Proposed Research on suspected novel Virus from Genomics studies on Sockeye salmon

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Determine the potential role of the suspected viral disease identified through genomic signatures in gill, brain and liver (and known to exacerbate survival of sockeye adults in the Fraser river) in the abnormally low returns of Fraser sockeye in 2009

- **Identify and sequence the virus and develop a molecular tool for screening (required for further analysis) (87K) (see details below)**
- Establish infectivity of fish containing the viral-signature and those containing brain lesions (28K)
- Characterize the levels of infection in the kidney (likely the primary tissue for the viral infection) of returning adults in 2005-2009 to determine if overall infection levels were unusually high in the 2005 adult returns (predicted >75% based on gill, liver, brain profiles) (45K)
- Characterize infection levels in smolts sampled in the Strait of Georgia in 2007 (5K pending sample availability)
- Determine whether the virus is vertically and/or horizontally transmitted (40K)
- Establish susceptibility of different species and life-history stages (70K over 2 years)
- To establish whether this disease could be a key factor in the general declines of Chinook, coho and sockeye salmon in BC and elsewhere, contrast prevalence levels between stocks and species that are in decline and those that are not (160K over 2-3 years)

THIS FISCAL YEAR JAN-MAR 2010

- **Identify and sequence the virus and develop a molecular tool for screening (required for further analysis) (87K)**
  January-March 2010 -- 23K required
  - Most data thus far point to the involvement of a virus in the retrovirus family, with a strong involvement of lymphocytes. Given this information, we expect that the kidney and blood may be the primary infective tissue and thus could contain the highest viral loads. We will confirm this by running arrays on blood and kidney contrasting individuals positive and negative in brain/liver to identify the most infective tissues. These tissues will be used for concentrating the virus. **(5K) Miller**
  - RT assays will be performed on positive signature tissues to determine the likelihood that we are dealing with a retrovirus (which affects the way the virus is concentrated and handled) **(3K) (Miller)**
  - The virus will be concentrated from positive signature tissues using three approaches:
    - low speed centrifugation,
      - flocculation with Polybrene, and
      - Amicon ultrafiltration membranes
      - **(5K) (Miller/Garver)**
  - The concentrated material will be used for:
    - Infection of cell lines to establish a continuous source of concentrated virus for challenge work (this may or may not work, as not all viruses are culturable, and retroviruses are known to be difficult to culture) **(5K) (Garver)**
    - Electron microscopy to visualize the virus (important for identifying presence of viral particles in the concentrate and identification of viral family) **(3K) (contracted out, as Bill in Histology is leaving soon)**
    - Direct 454 sequencing **(7K for one round) Genome Centre, Vancouver**
- **Full physiological assessment of 2007 sockeye smolts and 2009 return adults: 1) Test Dick's hypothesis that unusually poor feeding and growth of sockeye salmon smolts in the ocean may have led to large losses (liver and muscle) 2) Test hypothesis that**

unusual high incidence of "disease" associated with the viral-related fate signature was present in 2007 (we already know this is present in smolts in 2008) (Kidney)

- We have 20 sockeye smolts collected in Hecate Strait by Marc Trudel, and 20 Chilko smolts collected in the Fraser River by David Patterson. We will do a full physiological assessment (liver-feeding, white muscle-growth/starvation, kidney-disease) of these fish and compare them to Chilko smolts in 2008 (river and ocean). (**42K**)

NEXT FISCAL YEAR (if funds available and positive results achieved)

April-March 2011 (if previous methods do not yield a viral sequence)

- Hybridization of different batches of concentrated virus onto viral arrays to identify the best batches for sequencing, to identify viral family more with confidence, and to generate primers from top hits (**10K**) **Miller/Garver and BC Centre for Disease Control**
- Direct 454 sequencing **(14 K) Genome Centre, McGill University**
- Subtractive cDNA libraries could also be developed and sequenced, potentially providing us with clones of viral genes, however this approach would only be done depending upon the success of other approaches (**20K**) **Miller and/or Genome Centre, McGill University**
- Establish infectivity of tissues positive for the virus or the viral signature (**28K**) **Garver**
- Histology of positive matched tissues to determine pathogenic effects on tissues/cells (**20K**) **Contracted out**
- Characterize the levels of infection in the kidney (likely the primary tissue for the viral infection) of returning adults in 2005-2009 to determine if overall infection levels were unusually high in the 2005 adult returns (predicted >75% based on gill, liver, brain profiles) and the out-migrating juveniles (**45K**) **Miller/Garver**
- Enhance screening efforts to establish prevalence of the virus over stocks and species that are in decline versus those that are not (**120K**)
- Technical salary for 12 months (**65K**) **Miller**
- Co-op Student to aid in sampling and processing and organization of samples (**15K** for 6 months) **Miller**
- Postdoctoral Fellow to lead these analyses (**55K**)
- TOTAL **382K**
- DELIVERABLES:
  - Virus confirmation
  - Viral identity
  - Infectious determination
  - Establish prevalence over years and stocks in smolts and adults, including stocks performing poorly and those performing well
  - Option to also look at other species (signature present in Chinook and coho smolts)