

Introduction

The plant restriction enzyme Pore-C (RE-Pore-C) protocol is intended for the manipulation of plant nuclei suspensions to capture three-dimensional interactions of DNA within chromatin. We strongly recommend reading the [Restriction Enzyme Pore-C info sheet](#) prior to starting this protocol to understand how to choose the appropriate restriction enzyme, the sequencing kit for library preparation, and information regarding sample input.

This protocol has been developed based on research by Oxford Nanopore Technologies and published literature:

- Belaghzal, H., Dekker, J. and Gibcus, J. H. (2017) Hi-C 2.0: an optimized Hi-C procedure for high-resolution genome-wide mapping of chromosome conformation, *Methods*, **123**, pp. 56–65. doi: 10.1016/j.ymeth.2017.04.004.Hi-C.
- Belton, J.-M. et al. (2012) Hi-C: a comprehensive technique to capture the conformation of genomes, *Methods*, **58(3)**, pp. 1–16. doi: 10.1016/j.ymeth.2012.05.001.Hi-C.
- Comet, I. et al. (2011) A chromatin insulator driving three-dimensional Polycomb response element (PRE) contacts and Polycomb association with the chromatin fiber, *Proceedings of the National Academy of Sciences* **108(6)**, pp. 2294–2299. doi: 10.1073/pnas.1002059108.
- Folta, K. M. and Kaufman, L. S. (2006) 'Isolation of Arabidopsis nuclei and measurement of gene transcription rates using nuclear run-on assays', *Nature Protocols*, **1(6)**, pp. 3094–3100.
- Gavrillov, A. A., Golov, A. K. and Razin, S. V. (2013) Actual Ligation Frequencies in the Chromosome Conformation Capture Procedure, *PLoS ONE*, **8(3)**, pp. 1–6. doi: 10.1371/journal.pone.0060403.
- Lieberman-Aiden, E. et al. (2009) Comprehensive mapping of long range interactions reveals folding principles of the human genome, *Science*, **326**, pp. 289–293.
- Liu, C. (2017) 'Plant Gene Regulatory Networks', in *Plant Gene Regulatory Networks: Methods and Protocols, Methods in Molecular Biology*, pp. 155–166. doi: 10.1007/978-1-4939-7125-1.
- Nagano, T. et al. (2015) Comparison of Hi-C results using in-solution versus in-nucleus ligation, *Genome Biology*, **16(1)**, pp. 1–13. doi: 10.1186/s13059-015-0753-7.
- Ulahannan, N. et al. (2019) Nanopore sequencing of DNA concatemers reveals higher-order features of chromatin structure, *bioRxiv*, p. 833590; doi: 10.1101/833590.
- Workman, R. et al. (2018) 'High molecular weight DNA extraction from recalcitrant plant species for third generation sequencing', *Protocol Exchange*, **version 1**, pp. 1–15. doi: 10.1038/protex.2018.059.
- Zhang, M. et al. (2012) 'Preparation of megabase-sized DNA from a variety of organisms using the nuclei method for advanced genomics research', *Nature Protocols*. Nature Publishing Group, **7(3)**, pp. 467–478. doi: 10.1038/nprot.2011.455.

Materials

- 2 g of plant material
- An appropriate restriction enzyme and associated buffer; for example NlaIII restriction enzyme (or equivalent) at 10 U/μl (NEB, R0125L) and NlaIII reaction buffer
- 0.5 M EDTA, pH 8
- Formaldehyde at 36.5% v/v (Sigma, F8775)
- Pure ethanol
- Glycine (Sigma, 50046-250G)
- IGEPAL CA-630 (Sigma, I8896-50ML)
- 5 M NaCl (Sigma, 71386)
- 10X phosphate-buffered saline (PBS)
- Phenol:chloroform:isoamyl alcohol in a 25:24:1 ratio, saturated with 10 mM Tris.HCl pH 8.0, 1 mM EDTA (Sigma P3803-400ML)
- Polyvinylpyrrolidone (PVP) PVP-40 (Sigma PVP40)
- Protease Inhibitor Cocktail (Sigma, P8340)
- Proteinase K at 20 μg/μl (NEB, P8107S)
- Recombinant albumin at 20 μg/μl (NEB, B9200S)
- SDS at 10% v/v (Fisher, 10265153)
- 3 M sodium acetate, pH 5.5

- Spermidine trihydrochloride (Sigma, 85578)
- Spermine tetrahydrochloride (Sigma, 85605)
- Sucrose (Sigma, S0389)
- T4 DNA Ligase 400,000 U/ml (NEB M0202S/L)
- 1 M Tris-HCl pH 8.0 (Fisher, 10336763)
- ECOSURF™ EH-9 (Sigma, STS0006) or Triton X-100 (Sigma, X100)
- Trizma base (Sigma, 93362)
- Tween-20 (Sigma, P9416)
- β-mercaptoethanol at 14.3 M (Sigma, 63689)
- Qubit dsDNA HS Assay Kit (ThermoFisher Q32851)
- Liquid nitrogen
- 1 ml disposable syringe
- 1.5 ml Eppendorf DNA LoBind tubes
- 2 ml Eppendorf DNA LoBind tubes
- 5 ml centrifuge tubes
- 50 ml centrifuge tubes
- Centrifuge and rotor for 15 ml Falcon tubes
- Ice bucket with ice
- 4°C and -80°C freezer storage
- Microfuge
- Microfuge rotor for 5 ml tubes
- Microfuge rotor for 2 ml tubes
- Microfuge rotor for 50 ml tubes
- Thermomixer with temperature control and rotation
- 10, 20, 200 and 1000 µl filter pipette tips and 100, 200 and 1000 µl wide-bore pipette tips
- 2, 10, 20, 200 and 1000 µl pipettes
- 40 µm cell strainer (Fisher, 11587522)
- 100 ml glass measuring cylinder
- 500 ml borosilicate filtering flask
- 500 ml borosilicate round bottom flask
- Büchner funnel with sintered glass filter
- Class I hood with active charcoal filter
- Gyrotory rocker
- Rotary evaporator system (rotovap) with pressure-controlled vacuum
- Qubit fluorometer (or equivalent for QC check)
- Agilent Bioanalyzer and 12,000 bp reagents (or equivalent)

Method

Day 1: 1 hour hands-on time

Note: Formaldehyde crosslinking of chromatin is achieved with vacuum infiltration. In this protocol, a rotary evaporator system (rotovap) is used to limit boiling. We recommend using a rotovap for the vacuum infiltration procedure, however users may also apply vacuum to a static inside of a vacuum chamber or desiccator.

1. Prepare 10 ml of filtered 2.5 M glycine.
2. Prepare 400 ml of fresh vacuum infiltration buffer and store at 4°C prior to use.

Component	Stock conc.	Final conc.	For 400 ml
PBS pH 7.4	10X	1X	40 ml
Sucrose	-	100 mM	13.69 g
ddH ₂ O	-	-	Up to 400 ml
Total	-	-	400 ml

- Collect 2 g of plant material. We recommend selecting younger leaves.
Note: If the plant material is $>1 \text{ cm}^2$, dissect the sample into smaller pieces before proceeding to the next step. Frozen plant material may also be thawed for use, however we recommend fresh material
- In a 500 ml round-bottom flask, suspend the dissected plant material in 100 ml of vacuum infiltration buffer.
- To reduce the surface tension of the buffer and prevent the plant material from clumping together, add 100 μl of 10% v/v ECOSURF™ EH-9 to the suspension for a final concentration of 0.01% v/v.
- Continue all subsequent steps inside a class I hood with double gloves. Add 2910 μl of 36.5% v/v formaldehyde to the suspension for a final concentration of 1% v/v formaldehyde in $\sim 103 \text{ ml}$.
- Connect the round-bottom flask to the rotovap and apply a 16 kPa (160 mBar) vacuum with 100 rpm rotation for 15 minutes at room temperature.
- Release the vacuum to atmospheric pressure, then reapply a 16 kPa (160 mBar) vacuum with 100 rpm rotation for a further 15 minutes at room temperature.
- Release the vacuum to atmospheric pressure, then decant the suspension into a Büchner funnel with a sintered glass filter. Vacuum-filter the suspension over a 500 ml filtering flask.
- Remove the Büchner funnel from the filtering flask and discard the filtrate. Resuspend the plant material in 100 ml of fresh vacuum infiltration buffer. Decant the suspension into the 500 ml round-bottom flask.
- Add 5270 μl of 2.5 M glycine to the suspension for a final concentration of 1% w/v glycine (125 mM) in $\sim 105 \text{ ml}$ and mix by swirling.
- Connect the round-bottom flask to the rotovap and apply a 16 kPa (160 mBar) vacuum with 100 rpm rotation for 10 minutes at room temperature.
- Release the vacuum to atmospheric pressure, then decant the suspension into a Büchner funnel with a sintered glass filter. Rinse out the round-bottom flask with distilled water and also decant into the Büchner funnel. Vacuum-filter the suspension over a 500 ml filtering flask.
- Remove the Büchner funnel from the filtering flask and discard the filtrate. Wash the plant material retained in the Büchner funnel twice with 50 ml of fresh vacuum infiltration buffer for 5 minutes each prior to filtering.
- Remove the Büchner funnel from the filtering flask and discard the filtrate. Wash the plant material retained in the Büchner funnel with 50 ml of distilled water and filter again.
- Remove the Büchner funnel from the filtering flask and discard the filtrate. Using a spatula, transfer the plant material into a fresh 50 ml centrifuge tube.
- The crosslinked plant material may now be removed from the class I hood. We recommend continuing directly to cryogrinding. However, the crosslinked plant material may be snap-frozen in liquid nitrogen then stored at -80°C for later use.

Cryogrinding: 20 minutes hands-on time

- Pre-cool the mortar and pestle at -80°C for at least 30 minutes.
- Place the chilled mortar and pestle on ice. Add a small volume of liquid nitrogen into the mortar, then add the cross-linked plant

material briefly and allow to freeze until the liquid nitrogen has evaporated.

- Carefully grind the cross-linked plant material into a fine powder, working quickly to minimise thawing. If the material starts to thaw, freeze further by adding a small volume of liquid nitrogen to the mortar.
- Use a spatula to collect the cryo-ground powder into a chilled 50 ml centrifuge tube on ice. We recommend continuing directly to nuclei isolation.
- Optional Step** If needed, the cryo-ground cross-linked plant material may be snap-frozen in liquid nitrogen then stored at -80°C for later use.

Nuclei isolation: 1.5 hours hands-on time, 1.5 hours procedure

Note: Do not proceed any further unless it is possible to complete the remainder of the protocol consecutively without delay. At no point is it advisable to hold any step longer than stated in this protocol. Increasing the timing of steps will be detrimental to Pore-C data quality and sequencing performance.

- Pre-cool a 50 ml centrifuge to 4°C .
- Prepare the following buffers:
 - 10 ml of 1X PBS and store at 4°C
 - 10X homogenisation buffer:

Component	Stock conc.	Final conc.	Volume
Trizma-base	-	100 mM	0.61 g
KCl	-	800 mM	3.00 g
EDTA	0.5 M	100 mM	10 ml
Spermidine trihydrochloride	-	10 mM	0.13 g
Spermine tetrahydrochloride	-	10 mM	0.17 g
ddH ₂ O	-	-	Up to 50 ml
Total	-	-	50 ml

Note: Adjust the pH to 9.0 - 9.4 with NaOH. Store at 4°C prior to use.

- Nuclei isolation buffer:

Component	Stock conc.	Final conc.	Volume
10X homogenisation buffer	10X	1X	10 ml
Sucrose	-	500 mM	17.12 g
PVP-40	-	1% w/v	1.00 g
ECOSURF™ EH-9	100%	0.5% v/v	500 μl
ddH ₂ O	-	-	Up to 100 ml
β -mercaptoethanol	100% (14.3 M)	0.25% v/v final (35.75 mM)	250 μl
Total	-	-	100 ml

Note: Do not add the β -mercaptoethanol until ready to use. Store at 4°C prior to use. Alternatively to ECOSURF™ EH-9, Triton X-100 may be used.

1. Washing buffer:

Component	Stock conc.	Final conc.	Volume
10X homogenisation buffer	10X	1X	1 ml
Sucrose	-	500 mM	1.71 g
ddH ₂ O	-	-	Up to 10 ml
Total	-	-	10 ml

Note: Store at 4°C prior to use.

1. Resuspend 1.5–2 g of cryo-ground plant material in 20 ml of chilled nuclei isolation buffer in a 50 ml centrifuge tube.
Note: Ensure the buffer has been supplemented with β -mercaptoethanol to 0.25% v/v final.
2. Ensure the centrifuge tube lid is fastened and place the sample horizontally on ice and mix on a gyratory rocker at 50 rpm for 15 minutes.
3. Pass the suspension through a 40 μ m cell strainer into a fresh chilled 50 ml tube on ice.
4. Once the sample has passed through the filter, rinse the original sample tube with a further 5 ml of chilled nuclei isolation buffer and filter through the same 40 μ m cell strainer.
5. Centrifuge for 20 minutes at 4°C using a centrifugation force appropriate for the genome size (see Figure 1):

Genome size (Mb)	Centrifugation force (g)
25	4800
50	4370
100	3970
250	3500
500	3190
1,000	2900
2,500	2560
5,000	2330
10,000	2120
25,000	1870
50,000	1700

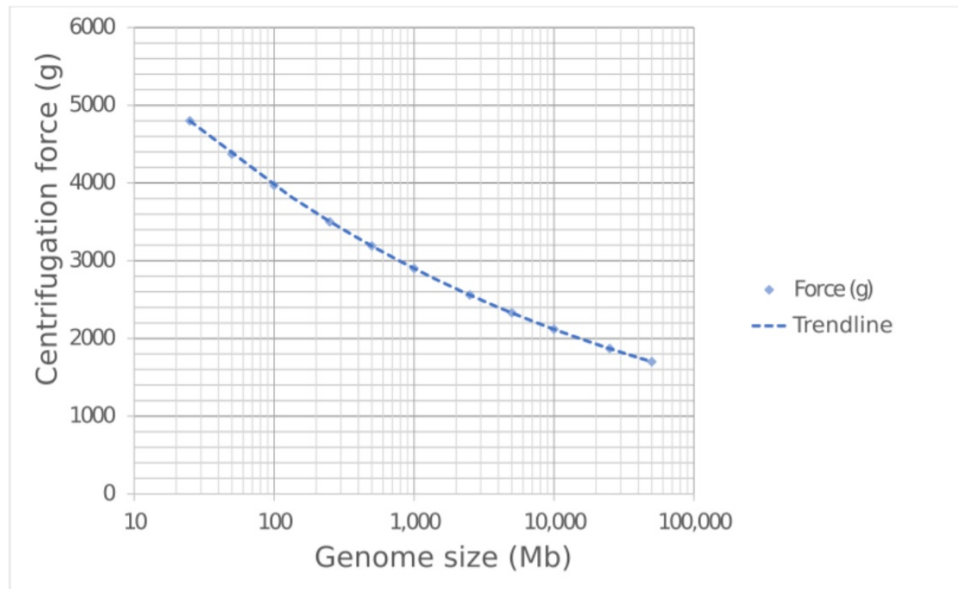


Figure 1. Recommended centrifugation force for nuclei isolation according to genome size.

- Carefully decant and discard the supernatant. Using a wide-bore pipette tip, gently resuspend the pellet in 1 ml of chilled nuclei isolation buffer.
- Add a further 19 ml chilled nuclei isolation buffer to the suspension and mix by swirling.
- Centrifuge for a further 10 minutes at 4°C using a centrifugation force appropriate for the genome size (see Figure 1).
- Repeat steps 29–31 one more time.
- Pre-cool a 2 ml centrifuge to 4°C.
- Carefully decant and discard the supernatant. Using a wide-bore pipette tip, gently resuspend the pellet in 1 ml of cold washing buffer. Transfer the sample to a fresh 2 ml centrifuge tube on ice. Rinse the original tube with a further 1 ml of cold washing buffer and transfer to the same 2 ml centrifuge tube.
- Centrifuge for 5 minutes at 4°C using a centrifugation force appropriate for the genome size.
- Aspirate and discard as much of the supernatant as possible without disturbing the pellet. Using a wide-bore pipette tip, gently resuspend the pellet in 2 ml of chilled 1X PBS.
- Centrifuge for 5 minutes at 4°C at 3000 g.
- Aspirate and discard the supernatant.

Chromatin denaturation: 10 minutes hands-on time, 40 minutes procedure

Note: Pre-heat thermomixer to 62°C.

- Resuspend the pellet of crosslinked plant nuclei in the 100 µl of 0.5% SDS and homogenise by gentle pipetting with a wide-bore pipette tip.
- Incubate at 62°C for 6 minutes without agitation, then cool to room temperature.
- Pre-heat the thermomixer to 37°C.
- Add 250 µl of nuclease-free water and 50 µl of 10% v/v ECOSURF™ EH-9 directly to the plant nuclei suspension for a final concentration of 1.25% v/v ECOSURF™ EH-9 in 400 µl. Mix by gentle pipetting with a wide-bore pipette tip.

Note: Alternatively to ECOSURF™ EH-9, Triton X-100 may be used.

5. Incubate at 37°C for 15 minutes without agitation.

6. Following incubation, the chromatin is ready for digestion. Add the following reagents to the plant nuclei suspension to achieve a final concentration of 1 U/μl for the selected restriction enzyme.

Example NlaIII digestion:

Component	Stock conc.	Final conc.	Volume
Plant nuclei suspension	-	-	400 μl
NEB CutSmart buffer	10X	1X	50 μl
NEB NlaIII	10 U/μl	1 U/μl	50 μl
Total	-	-	500 μl

7. Mix by gentle inversion and incubate the plant nuclei suspension in a thermomixer at 37°C for 18 hours without agitation before proceeding directly to proximity ligation.

Day 2: 5-10 minutes hands-on time, 6-hour procedure time + overnight

Depending on the restriction enzyme selected for the previous in situ digestion, follow one of the appropriate ligation reactions below then continue with the rest of the protocol for Day 2.

Do not attempt both ligation reactions using a single Pore-C extraction.

The proximity ligation reaction protocol selected will depend on the restriction enzyme used:

- If in situ digestion was carried out using a restriction enzyme that can be heat-denatured at <65°C, then proceed with proximity ligation A with heat denaturation.
- If the in situ digestion was carried out using an enzyme that cannot be heat-denatured at <65°C, then proceed with proximity ligation B with chemical denaturation.

It is not advisable to attempt heat denaturation at temperatures >65°C, as this may result in unintended crosslink reversal prior to proximity ligation.

Proximity ligation A with heat denaturation: 5 minutes hands-on time, 6-hour procedure

Note: Pre-heat the thermomixer to 65°C.

1. Heat-denature the restriction enzyme by incubating the plant nuclei suspension in a thermomixer at 65°C for 20 minutes at 300 rpm rotation.
2. Pre-cool the thermomixer to 16°C. Allow the plant nuclei suspension to cool to room temperature (~21°C) and set up the proximity ligation reaction according to the table below. Add the reagents in the following order directly to the plant nuclei suspension.

Component	Stock conc.	Final conc.	Volume
Digestion reaction	-	-	500 μl
Nuclease-free water	-	-	345 μl
NEB ligase buffer	10X	1X	100 μl
Recombinant albumin	20 μg/μl	0.1 μg/μl	5 μl
T4 DNA ligase	400 U/μl	20 U/μl	50 μl
Total	-	-	1000 μl

3. Mix by gentle pipetting with a wide-bore pipette tip, then incubate the plant nuclei suspension in a thermomixer at 16°C for 6

hours without agitation.

Note: Prolonged ligation may increase trans-chromosomal contacts in the Pore-C data.

4. Once the incubation is finished, completing ligation, continue directly to protein degradation and de-crosslinking.

Proximity ligation B with heat denaturation: 5 minutes hands-on time, 6-hour procedure

Note: Pre-heat the thermomixer to 65°C.

1. Chemically denature the restriction enzyme by adding 55 µl 1% SDS directly to the plant nuclei suspension for a final concentration of 0.1% SDS in a final volume of 555 µl. Mix by gently pipetting with a wide-bore pipette tip. Then incubate the plant nuclei suspension in a thermomixer at 65°C for 20 minutes without agitation.
2. Cool the thermomixer to 16°C. Allow the plant nuclei to cool to room temperature (~21°C) and set up the proximity ligation reaction according to the table below. Add the reagents in the following order directly to the plant nuclei suspension and mixing by gentle inversion after every addition.

Component	Stock conc.	Final conc.	Volume
Digestion reaction	-	-	555 µl
Nuclease-free water	-	-	190 µl
ECOSURF™ EH-9	10%	1%	100 µl
NEB ligase buffer	10X	1X	100 µl
Recombinant albumin	20 µg/µl	0.1 µg/µl	5 µl
T4 DNA ligase	-	~5%	50 µl
Total	-	-	1000 µl

Note: Alternatively to ECOSURF™ EH-9, Triton X-100 may be used.

1. Mix by gentle pipetting with a wide-bore pipette tip and incubate the plant nuclei suspension in a thermomixer at 16°C for 6 hours without agitation.
Note: Prolonged ligation may increase trans-chromosomal contacts in Pore-C data.
2. Once the incubation is finished, completing ligation, continue directly to protein degradation and de-crosslinking.

Protein degradation and de-crosslinking: 5 minutes hands-on time, 5 minutes procedure

Note: Pre-heat the thermomixer to 56°C.

1. To set up the protein degradation reaction, add the following reagents to the previous ligation reaction:

Component	Stock conc.	Final conc.	Volume
Ligation reaction	-	-	1000 µl
Nuclease-free water	-	-	300 µl
Tween-20	20%	5%	500 µl
SDS	10%	0.5%	100 µl
Proteinase K	20 µg/µl	1 µg/µl	100 µl
Total	-	-	2000 µl

2. Mix by gentle inversion. Incubate the plant nuclei suspension in a thermomixer at 56°C for 18 hours with periodic <1000 rpm

rotation (<30 seconds every 15 minutes) to prevent condensation inside the lid.

Note: Incubation at 56°C is an ideal compromise of enzyme activity over a prolonged period. It is not advisable to incubate at higher temperatures as enzyme activity will reduce over time.

Day 3: DNA extraction: 40 minutes hands-on time, 110 minutes procedure

Note: Pre-cool a 5 ml centrifuge to 15°C.

1. Cool the protein degradation reaction on ice, then transfer the entire volume to a 5 ml centrifuge tube. Rinse the original tube with a further 200 µl of nuclease-free water and transfer this to the 5 ml centrifuge tube.
2. The total sample volume should be approximately 2200 µl. Add an equal volume of chilled phenol:chloroform:isoamyl alcohol (25:24:1) saturated with 10 mM Tris.HCl pH 8.0 1 mM EDTA.
Note: Adjust this volume as needed to match that of the sample.
3. Mix with gentle inversion for 5 minutes to achieve a homogenous emulsion.
4. Centrifuge the sample at 15,000 g, 15°C for 15 minutes.
5. Carefully aspirate 2000 µl of the aqueous phase and transfer to a fresh 5 ml centrifuge tube. Add an equal volume of chilled phenol:chloroform:isoamyl alcohol (25:24:1) saturated with 10 mM Tris.HCl pH 8.0 1 mM EDTA.
Note: Adjust this volume as needed to match that of the sample.
6. Mix with gentle inversion for 5 minutes to achieve a homogenous emulsion.
7. Centrifuge the sample at 15,000 g, 15°C for 15 minutes.
8. Transfer the aqueous phase to a fresh 5 ml centrifuge tube and make a note of the recovered volume (~2000 µl).
Note: Be cautious not to take any of the interphase layer.
9. Transfer half of the recovered aqueous phase to a second 5 ml centrifuge tube to create two equal aliquots.
10. Add 0.02 volumes of 5 M NaCl (0.1 M final) and 0.1 volumes of 3 M sodium acetate pH 5.5 (0.3 M final) relative to the volume of the recovered aqueous phase in Step 8 to both 5 ml centrifuge tubes. Mix by gentle agitation and the solution will likely turn cloudy and then clear.
11. Add 3 volumes of 100% EtOH relative to the volume of the recovered aqueous phase in Step 8 to both 5 ml centrifuge tubes. Mix by gentle inversion and precipitate at -80°C for >1 hour or overnight at -20°C if possible.
12. Pre-cool the centrifuge to 4°C.
13. Centrifuge the samples in the 5 ml centrifuge tubes at 16,000 g, 4°C for 30 minutes.
14. Aspirate and discard the supernatant before washing the pellets in 4 ml 80% EtOH.
15. Centrifuge both samples at 16,000 g, 4°C for 5 minutes.
16. Aspirate and discard the supernatant before washing the pellets in 2 ml 70% EtOH.
17. Centrifuge the samples at 16,000 g, 4°C for 5 minutes.
18. Aspirate and discard the supernatant. Briefly spin down the tubes and aspirate any residual supernatant, then allow the pellets to air-dry for 5 minutes.
Note: After the pellets have air-dried, they may become loose in the tube.
19. Carefully resuspend both pellets individually in 75 µl TE buffer and incubate for 5 minutes at room temperature (~21°C). Mix by gentle agitation every few minutes.
20. Briefly spin down the tubes before transferring and pooling both samples into a 1.5 ml microcentrifuge tube.
21. Quantify the DNA concentration by using the Qubit dsDNA HS Assay Kit. Ensure a 1/10 dilution is used, as the Qubit reading will

be affected by high salt concentration.

22. Store the sample at 4°C until ready to begin the library preparation.

23. **Optional Step:** Analyse ~100 ng of the final DNA extract on an Agilent Bioanalyser 12,000 bp chip for average fragment size.

Library preparation

Please refer to the [Restriction Enzyme Pore-C info sheet](#) for guidance on which library prep kit to choose.

Sequencing experiment

When starting your sequencing experiment in MinKNOW, choose the SQK-PRC109 kit under Kit Selection.

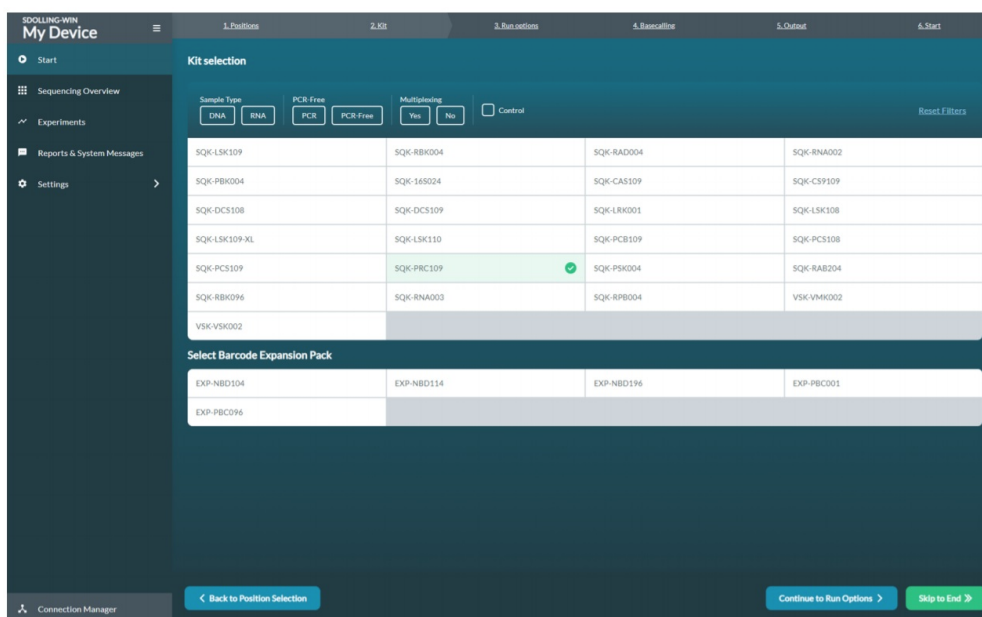


Figure 2. Select SQK-PRC109 in the Kit Selection tab in MinKNOW when setting up the sequencing run.

Results

Sample	DNA concentration, ng/μl	Total DNA mass, μg
<i>Arabidopsis thaliana</i>	24.2	3.63

Table 1. The yield of non-size selected RE-Pore-C DNA extract using NlaIII restriction enzyme.

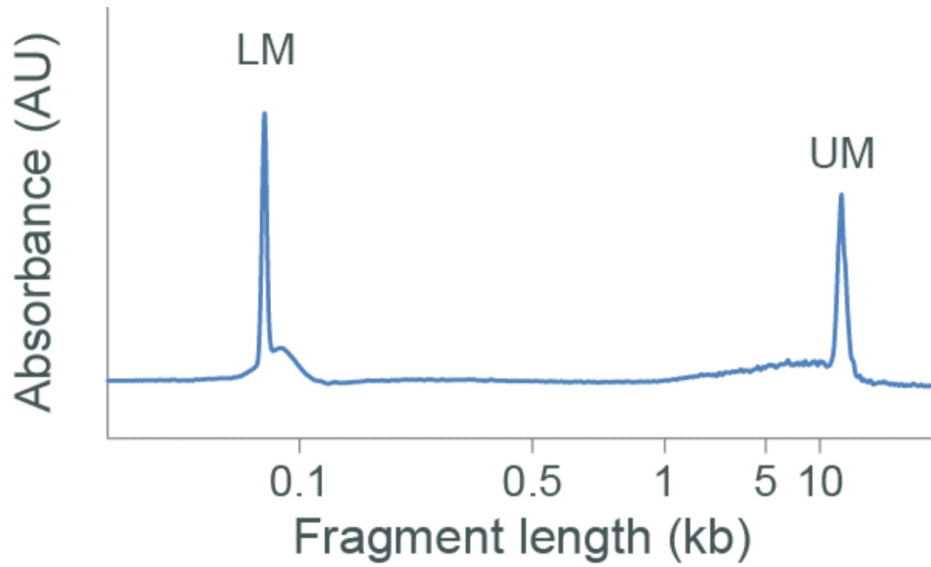
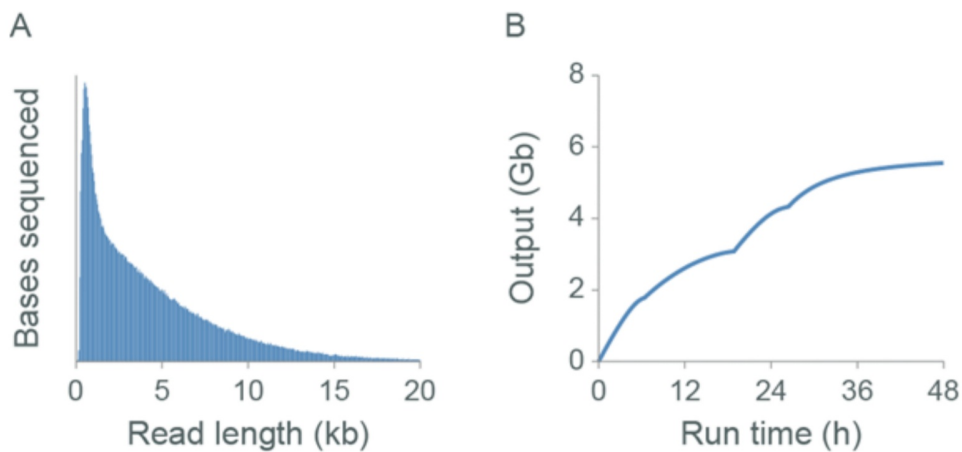


Figure 2. Agilent Bioanalyser DNA 12000 trace of non-size selected *Arabidopsis thaliana* RE-Pore-C DNA extract.



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Performance metric	Value
read N50 (kb)	~3
typical output (FLOMIN-106D) [‡] (Gb)	~5.8
estimated mean monomer length (kb)	~1.5
estimated monomers/N50 read	~2
contacts/Gb	~4 million
cis contacts (%)	~60%

[‡] Nuclease flushes were performed to optimise flow cell output

Figure 3. The sequencing and Pore-C output for libraries assessed on GridION Mk1. Libraries were generated as described using *Arabidopsis thaliana* Pore-C extract prepared with the NlaIII restriction enzyme. The read length distributions and output (Gbases) obtained from the libraries generated are shown in panel A and B, respectively. Panel C displays the Pore-C metrics obtained

Change log

Version	Change note
v4, 24th June 2022	Corrected the nuclei isolation buffer components
v3, 2nd March 2022	Updated step 10 of the day 3 prep to the correct volume of NaCl and the correct concentration
v2, 25th March 2021	Updated materials to include "ECOSURF™ EH-9 (Sigma, STS0006) or Triton X-100 (Sigma, X100)". Updated Day 1, step 5 to include ECOSURF™ EH-9. Updated Nuclei isolation buffer table to include ECOSURF™ EH-9. Updated section: Chromatin denaturation, step 4 to include ECOSURF™ EH-9. Updated Proximity ligation B with heat denaturation table to include ECOSURF™ EH-9.
v1, 30th July 2020	Initial protocol publication