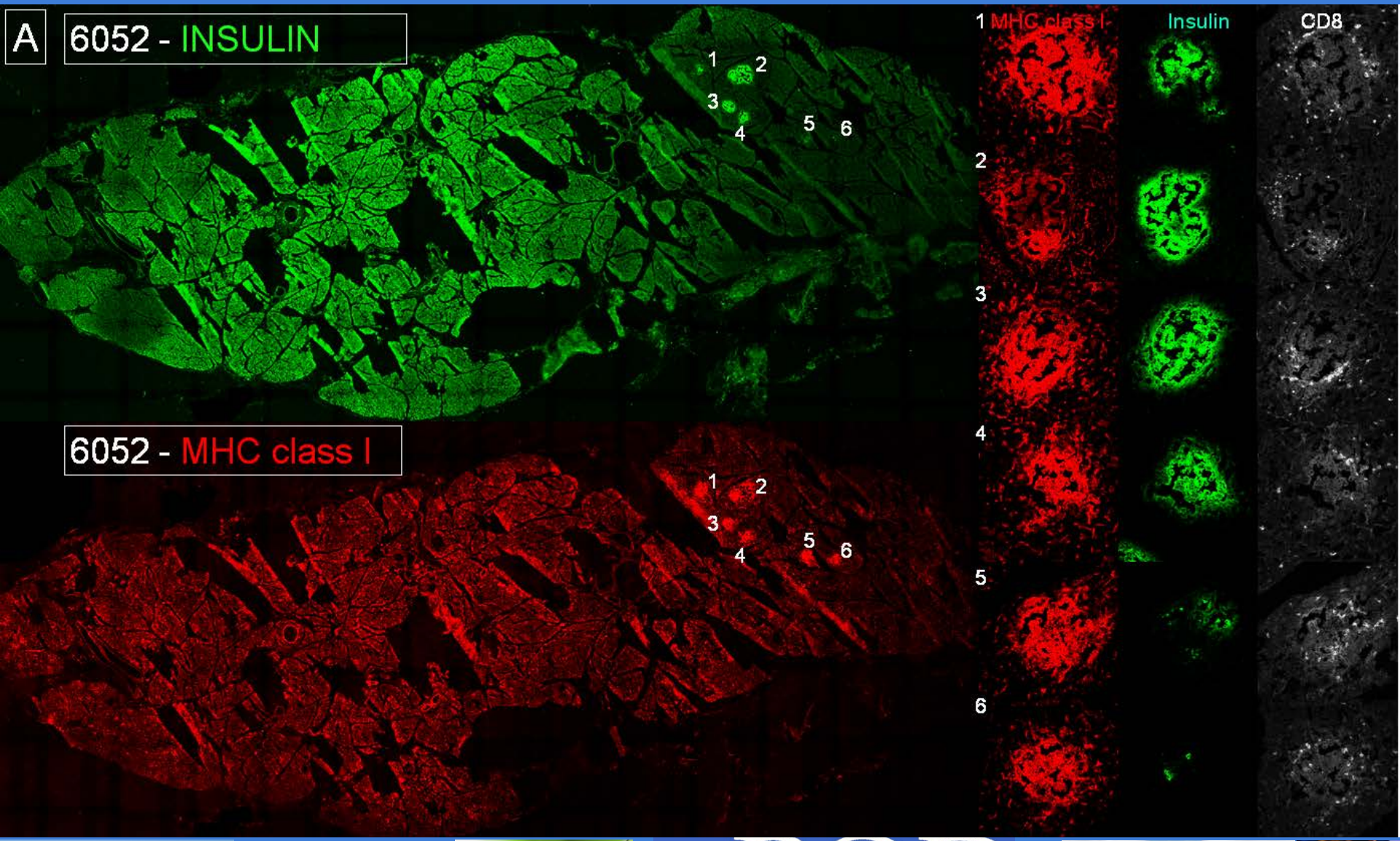
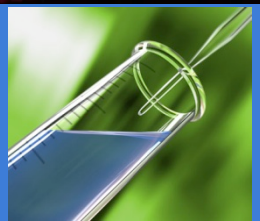
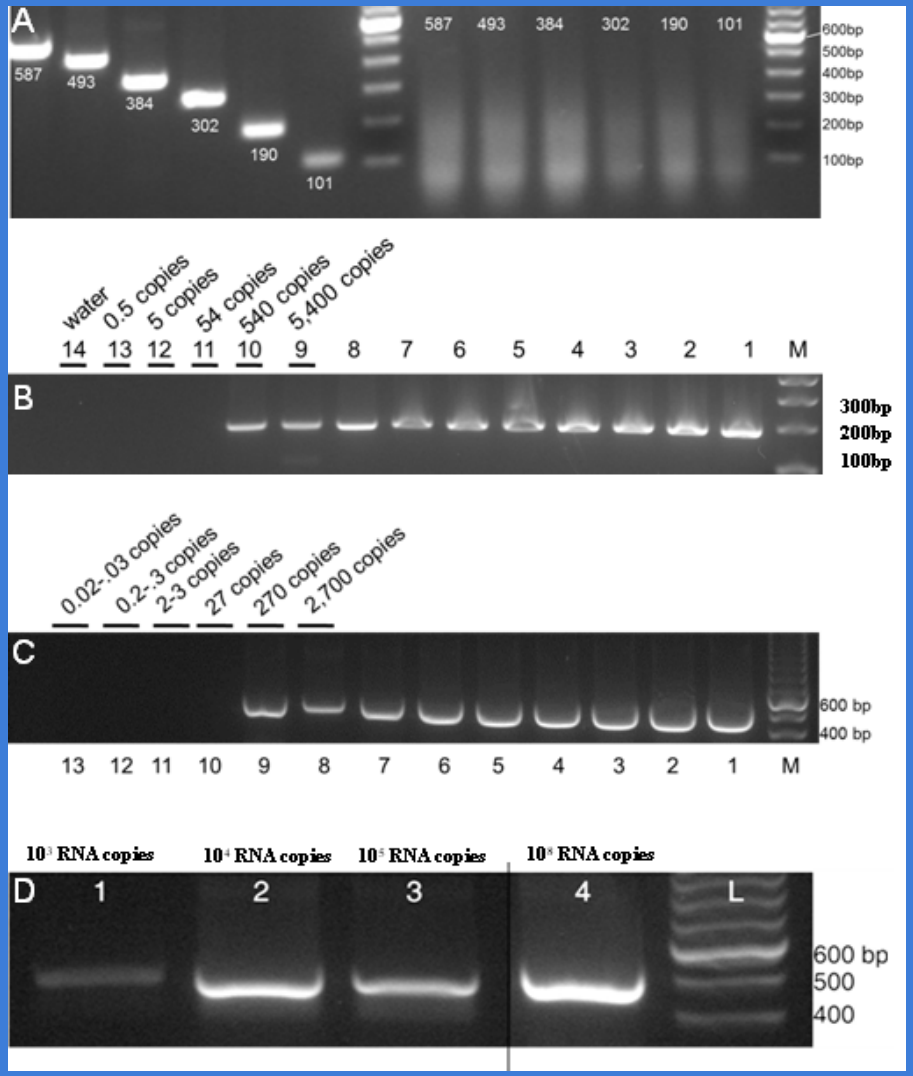
nPOD case 6052 – focal MHC class I hyperexpression and insulitis





nPOD
Network for Pancreatic Organ Donors with Diabetes

nPOD case 6052 – No evidence of viral infection



- Sections consecutive to the ones with MHC class I hyperexpression were independently probed by the Hyotty and Tracy for enterovirus species and by Tang for all known viral species (ViroChip)

- the RT-PCR system could detect less than one infected cells' equivalent of enterovirus RNA amidst a background of about 50,000 uninfected cells.

Optimization of PCR conditions for sensitive detection of enterovirus in nPOD samples.

(**A**) RNA quality analysis by amplifying cellular GAPDH mRNA up to an amplimer size of 600bp. Representative 100-600bp ladder was obtained from an nPOD nucleic acid sample (left panel). To exclude interference from genomic DNA, total nucleic acid content (RNA and DNA) instead of cDNA was used as the template material in the PCRs (right panel).
(**B, C**) To test PCR sensitivity, each lane contains nucleic acids from one individual PCR in which ten-fold decreasing copy numbers of linear viral cDNA template were used within a background nucleic acid content equivalent to 50.000 uninfected pancreas cells. (**B**) used primer pair E1 and E2, while (**C**) used primers E3 and E8.
(**D**) The yield of RT reactions containing ten-fold decreasing copy numbers of T7 transcribed CVB3 positive strand RNA were collected by ethanol precipitation and placed in a background nucleic acid mix equivalent to 50.000 uninfected pancreas cells.. One-half of each reaction used to template a separate PCR. Each PCR was then analyzed in its entirety in a single gel lane. Lane 1-4, detection of $1x10^3$ through $1x10^8$ RNA copies by PCR. Not shown are the detected bands for $1x10^6$ and $1x10^7$ copies. 100 bpladder is the marker

nPOD
Network for Pancreatic Organ Donors with Diabetes