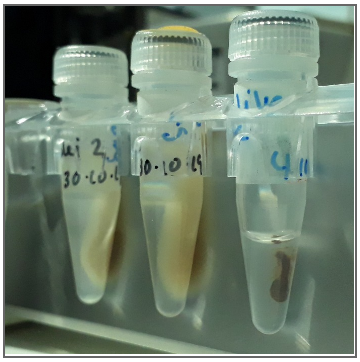

NOV 08, 2019

Plant nuclei enrichment for chromatin capture-based Hi-C library protocols

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High molecular weight DNA extraction from all kingdoms | Long Read Club

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ABSTRACT

Chromatin capture-based protocols to produce Hi-C libraries start with a crude nuclei extract or a total cell extract. Either way, plants can be challenging due to the high content of contaminants that could interfere at the chromatin capture step.
This protocol will help clean up the starting material needed for preparing Hi-C libraries. The nuclei yield varies, but should be enough for at least two preps. This protocol has been tested on blueberry, bilberry, pepino, Gillenia and rewarewa.

DOI: dx.doi.org/10.17504/protocols.io.8vehw3e

Protocol Citation: Elena Hilario 2019. Plant nuclei enrichment for chromatin capture-based Hi-C library protocols. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8vehw3e

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

Created: Oct 30, 2019

Last Modified: Nov 08, 2019

PROTOCOL integer ID:
29318

GUIDELINES

Consumables and equipment

- Fresh or flash frozen leaf tissue, 1-2 g
- 15 mL Corning™ Falcon tubes with rack
- 50 mL Corning™ Falcon tubes with rack
- Medium size mortar and pestle
- Disposable plastic Pasteur pipettes
- Scissors
- Glass Pasteur pipettes with dropper bulb
- Magnetic stirring plate
- Magnetic stirrer
- 100 mL glass beaker
- Miracloth sheets cut 10 x 10 cm
- Small funnel, washed and rinsed with ethanol
- Spatulas with flat and round end points, washed and rinsed with ethanol
- Cell strainers, 100 and 40 µm
- Dounce homogenizer, pestle B
- 1.5 mL screw capped tubes, sterile
- Wide bore pipette tips, 1 ml, 200 µL, with pipettes
- Benchtop centrifuge with swing bucket rotor, precooled at 10°C with adaptors for 15 and 50 mL conical tubes
- Benchtop microcentrifuge for 1.5 mL tubes
- Ice bucket with crushed ice

MATERIALS

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- Liquid Nitrogen
- PIPES KOH **Sigma Aldrich**
- D-Mannitol **Sigma Aldrich**
- Polyvinylpyrrolidone K40 **Sigma Aldrich**
- L-Lysine monohydrochloride **Sigma Aldrich**
- EGTA **Sigma Aldrich**
- Magnesium chloride hexahydrate **Sigma Aldrich**
- Sodium metabisulfite **Sigma Aldrich**
- 2-Mercaptoethanol **Sigma Aldrich**
- Triton X-100 **Sigma Aldrich Catalog #T8787-50ML**
- Percoll **Sigma Aldrich Catalog #17-0891-01**
- Miracloth **Merck Millipore Catalog #475855**
- Cell Strainer 40 µm **Sigma Aldrich**
- Cell strainer 100 µm **Sigma Aldrich**

SAFETY WARNINGS

- Work in a fume cabinet
- Consult your institution's policy on chemical waste collection and management

BEFORE START INSTRUCTIONS

Solutions and Reagents

- **NEB (Nuclei Extraction Buffer)** 0.5 M Mannitol, 10 mM PIPES-KOH, 10 mM $MgCl_2$

$6H_2O$, 2% PVP K40, 200 mM L-lysine monohydrochloride, 6 mM EGTA, pH 6

Reagent	1 L
Mannitol	91 g
PIPES-KOH	3.78 g
MgCl2 6H2O	2.03 g
PVP K40	20 g
L-lysine monohydrochloride	36.52 g
EGTA	2.28 g

Heat up 800 mL of deionized water, 2-4 min in microwave. Pour into a glass beaker with a magnetic stirrer. Add PVP K40, three spoonfuls at a time, stir vigorously. To speed up this process you can use two flat spatulas and “cut” the PVP blobs as if it was a steak.
Add mannitol and dissolve completely before adding PIPES, magnesium, lysine, and EGTA. Adjust the pH to 6.0 with 10 M NaOH. Adjust the volume to 1 L. Split the buffer into two 1 L Schott bottles. Autoclave and cool down to room temperature. Store at 4°C until ready to use.

- **NEB -PVP/M/T:** 0.5 M Mannitol, 10 mM PIPES-KOH, 10 mM $MgCl_2$, 200 mM L-lysine monohydrochloride, 6 mM EGTA, pH 7.0, sterile. Prepare 1 L

- Prepare the following buffers. Use a wide bore tip to pipette the Triton X-100. To dissolve the Triton X-100 use a magnetic stirrer.

Solution/chemical	NEB-complete +Triton X-100	NEB-βME	NEB-A
NEB	50 mL	50 mL	--
NEB-PVP/M/T	--	--	50 mL
β-mercaptoethanol	20 µL	--	100 µL
sodium metabisulfite	0.1 g	0.1 g	0.1 g
Triton X-100	250 µL	--	250 µL

- Prepare 10 ml 75% Percoll® in NEB-A and split into two 15 mL Falcon tubes. The two tubes should weigh exactly the same. Keep both tubes in the refrigerator until ready.
- Set up a small ice box on a magnetic stirrer plate and place a 100 mL glass beaker

with stirrer and the 50 mL of NEB-complete solution. Keep covered until ready to add the ground tissue.

Acknowledgements

Thanks to Blue Plunkett and Caitlin Elborough for testing this protocol on bilberry and blueberry. And to Marcela Martinez-Sanchez, Hilary Ireland and Jesse Prebble, for providing samples of pepino, Gillenia and rewarewa, for me to test.

Nuclei isolation

1 Weight 1-2 g of young leaves, either fresh or frozen.

2 Pre-cool the mortar and pestle by pouring liquid nitrogen to the rim of the mortar. Once the liquid nitrogen is evaporated, repeat the pre-cooling step.

3 Add the leaves to the mortar and pour liquid nitrogen over them to freeze them immediately. You can crush the leaves slightly to make them fit.

4 When the liquid nitrogen has evaporated, grind the leaves with circular motion, and also by pushing from the rim to the center of the mortar. Retrieve the powder from the pestle with a pre-cooled spatula. Add more liquid nitrogen and repeat this step until a fine powder is obtained. For most plants it takes 3 rounds of grinding. The powder should resemble icing sugar.

5 Transfer the powder to the beaker containing 50 mL of NEB-complete +Triton X100. Stir at moderate speed until all the powder is dissolved. Any large clumps can be broken with the spatula against the beaker's internal wall. 00:10:00 Organellar lysis 4 °C Ice box on magnetic stirring plate .

Note

If you go overtime, do not worry. The amount of Triton-X100 will not lyse the nuclei, but keep in mind that the crude lysate has large cell wall debris that might break them.

6 Filtrate the sample through the Miracloth sheet. Very carefully pipette the extract in the funnel to detach the residue stuck in the Miracloth.

6.1 Assemble the 100 µm strainer on a 50 mL Falcon tube. Transfer the filtrated extract to the strainer. This filtration step should flow more or less quickly.

6.2 Assemble the 40 µm strainer on a 50 mL Falcon tube and transfer the filtrated extract to the strainer. This step might take about 10 min. Place the assembled tube on ice.
You can help the filtration by pipetting the filtrate in the strainer very gently with a disposable Pasteur pipette (cut the tip to make it wide bore). If the strainer becomes clogged half way through the filtration, replace it with a new one.

7 Collect the nuclei by centrifugation 1800 x g , Nuclei pellet 00:15:00 Nuclei pellet 10 °C Centrifugation . Discard the supernatant and dispose it according to your institution's waste disposal guidelines.

> **Note**
>
> The centrifugal force depends on the genome size but also on the medium's density. The osmoticum used in this buffer (mannitol) is at 0.5 M and seems to work well with genomes upto 3 Gb (N. benthamiana), at 1800 x g, but please see recommendations here. Other cellular material such as starch might contribute to the sedimentation of nuclei of large genomes such as the N. benthamiana.

8 Add 30 mL NEB-βME to the pellet and resuspend it gently by inverting the tube, in a rocking movement. Collect the nuclei by centrifugation go to step #7 Nuclei pellet

9 Add 7 mL of NEB-A buffer to the pellet and resuspend it gently. Transfer the mixture to the Dounce homogenizer and place it on the ice box. Use the "B" pestle to homogenize the nuclei sample by pulling and pushing the pestle very gently. Take care not to lift it out of the liquid or creating too many bubbles.

Percoll gradient

10 Carefully overlay 3.5 mL filtrated nuclei suspension over each 75% Percoll® solution. The loaded tubes should weigh **exactly** the same.

11 Collect the nuclei at the Percoll® gradient interphase by centrifugation 650 x g , Percoll gradient 00:30:00 Percoll gradient 10 °C Percoll gradient . Adjust the acceleration = 9 (max), and deceleration = 1 (min).

12 With a light source behind the gradient, collect the sample found at the interphase of the Percoll® gradient using a glass Pasteur pipette. Do this step very slowly. Transfer sample to a new 15 mL Falcon tube and keep on ice. You will obtain about 4 mL of nuclei solution.

> **Note**
>
> The interphase can form as a tight band between the two solutions of different density, or as a fluffy band. In either case, collect as much as possible. Most contaminants will show as a dense pellet at the bottom of the gradient, or as a colored solution on the top layer.

13 Add enough NEB-A solution to the nuclei to have a final volume of 10 mL. Collect nuclei by centrifugation go to step #7

14 Discard the supernatant . Retrieve the nuclei pellet by adding ~ 500 µL NEB-A and gently resuspend it with a wide bore pipette tip. Transfer it to a 1.5 mL screw-capped tube. Add another ~ 500 µL NEB-A to the Falcon tube to retrieve any remaining nuclei and transfer it to the screw-capped tube. Make sure the solution is homogeneous. Transfer half of it to another 1.5 mL screw-capped tube and add enough NEB-A to each tube to have a final volume of 1 mL. Collect the nuclei by centrifugation

5200 rpm , Enriched nuclei sample 00:02:00 Enriched nuclei sample Room temperature .
Discard the supernatant.

15 Estimate the yield by weighting the tubes (subtract the empty tube weight). Store the enriched nuclei sample -80 °C Storage unitl ready to start the crosslinking steps of the Hi-C protocol.