(1)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: May 20, 2003 9:16 AM
To: JonesS@pac.dfo-mpo.gc.ca
Subject: ASK-2 cell line and viral RNA

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

Today I shipped three 75 cm*2 flasks with ASK-2 cell monolayers.
The cells are 2 days old today.

To use, we split them 1:2 when the monolayer is confluent. Growth medium is L-15 + 1 mM L-glutamine + 10% FBS (from Sigma ..
Hybridoma tested) + 1x antibiotics.
Maintenance medium is same with FBS reduced to 5%.

These cells grow very slowly but once confluent they are very susceptible to ISAV (I have seen CPE come up in 2 days!).
However, I do not know how good they are for primary virus isolation.

I have also include ISAV isolate NBISA01 RNA in formamide.
To use, precipitate with 2.5 x volume 100% ethanol in high salt and then resuspend in RNase-free water.

The shipment was sent Federal Express. The FedExp track number is as follows: 4967 8015 7201.

Let me know when you receive it.

Regards,

Fred.

(2)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: October 3, 2003 10:18 AM
To: JonesS@pac.dfo-mpo.gc.ca
Subject: RE: visit

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

Good to hear from you. I would be happy to run those samples through our RT-PCR system here. We also use the segment 8
F5/R5 primers. We clone our products using the Invitrogen TOPO TA Cloning kit and use a commercial company for sequencing.
Feel free to send the samples along and I will let you know what we find.

Best regards.
Fred.

(by the way, I plan to be in Nanaimo for the Thanksgiving weekend.
Hopefully I will see you then?).

Date sent: Fri, 03 Oct 2003 10:08:55 -0700
From: JonesS@pac.dfo-mpo.gc.ca
Subject: RE: visit
To: kibenge@upei.ca

> Hi Fred:
> I hope all is going well. I am hoping you will be interested in
> collaborating with us on our virus surveillance studies. We have been
> screening Pacific salmon for viral pathogens and have some samples
> that we would like to have confirmed for ISA virus. We noticed a
> positive RTPCR reaction using the segment 8 (f5/r5) primers and were
> interested in having these observations confirmed, with sequencing of
> the reaction products. I have 20 samples that I would like to send you.
> Looking forward to your reply.
> Best wishes,
> Simon
>
> Dr. Simon R.M. Jones
> Department of Fisheries and Oceans
> Pacific Biological Station
> 3190 Hammond Bay Road
> Nanaimo, British Columbia
> V9T 6N7, Canada
>
> tel: 250 729 8351
> fax: 250 756 7053
> joness@pac.dfo-mpo.gc.ca
> http://www.pac.dfo-mpo.gc.ca/sci/aqua/profiles/jones_e.htm
>
>
>
>
>
> -----Original Message-----
> From: Dr. Fred Kibenge [mailto:kibenge@upei.ca]
> Sent: July 9, 2003 8:27 AM
> To: JonesS@pac.dfo-mpo.gc.ca
> Subject: Re: visit

(3)

>
>
> Hi Simon,
>
> Good to hear from you. I am still interested in presenting a seminar
> and further discussions on collaboration.
>
> The Seminar title is "Biochemistry, Aetiopathogenesis and Immunology
> of Infectious Salmon Anaemia Virus".
>
> All the best.
>
> Fred.

(4)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: October 7, 2003 6:36 AM
To: JonesS@pac.dfo-mpo.gc.ca
Subject: RE: visit

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

I just wanted to let you know that I received the 20 samples (1A-10A and 1B-10B) in good condition, and will soon start working on them.

All the best.

Fred.

From: Dr. Fred Kibenge <kibenge@ACAD1.CS.UPEI.CA>
To: JonesS@pac.dfo-mpo.gc.ca
Subject: RE: visit
Send reply to: kibenge@upei.ca
Date sent: Fri, 3 Oct 2003 17:17:48 ADT

> Hello Simon,
>
> Good to hear from you. I would be happy to run those samples through
> our RT-PCR system here. We also use the segment 8
> F5/R5 primers. We clone our products using the Invitrogen TOPO TA
> Cloning kit and use a commercial company for sequencing.
> Feel free to send the samples along and I will let you know what we
> find.
>
> Best regards.
>
> Fred.
>
> (by the way, I plan to be in Nanaimo for the Thanksgiving weekend.
> Hopefully I will see you then?).
>
> Date sent: Fri, 03 Oct 2003 10:08:55 -0700
> From: JonesS@pac.dfo-mpo.gc.ca
> Subject: RE: visit
> To: kibenge@upei.ca
>
> > Hi Fred:
> > I hope all is going well. I am hoping you will be interested in
> > collaborating with us on our virus surveillance studies. We have
> > been screening Pacific salmon for viral pathogens and have some
> > samples that we would like to have confirmed for ISA virus. We
> > noticed a positive RTPCR reaction using the segment 8 (f5/r5)
> > primers and were interested in having these observations confirmed,
> > with sequencing of the reaction products. I have 20 samples that I would like to send you.
> > Looking forward to your reply.
> > Best wishes,
> > Simon
> >
> > Dr. Simon R.M. Jones
> > Department of Fisheries and Oceans
> > Pacific Biological Station
> > 3190 Hammond Bay Road

(5)

> > Nanaimo, British Columbia
> > V9T 6N7, Canada
> >
> > tel: 250 729 8351
> > fax: 250 756 7053
> > joness@pac.dfo-mpo.gc.ca
> > http://www.pac.dfo-mpo.gc.ca/sci/aqua/profiles/jones_e.htm
> >
> >
> >
> >
> >
> > -----Original Message-----
> > From: Dr. Fred Kibenge [mailto:kibenge@upei.ca]
> > Sent: July 9, 2003 8:27 AM
> > To: JonesS@pac.dfo-mpo.gc.ca
> > Subject: Re: visit
> >
> >
> > Hi Simon,
> >
> > Good to hear from you. I am still interested in presenting a
> > seminar and further discussions on collaboration.
> >
> > The Seminar title is "Biochemistry, Aetiopathogenesis and Immunology
> > of Infectious Salmon Anaemia Virus".
> >
> > All the best.
> >
> > Fred.
>
>

6

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: October 21, 2003 1:57 AM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: gels

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

Attached are some gels of the testing on the 20 samples.
My pictures are clearer than this (clear enough to cut the bands out) but I thought you might want to have an idea of the intensity of the bands compared to the positive controls?

All the best.

Fred.

(7)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: October 21, 2003 1:57 AM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: gels

Follow Up Flag: Follow up
Flag Status: Red

Attachments: DFO gels.doc

DFO gels.doc (22 KB)

```
* This message contains the file 'DFO gels.doc', which has been
* uuencoded. If you are using Pegasus Mail, then you can use
* the browser's eXtract function to lift the original contents
* out to a file, otherwise you will have to extract the message
* and uudecode it manually.
```

(8)

High Sea Chinnok Salmon samples checked for ISAV using RT-PCR (samples received from DFO, PBS, Dr. Simon Jones/Dr. Molly Kibenge).

Figure 1: Segment 8 primers (F5/R5). All positive samples ran on one gel.

Lanes:
1. 1kb ladder (Invitrogen)
2. Sample 3B
3. Sample 4B
4. Sample 10B
5. NBISA01 +ve control
6. Sample 1A
7. Sample 2A
8. Sample 3B
9. Sample 4B
10. NBISA01 +ve control

Figure 2: Segment 7 ORF1 primers

Lanes:
1. 1kb ladder (Invitrogen)
2. Sample 1A -ve
3. Sample 2A +ve (400bp)
4. Sample 3B -ve
5. Sample 4B +ve (400bp)
6. Sample 5B -ve
7. Sample 6B +ve (400bp)
8. Sample 10B +ve (400bp)
9. NBISA01 +ve control (903bp)

(9)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: October 21, 2003 1:57 AM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: gels

Follow Up Flag: Follow up
Flag Status: Red

Attachments: DFO 100703 gels.ppt

DFO 100703 gels.ppt (140 KB)

```
* This message contains the file 'DFO 100703 gels.ppt', which has been
* uuencoded. If you are using Pegasus Mail, then you can use
* the browser's eXtract function to lift the original contents
* out to a file, otherwise you will have to extract the message
* and uudecode it manually.
```

High Sea Chinnok Salmon samples checked for ISAV using RT-PCR (samples received from DFO, PBS, Dr. Simon Jones/Dr. Molly Kibenge).

Figure 1: Segment 8 primers (F5/R5). All +ve samples ran on one gel.

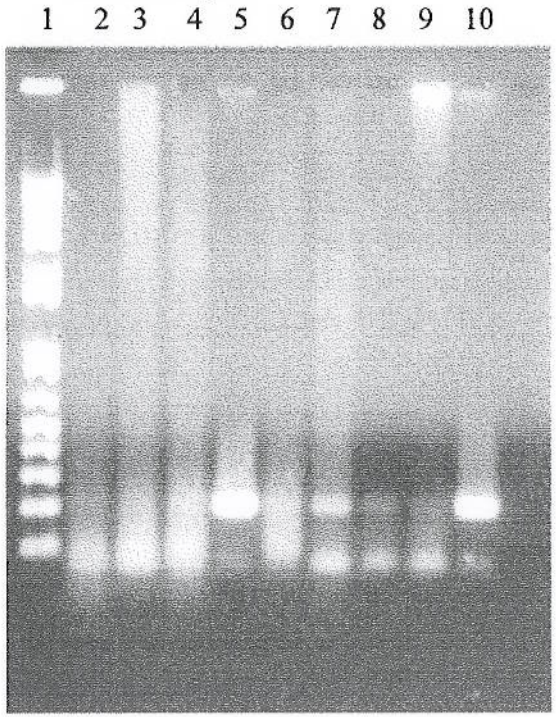
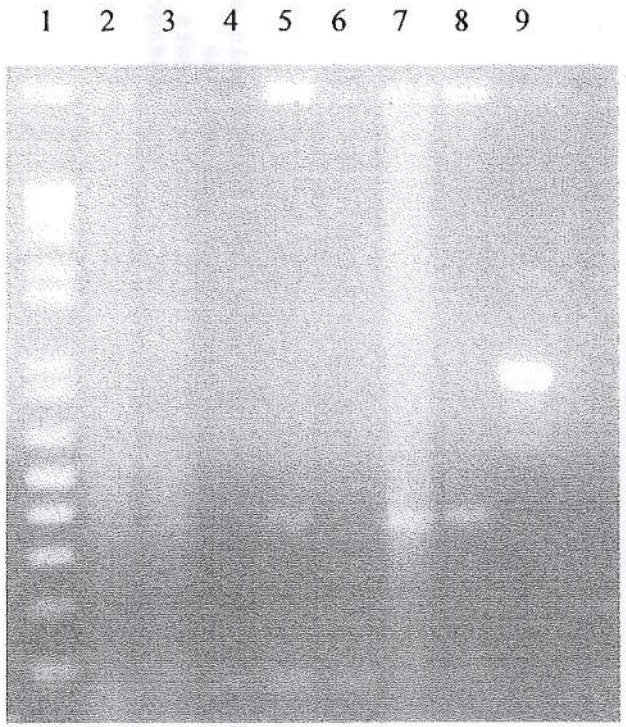


Figure 2: Segment 7 ORF1 primers



(11)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: November 5, 2003 1:34 PM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: sequence analysis of segment 7 products

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

Attached is the information I have obtained on the segment 7 clones. You will note that I am being very cautious.

All the best.

Fred.

(12)

High Sea Chinnok Salmon samples labeled 1A to 10A and 1B to 10B, checked for ISAV using RT-PCR (samples received from DFO, PBS, Dr. Simon Jones/Dr. Molly Kibenge).

Sample ID	Segment 8 primers (F5/R5)	Segment 7 ORF1 primers
1A	+ ve (~220bp)	- ve
2A	+ ve (~220bp)	+ ve (~400bp)
3A	weak + ve (~220bp)	not done
4A	- ve	not done
5A	- ve	not done
6A	- ve	not done
7A	- ve	not done
8A	- ve	not done
9A	- ve	not done
10A	- ve	not done
1B	- ve	not done
2B	- ve	not done
3B	+ ve (~220bp)	- ve
4B	+ ve (~220bp)	+ ve (~400bp)
5B	- ve	- ve
6B	- ve	+ ve (~400bp)
7B	- ve	not done
8B	- ve	not done
9B	- ve	not done
10B	+ ve (~220bp)	+ ve (~400bp)
NBISA01 +ve control	+ ve (~220bp)	+ ve (903bp)

- ve denotes no PCR product seen; + ve denotes PCR product (size in base pairs) seen.

Sequence Analysis of the RT-PCR products of the Segment 7 ORF1 primers:

All 5 RT-PCR products obtained were subcloned using the TOPO TA cloning kit. The DNA inserts were then sequenced.

The NBISA01 +ve control DNA insert was 903 bp long.
All inserts of the DFO samples were 377 bp long.

The DFO samples were 99.7% identical and corresponded to the ISAV Segment 7 ORF2 product. They had a 95.8% identity with the NBISA01 +ve control, and 99.7% identity with ISAV strain 810/9/99 from Norway. The sequences are given on page 3, and sequence comparisons using the FASTA program are attached in the next 6 pages (pages 4-9).

[These particular DFO samples were processed separately by my self together with ISAV isolate NBISA01 as positive control. So, I can rule out cross contamination during RNA extraction. However, I also have a graduate student working with both New Brunswick and European (Norway, Scotland, Nova Scotia) ISAV isolates, and cannot conclusively rule out the possibility (however small!) of reagent contamination. Reagent contamination is unlikely since the RT-PCR product was not in all samples tested. For this reason, I am sending you the sequence of the Segment 7 ORF1 primers that I used so that you can try them on your samples. Also, I wish to encourage you to send other blind samples to me for RT-PCR testing so that you can confidently interpret your results].

Segment 7 ORF1 primers:

For the purpose of evaluating individual ORFs, we separated the otherwise partial overlapping ORFs of segment 7 based on the gene expression model suggested by Ritchie *et al.* (2002), of partial splicing of ORF1 mRNA and a frameshift such that ORFs 1 and 2 products share the first 22 amino acid residues.

ISAV SEG7 (ORF1) FOR (23 mer) 5'-ATGGATTTCACCAAAGTGTATGG-3'	1-23,	AF328627
ISAV SEG7 (ORF1) REV (23 mer) 5'-TCACATTCTGAAGTGAAGTCCAG-3'	881-903,	AF328627

ISAV SEG7 (ORF2) FOR (82 mer) 5'-ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAA AACTTCACGGAAAAGACAAGGTGGCTTCTTTCCTGTCGG-3' 1-63/590-608, AF328627

Ritchie, R. J., A. Bardiot, K. Melville, S. Griffiths, C. O. Cunningham, and M. Snow. 2002. Identification and characterization of the genomic segment 7 of the infectious salmon anaemia virus genome. Virus Res. **84:**161-70.

>2A DFO Seg 7 ORF1 reverse (2A-2)
ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAA
GACAAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCA
AGTACGTCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAAC
GATTCTATGACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAA
ATGGACAGAGACGGCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGAT
GGGTGCACTGGGACAGCATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGG
AGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA

>4B DFO Seg 7 ORF1 (4B-1)
ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAA
GACAAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCA
AGTACGTCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAAC
GATTCTATGACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAA
ATGGACAGAGACGGCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGAT
GGGTGCACTGGGACAGCATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGG
AGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA

>6B DFO Seg 7 ORF1 (6B-2)
ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAA
GACAAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCA
AGTACGTCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAAC
GATTCTATGACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAA
ATGGACAGAGACGGCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGAT
GGGTGCACTGGGACAGCATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGG
AGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA

>10B DFO Seg 7 ORF1 (10B-1)
ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAA
GACAAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCA
AGTACGTCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAAC
GATTCTATGACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAA
ATGGACAGAGACGGCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGAT
GGGTGCACTGGGACAGCAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTG
GAGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA

```
A:DFO4B.SEQ, 377 nt vs A:DFO10B.SEQ library
using DNA matrix

   377 residues in     1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn init1   opt
>10B DFO Seg 7 ORF1 (10B-1)                                        1501  1501  1501

>10B DFO Seg 7 ORF1 (10B-1)                                        1501 1501 1501
  99.7% identity in 377 nt overlap

                10        20        30        40        50        60
4B DFO ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
       X::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
                10        20        30        40        50        60

                70        80        90       100       110       120
4B DFO AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
                70        80        90       100       110       120

               130       140       150       160       170       180
4B DFO TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
               130       140       150       160       170       180

               190       200       210       220       230       240
4B DFO ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
               190       200       210       220       230       240

               250       260       270       280       290       300
4B DFO GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
               250       260       270       280       290       300

               310       320       330       340       350       360
4B DFO CATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
       :: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    CAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
               310       320       330       340       350       360

               370
4B DFO TTCACTTCAGAATGTGA
       ::::::::::::::::X
10B    TTCACTTCAGAATGTGA
               370

Library scan:  0:00:00  total CPU time:  0:00:19
```

```
A:DFO6B.SEQ, 377 nt vs A:DFO10B.SEQ library
using DNA matrix

   377 residues in     1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn init1   opt
>10B DFO Seq 7 ORF1 (10B-1)                                1501  1501  1501

>10B DFO Seq 7 ORF1 (10B-1)                                1501 1501 1501
  99.7% identity in 377 nt overlap

                10        20        30        40        50        60
6B DFO ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
       X::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
                10        20        30        40        50        60

                70        80        90       100       110       120
6B DFO AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
                70        80        90       100       110       120

               130       140       150       160       170       180
6B DFO TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
               130       140       150       160       170       180

               190       200       210       220       230       240
6B DFO ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
               190       200       210       220       230       240

               250       260       270       280       290       300
6B DFO GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
               250       260       270       280       290       300

               310       320       330       340       350       360
6B DFO CATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
       :: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
10B    CAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
               310       320       330       340       350       360

               370
6B DFO TTCACTTCAGAATGTGA
       ::::::::::::::::X
10B    TTCACTTCAGAATGTGA
               370

Library scan:  0:00:00  total CPU time:  0:01:46
```

```
a:dfo10b.seq, 377 nt vs a:dfo2ar.seq library
using DNA matrix

   377 residues in     1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn init1   opt
>2A DFO reverse                                           1501  1501  1501

>2A DFO reverse                                           1501 1501 1501
  99.7% identity in 377 nt overlap

                10        20        30        40        50        60
10B DF ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
       X::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
                10        20        30        40        50        60

                70        80        90       100       110       120
10B DF AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
                70        80        90       100       110       120

               130       140       150       160       170       180
10B DF TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
               130       140       150       160       170       180

               190       200       210       220       230       240
10B DF ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
               190       200       210       220       230       240

               250       260       270       280       290       300
10B DF GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
               250       260       270       280       290       300

               310       320       330       340       350       360
10B DF CAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
       :: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
2A     CATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
               310       320       330       340       350       360

               370
10B DF TTCACTTCAGAATGTGA
       :::::::::::::::X
2A     TTCACTTCAGAATGTGA
               370


Library scan:  0:00:00  total CPU time:  0:00:16
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(18) X

```
A:DFO10B.SEQ, 377 nt vs A:810M2.SEQ library
using DNA matrix

   485 residues in    1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn init1   opt
>810 SEG. 7 ORF2 (SE5058)                                  1501  1501  1501

>810 SEG. 7 ORF2 (SE5058)                                  1501 1501 1501
  99.7% identity in 377 nt overlap

              10        20        30        40        50        60
10B DF ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
       X::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
              10        20        30        40        50        60

              70        80        90       100       110       120
10B DF AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
              70        80        90       100       110       120

             130       140       150       160       170       180
10B DF TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
             130       140       150       160       170       180

             190       200       210       220       230       240
10B DF ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
             190       200       210       220       230       240

             250       260       270       280       290       300
10B DF GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
             250       260       270       280       290       300

             310       320       330       340       350       360
10B DF CAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
       :: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
810    CATCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
             310       320       330       340       350       360

             370
10B DF TTCACTTCAGAATGTGA
       ::::::::::::::::X
810    TTCACTTCAGAATGTGATTGGCTGAAAACATGTTTTGTAAACAAGAATTTTGTGTTTTTG
             370       380       390       400       410       420

Library scan:  0:00:00  total CPU time:  0:00:37
```

(19)

X

```
a:dfo10b.seq, 377 nt vs a:nbisadfo.seq library
using DNA matrix

   903 residues in     1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn initl   opt
>NBISA-1 (SF2912; DFO Seq 7 ORF1 NBISA-1)                  1152  1152  1152

>NBISA-1 (SF2912; DFO Seq 7 ORF1 NBISA-1)                   1152 1152 1152
  94.9% identity in 316 nt overlap

              40        50        60        70        80        90
10B DF TTGACCAACTAAAACTTCACGGAAAAGACAAGGTGGCTTCTTTCCTGTCGGGCTCAAAGG
                                          X:::::::::::::::::::: ::::::::
NBISA- TTTCCTTGATGAACTTGCTACTGTTGTTACAGGTGGCTTCTTTCCTGTCGGACTCAAAGG
       560       570       580       590       600       610

             100       110       120       130       140       150
10B DF TTCCTGGGGAGGATGGTATCTCAAGTACGTCAGGTATGCTGGACCTCTTGAAGGATCAAG
       ::::::::::::::::: :::::::::::::::::::::::::::::::  ::::::::
NBISA- TTCCTGGGGAGGATGGTACCTCAAGTACGTCAGGTATGCTGGACCTCTTGCGGGATCAAG
       620       630       640       650       660       670

             160       170       180       190       200       210
10B DF TGGGTTCATTGTCAATCAACGATTCTATGACAGAGCCCAAAACAGAGCTGGATCCAGGGT
       ::: ::::::::::::::::::::::: ::::::::::::::::   ::::::::::::
NBISA- TGGATTCATTGTCAATCAACGATTCTACGACAGAGCCCAAAACAAGACTGGATCCAGGGT
       680       690       700       710       720       730

             220       230       240       250       260       270
10B DF TGTATCCATGGTTGAAATGGACAGAGACGGCGTATCATTCATCTACGAGAAGCCTAGCAT
       ::::::::::::::::::::::: :::::::: :::: :::::::::::::::::::: :
NBISA- TGTATCCATGGTTGAAATGGACGGAGACGGCTTATCGTTCATCTACGAGAAGCCTAGCGT
       740       750       760       770       780       790

             280       290       300       310       320       330
10B DF CTACCATAGTGATGGGTGCACTGGGACAGCAGCGAGGGTCTGGAGACGGGATCACAATGA
       ::::::::::::::::::::::: :::::::::: :::::::::::::: ::::::
NBISA- CTACCATAGTGATGGGTGCACTGGGTCAGCAGCGAGGTTCTGGAGACGGGATCGCAATGA
       800       810       820       830       840       850

             340       350       360       370
10B DF GAGAGCTGGAGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA
       ::::::::::::::::::::::::::::::::::::::::::::::X
NBISA- GAGAGCTGGAGTTGAGCTTAGGGCTGGACTTCACTTCAGAATGTGA
       860       870       880       890       900

Library scan:  0:00:00  total CPU time:  0:00:28
```

(20)

X

```
A:DFO10B.SEQ, 377 nt vs A:NBISAM2.SEQ library
using DNA matrix

   485 residues in     1 sequences
   1 scores better than 59 saved, ktup: 4
The best scores are:                                      initn initl   opt
>NBISA01 SEG. 7 ORF2 (SE5060)                             1396  1396  1396

>NBISA01 SEG. 7 ORF2 (SE5060)                             1396 1396 1396
  95.8% identity in 377 nt overlap

                10        20        30        40        50        60
10B DF ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAAAAGAC
       X:::::::::::::::::::::::::::::::::::::::::::::::::::::::::: ::::
NBISA0 ATGGATTTCACCAAAGTGTATGGTGTGCTGGTTGACCAACTAAAACTTCACGGAACAGAC
                10        20        30        40        50        60

                70        80        90       100       110       120
10B DF AAGGTGGCTTCTTTCCTGTCGGGCTCAAAGGTTCCTGGGGAGGATGGTATCTCAAGTACG
       :::::::::::::::::::::: :::::::::::::::::::::::::::: ::::::::::
NBISA0 AAGGTGGCTTCTTTCCTGTCGGACTCAAAGGTTCCTGGGGAGGATGGTACCTCAAGTACG
                70        80        90       100       110       120

               130       140       150       160       170       180
10B DF TCAGGTATGCTGGACCTCTTGAAGGATCAAGTGGGTTCATTGTCAATCAACGATTCTATG
       :::::::::::::::::::::  :::::::::: :::::::::::::::::::::::: :
NBISA0 TCAGGTATGCTGGACCTCTTGCGGGATCAAGTGGATTCATTGTCAATCAACGATTCTACG
               130       140       150       160       170       180

               190       200       210       220       230       240
10B DF ACAGAGCCCAAAACAGAGCTGGATCCAGGGTTGTATCCATGGTTGAAATGGACAGAGACG
       ::::::::::::::   :::::::::::::::::::::::::::::::::: ::::::
NBISA0 ACAGAGCCCAAAACAAGACTGGATCCAGGGTTGTATCCATGGTTGAAATGGACGGAGACG
               190       200       210       220       230       240

               250       260       270       280       290       300
10B DF GCGTATCATTCATCTACGAGAAGCCTAGCATCTACCATAGTGATGGGTGCACTGGGACAG
       :: :::: :::::::::::::::::::: :::::::::::::::::::::::::::: :::
NBISA0 GCTTATCGTTCATCTACGAGAAGCCTAGCGTCTACCATAGTGATGGGTGCACTGGGTCAG
               250       260       270       280       290       300

               310       320       330       340       350       360
10B DF CAGCGAGGGTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
       :::::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::
NBISA0 CAGCGAGGTTCTGGAGACGGGATCACAATGAGAGAGCTGGAGTTGAGCTTAGGGCTGGAC
               310       320       330       340       350       360

               370
10B DF TTCACTTCAGAATGTGA
       ::::::::::::::::X
NBISA0 TTCACTTCAGAATGTGATTGGTTGAAAACTTGTTATGTAAACAAGAATTTTGTGTTTTTG
               370       380       390       400       410       420

Library scan:  0:00:00  total CPU time:  0:00:22
```

(21)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: November 10, 2003 8:41 AM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: sequence analysis of segment 8 products

Follow Up Flag: Follow up
Flag Status: Red

Hello Simon,

Attached is the information I have obtained on the segment 8 clones. I suppose this result rules out the possibility of "reagent contamination" with lab. virus strains!

All the best.

Fred.

(22)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: November 10, 2003 8:41 AM
To: JonesS@pac.dfo-mpo.gc.ca
Cc: KibengeM@pac.dfo-mpo.gc.ca
Subject: sequence analysis of segment 8 products

Follow Up Flag: Follow up
Flag Status: Red

Attachments: DFO 100703 results.doc

DFO 100703 esults.doc (108 KB..

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* This message contains the file 'DFO 100703 results.doc', which has been
* uuencoded. If you are using Pegasus Mail, then you can use
* the browser's eXtract function to lift the original contents
* out to a file, otherwise you will have to extract the message
* and uudecode it manually.
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High Sea Chinnok Salmon samples labeled 1A to 10A and 1B to 10B, checked for ISAV using RT-PCR (samples received from DFO, PBS, Dr. Simon Jones/Dr. Molly Kibenge).

Sample ID	Segment 8 primers (F5/R5)	Segment 7 ORF1 primers
1A	+ ve (~220bp)	- ve
2A	+ ve (~220bp)	+ ve (~400bp)
3A	weak + ve (~220bp)	not done
4A	- ve	not done
5A	- ve	not done
6A	- ve	not done
7A	- ve	not done
8A	- ve	not done
9A	- ve	not done
10A	- ve	not done
1B	- ve	not done
2B	- ve	not done
3B	+ ve (~220bp)	- ve
4B	+ ve (~220bp)	+ ve (~400bp)
5B	- ve	- ve
6B	- ve	+ ve (~400bp)
7B	- ve	not done
8B	- ve	not done
9B	- ve	not done
10B	+ ve (~220bp)	+ ve (~400bp)
NBISA01 +ve control	+ ve (~220bp)	+ ve (903bp)

- ve denotes no PCR product seen; + ve denotes PCR product (size in base pairs) seen.

(23)

Sequence Analysis of the RT-PCR products of the Segment 8 F5/R5 primers:

Attempts were made to clone some of the RT-PCR products obtained using the TOPO TA cloning kit. The DNA inserts were then sequenced.

The NBISA01 +ve control DNA insert was 211 bp long.
Inserts of the DFO samples were variable in size and ranged from 116 bp long in clone 3B to 211 bp long in clone 2A. Of these clones, the most representative (based on sequence analysis) was clone 10B with a DNA insert of 187bp (since as shown in the alignment on page 13, clone 10B lines up very well with clone 2A; both 3B and 4B are missing internal sequences between the F5 and R5 primers because of an internal mis-priming or duplicate sequence; in clone 4B the F5 primer sequence is also duplicated, in tandem).

The DNA insert in clone 2A was the most similar to the NBISA01 +ve control DNA insert with 94.3% sequence identity. The other DFO samples sequenced (3B, 4B, and 10B) were only similar to the NBISA01 +ve control DNA insert in the primer regions, whereas they had considerable areas of sequence identity among themselves: 100% identity in 72 nucleotide overlap between 3B and 4B; 97.9% identity in 97 nucleotide overlap between 3B and 10B; and 95.9% identity in 97 nucleotide overlap between 4B and 10B. **Thus the "DFO B samples" would seem to contain a unique sequence, represented as clone 10B with a DNA insert of 187bp, amplified using the ISAV segment 8 F5/R5 primers. The smaller RT-PCR product of DFO 10B compared to ISAV NBISA01 is due to internal deletions in DFO 10B. I suppose this result rules out the possibility of "reagent contamination" with laboratory virus strains! For specific amplification of the virus in your samples, I suggest that you design new primers based on the DFO 10B clone sequence but excluding the F5 and R5 sequences. It would be interesting to see if these new primers amplify the original ISAV.**

The sequences are given on page 11, the sequence alignment of the 4 sample is shown on page 12, and the sequence comparisons using the FASTA program are given on pages 13-15.

This completes the analysis of the 20 High Sea Chinnok salmon samples. I look forward to more blind samples from you.

All the best.

Fred.

>2A DFO F5/R5 2A-#1 10/17 (SF3322)
GAAGAGTCAGGATGCCAAGACGCGGATGGTGGAGAGGAAAAGTGGGCAATGGTGT
ATGGTATGATTTCACCAGACATGGCGGAGGAGAAGACGATGTTGAAGGACCTGAAG
ACAATGCTACACAGCAGGATGCAGATGTATGCTCTAGGAGCGAGTTCGAAAGCCCT
GGAAACTTTAGAAAAGGCCATCGTCGCTGCAGATCATCGACTTC

>3B DFO F5/R5 3B-#2 10/9 (SF3323)
GAAGAGTCAGGATGCCAAGACGGAAGTCGCTGCAGATCATCGACTTCACTGAAGAG
TCAGGATGCCAAGACGGAAGTCGCTGCAGATCATCGACTTCGCTGCAGATCATCGA
CTTC

>4B DFO F5/R5 4B-#1 10/9 (SF3324)
GAAGAGTCAGGGATGCCAAGACG
GAAGAGTCAGGATGCCAAGACGAAGAGTCAGGATGCCAAGACGGAAGTCGCTGCA
GATCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGCTCTCTCTCTCTCCCTCT
CTCTCTCTCTCTCTCTCTCTCTCGCTGCAGATCATCGACTTC

>10B DFO F5/R5 10B-#2 10/9 (SF3325)
GAATAGTCAGGATGCCAAGACGGAAGAGTCAGGATGCCAAGACGACTCGCTGCAGA
TCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGAGTCAGGATCAAGACG
GAAGTCGCTGCAGATCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGTC
GCTGCAGATCATCGACTTC

```
Page 1.1
          1             15 16            30 31            45 46            60 61            75 76            90
    1 3B  --------------- --------------- --------------- -GAAGAGTCAGGATG CCAAGACGGAAGTCG CTGCAGATCATCGAC     44
    2 10B --------------- --------GAATAGT CAGGATGCCAAGACG -GAAGAGTCAGGATG CCAAGACG--ACTCG CTGCAGATCATCGAC     64
    3 4B  GAAGAGTCAGGGATG CCAAGACGGAAGAGT CAGGATGCCAAGAC- -GAAGAGTCAGGATG CCAAGACGGAAGTCG CTGCAGATCATCGAC     88
    4 2A  --------------- --------GAAGAGT CAGGATGCCAAGACG CGGATGGTGGAGAGG AAAAGTGGGCAATGG -TGTATGGTAT-GAT     65

Page 2.1
          91          105 106         120 121         135 136         150 151         165 166         180
    1 3B  TTCACTGAAGAGTCA GGATGCCAAGACGGA AG-----TCGCTGCA G---ATCATCGACT- -----------TCGC TGCAG-ATCATCGAC    113
    2 10B TTCACTGAAGAGTCA GGATGCCAAGACGGA AG-----AGTCAGGA TC--AAGACGGAAG- -----------TCGC TGCAG-ATCATCGAC    134
    3 4B  TTCACTGAAGAGTCA GGATGCCAAGACGCT CTCTCTCTCTCTCCC TCT-CTCTCTCTCTC TCTCTCTCTC-TCGC TGCAG-ATCATCGAC    175
    4 2A  TTCACCAGACATGGC GGAGGAGAAGACGAT GTTGAAGGACCTGAA GACAATGCTACACAG CAGGATGCAGATGTA TGCTCTAGGAGCGAG    155

Page 3.1
          181         195 196         210 211         225 226         240 241         255 256         270
    1 3B  TTC------------ --------------- --------------- -----------    116
    2 10B TTCACTGAAGAGTCA GGAT---GCCAAGAC GGAAGTCGCTGCAGA TCATCGACTTC    187
    3 4B  TTC------------ --------------- --------------- -----------    178
    4 2A  TTCGAAAGCCCTGGA AACTTTAGAAAAGGC CATCGTCGCTGCAGA TCATCGACTTC    211
```

(26)

```
A:DFO2A.SEQ, 211 nt vs A:DFONBISA.SEQ library
using DNA matrix

   211 residues in     1 sequences
   1 scores better than 57 saved, ktup: 4
The best scores are:                                      initn initl   opt
>NBISADFO Positive control F5/R5 NBISA #1 10/17 & 10/9  760   760   760

>NBISADFO Positive control F5/R5 NBISA #1 10/17 & 10/9  760  760  760
  94.3% identity in 211 nt overlap

               10        20        30        40        50        60
2A DFO GAAGAGTCAGGATGCCAAGACGCGGATGGTGGAGAGGAAAAGTGGGCAATGGTGTATGGT
       X:::::::::::::::::::::::::::::::::::::::: ::::::::::::::::::
NBISAD GAAGAGTCAGGATGCCAAGACGCGGATGGTGGAGAGGAAAAATGGGCAATGGTGTATGGT
               10        20        30        40        50        60

               70        80        90       100       110       120
2A DFO ATGATTTCACCAGACATGGCGGAGGAGAAGACGATGTTGAAGGACCTGAAGACAATGCTA
       :::::::::: :::::::: :::::::::::::: ::::::: ::::: :::::::::
NBISAD ATGATTTCACCCGACATGGCAGAGGAGAAGACGATGCTGAAGGAGCTGAAAACAATGCTA
               70        80        90       100       110       120

              130       140       150       160       170       180
2A DFO CACAGCAGGATGCAGATGTATGCTCTAGGAGCGAGTTCGAAAGCCCTGGAAACTTTAGAA
       :::::::::::::::::::::::::: :: :: :::::::::::::: :: : :::::::
NBISAD CACAGCAGGATGCAGATGTATGCTCTGGGTGCAAGTTCGAAAGCCCTAGAGAATTTAGAA
              130       140       150       160       170       180

              190       200       210
2A DFO AAGGCCATCGTCGCTGCAGATCATCGACTTC
       ::::::::::::::::::::::::::::::X
NBISAD AAGGCCATCGTCGCTGCAGATCATCGACTTC
              190       200       210

Library scan:  0:00:00  total CPU time:  0:00:15
```

```
A:DFO10B.SEQ, 187 nt vs A:DFO3B.SEQ library
 using DNA matrix

    116 residues in     1 sequences
    1 scores better than 57 saved, ktup: 4
The best scores are:                                      initn init1   opt
>3B DFO F5/R5 3B-#2 10/9 (SF3323)                          328   328   364

>3B DFO F5/R5 3B-#2 10/9 (SF3323)                          328   328   364
  97.9% identity in 97 nt overlap

            70        80        90       100       110       120
10B DF ACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGAGTCAGGAT--CAAGACGGAAGTCGC
                                        :::::::::::: X::::::::::::
3B                                      GAAGAGTCAGGATGCCAAGACGGAAGTCGC
                                              10        20        30

              130       140       150       160       170       180
10B DF TGCAGATCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGTCGCTGCAGATCAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
3B     TGCAGATCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGTCGCTGCAGATCAT
              40        50        60        70        80        90


10B DF CGACTTC
       ::::::X
3B     CGACTTCGCTGCAGATCATCGACTTC
              100       110

Library scan:  0:00:00  total CPU time:  0:00:14
```

(28) X

```
 A:DFO4B.SEQ, 178 nt vs A:DFO10B.SEQ library
using DNA matrix

   187 residues in     1 sequences
   1 scores better than 57 saved, ktup: 4
The best scores are:                                      initn init1  opt
>10B DFO F5/R5 10B-#2 10/9 (SF3325)                         352   228   341

>10B DFO F5/R5 10B-#2 10/9 (SF3325)                          352  228  341
  95.9% identity in 97 nt overlap

                         10        20        30        40
4B DFO            GAAGAGTCAGGGATGCCAAGACGGAAGAGTCAGGATGCCAAGAC-GAAGA
                                    :: :::::::::::::::::::: :::::
10B    CAAGACGACTCGCTGCAGATCATCGACTTCACTGAAGAGTCAGGATGCCAAGACGGAAGA
        40        50        60        70        80        90

      50        60        70        80        90        100
4B DFO GTCAGGATGCCAAGACGGAAGTCGCTGCAGATCATCGACTTCACTGAAGAGTCAGGATGC
       ::::::::  X:::::::::::::::::::::::::::::::::::::::::::::::::
10B    GTCAGGAT--CAAGACGGAAGTCGCTGCAGATCATCGACTTCACTGAAGAGTCAGGATGC
       100          110       120       130       140       150

    110       120       130       140       150       160
4B DFO CAAGACGCTCTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCGCTGCAGATC
       ::::::X
10B    CAAGACGGAAGTCGCTGCAGATCATCGACTTC
         160       170       180

Library scan:  0:00:00  total CPU time:  0:00:15
```

(29)

Jones, Simon

From: Dr. Fred Kibenge [kibenge@upei.ca]
Sent: December 8, 2003 6:59 AM
To: KibengeM@pac.dfo-mpo.gc.ca
Cc: JonesS@pac.dfo-mpo.gc.ca
Subject: ACGT information

Follow Up Flag: Follow up
Flag Status: Red

Hi Molly,

Here is the ACGT information you wanted:

They sequence PCR products.

Sequencing can be done with PCR primers used to generate the PCR product, but you would miss the primer sequence (i.e., the
M13 Forward primer sequence or the M13 Reverse primer sequence would not show). If you want complete sequence then you have to ask to sequence with both primers seperately.

In 10% of the sequencing reactions (that do not work) they may want to try internal primers, i.e., your F5 or R5 primers; but they would let you know if this is necessary.

To sequence a 500bp product, they need 50 ng per reaction. It is recommended that you pass your PCR product through the QIAGEN PCR purification kit, and then send them the purified material as is. They prefer that you also send them a photo of the gel (together with the sample for sequencing) indicating the volume of the PCR product that was loaded.

They charge $30 per sequencing reaction. For a 500bp fragment, you need only one reaction.

Here is the contact information:
Ask for Chi or Philip.

ACGT Corporation
100 Bay Street, 11th Floor
Toronto, Ontario
M5G 1Z6

Tel: 1-800-735-0847 or
1-416-977-2228.

Fax: 1-416-977-7122.

You already have their e-mail address: order@acgtcorp.com.

Let me know if you need more information.

Fred.