mu$ ls)

- Set a P1000 pipette to 200  $\mu$ l.
- Insert the tip into the priming port.
- Turn the wheel until the dial shows 220-230  $\mu$ l, or until you can see a small volume of buffer entering the pipette tip.

- Prepare the flow cell priming mix: add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FLB), and mix by pipetting up and down.
- 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.
- Thoroughly mix the contents of the SQB and LB tubes by pipetting.

In a new tube, prepare the library for loading as follows:

**Important** the Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

34 µl Sequencing Buffer (SQB)  
 25.5 µl Loading Beads (LB), mixed immediately before use  
 4.5 µl Nuclease-free water  
 11 µl DNA library

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.
- 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.
- Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION lid.

## **STARTING A SEQUENCING RUN 5 minutes**

- Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI.
- If your MinION was disconnected from the computer, plug it back in.
- Choose the flow cell type from the selector box. Then mark the flow cell as "Selected".
- Click the "New Experiment" button at the bottom left of the GUI.
- Select the appropriate protocol script
- Experiment type: Choose Sequencing Run under "Choose Operation"
- Flow Cell product code: Choose the Flow Cell type under "Flow cell product code"
- Sequencing kit: Choose the kit used.
- Choose whether or not live basecalling is enabled.
- The most appropriate script will appear in the drop-down menu.
- Start run.