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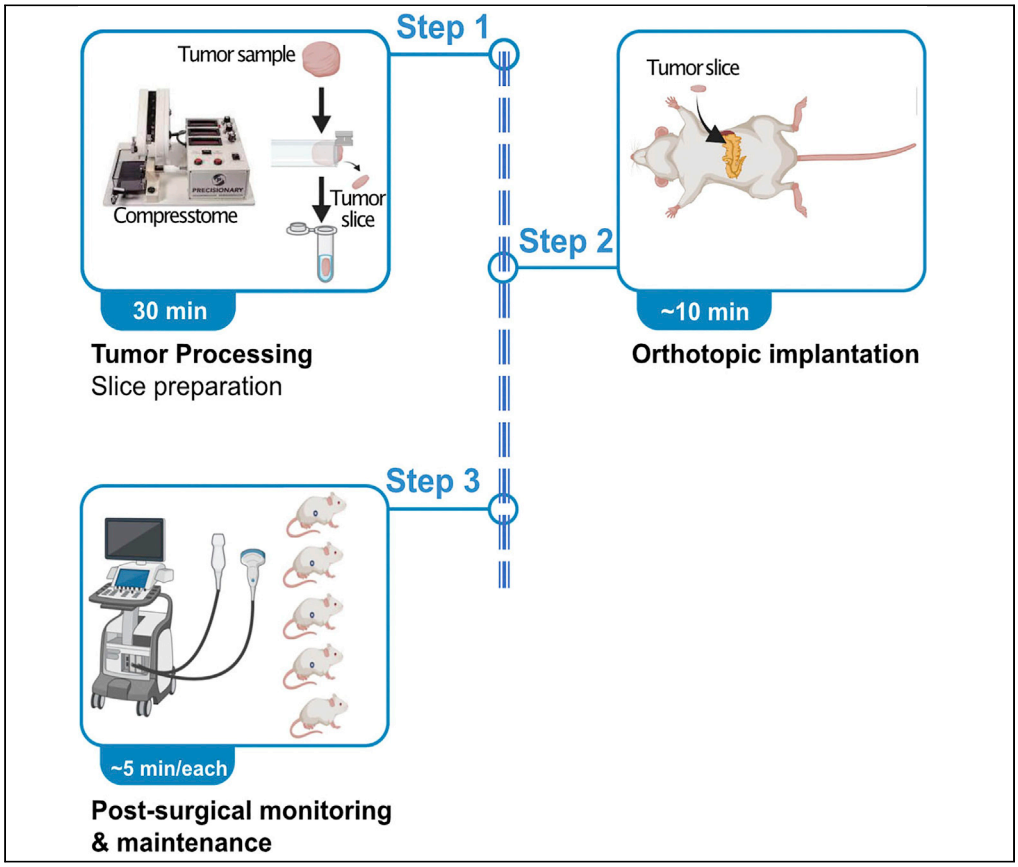
# Generation of orthotopic patient-derived xenograft models for pancreatic cancer using tumor slices




Orthotopic patient-derived xenograft models recapitulate the genomic complexity of the original tumor and some aspects of local microenvironment, useful for rapid development and validation of personalized treatment strategies. Here, we precisely describe a protocol for generating tumor slices from human or murine-derived pancreatic cancer. They are then implanted directly into the murine pancreas, monitored using ultrasound, with a 90% success rate. This assay creates a clinically relevant *in vivo* model facilitating personalized treatment development.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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## Highlights

A highly efficient protocol for patient-derived xenograft (PDX) mouse model generation

Tumor slice preparation from human or murine-derived pancreatic tumor

Direct orthotopic implantations of tumor slices into mouse pancreas

Tumor slices, compared to cell lines or fragments, improves engraftment to about 90%

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# Generation of orthotopic patient-derived xenograft models for pancreatic cancer using tumor slices

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## SUMMARY

**Orthotopic patient-derived xenograft models recapitulate the genomic complexity of the original tumor and some aspects of local microenvironment, useful for rapid development and validation of personalized treatment strategies. Here, we precisely describe a protocol for generating tumor slices from human or murine-derived pancreatic cancer. They are then implanted directly into the murine pancreas, monitored using ultrasound, with a 90% success rate. This assay creates a clinically relevant *in vivo* model facilitating personalized treatment development.**

## BEFORE YOU BEGIN

The methods presented required administrative approval for human and animal research.

All animal research procedures were approved by the Columbia University Irving Medical Center Institutional Animal Care and Use Committee (IACUC). Mouse colonies were bred and maintained under a standard 12 h light / 12 h dark cycle. Standard mouse chow and water were provided *ad libitum*.

All human research procedures were approved by the Columbia University Irving Medical Center Institutional Review Board (IRB) and informed consent was attained for all research subjects.

This protocol assumes familiarity with basic small animal surgical techniques, such as suturing.

### Compresstome preparation

Timing: 30 min

1. Place a bottle of Hank's Balanced Salt Solution and the Compresstome chilling block in a −20°C freezer at least 30 min prior to collection of bulk tissue.
2. Weigh out 0.25 g low-melting agarose into 50 mL flask.
3. Prepare the sectioning blade.
   a. Clean the Compresstome blade holder in an acetone bath and scrape to remove any super glue residue (if necessary).

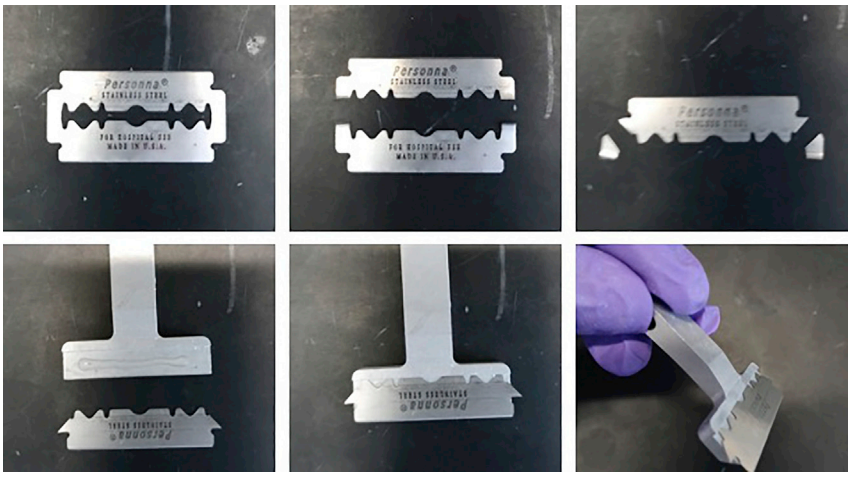

**Figure 1. Sectioning blade preparation**
Visual representation of step 3.

b. Prepare a razor blade by first cutting in half horizontally to create a single sided blade. Then trim the top corners of the blade down to allow for a tight fit of the blade onto the holder (Figure 1).
c. Place a small amount of super glue onto the flat side of the blade holder and place the prepared razor blade flush against the lip of the blade holder (Figure 1). Allow at least 20 min to dry and set.

⚠ CRITICAL: To prevent injury while preparing the sectioning blade, leave the blade edge wrapped in the wax-paper wrapper while cutting. Use forceps to apply the trimmed blade to the blade holder.

⚠ CRITICAL: To maintain sterility throughout the protocol, all tools should be sterilized via autoclaving and 70% ethanol spray and users should wear all relevant sterile PPE (sterile gown or lab coat, mask, and surgical gloves).

## Surgery preparation

Timing: 10 min

4. Prepare suture loops (one loop per anticipated surgery).
   a. Using sterile scissors, cut lengths of absorbable sutures approximately 6 cm (or 2 inches) for each loop.
   b. Bring the two ends together to create a circle, loosely tie a simple knot (half-hitch), leaving a small overhang of the ends to tighten later (Figure 2).

## Equipment setup

### *Compresstome setup*

Reference the Compresstome User Manual[1] for more detailed setup and operating instructions. For Pancreatic Ductal Adenocarcinoma (PDAC), section tissue using the following Compresstome settings: Continuous, speed – 8.4, oscillation – 4.6, slice thickness – 300 μm. These speed and oscillation settings will depend on the characteristics of the bulk tissue. The settings listed above are appropriate for pancreatic ductal adenocarcinoma but may need to be adjusted for other tissue types.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Biological samples** | | |
| Patient tumor samples. Pancreatic cancer: adults (>18) from both sexes. | NewYork-Presbyterian Columbia University Irving Medical Center | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Antibiotic (Baytril; enrofloxacin) | MWI Animal Health | 018675 |
| Buprenorphione extended-release | Fidelis | Ethiqa XR |
| Eye ointment | Alcon | Systane |
| Iodine solution (Povidone) | Avrio Health | Betadine |
| 70% ethanol | Fisher Scientific | BP82031 |
| Penicillin-streptomycin | Caisson Labs | PSL01 |
| Cyanoacrylate (Super Glue) | Bazic Products | 2007 |
| Depilatory Cream with Aloe (Nair) | Church and Dwight Co INC | Nair |
| Hank's Balanced Salt Solution (HBSS) | Thermo Fisher | 14-025-092 |
| Isoflurane | Henry Schein | 029405 |
| Low-melting agarose | Sigma-Aldrich | A9414 |
| Phosphate buffered saline | Gibco Life Technologies | 10010-023 |
| Matrigel | Corning | 356231 |
| RPMI 1640 | Gibco Life Technologies | 21870-076 |
| DMEM | Gibco Life Technologies | 12430062 |
| Saline | Fisher Scientific | 50-843-140 |
| **Experimental models: Organisms/strains** | | |
| Mouse: Athymic nude (J:Nu) male or female mice for pancreatic cancer; 4–6 weeks | Jackson Labs | 007850 |
| Mouse: NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) male or female mice for pancreatic cancer; 4–6 weeks | Jackson Labs | 005557 |
| **Other** | | |
| Compresstome® Vibrating Microtome | Precisionary Instruments, LLC | VF-310-0Z |
| Isoflurane vaporizer | Vet Equip | RL2-922100 |
| VEVO ultrasound | VisualSonics | Vevo3100 |
| 20-mm specimen tubes | Precisionary Instruments, LLC | VF-SPS-VM-20-BOS |
| Chilling block | Precisionary Instruments, LLC | VF-VM-CB-20-BOS |
| Dissection scissors (1) | Roboz Surgical Instruments | RS-5882 |
| Double-edge prep blades | AccuTec Blades, Inc. | 74-002 |
| Forceps (2) | Roboz Surgical Instruments | RS-8254 |
| Microscissors (1) | Roboz Surgical Instruments | RS-5912 |
| Removable blade holder | Precisionary Instruments, LLC | VF-BH-VM-310-0Z-BOS |
| Surgical needle driver (1) | Roboz Surgical Instruments | RS-7841 |
| Wound clips | Roboz Surgical Instruments | RS-9258 |
| Wound clip applicator | Roboz Surgical Instruments | RS-9250 |
| Wound clip remover | Roboz Surgical Instruments | RS-9263 |
| Scalpel | Exel International | 29550 |
| Heated pad | BN-LINK | RH-0806 |
| Shaver | Oster | 078997-000-000 |
| 2-mL Eppendorf tubes | Fisher Scientific | 14-666-313 |
| Sterile cotton swabs | Fisher Scientific | 23-400-100 |
| Sutures (absorbable) | eSutures | G500N |
| Sutures (non-absorbable) | eSutures | J8661N |
| Transfer pipet | Samco Scientific | 12430857 |
| Petri dish | Thermo Fisher | 172931 |

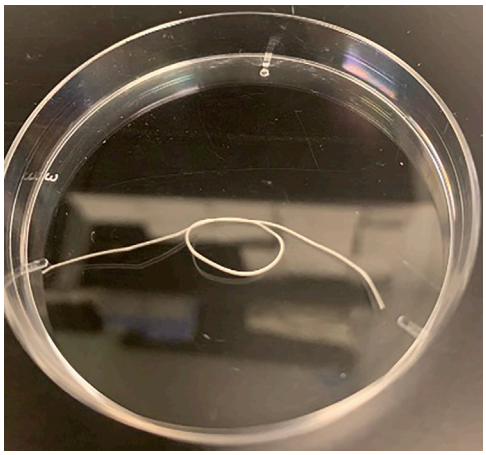

**Figure 2. Suture loop preparation**
Visual representation of step 4.

## STEP-BY-STEP METHOD DETAILS

### Tissue collection and tissue slice generation

Timing: 30 min

Bulk tissue, the full tumor sample removed directly from a tumor bearing mouse or patient, is sectioned into thin cross-sectional slices suitable for surgical implantation. These slices contain both epithelial and stromal tissue in its native tissue architecture to facilitate engraftment and support tumor initiation and growth. These flexible slices are more amenable to folding into a pocket of pancreatic tissue for orthotopic implantation compared to a rigid cube.

The protocol may be used for either implantation of primary tissues from human patients, or for expansion of established PDX lines.

*Note:* Just prior to tissue collection, fill wells of a 6-well plate halfway with ice cold Hank's Balanced Salt Solution. Keep plate on ice.

1. Collecting tissue sample from:
   a. Primary human (biopsy).
      i. Two sample cores should be collected from each biopsy and placed into cold, sterile PBS. Biopsy cores are generally thin strings of tissue, with a <1 mm diameter.
      ii. Trim core samples with a scalpel under sterile conditions into approximately 2 mm long fragments.
      iii. Place individual slices from each core into 2 mL Eppendorf tubes with 50 μL PDX media (RPMI + 1% penicillin/streptomycin) and 50 μL Matrigel.

   ⚠ CRITICAL: Store on icy slush (ice half filled with water).

      iv. Proceed directly to step 4 (orthotopic implantation).
   b. Primary human (resection).
      i. Following resection, tissue should be placed in cold, sterile DMEM – or any standard collection media - and kept on ice until embedded (step 2).

      If possible, the optimal size and shape of resected patient tissue should be in the shape of a cylinder or wedge, with 2–3 mm width and a longer axial dimension (> 1 cm if possible) in order to facilitate stable embedding into the sample holder.
   c. Established PDX line (mouse).

**Figure 3. Embedding specimen in agarose**

Fully assembled specimen holder with cooling block.

Notice the change in color and opacity of the agarose from clear (middle, newly embedded sample) to a hazy gray (right, cooled and solidified sample).

i. Humanely euthanize the animal as per your approved IACUC protocol.
ii. Place the mouse on a dissection stage in a supine position and spray torso with 70% ethanol to sterilize.
iii. Using dissection scissors, cut through and peel away the skin and peritoneum to expose the abdominal cavity.
iv. Locate the pancreas (typically behind intestinal loops, below the stomach, extending laterally to the spleen) and locate the tumor.
v. Use dissection scissors and/or scalpel to remove the tumor from the surrounding tissue. Place in a sterile petri dish.

d. If necessary, trim any healthy pancreas tissue from the borders of the tumor, then transfer to the 6-well plate with ice-cold Hank's Balanced Salt Solution and store on ice prior to moving on to step 2.

2. Embed tissue in specimen tube.
   a. Add 10 mL distilled $H_2O$ to pre-measured low-melting agarose (2.5% w/v) and microwave on high for 30 s.
      i. Let cool until the flask is still hot, but comfortable to touch (approximately 1 min to around 50°C/120°F).
   b. Insert Compresstome white plastic plunger into metal casing, leaving an approximately 2–3 cm chamber.
   c. Tape casing to plunger with lab tape (Figure 3) and place the completed construct vertically in ice.
   d. Fill the chamber of the specimen tube with cooled agarose using a transfer pipet.
   e. Transfer the bulk tissue into the specimen tube using forceps and submerge until fully covered by agarose.
   f. Place the frozen chilling block around the specimen tube and let sit on ice for several minutes until the agarose solidifies.

*Note:* The agarose will solidify and turn slightly opaque. However, remove the sample from the cooling block before the agarose freezes, indicated by crystals forming on the edges of the agarose.

3. Section bulk tissue.
   a. Insert specimen tube into Compresstome tray until flush, then remove tape around the specimen tube to release the plunger.
   b. Screw the tray into the Compresstome base.
   c. Fill the tray halfway with ice cold Hanks' Balanced Salt Solution.
   d. Screw in blade holder with blade-side facing the specimen holder using the provided Allen wrench.

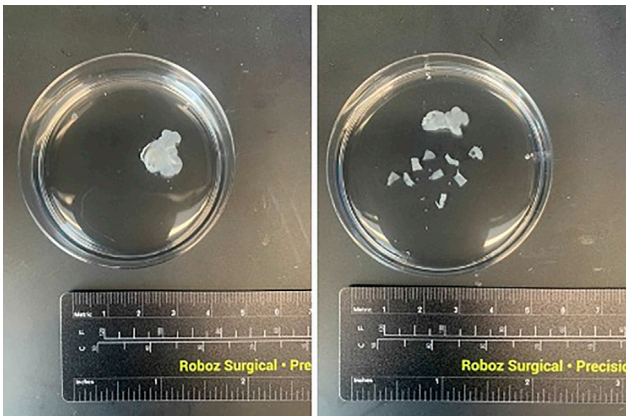


**Figure 4. Tumor slice trimming**
Representative images of a full tumor slice and appropriately trimmed implantation-sized pieces (step 3h).

e. Advance the Compresstome piston until in contact with specimen holder using 'fast forward' button and continue advancing until agarose begins to protrude from the edge of the specimen holder into the tray.
f. Section tissue using the Compresstome settings as described in equipment setup section). Collect slices into cold Hank's Balanced Salt Solution in a 6-well plate on ice.
g. Use a scalpel to further trim slices into pieces approximately 2 × 3 mm (Figure 4).
h. Place individual slices into 2 mL Eppendorf tubes with 50 μL PDX media (RPMI + 1% penicillin/streptomycin) and 50 μL Matrigel. Store on icy slush (ice half filled with water).

⚠ CRITICAL: The ice-water slush mix is critical. Using only ice may freeze the slices, drastically reducing the engraftment viability.

### Orthotopic implantation

**Timing: 7–10 min/mouse**

Methods video S1 provides a step-by-step visualization of the implantation surgery.

4. Use an isoflurane chamber primed with 2% isoflurane in oxygen to induce anesthesia in 4–6 weeks old mice.
   a. Ensure adequate depth of anesthesia by toe pinch reflex check and continue to check every 10 min for duration of procedure.
5. Place the anesthetized mouse lying on its right flank on a heated pad with head in an anesthesia nosecone, administering 2% isoflurane.
6. Administer 3.25 mg/kg Buprenorphine* extended-release (~50 μL) followed by 20 mg/kg antibiotic enrofloxacin (Baytril; diluted 1:10 in sterile saline, ~50 μL) subcutaneously (preferably under the neck scruff).

⚠ CRITICAL: Buprenorphine is a Class III Controlled Substance and must be stored, dispensed, and used according to all relevant regulatory requirements. Extended-release formulations (Buprenorphine extended release) are preferred as to simplify the number, frequency and volume of analgesic administrations.

*Note:* User should use the analgesic and antibiotic treatments approved for their protocols.

7. Apply ophthalmic ointment to mouse's eyes to prevent the eyes from drying during the surgery.

8. If the recipient animals have fur, shave the left abdominal flank and remove fur stubble with depilatory cream (e.g., Nair). Clean off excess cream and fur with gauze soaked in PBS or isotonic saline.

*Note:* This step could also be done as part of surgery prep to save time.

9. Disinfect the skin three times with povidone-iodine and 70% ethanol in alternating swabs.
10. Identify the location of the spleen on the left abdominal flank.
    a. Use scissors to make a lateral ("head to tail" axis) skin incision ($\leq$ 15 mm) on the left upper quadrant of the abdomen slightly ventral (toward the belly) from the distal point of the spleen.
    b. Gently blunt dissect the skin away from the peritoneal muscle.
11. Use another pair of sterile scissors to make a lateral incision in the peritoneal wall above the spleen, parallel to the skin incision.

*Note:* Take care to avoid injury to the spleen.

12. Identify the pancreatic tissue in the vicinity of the distal tail of the spleen and the left kidney.
    a. Using a sterile cotton swab (rather than forceps), externalize the pancreas by touching the swab to the pancreas.
    b. Gently lift the pancreas through the incisions, and rest it on top of the abdomen.

*Note:* Pancreatic tissue is extremely fragile and prone to injury. Thus, the use of forceps to manipulate the pancreas is not advised.

13. Place a suture loop underneath the pancreas, with the suture ends above the pancreas.
14. With the tail of the pancreas oriented to point distally, use microscissors to make a coronal incision ("body cross section" axis) in the pancreatic parenchyma of approximately 2–3 mm, 1 mm below the line of the suture loop and create a "pocket" by gently expanding scissors or tongs within the parenchyma.
15. Remove the cut tissue slice from the media/Matrigel mix and carefully insert into the pancreatic pocket.
    a. Very carefully fold the tail of the pancreas over the pocket and inside the suture loop, such that the tails of the suture loop are underneath the pancreas.
    b. Gently pull close the suture loop to close the pocket and firmly hold the tumor fragment in place.

*Note:* The closed suture should be tight enough to maintain the integrity of the pocket such that the tissue will remain folded over once released, and loose enough to not risk laceration of restriction of blood flow that will induce ischemia. If the tissue is distorted around the suture loop (puckered or noticeably cinched), this indicates that the suture is pulled too tight.

16. Pull the suture loop tails around to the front of the pancreas and tie a double knot to fix the pocket closed.
17. Carefully return the tail of the pancreas to the abdominal cavity, making sure that the pocket is disturbed as little as possible.

*Note:* Take care that the pancreas is entirely within the abdomen space and not protruding between peritoneum and skin layer.

18. Suture the abdominal incision closed with sterile absorbable suture.

**Table 1. Surgery success metrics – Four independent rounds of implantation surgeries demonstrated that approximately 90% of surgeries resulted in tumor formation**

| | # Mice implanted | # Tumors developed | % Successful Surgeries |
|---|---|---|---|
| Sample 1 | 35 | 30 | 85.7 |
| Sample 2 | 35 | 31 | 88.5 |
| Sample 3 | 35 | 32 | 91.4 |
| Sample 4 | 35 | 32 | 91.4 |

*Note:* Take care to avoid suturing the pancreas onto the incision wound.

19. Use wound clips to close the skin incision.
20. Apply povidone-iodine disinfectant to the closed wound and return animal to a cage on a heated pad for recovery. Monitor until animal is alert and independently mobile.
21. Return mice to the cage and ensure full recovery.

### Post-surgical monitoring & maintenance

22. Monitor mice daily for satisfactory wound healing, administering post-operative analgesia in accordance with IACUC protocol and veterinary guidelines.
23. Approximately 14 days after surgery, the surgical wound should be healed enough to remove the wound clips using a clip remover.
24. After wound clips are removed, implanted mice should be monitored at least twice a week for tumor development.
    a. Primary screening for tumor development may be performed by abdominal palpation.
        i. Half suspend the mouse by holding the base of the tail with one hand while allowing the mouse to grip onto a surface with its front paws.
        ii. With the other hand, place thumb and index finger on the animal just caudal (below) the ribcage.
        iii. Gently press fingers together and gently push underneath the ribcage.

*Note:* Presence of a hard lump suggests that a tumor has likely developed.

    b. An animal identified via palpation should then be examined with ultrasound as previously described by Sastra et al.,[2] to confirm tumor presence and size.

## EXPECTED OUTCOMES

Using this method, approximately 90% of all mice implanted with tumor slices are expected to develop PDAC tumors (Table 1). Tumor lesions should be observable by abdominal ultrasound within 14–21 days following implantation (Figure 5). Tumor growth kinetics are expected to be variable and highly dependent on the state of the primary tissue. Tissue with areas of necrosis will have lower success rates.

Passaged PDX tumors are observed to maintain similar tissue architecture (Figure 6), demonstrating maintenance of both cancerous ductal and non-cancerous stromal compartments. Furthermore, previous studies have demonstrated that tumor genetic signatures remain comparable to the original parental tissue.[3] No engraftment differences due to mice gender were noticed using this protocol.

## LIMITATIONS

The protocol described here has only been validated using fresh (non-frozen, non-fixed) tissue. However, we have performed similar PDX implantation surgeries with cryopreserved tissue fragments

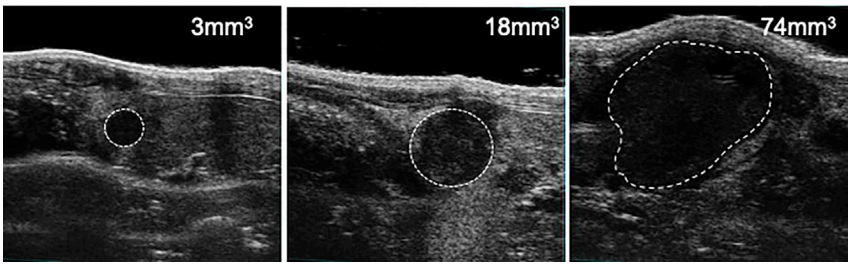


**Figure 5. Tumor monitoring via ultrasound**
Example time course of tumor growth. Tumors will present at discrete, hypoechoic (dark) regions within pancreatic tissue.

with comparable success, suggesting that this protocol could be performed using previously frozen tissue, expanding the scope of possible parental samples and studies.

As with most *in vivo* animal work, the success of this protocol is heavily reliant on the general health status of the recipient animals according to the animal health status protocol approved by your laboratory/center. User should confirm the animal health status protocol approved by its laboratory/center in order to proceed correctly. Recipient animals should undergo a careful evaluation of overall health prior to implantation in order to optimize surgery outcomes. The recipient animals used for these surgeries are approximately 4–6 weeks old, between 18–22 g, and are sex matched to the individual patient to minimize rejection by the host animal.

Finally, as this protocol relies on the use of live tissue, the biggest consideration should be to complete the entire protocol (tissue collection to completed surgeries) in less than two hours. If the recipient cohort is larger than 5–10 animals, it is recommended that multiple surgical groups work simultaneously to speed up the process.

## TROUBLESHOOTING

### Problem 1

The bulk tumor tissue may dislodge from the agarose and fall out of the specimen holder during sectioning (step 3).

### Potential solution

The tissue can be re-embedded using fresh melted agarose (return to step 2), with careful attention to the orientation of the tissue when embedded, which will help to anchor the tissue. The sample

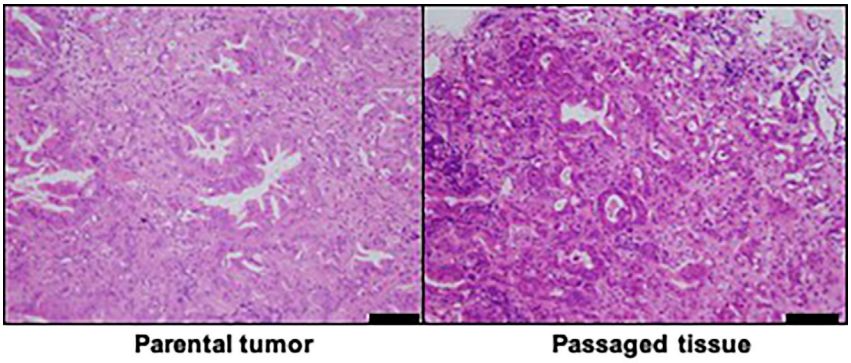


**Figure 6. H&E comparing parental tumor and passaged tissue structures**
H&E staining demonstrates preservation of the original parental tissue histology in a passaged tumor (First passage – P0). Scale bar = 100 μm.

should be oriented such that the longer dimension of the tissue is vertical (e.g., a tin can standing up rather than on its side). If this is a recurring issue, it may be helpful to increase the percentage of agarose to 3% or replace the razor blade.

### Problem 2

Significant bleeding from pancreatic tissue during the implantation surgery.

### Potential solution

The pancreas is a delicate organ and should be handled with extreme care during the surgery. Most tissue damage happens related to the steps for exposing the pancreas (steps 10–12). Extreme care should be taken to ensure that the tissue layers (skin – peritoneal muscle – viscera) are well separated prior to each incision. Furthermore, the pancreas should not be handled with forceps to prevent laceration or crushing injuries.

### Problem 3

Rarely, spontaneous lymphoma may development in PDX mice due to the presence of Epstein-Barr virus (EBV+) as described in Facompre et al.[4]

### Potential solution

When the tumor is processed, a portion can be used for OCT embedding. While we are preparing the tissue slices for the surgeries, we recommend cutting one slide with 1–2 sections from the OCT block (5 μm thickness) to perform a rapid hematoxylin and eosin (H&E) stain while performing the embedding and sectioning steps. The presence of an abundance of "small, round, blue cells" within the tumor tissue is indicative of lymphoma. It is recommended that the surgeries do not move forward with this sample if there is any evidence of lymphoma.

Of note, as the lymphocyte transformation responsible for the development of lymphoma in PDX models occurs at the host animal level after implantation, rather than originating from the patient tissue directly, the development of lymphoma in one mouse does not indicate necessarily that the remaining implantations of the cohort are similarly compromised.

### Problem 4

Small tumor to begin with for making tumor slices due to high inflammation in the pancreas.

### Potential solution

Inflamed pancreas tissue can look similar to tumor tissue on ultrasound. Occasionally, the inflammation is severe enough that upon necropsy, the actual tumor tissue is smaller than anticipated and may not be sufficient size or shape to create tumor slices. As an alternative, the tumor can be cut into 3 × 3 mm3 pieces manually and proceed with the remaining protocol.

### Problem 5

The user does not have access to some of the instruments or equipment as described in this protocol.

### Potential solution

There are several alternatives to many of the steps described in this protocol. If the user does not have access to the Compresstome® described here, other vibratome models are likely suitable for creating the tumor slices. Additionally, if wound clips are not available, the outer incision can be closed using non-absorbable sutures or Vetbond. However, be sure that the resulting scar is not overlapping to avoid interfering with future ultrasound scans.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by K.P.O. (kenolive@columbia.edu).

### Materials availability

This study did not generate new unique agents.

### Data and code availability

This study did not generate new datasets or code.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101899.

## ACKNOWLEDGMENTS

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## AUTHOR CONTRIBUTIONS

A.C.G. managed the project and developed and optimized storage conditions and media composition. A.R.D. developed the tissue and slice preparation protocol. S.A.S. adapted the surgical procedure from Qiu and Su.[5] A.C.G. and A.R.D. contributed equally to manuscript preparation. K.P.O. contributed conceptualization, funding, and supervision.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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