(Web Link)	National Biobank Consortium of Taiwan Standard Operating Procedure	No.: NBCT SOP-004
	Title: NBCT Fresh Frozen Tissue Specimen Collection and DNA Extraction Process	Version/Total Pages: No. 1.1 / 17 pages
		Issue Date: 114-12-01

Document Revision History:

Version	Issuing Unit	Approval Date	Approved By	Issue Date
1.0	Central Office	110-07-14	Ministry of Health and Welfare	110-07-20
1.1	Central Office	114-09-16	Ministry of Health and Welfare	114-12-01

Table of Contents

1. Purpose

"National Biobank Consortium of Taiwan" (hereinafter referred to as the "Integration Platform") primarily integrates domestic human biobanks — their data, biospecimens, and related information (hereinafter referred to as "Integration Platform biospecimen data") — through a cloud-based system, making them available for application and use by medical, clinical, industry, and related biomedical research investigators nationwide. To achieve cross-institutional collaboration, it is necessary to establish consistent quality standards for dispensed biospecimens and consistent clinical data content. To maintain good specimen quality and to ensure consistency in the steps for fresh frozen tissue specimen collection and DNA handling, so as to facilitate future related research, this Standard Operating Procedure is hereby established.

2. Scope of Application

This SOP applies to the entire process from fresh frozen tissue specimen collection through tissue DNA extraction and completion of quality testing.

3.Responsibilities

All human biobanks that have joined the Integration Platform, when extracting DNA from human tissue they have collected, are recommended to follow this Standard Operating Procedure, or must otherwise achieve the same quality and yield.

4. Description

This SOP can be divided into five stages

1. Fresh frozen tissue specimen collection from surgical resection or biopsy
2. Frozen tissue embedding and section staining interpretation
3. Frozen tissue DNA extraction
4. Frozen tissue DNA purification (as needed)
5. Frozen tissue DNA quality testing (horizontal electrophoresis)

5. Operating Procedures

5.1 Fresh Frozen Tissue Specimen Collection from Surgical Resection or Biopsy

Materials and Equipment:

cryotubes, sterilized fine-tipped forceps (11cm), scalpel blade, aluminum foil, liquid nitrogen, thermos flask, freeze-resistant labels, -80℃ freezer or liquid nitrogen tank.

Procedure:

After obtaining case consent, first prepare cryotubes for the tissue to be preserved, affix pre-numbered freeze-resistant labels, and draw an appropriate amount of liquid nitrogen into a thermos flask beforehand. Under the guidance of a pathologist, cut the remaining tissue specimen from the surgical resection or biopsy after pathological examination, dividing it into tumor tissue and surrounding non-tumor tissue; place it on aluminum foil, and immediately cut it into small pieces using a scalpel blade (approximately 5x5x5 mm^3), then rapidly freeze it in liquid nitrogen. Using fine-tipped forceps, place the pieces into a cryotube (a single tube can hold many pieces), and store at -80℃ in a freezer or liquid nitrogen tank.

Precautions:

1. Because collecting fresh tissue specimens involves many variables, handling must be adapted to the actual situation.
2. Priority must be given to having sufficient tissue for pathological diagnosis; if the remaining tissue is insufficient, do not retain frozen tissue.
3. If the tumor tissue specimen cut is already very small, there is no need to cut it into smaller pieces.
4. Sometimes surgery finishes very late and same-day collection is not possible; if operating room staff can assist by first refrigerating the specimen at 4℃ (it must not be placed in formalin), it may be collected the next day (this should be noted as "collected the next day") — do not discard this specimen. If a fresh specimen is quickly placed in refrigeration, the DNA quality of some tissues can still be very good even if collected the next day.

5.2 Frozen Tissue Embedding and Section Staining Interpretation

Equipment:

-80℃ freezer, cryostat, stainer, coverslipper

Consumables:

1. Aluminum foil molds (Note: cut aluminum foil into squares of approximately 4 cm, use the back end of a whiteboard marker as a form to make the aluminum foil

molds.)
2. OCT
3. Sterilized fine-tipped forceps (11cm)
4. 100ml beaker
5. Specimen storage box (10x10 grid) (the inner grid dividers need to be removed, so that the grid space becomes 5x5, allowing 25 aluminum foil molds to be placed.)
6. 1.5ml microcentrifuge tube (eppendorf)
7. Foam box (for holding frozen tissue and dry ice)
8. Dry ice
9. Glass slides

Procedure:

1. Before extracting tissue DNA, first prepare a foam box (already loaded with dry ice) and the list of specimens to be retrieved. From the –-80℃ freezer, take out the 9x9 grid specimen storage box containing cryotubes and place it in the foam box (already loaded with dry ice); take out the frozen tissue pieces one by one and place them into the correspondingly pre-numbered microcentrifuge tubes. Then return the cryotubes to their places one by one. This step requires two people; the other person is responsible for verifying that the numbered microcentrifuge tubes are correctly filled.
2. Place the microcentrifuge tubes containing the tissue on the retrieval list into a 9x9 grid specimen storage box, and store it in the –-80℃ freezer until needed.
3. Write the specimen number on the side of each aluminum foil mold in advance.
4. Take out the specimen storage box containing the microcentrifuge tubes with the frozen tissue pieces, and place it into the foam box containing dry ice, keeping it frozen until needed.
5. In the foam box containing dry ice, place the aluminum foil molds labeled with specimen numbers on the dry ice. Using sterilized forceps, remove the tissue specimen from the microcentrifuge tube and quickly place it, largest surface facing down, into the bottom of the aluminum foil mold; add OCT to completely cover the specimen, avoiding thawing of the tissue as much as possible throughout the process.
6. Place the used forceps into a beaker containing water, to be cleaned and sterilized together afterward.
7. Once the OCT has frozen white, place it into a specimen storage box that has been broken down into a 5x5 grid. Then place it in the –-80℃ freezer; after freezing for half an hour, cryosectioning can be performed. The tissue number for each grid position must be noted.
8. Take –the OCT-embedded tissue specimen, now fully frozen, out of the -80℃ freezer, peel off the aluminum foil, and place it in the cryostat for sectioning at a thickness of 4 μm (the glass slide should already be labeled), and air-dry at room temperature for at least 2 hours before performing H&E staining. Air-drying for four hours or overnight is even better, to prevent the section from detaching.
9. Have the pathologist interpret and record the tumor diagnosis and percentage.

Precautions:

1. Throughout the entire process, ensure that the numbering is consistent.
2. During OCT embedding, the tissue may thaw slightly, which is more conducive to close adhesion with the OCT; this does not affect DNA quality. However, if RNA is to be extracted, the tissue must never have thawed. Therefore, for RNA extraction, it is best to select frozen tissue that has already been confirmed by sectioning, without needing to perform a new cryosection.

5.3 Frozen Tissue DNA Extraction

Equipment:

1. Water bath (Note: two units are needed, at temperatures of: 55℃ and 37℃ respectively.)
2. –-80℃ freezer
3. High-speed microprocessor-controlled large-capacity centrifuge
4. Benchtop high-speed refrigerated microcentrifuge (eppendorf centrifuge 5415 R or equivalent product)
5. Benchtop high-speed microcentrifuge
6. Ultra-micro spectrophotometer (Thermo NANODROP 2000 or equivalent product)
7. Vortex mixer(FN-CR95 or equivalent product)
8. Pipetman (pipetman) (1,000ul, 200ul, 10ul)
9. Water purification system
10. Chemical fume hood (chemical hood)

Reagents:

1. Cell lysis solution (QIAGEN, Cat.No.158908)
2. Proteinase K (Ambion, AM2546, 20mg/ml)
3. Ribonuclease (RNase) (AMRESCO CAT#0675-1G, 10mg/ml), aliquoted at 500ul into 1.5ml microcentrifuge tubes (eppendorf), stored frozen at –20℃ until use.
4. Protein Precipitation solution (QIAGEN, Cat.No.158912)
5. 100% isopropanol
6. 75% ethanol (75% EtOH)
7. Sterilized secondary distilled water, ddH_2O (abbreviated as “secondary water”)

Consumables:

1. Anti-contamination paper (Note: cut several squares of anti-contamination paper approximately 5 cm on each side)

2. Aluminum foil (Note: cut aluminum foil into squares approximately 5 cm on each side)
3. Parafilm (Note: cut parafilm into squares approximately 5 cm on each side)
4. Sterilized fine-tipped forceps (11cm)
5. Dry ice
6. No. 21 scalpel blade
7. 15ml conical centrifuge tubes (Note: each tissue specimen requires three 15ml conical centrifuge tubes)
8. 1.5ml microcentrifuge tube (eppendorf)
9. 3ml dropper
10. Filter (Filter tip) (1,000ul, 200ul, 10ul)
11. Specimen storage box (9x9 grid)

Procedure:

1. Prepare in advance the pre-numbered aluminum foil and parafilm, and the 15ml conical centrifuge tubes (three) with the specimen number written on the cap and side, as well as the 1.5ml microcentrifuge tube.
2. Turn on the water bath and set the temperature to 55℃.
3. Take the OCT-embedded frozen tissue block that has “confirmed tumor diagnosis and percentage” out of the -80℃ freezer, place it on aluminum foil, and let it thaw at room temperature (10 minutes).
4. While waiting for the specimen to thaw, add 3ml of cell lysis solution to the first 15ml conical centrifuge tube using a 3ml dropper.
5. Using sterilized forceps, remove the specimen from the thawed OCT and place it onto parafilm (use the forceps to remove OCT adhering to the outside of the specimen, minimizing residue as much as possible).Using a No. 21 scalpel blade,
6. mince the specimen placed on the parafilm, with a piece of anti-contamination paper cut to 5x5cm2 placed underneath. Using the blade, transfer the specimen into the conical centrifuge tube from step 4, and tighten the cap and gently shake to mix the specimen and cell lysis solution thoroughly.Add 20µl of proteinase K
7. using a 200ul pipetman, and mix thoroughly.Place the 15ml conical centrifuge tube in the 55℃ water bath
8. overnight. (Because the tissue specimen will settle at the bottom of the conical tube, occasionally shake the conical centrifuge tube to accelerate dissolution of the specimen.)The next day, turn on the second water bath and set the temperature to

37℃.Take the 500µl-per-tube aliquots of ribonuclease (RNase) out of the 20℃ freezer and place them in the 4℃

9. freezer, allowing them to thaw gradually. Once the specimen in the 15ml conical centrifuge tube is confirmed to be fully dissolved (completely clear)
10. , add –6µl of ribonuclease (RNase) using a 10ul pipetman, mix gently and thoroughly,
11. then place it in the 37℃ water bath and let stand for 60 minutes.Remove the conical centrifuge tube from the 37℃ water bath and let it cool at room temperature. In a chemical fume hood, add 2ml of protein precipitation solution using a 3ml dropper and mix thoroughly. Then move it into the 4℃ freezer and let stand for
12. 30 minutes.Remove the conical centrifuge tube from the 4℃ freezer, and shake it so that the white precipitate is evenly dispersed throughout the tube. Then place the conical centrifuge tube into the high-speed microprocessor-controlled large-capacity centrifuge and centrifuge at 3,200rpm for 25 minutes.Using a 3ml dropper, draw off
13. the supernatant from the centrifuged 15ml conical centrifuge tube, and transfer it into a second 15ml conical centrifuge tube.Place the first
14. 15ml conical centrifuge tube, from which the supernatant has already been drawn off, into the high-speed microprocessor-controlled large-capacity centrifuge, and centrifuge at 3,200rpm for 1 minute, then use a 200µl pipetman
15. to transfer the remaining supernatant once more into the second 15ml conical centrifuge tube.To the second 15ml conical centrifuge tube, now containing the supernatant, add 4ml of 100% isopropanol (this must be done in a chemical fume hood) using a 3ml dropper, close the cap tightly and mix thoroughly, then seal the cap with parafilm. Then place the conical centrifuge tube on the vortex mixer and mix at room temperature for 50
16. minutes. (Note: a fibrous strand may form at this point.)Place the second 15ml conical centrifuge tube into the high-speed microprocessor-controlled large-capacity centrifuge, and centrifuge at 3,200rpm for 25 minutes.Transfer the supernatant from the second 15ml conical centrifuge tube in step 17 into a third 15ml conical centrifuge tube for retention. (Note: this supernatant is retained for the time being and may be discarded once the extracted DNA is confirmed to be satisfactory.)
17. Using a 1,000ul pipetman, transfer the precipitate from the tube

18. in step 18 into a 1.5ml microcentrifuge tube, and add 1ml of 75% EtOH, and mix
19. gently and thoroughly.Place the microcentrifuge tube into a pre-chilled high-speed refrigerated microcentrifuge (4℃), and centrifuge at 16,100xg for 10 minutes.Discard the supernatant, retain
20. the precipitate, and, using a benchtop high-speed microcentrifuge (or high-speed refrigerated microcentrifuge), centrifuge at 16,100xg for 30 seconds. Finally, use a 200ul
21. pipetman to remove the remaining supernatant.Air-dry at room temperature, visually estimating the size of the pellet (pellet) in order to determine the volume of water needed for resuspension.
22. Add the required amount of secondary water using a 200ul pipetman.Once the pellet is completely dissolved, gently tap the bottom of the 1.5 ml microcentrifuge tube to mix the tissue DNA within thoroughly.
23. Using the benchtop microcentrifuge, centrifuge at 16,100xg for 30 seconds. Place the 1.5 ml microcentrifuge tube in an ice bucket containing crushed ice; using a 10ul pipetman, draw 2ul of tissue DNA , and use the ultra-micro spectrophotometer (Nanodrop) to measure the DNA concentration and O.D. value: 260/280 & 260/230 (see Attachment 1).If, according to the ultra-micro spectrophotometer measurement results, the O.D. value at either
24. 260/280 or 260/230 is below 1.8, DNA purification (DNA Purification) is required.If the O.D. value is satisfactory, then the tissue DNA microcentrifuge tube, once its concentration has been measured, can be placed in numerical order into a specimen storage box (9x9
25. grid) (with the specimen number and specimen type noted on the lid and side of the box), and temporarily stored in the 4℃ freezers until the horizontal electrophoresis gel run is complete, after which it is moved to the -80℃ freezer for storage.

Note: Because specimens are precious, if the total tissue DNA quantity is less than 30ug, it is recommended not to purify further. If the total tissue DNA quantity is less than 50ug, it is recommended to discuss with the biomedical supervisor whether purification is needed, because the DNA purification process results in the loss of one-third to more than one-half of the DNA.

5.4 Frozen Tissue DNA Purification (as needed) (must be performed entirely in a chemical fume hood)

Reagents:

1. Phenol:chloroform:isoamyl alcohol (25:24:1)
2. Chloroform
3. 100% ethanol (100% EtOH)
4. 75% ethanol (75% EtOH)
5. 5M sodium chloride (NaCl)
6. Sterilized secondary distilled water, ddH_2O (abbreviated as “secondary water”)

Procedure:

1. To the tissue DNA in the 1.5ml microcentrifuge tube, add secondary water to bring the volume up to 500µl, then add 500µl of phenol/chloroform, and shake vigorously for approximately 20 seconds to thoroughly mix the organic solvent and aqueous layers.
2. Centrifuge the thoroughly mixed 1.5ml microcentrifuge tube in the high-speed refrigerated microcentrifuge at 25℃, 16,100xg, for 5 minutes.
3. Carefully transfer the supernatant from the centrifuged 1.5ml microcentrifuge tube into a second new 1.5ml microcentrifuge tube.
4. To the second 1.5ml microcentrifuge tube, add an equal volume (approximately 500µl) of phenol/chloroform, and shake vigorously for approximately 20 seconds to thoroughly mix the organic solvent and aqueous layers.
5. Centrifuge the thoroughly mixed second 1.5ml microcentrifuge tube in the high-speed refrigerated microcentrifuge at 25℃, 16,100xg, for 5 minutes.
6. Carefully transfer the supernatant from the centrifuged 1.5ml microcentrifuge tube into a third new 1.5ml microcentrifuge tube.
7. To the third 1.5ml microcentrifuge tube, add 500µl of chloroform, and shake vigorously for approximately 20 seconds to thoroughly mix the organic solvent and aqueous layers.
8. Centrifuge the third 1.5ml microcentrifuge tube in the high-speed refrigerated microcentrifuge at 25℃, 16,100xg, for 5 minutes.
9. Carefully transfer the supernatant from the centrifuged 1.5ml microcentrifuge tube into a fourth new 1.5ml microcentrifuge tube.
10. To the fourth 1.5ml microcentrifuge tube, add 1,000µl of 100% ethanol and 50µl of 5M NaCl, and mix gently and thoroughly.

11. Place this microcentrifuge tube into the -20℃ freezer for 30 minutes to allow the reaction to go to completion.
12. Place the microcentrifuge tube into a pre-chilled high-speed refrigerated microcentrifuge (4℃), and centrifuge at 16,100xg for 10 minutes.
13. Discard the supernatant, retain the precipitate, add 1,000µl of 75% ethanol, and centrifuge in the high-speed refrigerated microcentrifuge (4℃), at 16,100xg for 10 minutes.Centrifuge in the benchtop microcentrifuge
14. , at 16,100xg for 30 seconds. Finally, use a 200µl pipetman to remove the remaining supernatant.
15. Air-dry at room temperature, while visually estimating the size of the pellet (pellet) in order to determine the volume of water needed for resuspension. Add the required amount of secondary water using a 200ul pipetman.
16. Once the pellet is completely dissolved, gently tap the bottom of the 1.5 ml microcentrifuge tube to mix the DNA within thoroughly.Using the benchtop high-speed microcentrifuge, centrifuge at 16,100xg for 30 seconds.Place the 1.5 ml microcentrifuge tube in an ice bucket containing crushed ice; using a 10ul pipetman, draw 2ul of tissue DNA, and use the ultra-micro spectrophotometer to measure the tissue DNA concentration and O.D. value. Because specimens are precious, if the O
17. .D. value is still not satisfactory, discuss with the biomedical supervisor whether further purification is needed.Once the tissue DNA concentration has been measured, place it in numerical order by specimen number into a specimen storage box (9x9 grid) (with the specimen number and specimen type noted on the lid and side of the box), and temporarily store it in the 4℃ freezer until the horizontal electrophoresis gel run is complete, after which it is moved to the -80℃ freezer for storage.

5.5 Frozen Tissue DNA Quality Testing (Horizontal Electrophoresis)

Equipment:

1. High-sensitivity gel imaging system (SYNGENE In Genius 3 or equivalent product)
2. Microwave oven
3. Mini electrophoresis tank
4. Micro analytical balance

5. Benchtop horizontal shaker (DSR-2800D-N or equivalent product)

Reagents:

1. UltraPure™ TAE Buffer, 10X (Thermo Cat no.15558042)
2. Agarose gel powder, Agarose – Molecular Biology Grade (Invitrogen Cat no.75510-019)
3. Ethidium bromide (EtBr)

Consumables:

1. Weighing paper (12x12cm), spatula
2. Plastic wrap
3. 500ml sterilized Erlenmeyer flask
4. 1L sterilized serum bottle
5. Graduated cylinders (50ml and 1L)

Procedure:

1. Preparing the Agarose Gel (0.8% gel)

1. Prepare 1X TAE Buffer: using a 1L graduated cylinder, pour 100ml of UltraPure™ TAE Buffer (10X) into a 1L serum bottle, then add 900ml of secondary water (ddH_2O), shake up and down to mix thoroughly, and store at room temperature.
2. Using a 50ml graduated cylinder, pour 40ml of the 1X TAE Buffer into the sterilized Erlenmeyer flask.
3. Zero the microbalance with weighing paper on it, then use a spatula to weigh out Agarose (agarose gel powder) 0.32g, and add it into the Erlenmeyer flask containing the 1X TAE Buffer, mixing them together.
4. Cover the Erlenmeyer flask with plastic wrap, poke several small holes in the plastic wrap using a filter tip, place it in the microwave, and microwave for 30 seconds, then shake to mix thoroughly. This step must be repeated until the agarose gel powder (Agarose) is completely dissolved in the 1X TAE Buffer and appears completely transparent. (Repeat at least three times.)
5. Take the thoroughly mixed agarose (Agarose) solution out of the microwave and place it on the benchtop horizontal shaker, shaking slowly for 5 minutes.
6. Using a 2ul pipetman, draw up 2ul of EtBr and add it to the Erlenmeyer flask, mixing thoroughly.
7. Pour into the casting tray, and let cool for 1 hour to solidify into the agarose gel (gel).

2. Performing Horizontal Electrophoresis

1. Place the solidified agarose gel (gel) into the mini electrophoresis tank, and pour in 450ml of TAE Buffer (1X).
2. Mix 1.2ug of DNA with 2ul of 6X Loading dye, then add sterilized secondary water (ddH_2O) to bring the total volume to 10ul.
3. Using a 10ul pipetman, inject the entire volume of the above thoroughly mixed liquid into a well of the agarose gel (gel) in the mini electrophoresis tank.
4. Perform horizontal electrophoresis for 35 minutes at a voltage of 85 volts (current of 500 mA).
5. Remove the agarose gel (gel) from the mini electrophoresis tank, and take it to the high-sensitivity gel imaging system to confirm whether a major band is present (see Attachment 2). Save the file and keep a photograph on record.

5.6 Preparing Frozen Tissue Fragments for Protein Extraction

Equipment:

-80°C freezer, sterilized 4-inch mortar and pestle

Consumables:

1. Aluminum foil
2. Sterilized fine-tipped forceps (11cm)
3. Liquid nitrogen
4. Thermos cup capable of holding liquid nitrogen (stainless steel preferred)
5. Specimen storage box (10x10 grid)
6. 1.5ml microcentrifuge tube (eppendorf)
7. Foam box (for holding frozen tissue and dry ice)
8. Dry ice

Procedure:

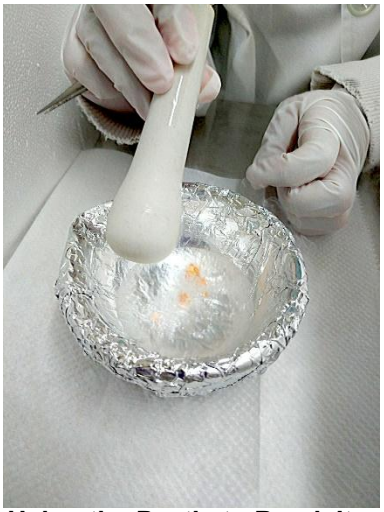
1. Completely line the inside of the mortar with aluminum foil.
2. First prepare a foam box (already loaded with dry ice) and the list of specimens to be retrieved. From the -80°C freezer, take out the 9x9 grid specimen storage box containing the frozen tissue with “confirmed tumor diagnosis and percentage,” and place it in the foam box (already loaded with dry ice).9x9
3. Pour a small amount of liquid nitrogen from the thermos cup (filled 80% full) into the mortar.
4. Take one large frozen tissue piece out of the cryotube containing the frozen tissue

pieces and place it in the mortar. Use the pestle to break the frozen tissue piece into small fragments, then use the fine-tipped forceps to pick up the small tissue fragments and place them into a pre-numbered microcentrifuge tube. The mortar must be kept supplied with sufficient liquid nitrogen throughout, in order to keep the tissue frozen. Then place the microcentrifuge tube into the 9x9 grid specimen storage box (kept in the foam box containing dry ice).

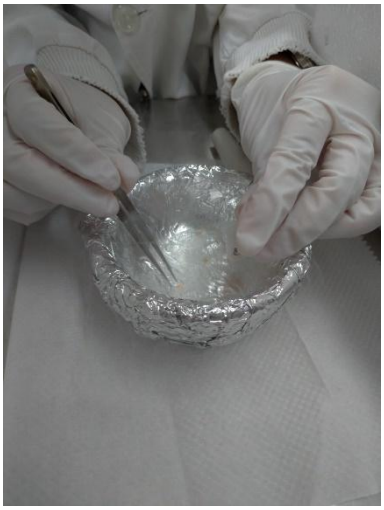
5. This procedure requires three people: one person is responsible for taking out the frozen tissue, breaking it into small fragments, and placing it into the pre-numbered microcentrifuge tube; one person prepares the microcentrifuge tubes and verifies that the correct tube is being filled. The third person is responsible for replenishing the liquid nitrogen, and for replacing the mortar and pestle (each frozen tissue piece requires its own freshly aluminum-foil-lined mortar and pestle) and the forceps.

Note: Because frozen tissue specimens are precious, a single piece of 5x5x5 mm^3 or larger can yield a considerable amount of DNA or RNA, which can be provided to many applicants. It would be wasteful to give an entire piece to just one applicant. However, some researchers need tissue protein for their experiments. It is therefore recommended to first break the tissue into small fragments before providing it to applicants for protein extraction, with the goal of conserving the specimen. However, since it is difficult to break tissue into pieces of exactly uniform size, it is also acceptable to combine small fragmented pieces into a single tube (depending on total volume). If the originally retained tissue piece is already very small (<2x2x2 mm^3), there is no need to break it into smaller fragments.

Removing the Frozen Tissue and Placing It in the Mortar



Using the Pestle to Break It into Small Fragments



Using Fine-Tipped Forceps to Remove the Tissue and Place It into a Microcentrifuge Tube

6. Quality Standards for Dispensing

Tissue DNA dispensed by the Integration Platform shall be accompanied by the following information:

1. Tumor component (must be interpreted and confirmed by a pathologist)
   -- Tumor tissue: the tumor component (tumor %) of the original tissue specimen should, in principle, be >50%; the higher the better.
   -- Non-tumor tissue surrounding the tumor: absence of tumor cell contamination must be confirmed.
   Note: Because the nature of each specimen differs, and some are difficult to obtain, the tumor component requirement may be lowered for such specimens, but it must still be at least 30% or above to have good research value. The tumor component level may be adjusted in accordance with the applicant's requirements.
2. Tissue DNA optical density (O.D.) value:
   -- 260/280 ratio should fall between 1.6-2.2
   -- 260/230 ratio should fall between 1.6-2.5

Note: (1) Because some specimens are difficult to obtain or very scarce, the amount of tissue DNA that can be extracted may be very small and unable to be purified. This situation may be discussed with the applicant; if the applicant is able to accept it, the specimen may be dispensed even if the O.D. value is not ideal, but this must be noted and the applicant must be fully informed.
(2) It is recommended that the 260/280 ratio and the 260/230 ratio preferably both be >1.8, to confirm that the DNA

3. does not contain significant protein contamination.
   Whether the horizontal electrophoresis gel image shows a major band (as needed)-- If a major band is present for the tissue DNA, this indicates good DNA quality, but it is not a mandatory condition for dispensing; it is required only when specifically requested by the applicant.

Frozen tissue fragments dispensed by the Integration Platform shall be accompanied by the following information:

1. Tumor component (must be interpreted and confirmed by a pathologist)
2. Confirmation that the tissue has never thawed since being frozen.

7. References

1. Hoyo C. Methylation variation at IGF2 Differentially Methylated Regions and Maternal Folic Acid Use before and during Pregnancy. Epigenetics 2011;7(6):928- https://www.ncbi.nlm.nih.gov/pubmed/21636975

2. Handbook: https://www.qiagen.com/mx/resources/resourcedetail?id=a9e6a609-4600-4b03-afbd-974318590ce5&lang=en

8. Attachments

Attachment 1: Example of Measuring Frozen Tissue DNA Concentration and O.D. Value Using an Ultra-Micro Spectrophotometer

Table 1: Optical density (O.D) values are good, all >1.8, with concentrations maintained below 2000ng/μl.

Tissue DNA O.D. Value				
No.	Concentration (ng/μl)	O.D 260/280	O.D 260/230	Volume of DNA to Load for Gel Run (μl)
1T	1495.9	1.86	2.27	0.84
1N	1094.9	1.86	2.25	1.14
2T	1018.7	1.86	2.31	1.23
2N	1079.2	1.87	2.32	1.16
3T	1344.8	1.85	2.01	0.93
3N	1496.7	1.86	2.26	0.84
4T	1313.5	1.85	2.23	0.95
4N	1085.5	1.85	2.27	1.15
5T	1871.3	1.86	2.23	0.67
5N	1144.2	1.86	2.24	1.09

Note: Concentration and O.D. detected by Thermo NANODROP 2000

Table 2: Optical density (O.D) values are, in some cases, somewhat lower; consider re-purification.

Tissue DNA O.D. Value				
no.	Concentration (ng/µl)	O.D 260/280	O.D 260/230	Volume of DNA to Load for Gel Run (ul)
1T	471.3	1.86	**1.65**	3.4
1N	915.9	1.86	**1.64**	1.7
2T	2554.5	1.84	2.12	0.6
2N	817.9	1.85	**1.46**	2.0
3T	1232.4	1.84	1.89	1.3
3N	1133.0	1.86	**1.74**	1.4
4T	1237.5	1.85	**1.65**	1.3
4N	1197.7	1.87	**1.75**	1.3
5T	981.1	1.84	2.17	1.6
5N	1266.7	1.88	1.92	1.3

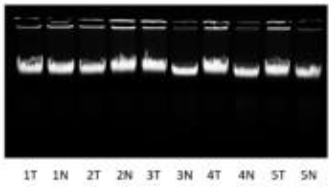
Note: Concentration and O.D detected by Thermo NANODROP 2000

Attachment 2: Example of Frozen Tissue Extracted DNA Concentration, O.D. Value, and Horizontal Electrophoresis Gel Image

Frozen Tissue Extracted DNA Concentration, O.D. Value, and Horizontal Electrophoresis Gel Image — Example 1.

tissue DNA O.D值

no.	濃度 (ng/μl)	O.D 260/280	O.D 260/230	跑膠所需load DNA的量(ul)
1T	1495.9	1.86	2.27	0.84
1N	1094.9	1.86	2.25	1.14
2T	1018.7	1.86	2.31	1.23
2N	1079.2	1.87	2.32	1.16
3T	1344.8	1.85	2.01	0.93
3N	1496.7	1.86	2.26	0.84
4T	1313.5	1.85	2.23	0.95
4N	1085.5	1.85	2.27	1.15
5T	1871.3	1.86	2.23	0.67
5N	1144.2	1.86	2.24	1.09

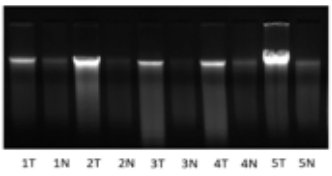


Note: Optical density (OD) values are good; all samples have a major band.

Frozen Tissue Extracted DNA Concentration, O.D. Value, and Horizontal Electrophoresis Gel Image — Example 2.

tissue DNA O.D.值

no.	濃度 (ng/μl)	O.D. 260/280	O.D. 260/230	跑膠所需load DNA的量(ul)
1T	471.3	1.86	1.65	3.4
1N	915.9	1.86	1.64	1.7
2T	2554.5	1.84	2.12	0.6
2N	817.9	1.85	1.46	2.0
3T	1232.4	1.84	1.89	1.3
3N	1133	1.86	1.74	1.4
4T	1237.5	1.85	1.65	1.3
4N	1197.7	1.87	1.75	1.3
5T	981.1	1.84	2.17	1.6
5N	1266.7	1.88	1.92	1.3



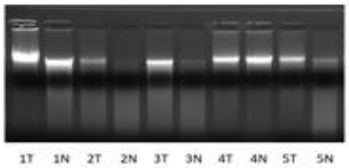
Note: Optical density (OD) values < 1.8 are suboptimal; some samples lack a major band.

Even if the material lacks major band, it remains suitable for various experiments; its applicability depends on the applicant's specific requirements.

Frozen Tissue Extracted DNA Concentration, O.D. Value, and Horizontal Electrophoresis Gel Image — Example 3.

tissue DNA O.D. 值

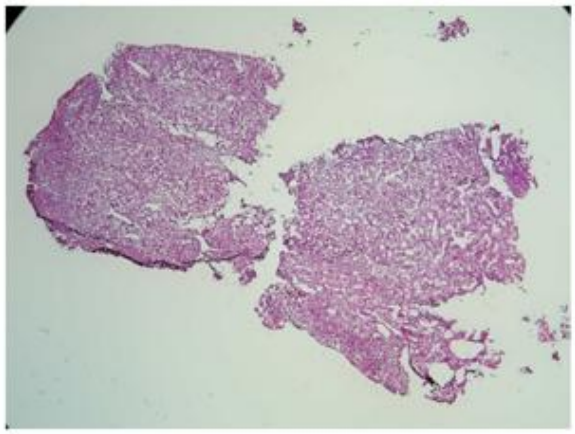
no.	濃度 (ng/μl)	O.D. 260/280	O.D. 260/230	跑膠所需load DNA的量(ul)
1T	2337.1	1.83	1.97	0.53
1N	2752.1	1.84	1.95	0.45
2T	1859.3	1.9	2.13	0.67
2N	1449.2	1.88	1.79	0.86
3T	2559.1	1.83	1.94	0.49
3N	1548.1	1.89	2.06	0.81
4T	2352.0	1.82	2.07	0.53
4N	2139.0	1.87	1.97	0.58
5T	2489.2	1.84	2.19	0.5
5N	1382.2	1.86	1.93	0.9



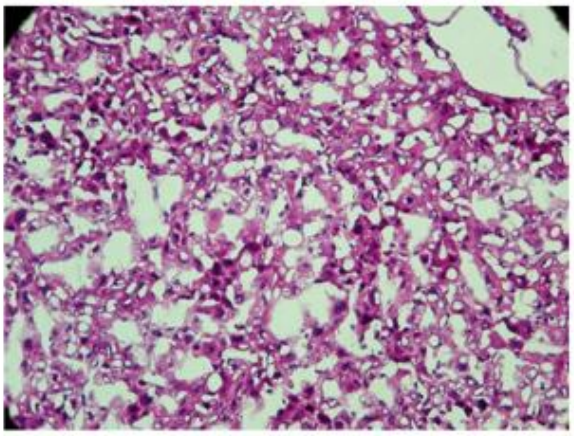
Note: Although the OD value is within the acceptable range, there is no major band (2N), or the band is very faint (3N).

Attachment 3: Example of Frozen Tissue Tumor Component Interpretation

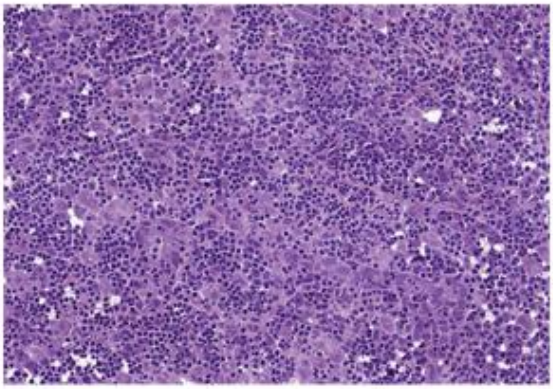
All frozen-collected tumor tissue must first undergo frozen sectioning to determine whether tumor cells are present.



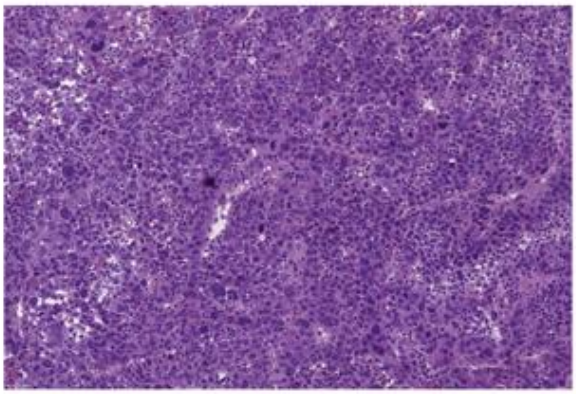
99% are liver cancer tumor cells.



99% are liver cancer tumor cells.

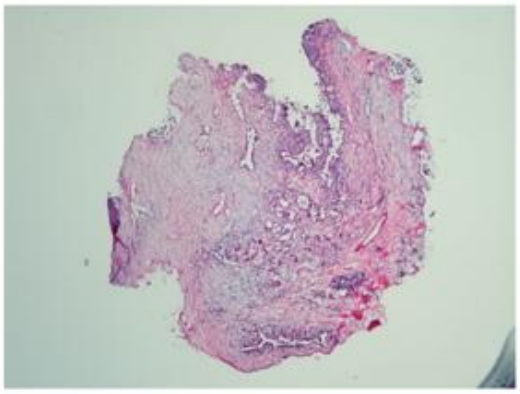


Only 50% are liver cancer tumor cells; there is a large number of inflammatory cells.

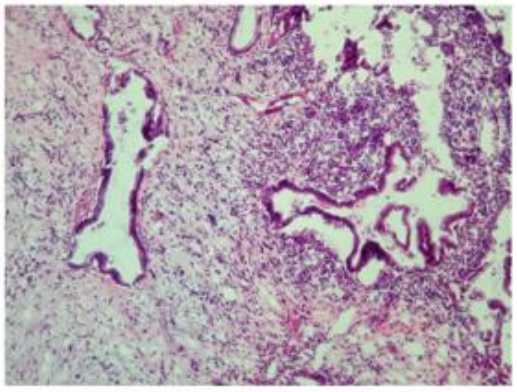


99% are liver cancer tumor cells.

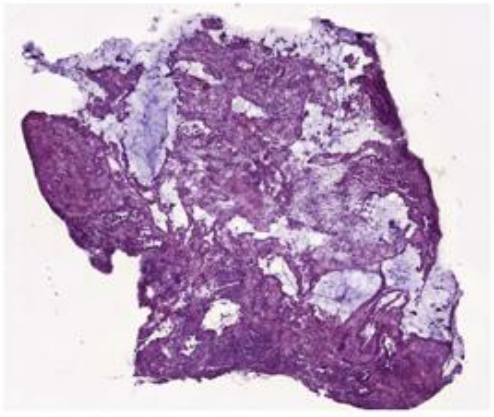
A fresh frozen tumor tissue specimen and its frozen-section image



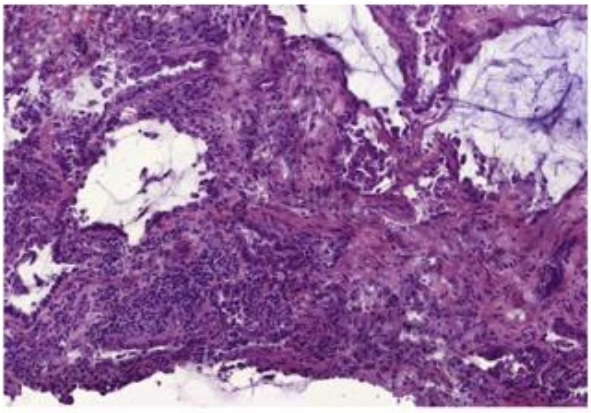
Cholangiocarcinoma; tumor cells <20%.



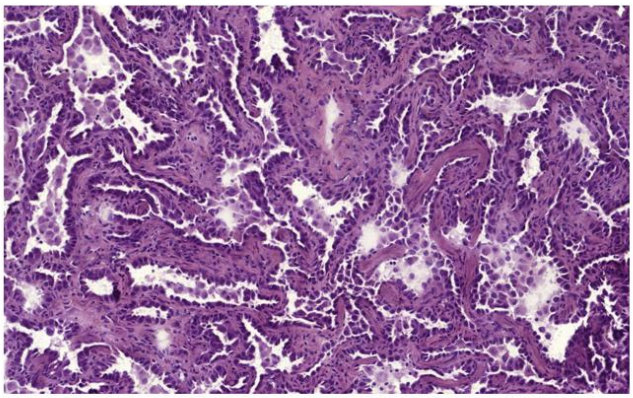
A magnified view of a section of the image



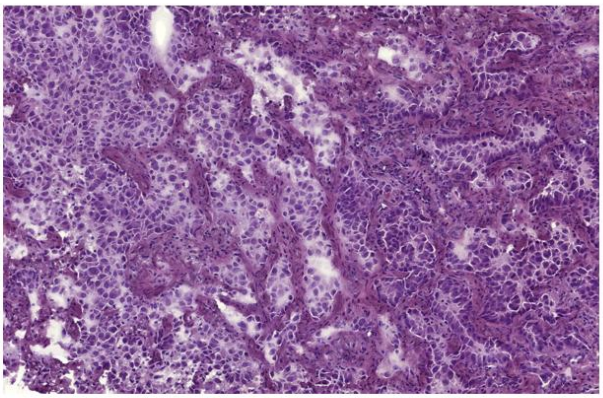
Lung adenocarcinoma; tumor cells <50%.



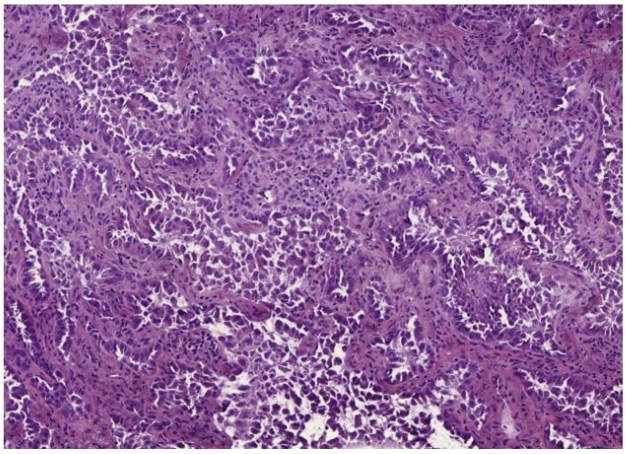
A magnified view of a section of the image



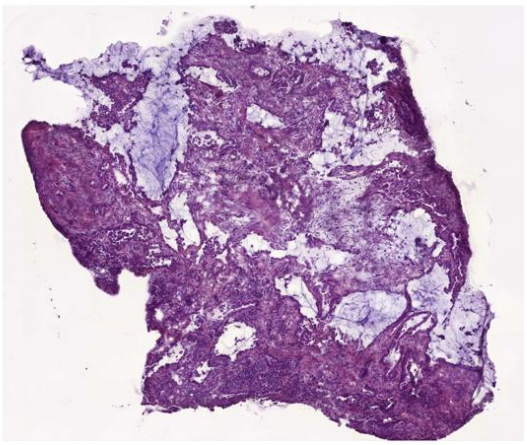
Adenocarcinoma, 50% tumor cells



Adenocarcinoma, 90% tumor cells

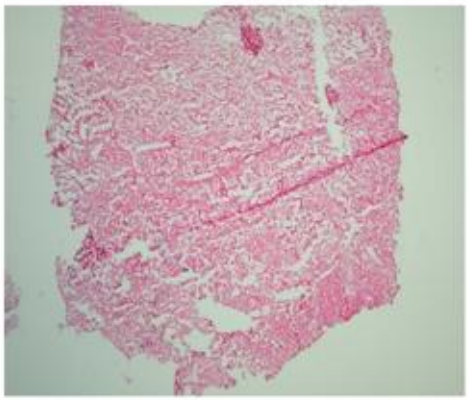


Adenocarcinoma, 70% tumor cells

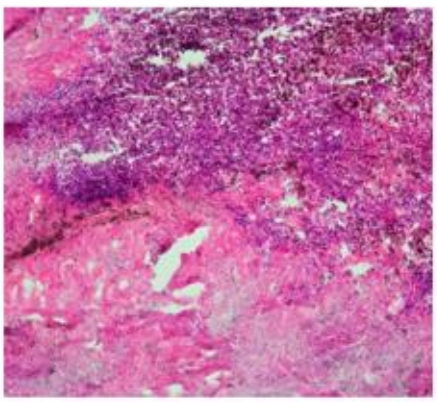


Adenocarcinoma, <50% tumor cells

None of these tumor tissues can be used.



Tissue completely necrotic.



No tumor cells present.