



Version 2 ▾

Jul 14, 2020

Cold Spring Harbor - Intraductal Injection V.2

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Works for me

dx.doi.org/10.17504/protocols.io.bijnkcme

NCI PDMC consortium



Shree Bose

ABSTRACT

from Xiao et al., Nat Prot 2014, modified by KM.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Xiao, X., Guo, P., Prasad, K., Shiota, C., Peirish, L., Fischbach, S., Song, Z., Gaffar, I., Wiersch, J., El-Gohary, Y. and Husain, S.Z., 2014. Pancreatic cell tracing, lineage tagging and targeted genetic manipulations in multiple cell types using pancreatic ductal infusion of adeno-associated viral vectors and/or cell-tagging dyes. *nature protocols*, 9(12), p.2719.

DOI

dx.doi.org/10.17504/protocols.io.bijnkcme

PROTOCOL CITATION

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Jul 14, 2020

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39246

MATERIALS

NAME	CATALOG #	VENDOR
S&T Vascular Clamp (7mm)	00396-01	Agnthos
Ketoprofen	K1751	Sigma Aldrich

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Ketoprofen	K1751	Sigma Aldrich
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Anesthesia and Preparation 2m

- 1 Anesthetize mice with 2.5–3% (wt/vol) isoflurane by inhalation.

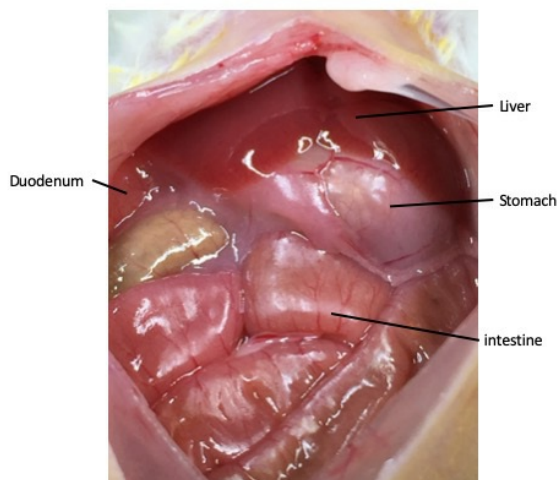
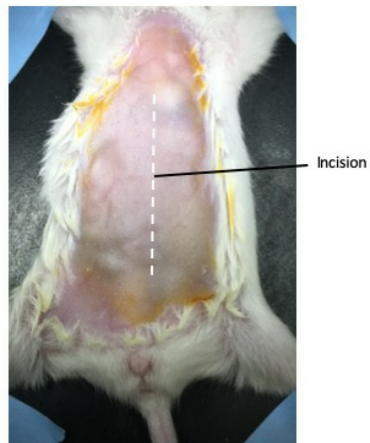


The inhalation should be closely adjusted, according to the breathing and heart rate of the mice during surgery. A typical infusion surgery takes 15-20 min. Unnecessary delays in the surgery should be avoided. (use Nair to remove hair from the lower chest and abdomen.)

- 2 Put the mouse onto the 37 °C warming pad. Immobilize the mouse on the head pad using surgical tape and Clean and disinfect the now hairless area with Betadine.

Laparotomy 1m

- 3 Use scissors to incise the skin at the midline of the abdomen.

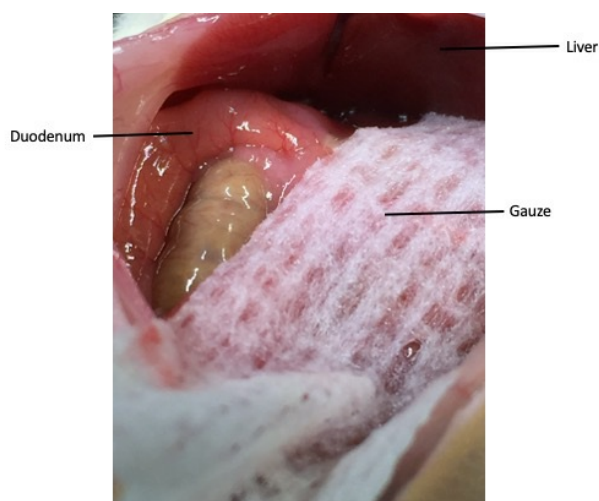


- 4 Next, cut the abdominal muscle along the linea alba to create a midline upper abdominal laparotomy incision of 1.5 cm. Avoid injuring any underlying organs when performing the laparotomy.
- 5 Clamp skin and peritoneum and rest the clamps on paper rolls.

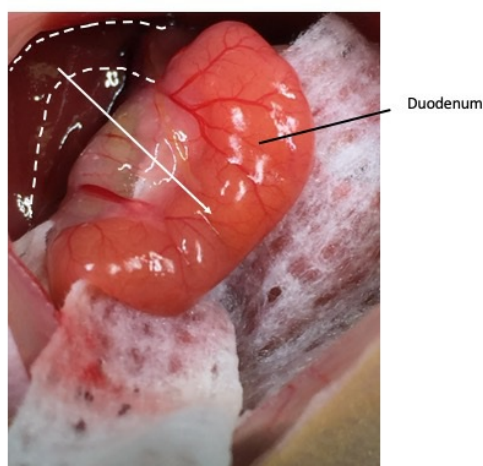
Exposure of the Pancreatic Duct

30s

- 6 Use wet cotton stick and ring forceps to touch the organs as follows:

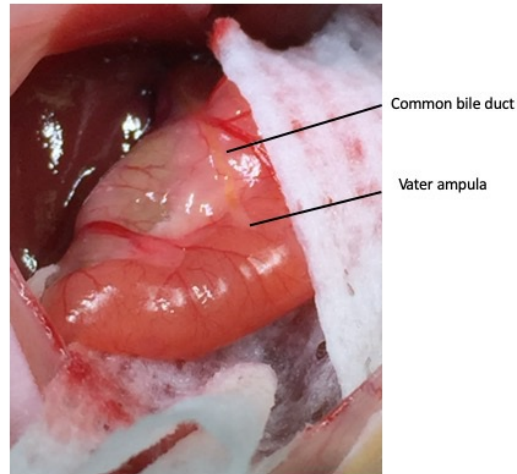


Use Cotton stick to gently pull out the stomach and position a wet gauze (with PBS) on the stomach and other organs.



Rotate and stretch the duodenum and position it on the gauze to expose the biliary-pancreatic duct and its junction with the duodenum (the sphincter of Oddi), which appears pale.

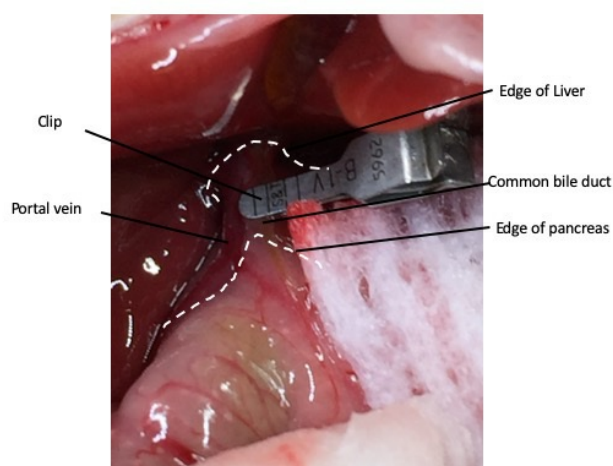
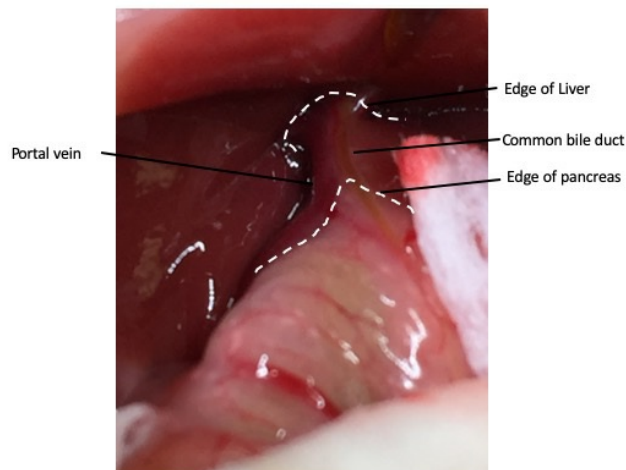
- 7 Cover the duodenum with moistened gauze (with PBS) to keep the position and to avoid substantial heat loss and drying.



Cannulation of the Duct

3m

- 8 A microclamp must be applied the common bile duct above the branch-point of the pancreatic duct, to prevent infusion into the liver.





S&T Vascular Clamp (7mm)

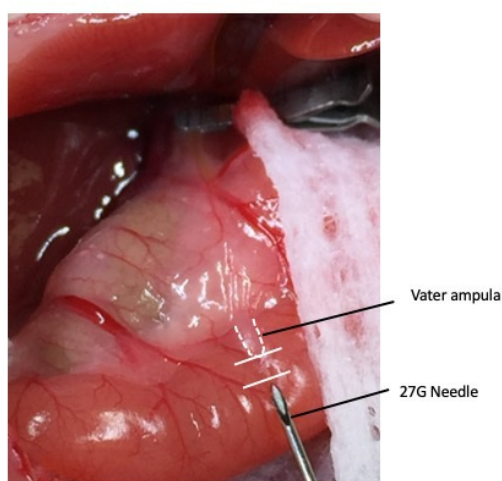
by Agnthos

Catalog #: 00396-01

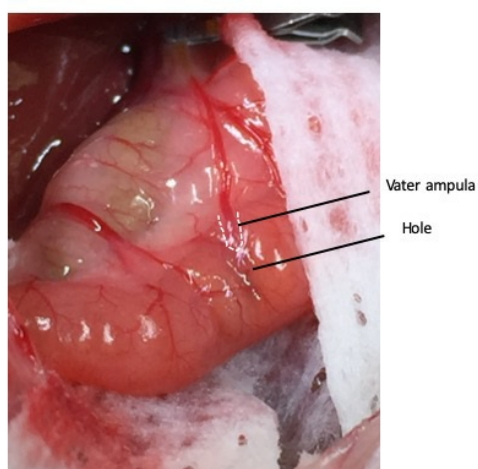


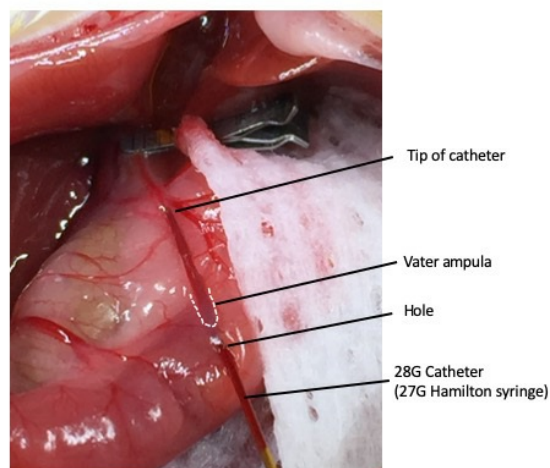
When the first clamp is placed, clamping of the portal vein located immediately below the common bile duct should be avoided.

- 9 Use a 30-gauge needle for B6J, 27-gauge for Nude and NSG to make a small hole opposite to the sphincter of Oddi, 1–2 mm away from the ampulla of Vater.



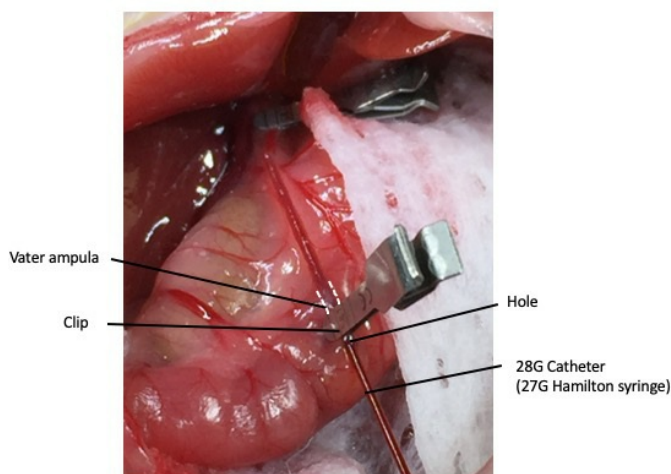
- 10 Next, pass a 31(B6J) or 27(Nude/NSG)-gauge catheter or Hamilton syringe (50 μ L, Model 705 SN SYR, Cemented NDL, 27gauge, 3 point style) through the small hole that has been created by the 30/27-gauge needle in the duodenal wall and then the sphincter of Oddi.





The tip of the catheter should be positioned at the origin of the pancreatic duct branch in the biliary-pancreatic duct. If the anatomy is unclear, the tip of the catheter can be placed just past the sphincter of Oddi.

- 11 Clamp the papilla around the catheter with another microclamp at the sphincter of Oddi to prevent backflow or leaking during the infusion. The microclamp is of low pressure to avoid injury of the duodenum.



S&T Vascular Clamp (7mm)

by Agnthos

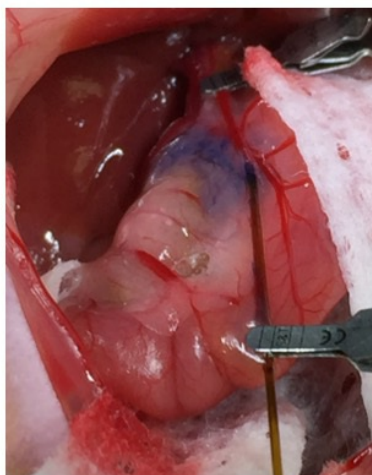
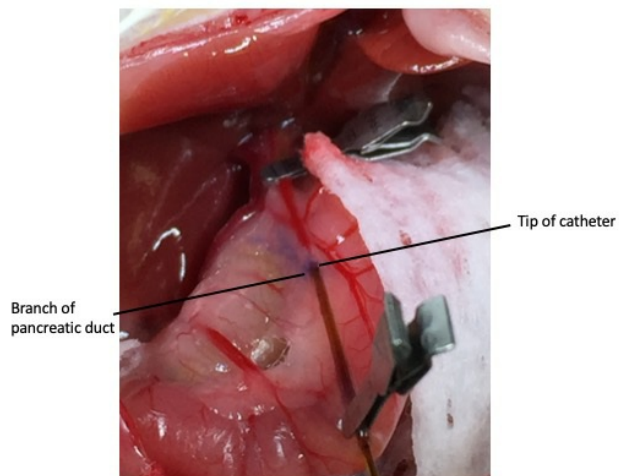
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Infusion

2m

12 Inject tumor volume with total amount **50 μ l** (50 ul of cell suspension)

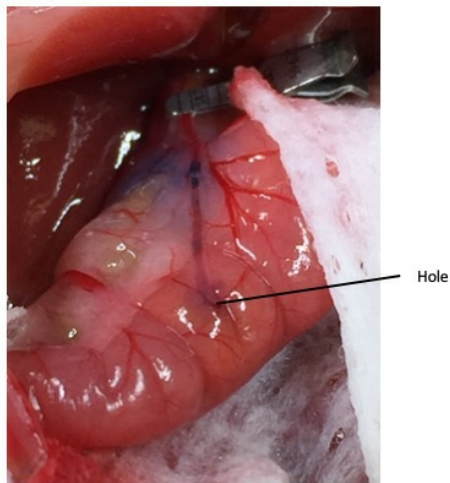
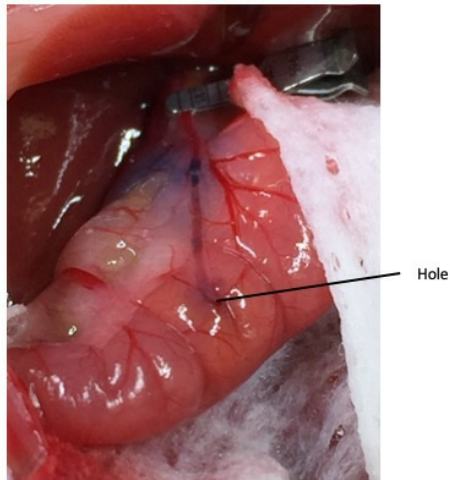
Rate **50 μ l** / **00:02:00** manually



Removal of the Cannula and Microclamps

30s

13 After the infusion is completed, remove the microclamp on the sphincter of Oddi and withdraw the syringe or catheter gently from the duct.

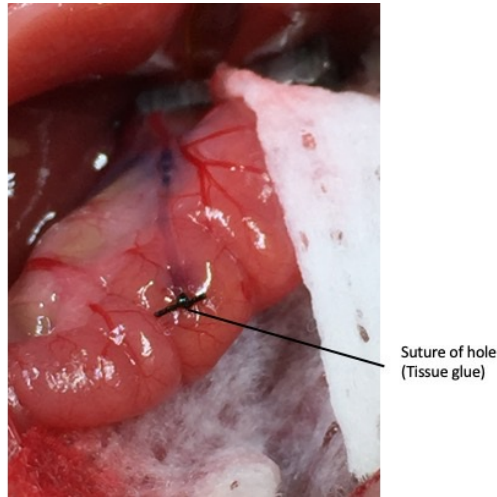


Closure 2m

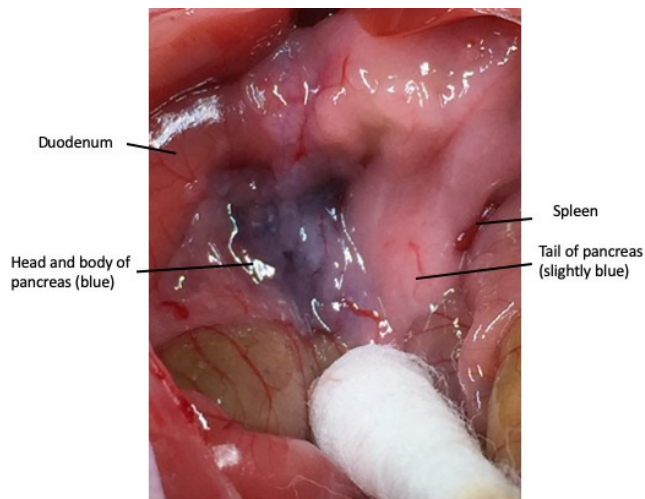
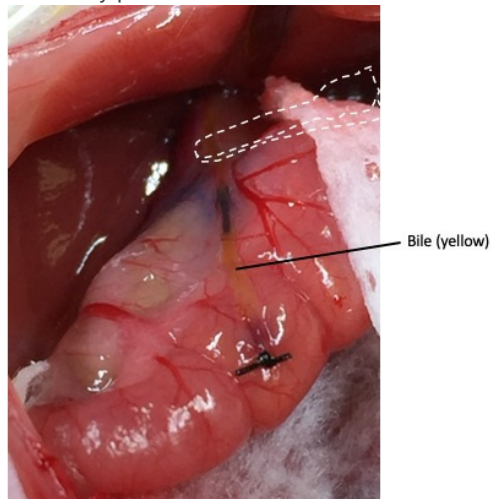
- 14 After infusion, close the hole in the duodenum created by the catheter with a 6-0 suture or a tissue glue for Nude and NSG.



For B6J, this suture does not seem to affect the experimental results, nor does it seem to affect survival of the mice.

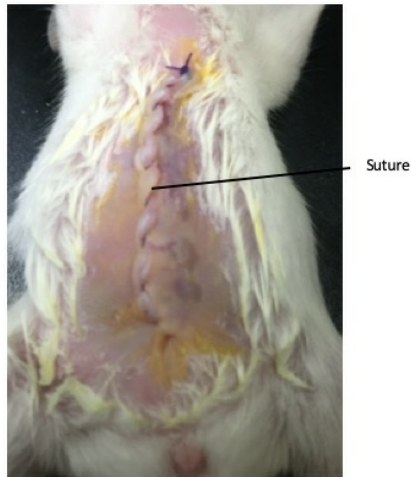


- 15 Remove the microclamp on the biliary-pancreatic duct.



Check if the tumor injection is performed correctly by checking blue dye in the pancreas

- 16 Return the duodenum to the right position and suture the abdominal muscles with a 4-0 suture using a running stitch.



Postoperative Care

5m

- 17 Return the mouse to its cage on a heated pad (37°C) until it fully recovers.
- 18 Give ketoprofen at a dose of 5 mg/kg s.c. once per day continuously for 3 d after surgery for analgesia.



Ketoprofen

by Sigma Aldrich

Catalog #: K1751

CAS Number: 22071-15-4