Islet isolation

PREP WORK
1) Add 1 mg/ml Collagenase P to islet buffer for digestion (7 mls per mouse)
2) Fill 5 ml syringe with a 30G needle and store on ice.
3) Add remaining 2 mls collagenase mixture to collection tube (15 ml conical)

MOUSE WORK
4) Cut open the peritoneal cavity of the mouse, place kimwipe or gauze over the rib cage, then push down to flip over liver onto kimwipe and cover to keep in place.
5) At this point a visible ‘Y’ or bifurcation of the common bile duct should be visible under the dissecting microscope (between the lobes of the liver)
6) Follow the duct to the duodenum and gently clamp the duct where it enters the intestine to block flow of collagenase solution into the intestine
7) Cannulate the common bile duct with preloaded 5ml syringe at the Y junction between the liver lobes. Inject solution into duct (liver should turn whitish and pancreas should begin to inflate if duct is properly accessed).
8) After perfusion, carefully dissect out the inflated pancreas and place in collection tube with 2 mls collagenase mixture, store on ice until all pancreata have been perfused.

AT THE BENCH
9) Digest pancreata at 37 degrees for 15-25 minutes in shaker bath (digestion time depends on specific lot of collagenase, age and strain of mice)
10) Following digestion shake vials for 1 minute by hand (wrap in tin foil to shake all at once)
11) Pipet 10 mls cold ‘wash buffer’ and briefly centrifuge to pellet (1000 rpm/30s-2 min)
12) Carefully decant supernatant and resuspend with 10 mls wash buffer and briefly centrifuge to pellet (1000 rpm/30s-2 min). Repeat for a total of 3 washes.
13) After final wash resuspend islets in 10 mls wash buffer. Pipet contents through 70 or 100 micron cell strainer into 50 ml conical. (Islets will remain on top of the strainer while digested tissue and debris should flow through)
14) Wash strainer with another 35 mls of wash buffer.
15) Transfer cell strainer to a new 50 ml conical and wash with another 25 mls wash buffer to wash residual debris away from islets
16) Next, invert cell strainer and rinse into a petri dish with 25 mls RPMI 1640 10% FCS + glutamine + Pen/Strep (R10F)
17) Using a pipet tip or forceps remove larger/undigested pancreas and discard.
18) Under stereo microscope gently swirl petri dish in a circular motion to center islets
19) Handpick islets into an eppendorf tube containing R10F.

SOLUTIONS
Islet buffer 500 mls
50 mls 10x Hanks buffered Salt Solution (without CaCl$_2$, MgCl$_2$, MgSO$_4$, NaHCO$_3$ and phenol red)
5 mls 1M Heps (10 mM final)
0.5 mls 1M MgCl$_2$ (1mM final)
444.5 mls MQ H2O
pH to 7.4

Wash Buffer
Mouse buffer with 1mM CaCl$_2$