Sep 08, 2021

FindingNemo Library 1: Modified ULK001

Inswasti Cahyani[1], John Tyson[2], Nadine Holmes[1], Josh Quick[3], Nicholas Loman[3], Matthew Loose[1]

[1]University of Nottingham; [2]University of British Columbia; [3]University of Birmingham

1 Works for me Share

dx.doi.org/10.17504/protocols.io.bxhhpj36

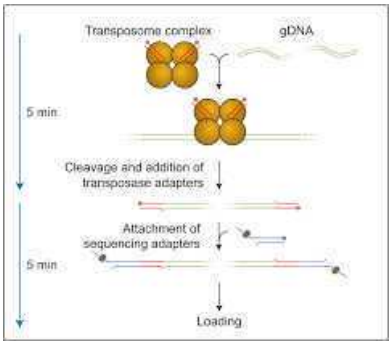
Inswasti Cahyani

ABSTRACT

This sub-protocol is designed to prepare library from extracted ultra-high molecular weight (UHMW) DNA to obtain ultra-long (UL) reads on Nanopore sequencers. The UL library protocols we tested are based on ONT's rapid kit, *i.e.,* **SQK-ULK001**, a transposase based adapter ligation kit.

Modified ULK001 protocol consistently produced N50 > 100 kb from a good input quality of UHMW DNA and is *our recommended route* for best output as it is also the most-cost effective.
Transposase-based reaction is done in a large volume of up to 1 ml.

The working principle of the ULK001 protocol is shown in the diagram below:



source: https://store.nanoporetech.com/uk/rapid-sequencing-kit.html

DOI

dx.doi.org/10.17504/protocols.io.bxhhpj36

PROTOCOL CITATION

Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose 2021. FindingNemo Library 1: Modified ULK001. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bxhhpj36

KEYWORDS

ultra-long sequencing, cohex, glass bead, nanopore, MinION, UHMW DNA, Monarch, Circulomics, phenol, SDS, CTAB, GM12878, Whatman, PromethION, Nanobind

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 19, 2021

LAST MODIFIED

Sep 08, 2021

OWNERSHIP HISTORY

Aug 19, 2021 Inswasti Cahyani

Sep 01, 2021 Inswasti Cahyani

PROTOCOL INTEGER ID

52489

PARENT PROTOCOLS

In steps of

FindingNemo in OneDay: Ultra-Long ONT Library Preparation from Cell to Flowcell in One Day

FindingNemo: A Toolkit of CoHex- and Glass Bead-based Protocols for Ultra-Long Sequencing on ONT Platforms

GUIDELINES

Acknowledgements

The FindingNemo protocol series was developed by Inswasti Cahyani for the Long Read Club with significant contributions from John Tyson and Nadine Holmes, also Josh Quick, and continuous discussion and support of Matt Loose and Nick Loman. We would also like to thank Giron Koetsier (NEB) and Kelvin Liu (Circulomics) for lending their expertise and advance product trials.

Please follow on Twitter for latest updates and results:
@NininUoN
@mattloose

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

MATERIALS TEXT

Chemicals/Compounds

- ☒ Nuclease-free
- Water **Thermofisher Catalog #AM9920**
- ☒ 1M Tris-HCl (pH 8.0) **Thermo Fisher**
- **Scientific Catalog #15568025**

Kits

- ☒ Ultra-Long DNA Sequencing Kit (SQK-ULK001) **Oxford Nanopore**
- **Technologies Catalog #SQK-ULK001**

Disposables

- ☒ DNA LoBind Tubes, 1.5
- mL **Eppendorf Catalog #0030108051**
- ☒ DNA LoBind 2.0ml PCR Clean Eppendorf
- Tubes **Eppendorf Catalog #0030 108.078**
- Wide-bore (or cut off) P1000 and P200 tips

SAFETY WARNINGS

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

When handling phenol always wear PPE, keep a solution of 50% (w/v) PEG-400 nearby to treat the burn in the case of accidental splashes.

BEFORE STARTING

Things to observe at all times:

- Excessive and vigorous pipetting and vortexing should be avoided as these may shear the DNA.
- Make up buffers with nuclease-free water to avoid introducing nucleases to solutions.
- Avoid unnecessary heating and freezing; isolated DNA should be stable for storage in the fridge for months

Library Prep Notes

1 Extracted UHMW DNA is often difficult to quantify due to its viscosity. However, accurate measurement of DNA concentration is crucial for calculating optimum ratio of the transposase enzyme to the DNA molecules.
We provide a protocol section for quantifying UHMW DNA in our 'FindingNemo' protocol master file.
Properly quantified DNA can then be processed for this library prep.

Both cell number and DNA concentration/amount are used to calculate the amount of transposase (FRA) and adapter (RAP-F).

We follow the original SQK-ULK001 protocol for the optimum ratio of transposase amount to human genomic DNA:

6 µl FRA to 6 million human cells (or around 40 µg DNA)

For other species, the genome size has to be taken into account and the FRA to DNA ratio optimised, *e.g.,* we had optimised a non-human cell line of **6.2 Gb genome** at:

2.5 µl FRA to 1 million non-human cell (around 12-15 µg DNA)

Transposase Reaction 55m

2 ⚠

In a 2 ml tube, dilute UHMW DNA to a concentration of around 50 ng/µl in a total volume of 750 µl (with water or elution buffer if required).
Mix well with a P1000 wide-bore tip.

- DNA concentration can still range from 20-50 ng/µl to have optimum tagmentation reaction.
- If input DNA amount is less than 20 µg (1-3 million cells used), halve all the reaction volumes, *i.e.,* 375 µl total DNA volume instead of 750 µl as in the table below.
- It is important to have as homogeneous DNA as possible at this step so the transposase can access and cut the DNA solution with an even distribution. It is OK to pipette thoroughly but gently.

A	B	C	D	E
Cell No. (million)	**Approx. DNA amount (µg)**	**Total DNA volume (µl)**	**DNA concentration (ng/µl)**	**Total reaction volume (µl)**
6	>20-40	750	20-50	1000
5				
4				
3	5-20	375		500
2				
1				

3 In a 1.5 ml tube, dilute the corresponding amount of transposase (FRA) with the dilution buffer (FDB) to a total volume of 250 µl (or 125 µl if doing half-reaction). More details in the table below.

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

A	B	C	D	E
Cell No. (million)	Approx. DNA amount (µg)	FRA (µl)	FDB (µl)	Total reaction volume (µl)
6	>20-40	6	244	1000
5		5	245	
4		4	246	
3	5-20	3	122	500
2		2	123	
1		1	124	

4 Mix the diluted FRA by vortexing for 2-3 seconds.

5 Using a P1000 wide-bore tip, add the diluted FRA to the DNA sample.
Stir the reaction with the pipette tip whilst expelling the diluted FRA to ensure an even distribution.
Mix thoroughly by gentle pipetting.

On ice

6 ⚠ 25m

Incubate the reaction as follows:

23 °C **00:10:00**

70 °C **00:05:00**

Room temperature **00:10:00 at least**

It is important that the room temperature at the fragmentation step (first incubation step) does not fall below 20^{o}C to ensure optimum reaction condition. The use of a water bath or heating block is recommended.

Adapter Ligation 55m

7 Add the corresponding volume of sequencing adapter (RAP-F) as in the table below.

A	B	C	D	E
Cell No. (million)	Approx. DNA amount (µg)	FRA (µl)	RAP-F (µl)	Total reaction volume (µl)
6	>20-40	6	5	1000
5		5	4.2	
4		4	3.3	
3	5-20	3	2.5	500
2		2	1.7	
1		1	0.8	

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

- Use a P1000 wide-bore tip to pipette mix. Visually check to ensure the reaction is thoroughly mixed.
- Tube inversion can be used to aid mixing.

8 Incubate for 30 minutes at 23^{o}C. 30m

23 °C **00:30:00**

Citation: Inswasti Cahyani, John Tyson, Nadine Holmes, Josh Quick, Nicholas Loman, Matthew Loose (09/08/2021). FindingNemo Library 1: Modified ULK001. https://dx.doi.org/10.17504/protocols.io.bxhhpj36

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited