For over a decade, adult sockeye salmon from the Fraser River have experienced unprecedented levels of mortality (up to 95%) during migration through high water temperature regions of the river. Importantly, stocks that historically entered the river in the fall have been entering in mid summer, placing them in the river during peak summer temperatures; these are the fish experiencing the highest en route mortalities. The functional genomics program has uncovered a novel disease afflicting the migrating salmon stocks from the Fraser River that may drive them quickly into the river and diminish their ability to withstand the high water temperatures.

In 2006, genome-wide transcriptional profiling of salmon radio-tagged in the ocean at distances up to 800 km from the Fraser River revealed that over 55% of the migrating sockeye salmon were responding physiologically to an intracellular pathogen present in their system. These diseased fish were the first to enter the river and were 16 times more likely to succumb to high water temperature stress. Similar profiles were observed in 25% of migrating sockeye salmon in 2005, and 36% in 2007. The infection was originally uncovered in expression profiles from gill tissue, in which response to the pathogen escalated considerably once salmon entered the river. However, expression profiles of the brain indicated an even stronger pathogen response that did not change greatly during migration. Moreover, identification of the genes differentially expressed in both gill and brain revealed signals consistent with a retroviral infection, a class of viruses that often cause cancer in their host.

Although retroviruses have been observed in a variety of fish species, only two are believed to affect salmon: the Atlantic salmon swim bladder virus, a fully characterized and sequenced virus isolated from tumours in the swim bladders of farmed salmon both in Europe and Maine, and the Salmon Leukemia Virus (SLV), postulated in the late 1980s to be the cause of Marine Salmon Anemia, also called Plasmacytoid leukemia, in BC and Washington farmed Chinook salmon. The original research on the latter virus was conducted by the Fish Health group at the Pacific Biological Station (led at the time by Mike Kent), but unfortunately they did not isolate the virus nor obtain any DNA sequence from it. Furthermore, their research showed that a microsporidian parasite, *Nucleosporia salmonis*, elicited a highly similar histological profile and was often found as a co-infective agent. The parasite has been implicated in high mortalities in farmed and wild salmon in Europe and the US. Below we list the accumulating evidence that suggests the disease afflicting our sockeye salmon is retroviral in nature and could be plasmacytoid leukemia:

- Gene expression data indicate the salmon may be responding to a retrovirus, specifically a leukemia-type retrovirus
    - SLV is the **only previously suspected retrovirus in BC salmon**
- We have already conducted molecular screening and **ruled out common viruses** afflicting Pacific salmon, including ISAV, IHNV, VHSV, Herpes, IPNV, Picornavirus. We also screened for bacterial pathogens and myxosporidian parasites, all negative.
- Plasmacytoid leukemia was observed in the early 1990s in wild sockeye and Chinook salmon in the Strait of Georgia. In laboratory challenges, homogenates

containing SLV were apparently infective in Chinook, sockeye and Atlantic salmon, but it is not clear whether other Pacific salmon species were challenged.

- Fish with plamacytoid leukemia are **anemic**, and diseased Chinook salmon had **pale gills**
    - pale gills are often observed in dying sockeye salmon in the Fraser R.
- Anemia generally involves **iron deficiency**
    - very low transcription of ferritin in our diseased fish may indicate that they are low in iron
- Fish with plasmacytoid leukemia generally **look healthy**, and it takes a trained histologist to identify the presence of the disease
    - There are generally no external signs of disease in the sockeye salmon entering the river early
- Fish carrying SLV may be very **temperature sensitive;** cell lines that may carry the virus are only cytolytic between 15-20^{o}C, and anecdotal evidence indicates mortalities of farmed fish peak in late August early September, when temperatures are also their highest
    - Our data indicate that high temperatures in the interior Fraser River drainage contributes substantially to poor survivorship of infected sockeye salmon individuals
- Fish carrying SLV may be very **sensitive to salinity changes;** the highest mortality in Chinook salmon was observed upon transfer from FW to SW
    - Our data indicate that infected sockeye salmon may experience osmoregulatory difficulties in SW. This is one potential trigger pushing late run sockeye salmon into FW prematurely.
- Retroviruses, including those that cause leukemia in mammals, are well known for inducing **immunosuppression**
    - Signals consistent with immunosuppression were observed in infected sockeye in SW
- Organisms with leukemia often succumb to **secondary bacterial infections**; Chinook salmon with plasmacytoid leukemia also carried a higher incidence of Bacterial Kidney Disease
    - The sockeye salmon dying in the river are also afflicted with numerous other pathogens
- Leukemia is associated with **coagulation disorders**
    - Field researchers noted heavy bleeding of sockeye salmon sampled in 2003, coagulation dysfunction was noted in expression profiling of liver tissue of these fish
- Retroviruses are neoplastic viruses, hence **associated with cancer**. SLV was apparently concentrated from tumours behind the eyes of afflicted Chinook salmon
    - Numerous cancer biomarkers were up-regulated in the brains of afflicted sockeye salmon, two of which are markers specific to brain cancer in mammals
    - Expression of genes involved in visual perception and growth and proliferation of cells specific to the eye were also noted

- The timing of the first diagnosis of plasmacytoid leukemia (late 1980s, early 1990s) precedes the shift in river entry timing in sockeye salmon, first noted in 1996. Similar timing shifts have also been noted for Chinook and pink salmon, but these have thus far received less scientific focus.

It is possible that the causative pathogen has been present in BC salmonids for much longer without strong pathogenic effects if it is, indeed, highly temperature dependent. As further increases in temperature might be expected due to climate change, this disease could increase in virulence in the future. However, poor survivorship of afflicted migrating fish might also eventually diminish the incidence of the disease, especially if transfer is largely vertical (from mother to eggs), which is a main route of infection for many retroviruses. Vertical transmission of the virus would also introduce the possibility of effects at other developmental stages, such as smolts. On the Chinook salmon farms, disease was observed in smolts.

Given the potential devastating impacts of this disease on sockeye salmon, and possibly other Pacific salmon species, we propose research that will conclusively establish whether plasmocytoid leukemia or a similar retrivirus is, in fact, the primary cause of river entry timing shifts and higher susceptibilities of salmon to temperature stress. We propose to conduct this research through collaboration between Miller, who performed the genomic analysis implicating a viral etiology, Garver, the virologist at PBS, and Patterson, responsible for the Environmental Watch Program in the Fraser River. Tissue samples for histology and viral isolation have already been collected, but funding is required for Garver to proceed with analyses of these tissues. The proposed research would include four main objectives:

1) Use of molecular tools to isolate sequences of viral and/or parasitic origin from afflicted fish (diseased fish identified originally through genomic analyses). This would include traditional PCR amplification-based approaches and application of the viral microarray used to discover SARS (the Vancouver group that did this analysis has agreed to collaborate),

2) Histological analysis of fish dying in the river in 2008,

3) Isolation and characterization of the virus from the brain, kidney and gills of afflicted fish. This research would include the development of in vitro (cell culture) and in vivo (fish) challenge models for the virus, if the virus is culturable,

4) Development of molecular markers for use in broader surveys to determine infection rates in juveniles, smolts and adults from different stocks. Genomic research already indicates that this is not a stock or late-run specific disease (although the effects to date may be largest on the late-run sockeye), and samples for this analysis have already been collected. Eventually, these markers could also be used to survey additional species. This research would require 60K in funding for 2009/2010.