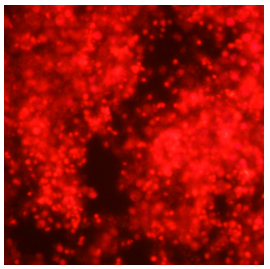
Sep 14, 2019 Version 3

Algal nuclei isolation for Nanopore sequencing of HMW DNA V.3



DOI
dx.doi.org/10.17504/protocols.io.7b7hirn

Robert Auber[1], Jennifer H Wisecaver[1]

[1]Purdue University

High molecular weight D... Wisecaver Lab

Robert Auber

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.7b7hirn

Protocol Citation: Robert Auber, Jennifer H Wisecaver 2019. Algal nuclei isolation for Nanopore sequencing of HMW DNA. **protocols.io https://dx.doi.org/10.17504/protocols.io.7b7hirn**

Manuscript citation:

Protocol status: Working
We use this protocol and it's working

Created: September 14, 2019

Last Modified: September 14, 2019

Protocol Integer ID: 27743

Keywords: Algae, DNA extraction, Nuclei isolation, Nanopore

Abstract

This protocol was developed for extraction of high molecular weight (HMW) DNA from *Prymnesium parvum,* a unicellular haptophyte alga, for the purpose of whole genome sequencing using Oxford Nanopore Technology (ONT) long reads. *P. parvum* is known to produce several specialized metabolic compounds that may compromise isolated DNA, leading to decreased sequencing yield. We found that separating intact nuclei from cellular debris prior to DNA isolation, improved read length and throughput. Isolated nuclei were processed using a Circulomics NanoBind kit to extract HMW DNA.

Guidelines

This protocol was adapated from the "Preparing Arabidopsis Genomic DNA for Size-Selected ~20 kb SMRTbell TM Libraries" protocol. (https://www.pacb.com/wp-content/uploads/2015/09/Shared-Protocol-Preparing-Arabidopsis-DNA-for-20-kb-SMRTbell-Libraries.pdf)

Take care to minialize pipetting of the sample as much as possible and never vortex to retain HMW DNA.

Materials

Prepared Buffers & Reagents

Nucleus Isolation Buffer (Must be cooled to 0°C for > 1 hour before use):
10 mM Tris pH 9.5
10 mM EDTA 100 mM KCL
500 mM Sucrose
4 mM Spermidine
1 mM Spermine
0.1% BME (add day of extraction)

Equipment

Refridgerated centrifuge with 50mL tube capacity
DynaMag Magnetic rack
ThermoMixer
Hula mixer
Epifluorescent microscope

	Item	Supplier	LOT/Catalog number
	50 mL conical tubes	Eppendorf	H180965P
	10 mL serological pipette	Fisher	13-676-10J
	LoBind 1.5 mL microcentrifuge tubes	Eppendorf	H177737J
	1000 µl and 100 µl wide bore pipette tips	Art	2069GPK/2079GPK
	Nylon mesh filters: 100 µm, 70 µm and 40 µm	Cell treat/Fisher/Biologix	180321-299/22363548/15-1040
	Liquid nitrogen	n/a	n/a
	Tris pH 9.5	Alfa Aesar	Q08F508
	EDTA	Milipore	3070822
	KCl	Fisher	177592
	Sucrose	Sigma	SLBW6518
	Spermidine trihydrochloride	Sigma	334-50-9
	Spermine tetrahydrochloride	Sigma	306-67-2
	2-Mercaptoethanol (BME)	Sigma	SHBH5561V
	Triton X-100	Sigma	SLBW7103
	Isopropanol	Fisher	175275
	100% Ethanol	Acros organics	B0536196

	Propidium iodide	eBioscience	BMS500PI
	Microscope slides	Thermo	3050
	Microscope slide covers	Thermo	3306

Safety warnings

All handling of β-mercaptoethanol (BME) and solutions containing BME should be done in a chemical fume hood.

Before start

Have sufficient liquid nitrogen on hand to snap freeze your samples.

Prepare equipment and reagents

1 Add BME to premade NIB. Per sample add 35 µL BME into 35 mL premade NIB.

2 Make 10% Triton X-100 NIB solution. Per sample, aliquot 1.8 mL NIB prepared in previous step and add 200 µL Triton X-100.

3 Cool solutions to 0 °C on ice.

4 Set thermomixer to 55 °C

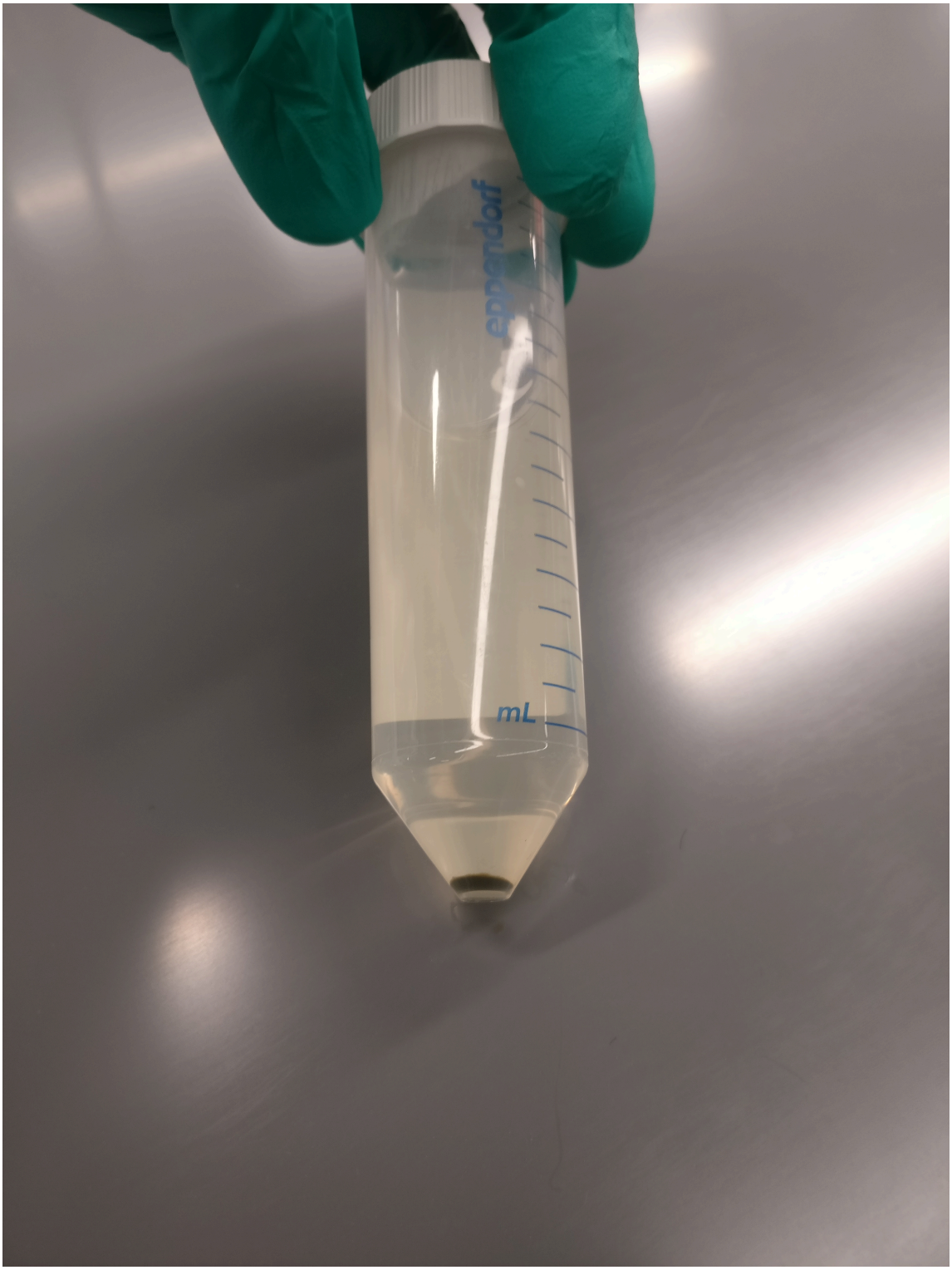
5 Chill centrifuge to 4 °C

6 Chill 50 ml conical tubes (4 per sample) and NIB buffers on ice.

Cell Lysis

7 Transfer 20 mL to 50 mL of culture to a pre-chilled 50 ml conical tube and centrifuge 2000 x g at 4 °C for 00:10:00 to pellet cells.

The amount of culture spun down as well as the centrifugation speed at which cultures are pelleted should be optimized for each organism. In our experience, the pellet size should be at least the size of a lentil for small (5 µm) for our alga.


eppendorf
mL

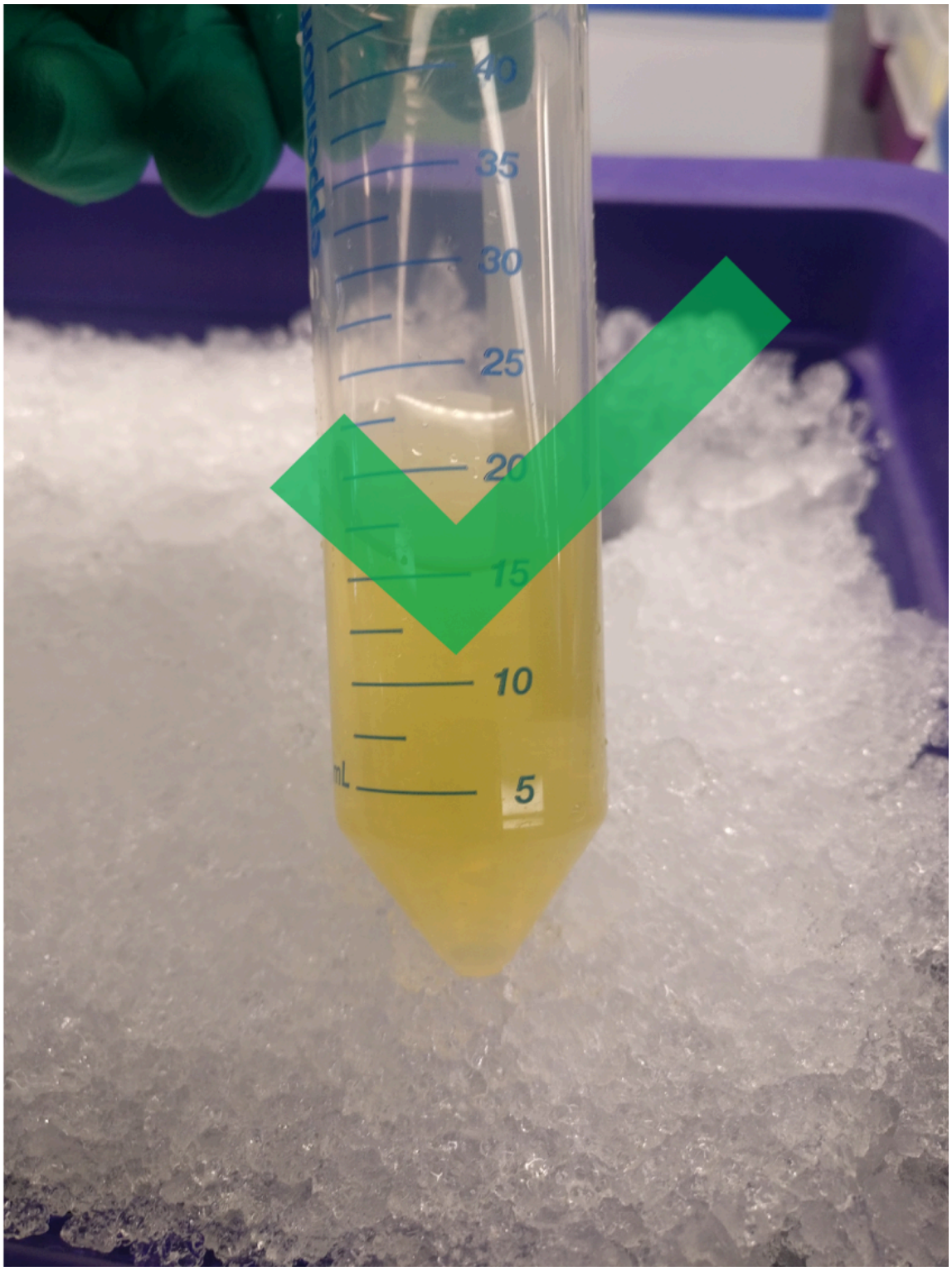
8 Discard the supernatant and snap freeze the conical tube in liquid nitrogen for at least 00:03:00 .

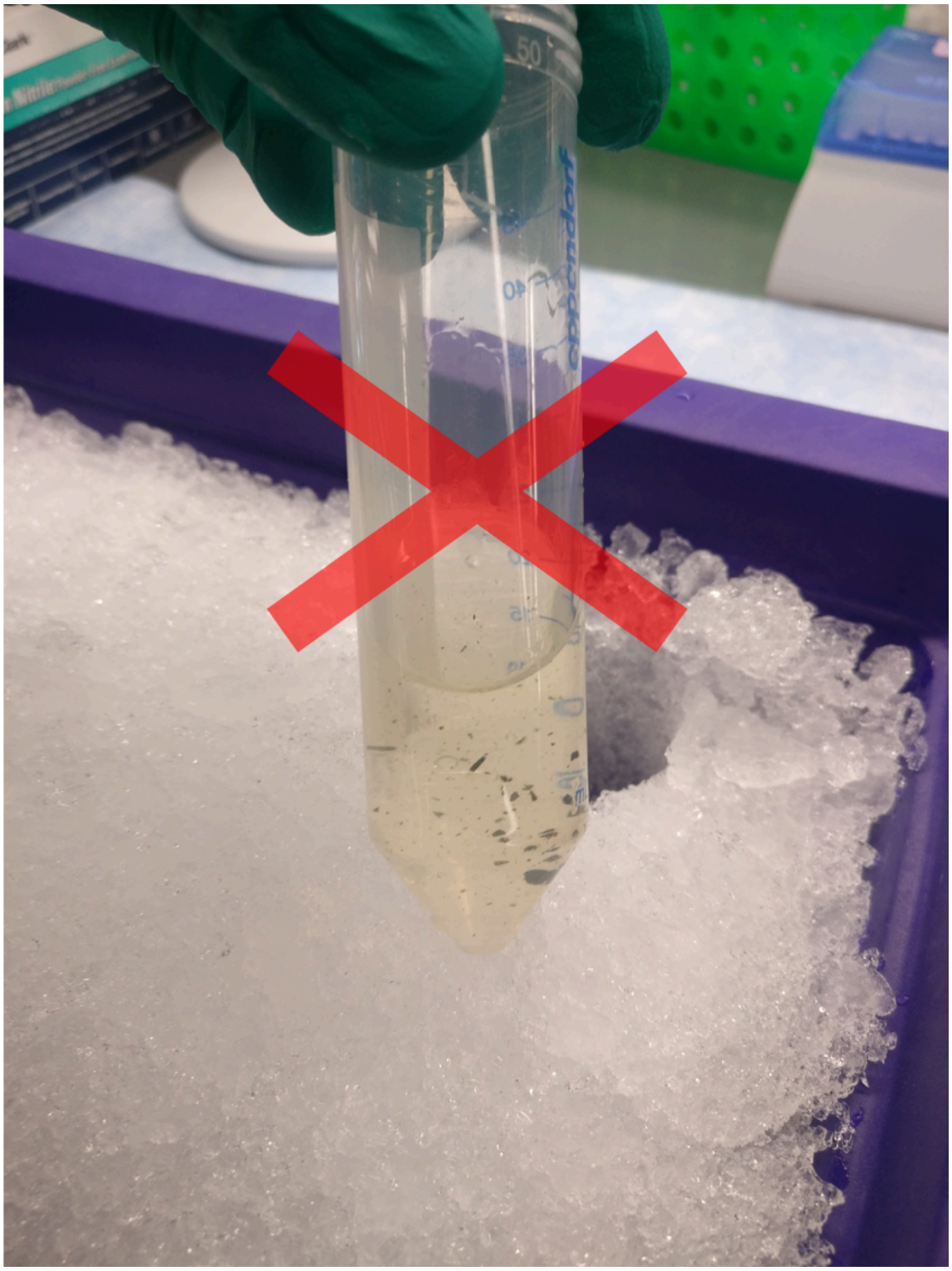
Some organisms will require some type of tissue disruption to lyse cells.

9 Place cell pellet on ice and add 10 mL of ice cold NIB.

If you know your pellet is not very soluble, only add 1mL of NIB at first and mix by pipetting up and down with a 1mL pipette. Add remaining 9mL NIB after clumps are broken up.

10 Mix by slowly pipetting up and down with a 10mL serological pipettor until the mixture is smooth and not clumpy.

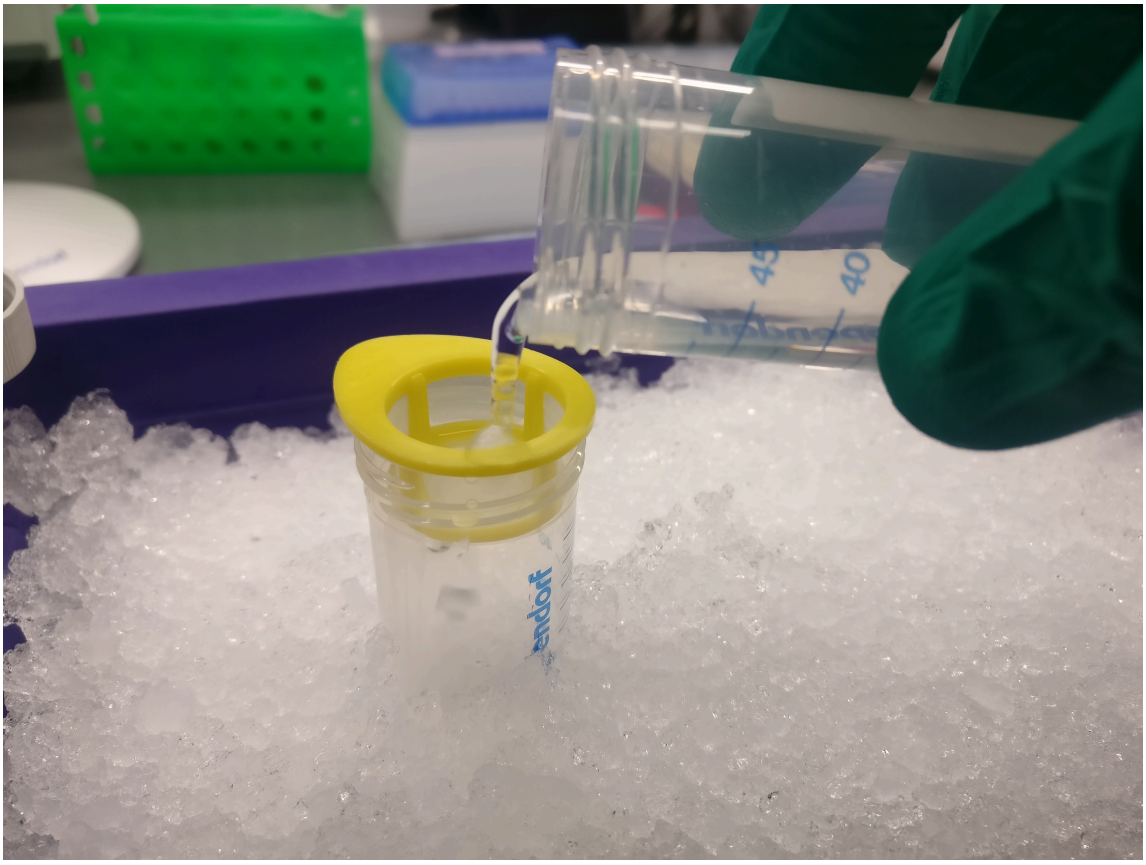

40
35
30
25
20
15
10
mL
5


50
40
eppendorf

Filter to remove particulate material

11 Place a 100 µm filter on top of a pre-chilled 50 ml conical tube.

12 Slowly pour the sample through the filter into the clean tube.



13 Use a serological pipettor to wash the filter with 10 mL of ice cold NIB.

14 Place a 70 µm filter on top of a second pre-chilled 50 ml conical tube.

15 Slowly pour the sample through the filter into the clean tube.

16 Use a serological pipettor to wash the filter with 10 mL of ice cold NIB.

17 Place a 40 µm filter on top of a third pre-chilled 50 ml conical tube.

18 Slowly pour the sample through the filter into the clean tube. (Do **not** wash with NIB)

Pellet nuclei

19 Add 1.5 mL of ice cold NIB + TritonX solution to filtrate.

20 Centrifuge 500 x g at 4 °C for 00:10:00 to pellet nuclei.

The speed at which the sample is spun down should be reevaluated for different organims based on genome size.

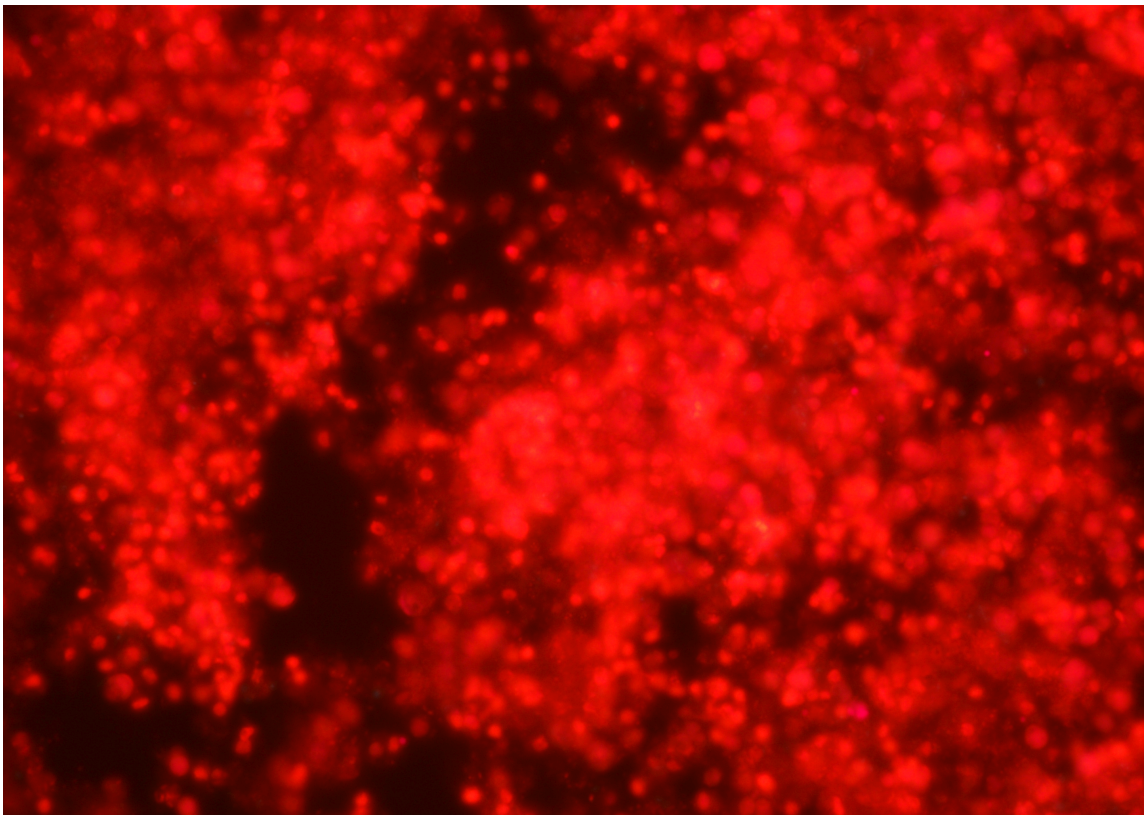
21 Discard the supernatant.

Confirm isolation via microscopy

22 Aliquot 1 µL of the pellet into a clean 1.5ml eppendorf tube. Add 18 µL of fresh NIB and 1 µL of propidium iodide to the tube.

23 Transfer the stained nuclei to a microscope and confirm presence of fluorescing nuclei under an epifluorescent light.

Image was taken on a Nikon Ts2R-FL microscope with a CFP LP cube (Excitation 448/23nm (436.5-459.5nm), Emission 472nm Long Pass)



Extract DNA from Nuclei

24 Extract DNA by referring to Circulomics Plant Nuclei Big DNA Kit protocol. https://www.circulomics.com/store/Nanobind-Plant-Nuclei-Big-DNA-Kit-Alpha-Version-p99924200