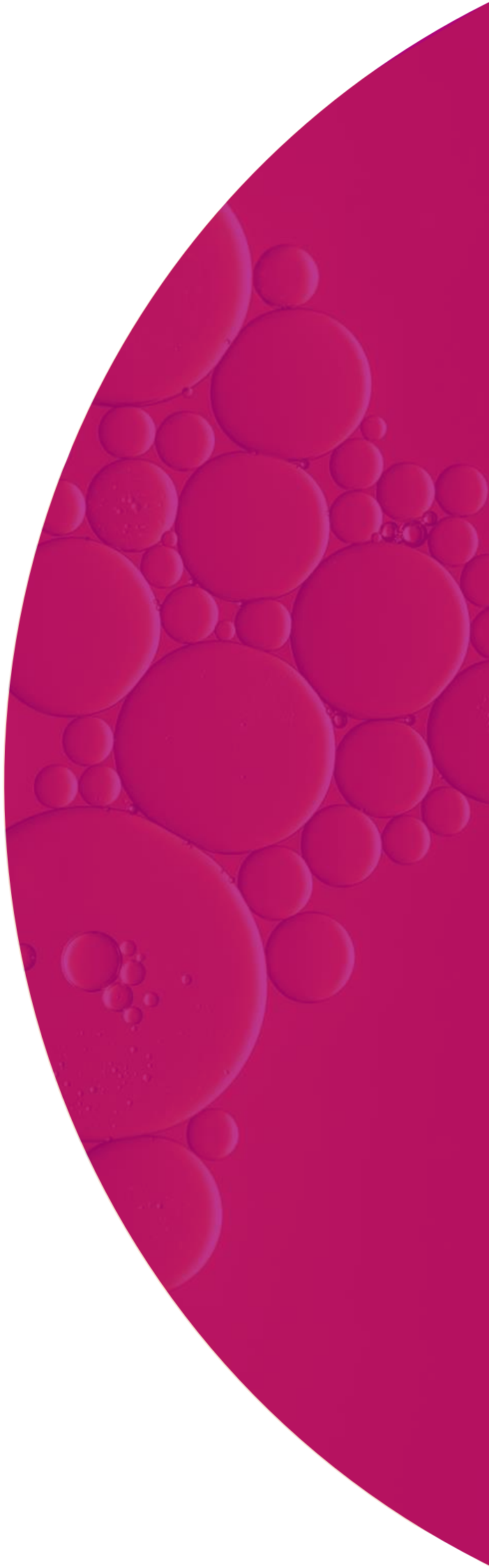


# Homogenizing tissue using cryoPREP

Procedure & checklist



## User supplied equipment and reagent list

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| Equipment                              | Model                 |
|--|-----------------------|
| Nanobind® tissue kit                   | PacBio® (102-302-100) |
| cryoPREP automated dry pulverizer CP02 | Covaris (500001)      |
| tissueTUBE extra thick TT05M XT        | Covaris (520140)      |
| milliTUBE 2 mL                         | Covaris (520132)      |
| tissueTUBE TT05 insertion tool         | Covaris (500231)      |
| Liquid nitrogen (LN2)                  |                       |
| Dry ice                                |                       |

# cryoPREP tissue homogenization

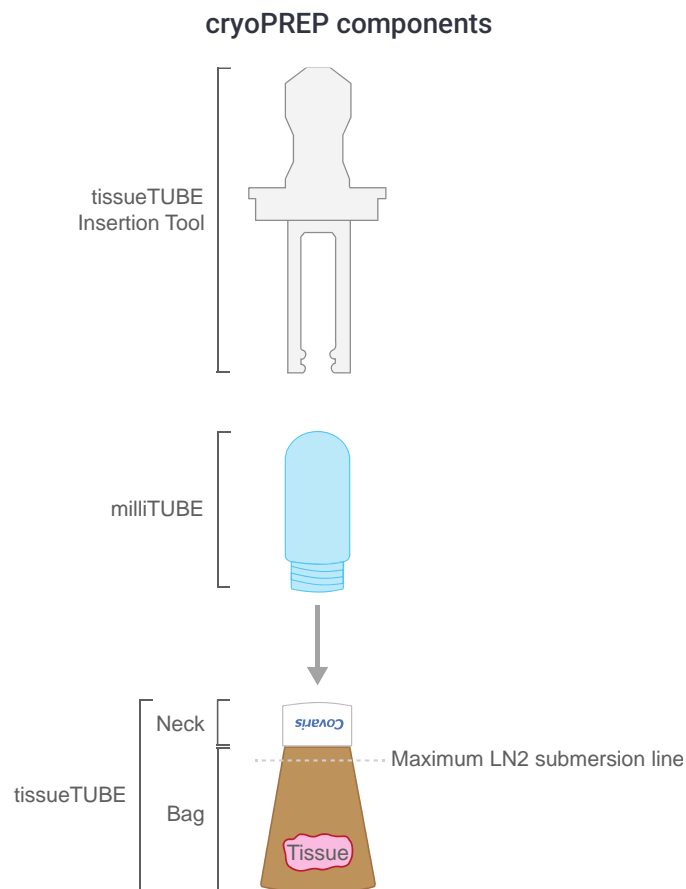
This Procedure & checklist describes tissue homogenization using the Covaris cryoPREP Automated Dry Pulverizer. Extraction data is provided from various human tissue samples.

## Sample notes

- This protocol has been tested using shrimp and various human tissues including skeletal muscle, pancreas, and colon.

## Protocol notes

- This Procedure & checklist is meant to be used with the **Nanobind tissue kit** (102-302-100).
- Tissue is placed in a tissueTUBE, secured with a milliTUBE, and placed on dry ice.
- Using the tissueTUBE Insertion Tool to hold the tissueTUBE, the tissue is incubated in liquid nitrogen.
- Tissue is pulverized with 10 impacts on maximum force interspersed with 10 second incubations in liquid nitrogen.
- Pulverized tissue is transferred to a 2 mL Protein LoBind tube.
- Sample can be imported into step 7 of either the **standard TissueRuptor** or **standard dounce homogenizer** protocol.



# Protocol

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1. Using clean tweezers, insert ~25 mg of tissue into the bag of an extra thick TT05M XT tissueTUBE.
  - The tissue should be placed approximately two-thirds of the way into the bag.
2. Screw in a 2 mL milliTUBE half way into the neck.
  - It is critical that the milliTUBE is not tightened all the way in order to permit gas to escape during the cryoPREP impacts.
3. Place the tissueTUBE on dry ice for  $\geq 5$  min.
  - This dry ice incubation is to chill the tissue and the plastic neck of the tissueTUBE.
  - If cryoPREP pulverizing more than one sample, place all tissueTUBEs (containing sample) on dry ice until ready to use.
4. Clasp the milliTUBE with the tissueTUBE insertion tool.
  - The tissueTUBE insertion tool functions as the handle for the tissueTUBE.
5. Submerge the bag of the tissueTUBE in liquid nitrogen (LN<sub>2</sub>) for  $\geq 30$  s.
  - Do not submerge the neck of the tissueTUBE in LN<sub>2</sub>, since this may cause it to crack.
  - Wait until the “sizzling” around the bag has diminished. This will take 30–90 s.
6. Move the tissueTUBE into the cryoPREP machine.
  - Make sure the milliTUBE is not tightened all the way.
7. Activate one impact on the maximum setting.
8. Remove the tissueTUBE and immediately pinch the bag with your fingertips to loosen the tissue pieces.
9. Repeat steps 5–8 nine times (for a total of ten impacts).
10. Remove tissueTUBE insertion tool, tighten the milliTUBE into the neck, and place the tissueTUBE on dry ice for  $\geq 1$  min.
11. Invert the tissueTUBE and flick the bag so that the pulverized tissue powder moves from the bag into the milliTUBE. Replace the tissueTUBE on dry ice for  $\geq 1$  min.
12. Repeat step 11 until all the pulverized tissue powder has moved from the bag into the milliTUBE.
13. Carefully unscrew the tissueTUBE off of the milliTUBE.
  - Sometimes pulverized powder becomes wedged in the neck and can fall out when the two components are separated.
14. Add 750  $\mu$ L of cold Buffer CT to the milliTUBE and pipette mix to resuspend powder.
  - Buffer CT should be kept on ice when removed from the refrigerator.
15. Transfer resuspended powder to a 2 mL Protein LoBind microcentrifuge tube.
  - If there is still tissue left in the milliTUBE, add another 750  $\mu$ L cold Buffer CT to rinse the milliTUBE and combine with the first 750  $\mu$ L.

## Quick tip

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DO NOT screw the milliTUBE all the way in. If the milliTUBE is tightened all the way, the bag may burst open during the cryoPREP impact in step 7.

## Quick tip

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Submerge only the bag of the tissueTUBE in LN<sub>2</sub> to avoid cracking the neck.

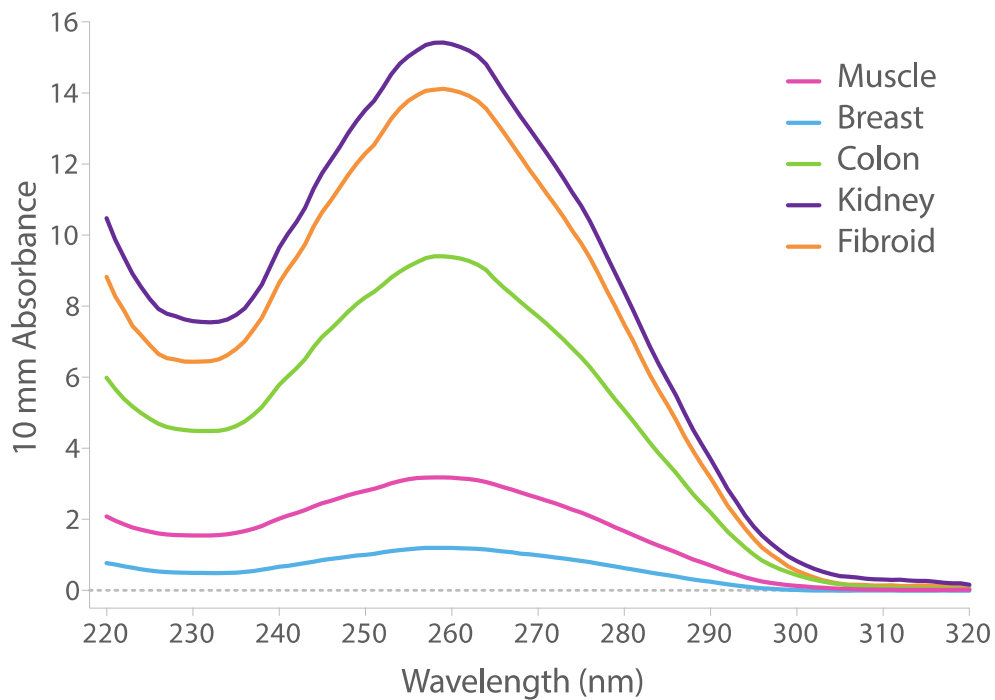
16. Proceed to step 7 in either the [Procedure & checklist – Extracting HMW DNA from animal tissue using TissueRuptor](#) or the [Procedure & checklist – Extracting HMW DNA from standard Dounce homogenizer tissue using Nanobind kits](#).

## DNA extraction yield and purity

- All samples were disrupted using cryoPREP.
- All tissues yielded DNA with good recovery and purities.

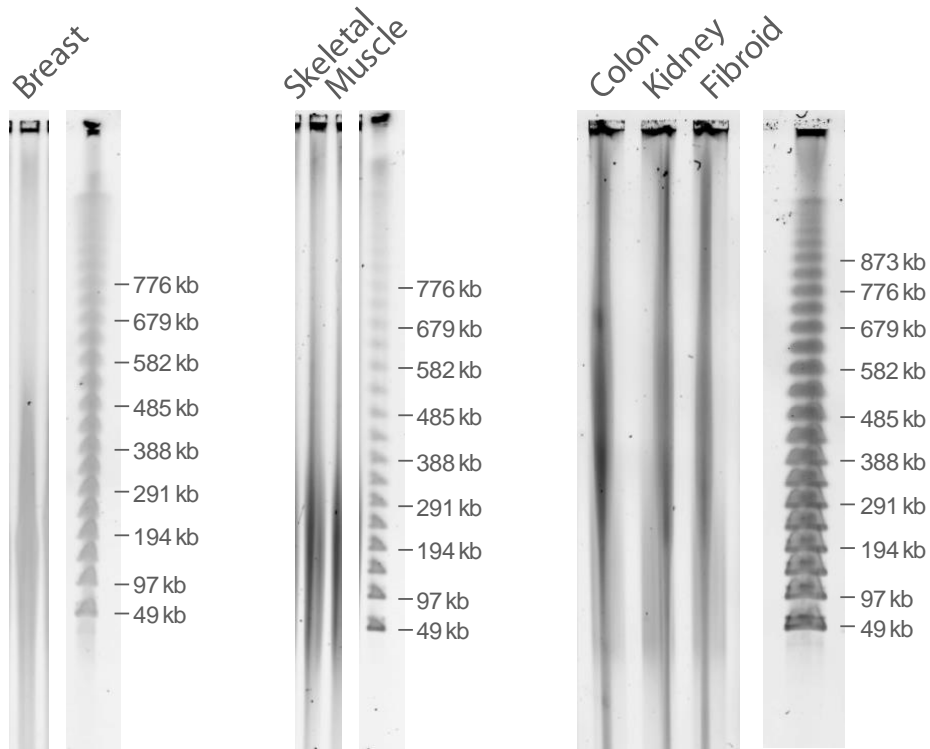
| Sample                | Sample input | dsDNA yield | % RNA | A260/A280 | A260/A230 |
|-----------------------|--------------|-------------|-------|-----------|-----------|
| Human colon           | 35 mg        | 39.8 µg     | 25    | 1.85      | 2.09      |
| Human kidney          | 47.5 mg      | 31.2 µg     | 22.3  | 1.83      | 2.03      |
| Human fibroid         | 34.5 mg      | 33.5 µg     | 44.6  | 1.88      | 2.19      |
| Human skeletal muscle | 26 mg        | 6.8 µg      | 16.1  | 1.90      | 2.05      |
| Human fibrous breast  | 35 mg        | 44.2 µg     | 7.4   | 1.85      | 2.06      |

cryoPREP DNA Nanodrop Spectra



# DNA size

- The DNA size was consistently 50–300+ kb for all tissue types.



PFGE of DNA extracted using the Nanobind tissue kit with various tissues disrupted with Covaris cryoPREP.

The samples (human breast, skeletal muscle, colon, kidney and uterine fibroid) were validated using third generation sequencing prior to HiFi sequencing (data not shown).

| Revision history (description) | Version | Date          |
|--------------------------------|---------|---------------|
| Initial release                | 01      | July 2022     |
| Minor updates throughout       | 02      | December 2022 |

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