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Oxford Nanopore Technologies SQK-LSK114 library preparation on Hamilton NGS STAR SOP

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Protocol status: In development

We are still developing and optimizing this protocol

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Disclaimer

*This protocol is still in development



Abstract

Library preparation for population-scale Oxford Nanopore long-read DNA sequencing SOP

At the NIH's Center for Alzheimer's and Related Dementias (CARD) <https://card.nih.gov/research-programs/long-read-sequencing> we will generate long-read sequencing data from thousands of patients with Alzheimer's disease, frontotemporal dementia, Lewy body dementia, and healthy subjects. With this research, we will build a public resource consisting of long-read genome sequencing data from a large number of confirmed people with Alzheimer's disease and related dementias and healthy individuals. To generate this large-scale nanopore sequencing data we have developed a protocol for processing and long-read sequencing human frontal cortex brain tissue, targeting an N50 of ~30kb and ~30X coverage.

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Acknowledgements:

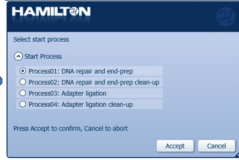
We would like to thank the Nanopore team (Jade Bartolo, Olivor Holman, Androo Markham & Jessica Anderson) and Hamilton team (Kristen Hise) and the whole CARD long-read team listed below ⇒ UCSC: Benedict Paten, Trevor Pesout, Paolo Carnevali, Mira Mastoras, Melissa Meredith, Jean Monlong, Ryan Lorig-Roach, Mobin Asri; NE: Miten Jain; NCI: Mikhail Kolmogorov; NHGRI: Adam Phillippy, Arang Rhie; Baylor: Fritz Sedlazeck, Farhang Jaryani; JHU: Winston Timp; NIA: Cornelis Blauwendraat, Kimberley Billingsley, Pilar Alvarez Jerez, Laksh Malik, Breeana Baker, Maysa Abdelhalim, Kensuke Daida, Rylee Genner, Abigail Miano-Burkhardt, Caroline Pantazis;

END-PREP

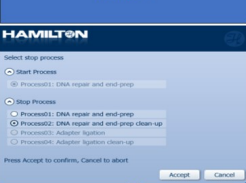
1. To start End-Prep process Click on the Methods manager and choose the ONT NGS LSK 114 v1.0 NIH Optimization Icon.



2. Click on process01: DNA repair and end prep and hit accept.



3. Click on process02: DNA repair and end prep clean-up and hit accept.



5. Make up the End-Prep Mastermix and continue with prompts.



4. Click 'Browse' to choose the Input File Worklist for the specific number of samples in the run and click 'OK'



LIGATION

1. To start Adapter Ligation process Click on the Methods manager and choose the ONT NGS LSK 114 v1.0 NIH Optimization Icon.



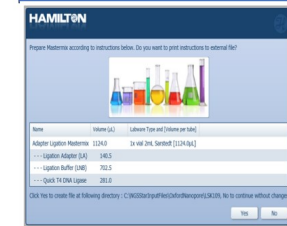
2. Click on process03: Adapter Ligation and hit accept.



3. Click on process04: Adapter Ligation clean-up and hit accept.



5. Make up the Ligation Mastermix and continue with prompts.



4. Click 'Browse' to choose the Input File Worklist for the specific number of samples in the run and click 'OK'



Figure 1: Overview of Hamilton SQK-LSK114 steps



Materials

Consumables

- SQK-LSK114-XL kit from ONT
- NEBNext FFPE DNA Repair Buffer
- NEBNext FFPE DNA Repair Mix
- Ultra II End-Prep Reaction Buffer
- Ultra II End-Prep Enzyme Mix
- Nuclease-free water
- TE Buffer
- Ethanol
- Agencourt AMPure XP Beads
- Hamilton 50 µl CO-RE tips with filter (Cat# 235948)
- Hamilton 300 µl CO-RE tips with filter (Cat# 235903)
- Hamilton 1000 µl CO-RE tips with filter (Cat# 235905)
- Hamilton 60 ml Reagent Reservoir, Self-Standing with Lid (Cat# 56694-01)
- Hamilton PCR ComfortLid (Cat# 814300)
- Bio-Rad Hard-Shell® 96-Well PCR Plates (Cat# HSP9601)
- Roche Diagnostics MagNA Pure LC Medium Reagent Tubs 20 (Cat# 03004058001)
- Sarstedt Inc Screw Cap Micro tube 2 ml, PP 1000/case (e.g. FisherScientific, Cat# NC0418367)
- Thermo Scientific™ Abgene™ 96 Well 0.8 ml Polypropylene Deepwell Storage Plate (Cat# AB-0859)

Equipment

- Hamilton NGS Star
- Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, #11766427)
- Hamilton On-Deck Thermal Cycler (ODTC)



1 Part 1: Sample Preparation and Instrument Initialization

Note: The CARD team has spent ~10 months optimizing this protocol for use with 48 brain or blood samples, so some optimizations may be specific to our samples and desired sequencing output. Therefore additional optimization is likely needed for new users.

Place all the necessary reagents on ice to thaw and the Agencourt AMPure XP Beads out at room temperature. Prepare samples on a 96-well HSP PCR plate (see Materials) and spin down.

Note: It is important that each well that will be used contains exactly  48 µL . Incorrect volumes can cause cascading failures during the run.

Carefully remove UV cover and open up the Method Manager application on the Hamilton computer and allow the Hamilton to initialize. Start Daily Maintenance. Once a week, also perform the Weekly Maintenance. Click through the prompts as they populate on the application.

Note: In our experience, AMPure XP beads and Short Fragment Buffer (SFB) perform best when thawed and used at room temperature rather than on ice.

2 Part 2: End-Prep (~1 hour 15 minutes)

20m

Start the LSK 114 v1.0 protocol and select "Process01: DNA repair and end-prep" for the start process, and "Process02: DNA repair and end-prep clean-up" for the stop process. Follow the prompts on the screen to select the number of samples to run and to begin setting up the deck. It is very important to never "lie" to the Hamilton. (add example)

Note: The LSK 114 v1.0 protocol is a slightly modified version of ONT's original protocol "OxfordNanopore_LSK114_MPH_v1.0". The only modification is the final incubation at the end, which has been changed to match the CARD manual library preparation protocol ( 00:20:00 at  37 °C at 300-450 x g).

3 Part 3: Adapter Ligation (~1 hour 45 minutes)

Start the LSK 114 v1.0 protocol and select "Process03: Adapter ligation" for the start process, and "Process04: Adapter ligation clean-up" for the stop process. Follow the prompts on the screen to select the number of samples to run and to begin setting up the deck.

4 Part 4: Instrument Clean-Up



Once Process04 has completed, the Hamilton will prompt the user to remove their samples so that it can begin cleaning-up.

The libraries can now proceed with sequencing. They can alternatively be stored at

 4 °C for ~2 weeks or  -20 °C for ~3 months.

When the instrument has finished, restock all consumables on deck so that instrument is prepared for the next run. Put UV cover back on, and start UV protocol for at least 3 minutes (our lab runs the UV for 30 minutes).