**Specific Aims.**

Pancreatic cancer has poor long term survival due to both late prognoses and the aggressiveness of the disease. Over 90% of cases are diagnosed late because there is no specific symptoms for the early stages of this disease and no diagnostic tool to distinguish malignant from non-malignant small pancreatic lesions. Recent advances in surgical techniques and treatments have increased average five years survival for patients diagnosed with pancreatic cancer from 5% to 15-40% when they are diagnosed with resectable tumors. An accurate, cost-effective, and easy to implement screening test for pancreatic cancer is needed to enable discovery of resectable tumors. One area under intense investigation is for blood based screening tests which are easy to apply and minimally invasive. Currently, blood based biomarkers utilizing various proteins, circulating tumor DNA, or miRNA levels are being investigated for early detection of pancreatic cancers. While blood level of the protein CA19-9 is conventionally used in the clinics to monitor pancreatic cancer progress after surgery, it have been found to have only up to 85% specificity and may not be applied to 10% of population who cannot make these proteins. An accurate blood based screening assay could help to determine treatment options and greatly increase the chance of survival for patients with pancreatic cancer.

We propose to apply a novel methylation haplotyping method, called MONOD, for identifying and classifying pancreatic lesions from circulating DNA. MONOD is based on the presence of common epigenetic abnormalities in cancer, regardless of the difference in driver mutations, and hence more robust in the presence of biological variability. It took advantage of clusters of tightly co-methylated CpG dinucleotides to improve the signal-to-noise ratio and technical sensitivity. Finally, it integrates methylation signatures across many markers from tumor cells and tumor-adjacent normal cells to detect and locate tumor simultaneously, and with sensitivity/specificity superior to using with tumor signature alone. The proof-of-concept for MONOD has been demonstrated on lung cancer and colorectal cancer in our recent Nature Genetics publication. Here we will apply this method to pancreatic cancer, based on specific signals we have identified from 10 pancreatic cancer plasma samples. Furthermore, we will take advantage of our expertise in developing targeted methylation assays to design and validate a highly efficient and sensitive assay covering the informative markers that could be eventually adopted as a routine clinical test for classifying pancreatic lesions (malignant or not) and identifying patients for resectional surgery, which would increase survival. We are proposing to accomplish this goal with the following specific aims:

**Aim 1**: **To identify and validate a set of MONOD markers for detecting pancreatic cancer and distinguishing benign/malignant cases, we will construct a highly comprehensive database of 73 normal human tissues, 12 cancer primary biopsies (including localized and metastatic pancreatic cancer biopsies), and perform a comprehensive identification/validation of informative tissue-of-origin markers on plasma samples.** Specifically, we will expand the current database constructed from 65 normal tissues WGBS data by generating 7 additional matched normal and pancreatic cancer tissues datasets, 1 chronic pancreatitis, and including 5 additional public cancer datasets. We will also collect and analyze >100 plasma sample from patients with four different stages of pancreatic cancer.

**Aim 2**: **To develop a plasma-based pancreatic cancer screening assay, we will develop two targeted methylation sequencing assays, perform technical benchmarking, and select one method for validation on ~200 clinical plasma samples from pancreatic cancer patients.** Specifically, we will compare the BSPP method that we first published on Nature Biotechnology and have optimized since 2009, with a novel methyl-AmpliSeq method that Thermo Fisher recently co-developed with us.

This project will have two key deliverables: (i) a set of MONOD markers identified and validated by ~300 clinical samples; (ii) a fully optimized targeted methylation sequencing assay that is ready for clinical implementation for early screening of pancreatic cancer in high-risk populations.