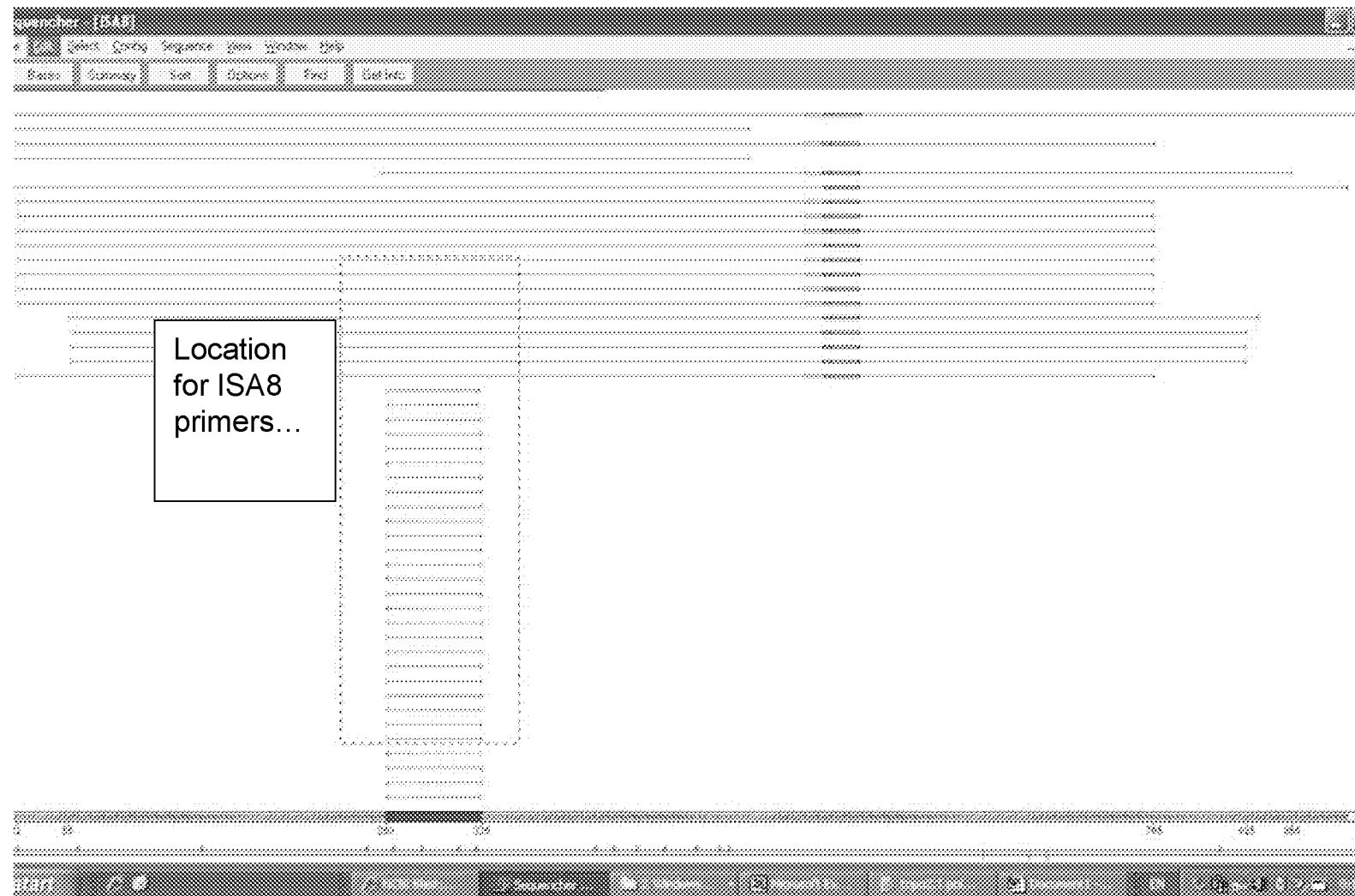


Presentation to Fish Health Group on status of molecular screening for Orthomyxoviruses performed by the Molecular Genetics Laboratory

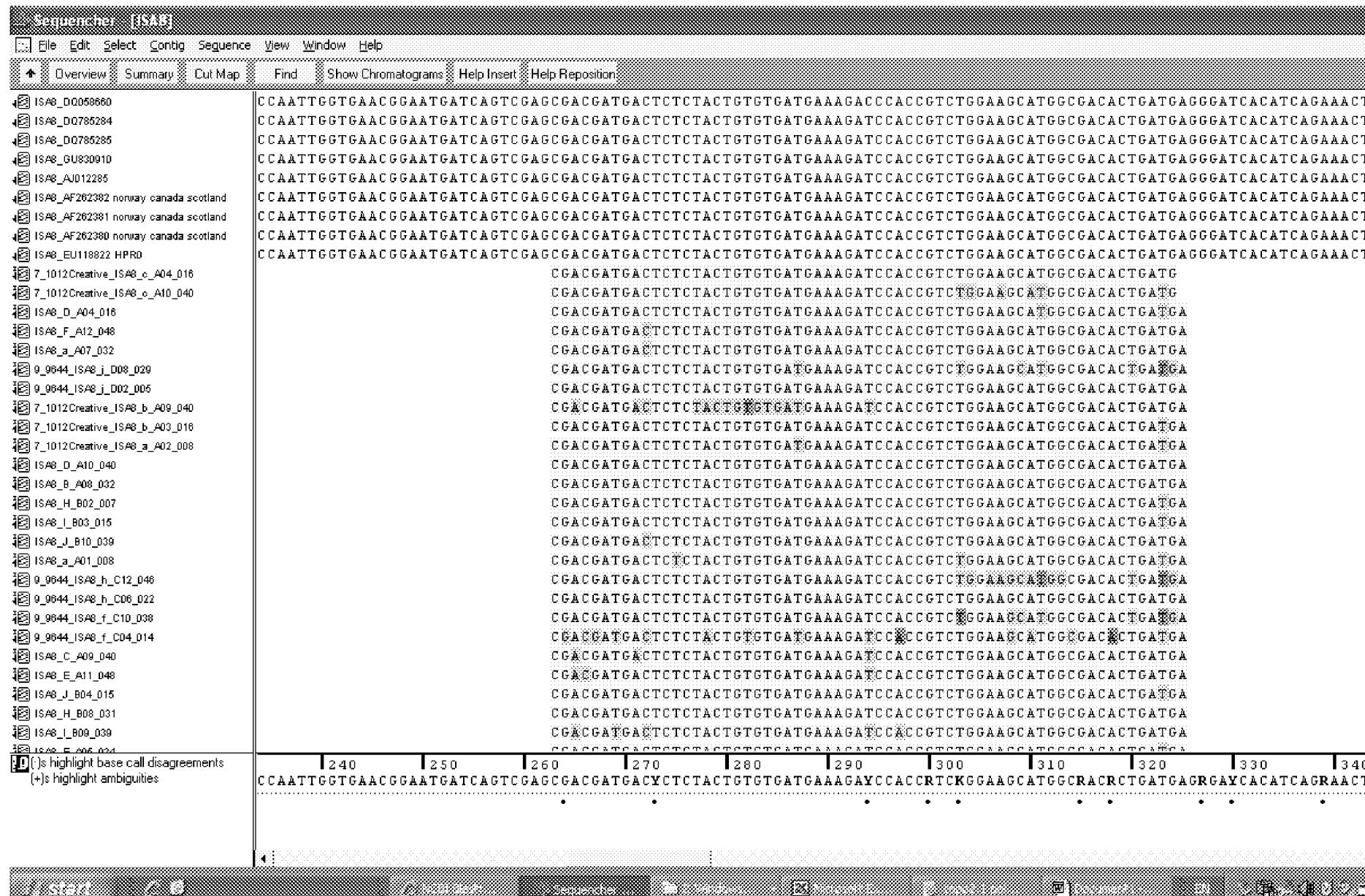
Nov 24, 2011

Present: Kristi Miller, Karia Kaukinen, Mark Saunders, Mark Higgins, Stewart Johnstone, Kyle Garver

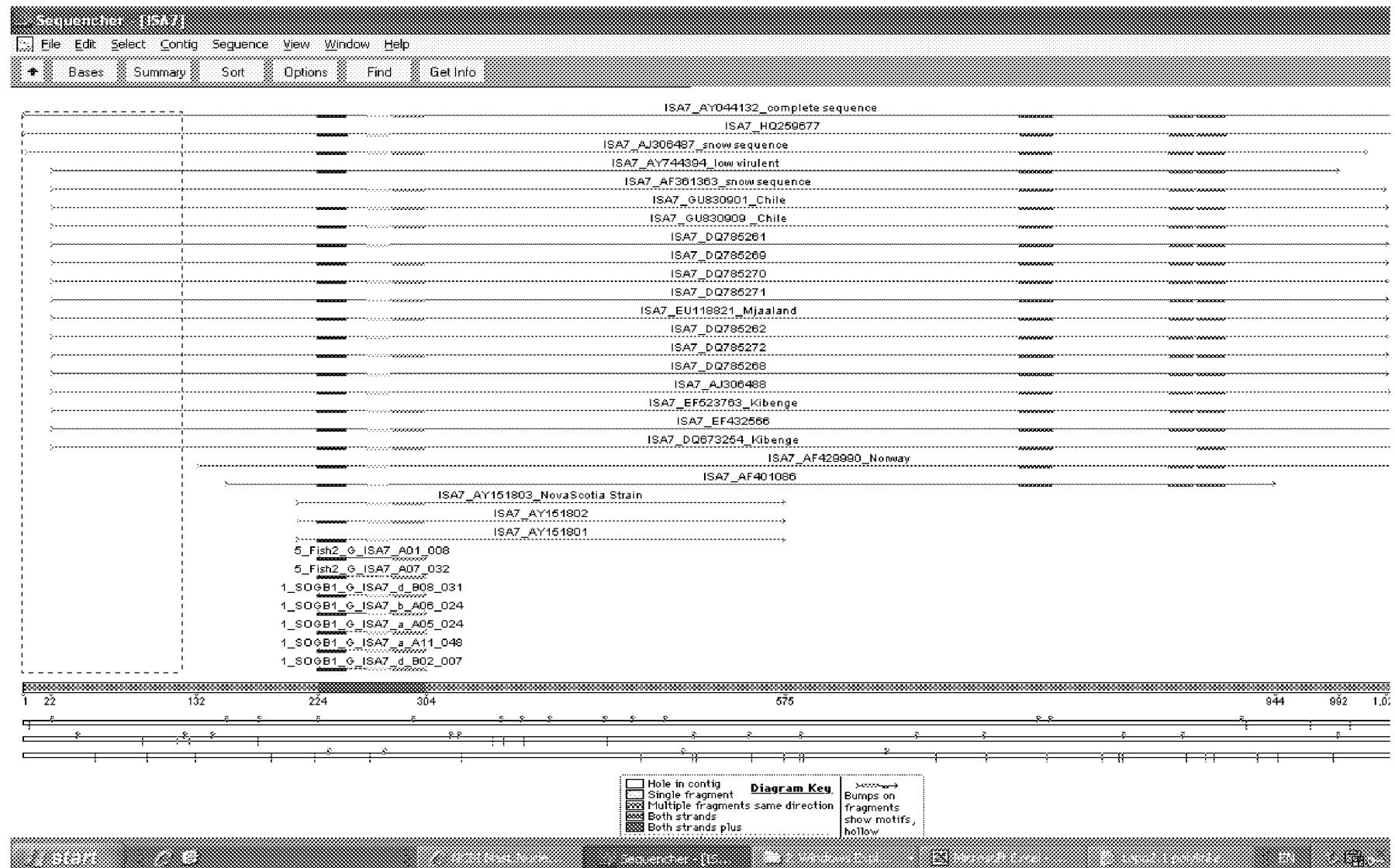
ISA8 quantitative RT-PCR (taqman) screened in wild sockeye salmon populations using published Plarre primers. Products have now been sequenced and align with ISA sequence variants



Products from 2 sockeye smolts and one chinook have been sequenced from ISA8 (Plarre) PCR (no probe used to generate sequence). 100% match to some European ISA isolates (very small fragment though)



ISA7 quantitative RT-PCR (taqman) screened in wild sockeye salmon populations using published Plarre primers. Products have now been sequenced and align with ISA sequence variants

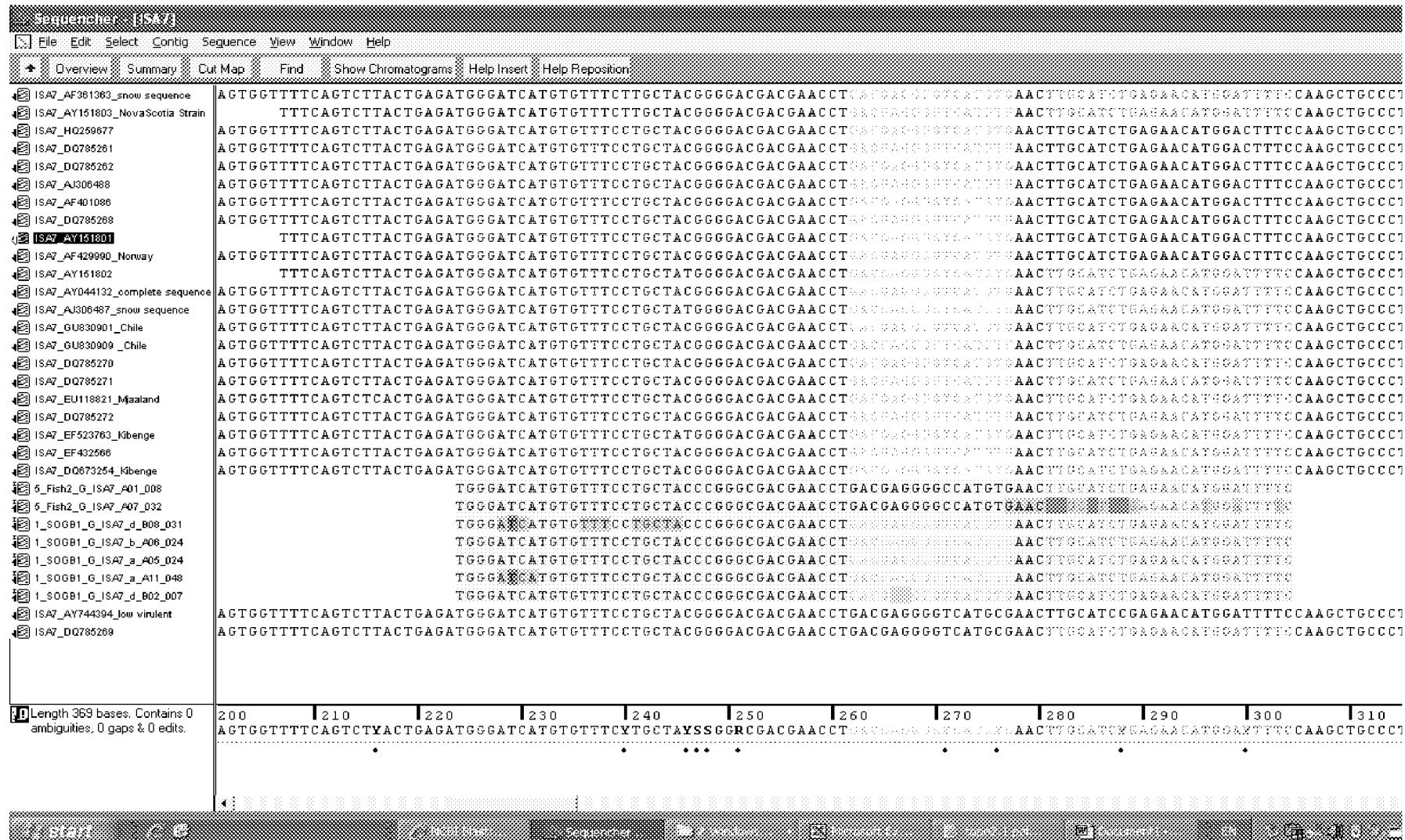


Top hit to ISA7 95% similar

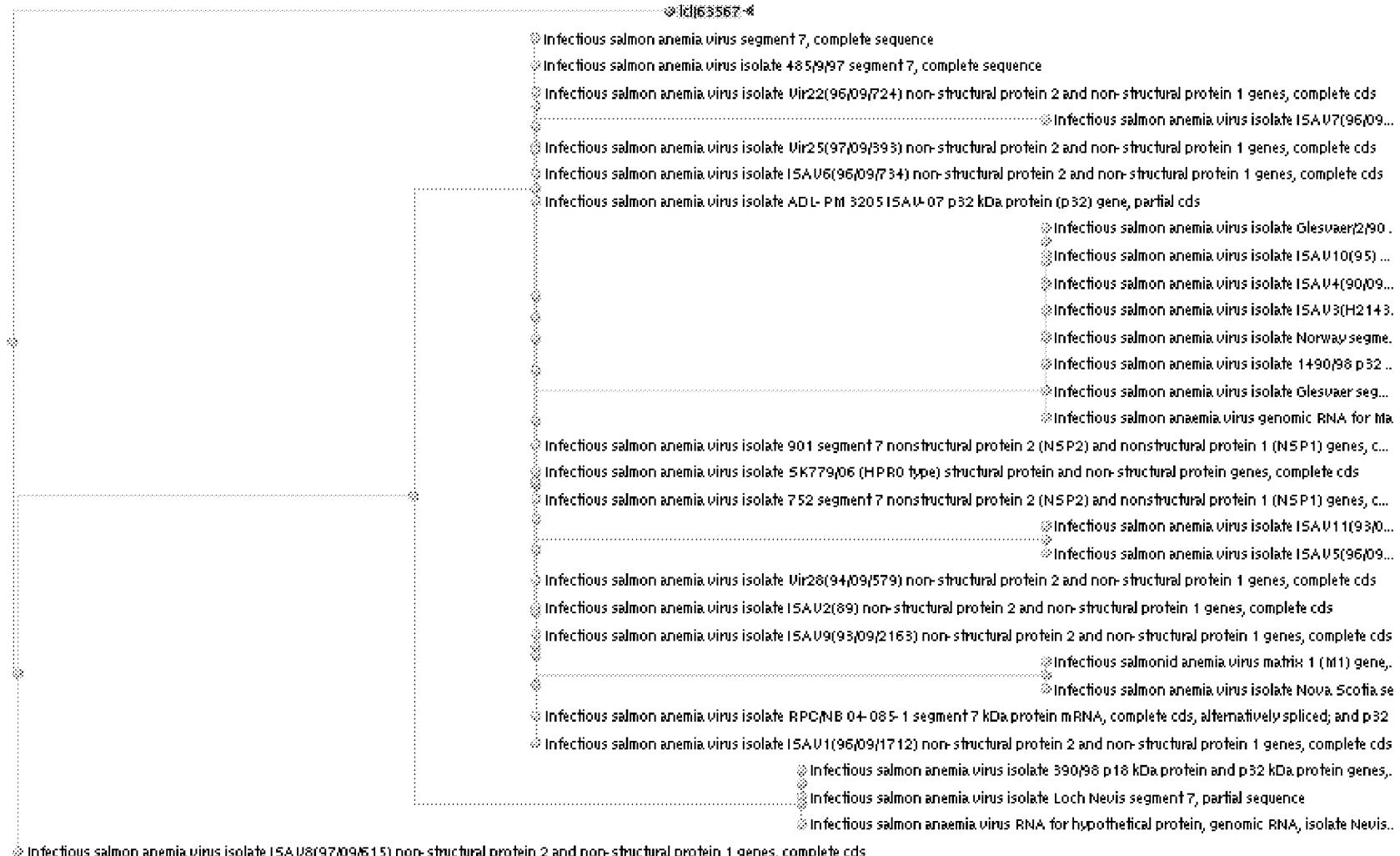
Accession	Description	Max score	Total score	Query cover	E value	Max identity
		e	age	age	ue	ink
<u>HQ011</u> <u>268.1</u> Infectious salmon anemia virus isolate ADL-PM 3205 ISAV-07 p32 kDa protein (p32) gene, partial cds		<u>128</u>	128	100%	4e-27	95%
<u>GU830</u> <u>909.1</u> Infectious salmon anemia virus isolate 901 segment 7 nonstructural protein 2 (NSP2) and nonstructural protein 1 (NSP1) genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>GU830</u> <u>901.1</u> Infectious salmon anemia virus isolate 752 segment 7 nonstructural protein 2 (NSP2) and nonstructural protein 1 (NSP1) genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>EU118</u> <u>821.1</u> Infectious salmon anemia virus isolate SK779/06 (HPRO type) structural protein and non-structural protein genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>272.1</u> Infectious salmon anemia virus isolate Vir22(96/09/724) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>271.1</u> Infectious salmon anemia virus isolate Vir28(94/09/579) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>270.1</u> Infectious salmon anemia virus isolate Vir25(97/09/393) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>267.1</u> Infectious salmon anemia virus isolate ISAV9(93/09/2163) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>264.1</u> Infectious salmon anemia virus isolate ISAV6(96/09/734) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>260.1</u> Infectious salmon anemia virus isolate ISAV2(89) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ785</u> <u>259.1</u> Infectious salmon anemia virus isolate ISAV1(96/09/1712) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>EF432</u> <u>566.1</u> Infectious salmon anemia virus isolate RPC/NB 04-085-1 segment 7 kDa protein mRNA, complete cds, alternatively spliced; and p32 kDa protein mRNA, complete cds		<u>128</u>	128	100%	4e-27	95%
<u>DQ673</u> <u>254.1</u> Infectious salmon anemia virus isolate 485/9/97 segment 7, complete sequence		<u>128</u>	128	100%	4e-27	95%
<u>AY044</u> <u>132.1</u> Infectious salmon anemia virus segment 7, complete sequence		<u>128</u>	128	100%	4e-27	95%
<u>HQ259</u> <u>677.1</u> Infectious salmon anemia virus isolate Glesvaer/2/90 segment 7, complete sequence		<u>122</u>	122	100%	2e-25	93%
<u>EF523</u> <u>763.1</u> Infectious salmon anemia virus isolate 390/98 p18 kDa protein and p32 kDa protein genes, complete cds		<u>122</u>	122	100%	2e-25	93%
<u>DQ785</u> <u>269.1</u> Infectious salmon anemia virus isolate ISAV11(93/09/2264) non-structural protein 2 and non-structural protein 1 genes, complete cds		<u>122</u>	122	100%	2e-25	93%

ISA7

2 Fraser River sockeye salmon sequenced and show 95% identity to European ISA. 3 fixed differences suggest that this Orthomyxovirus has been here for some time. More sequence data are required to determine how long



Tree showing sockeye ISAV7 sequence (yellow) relative to other ISAV isolates

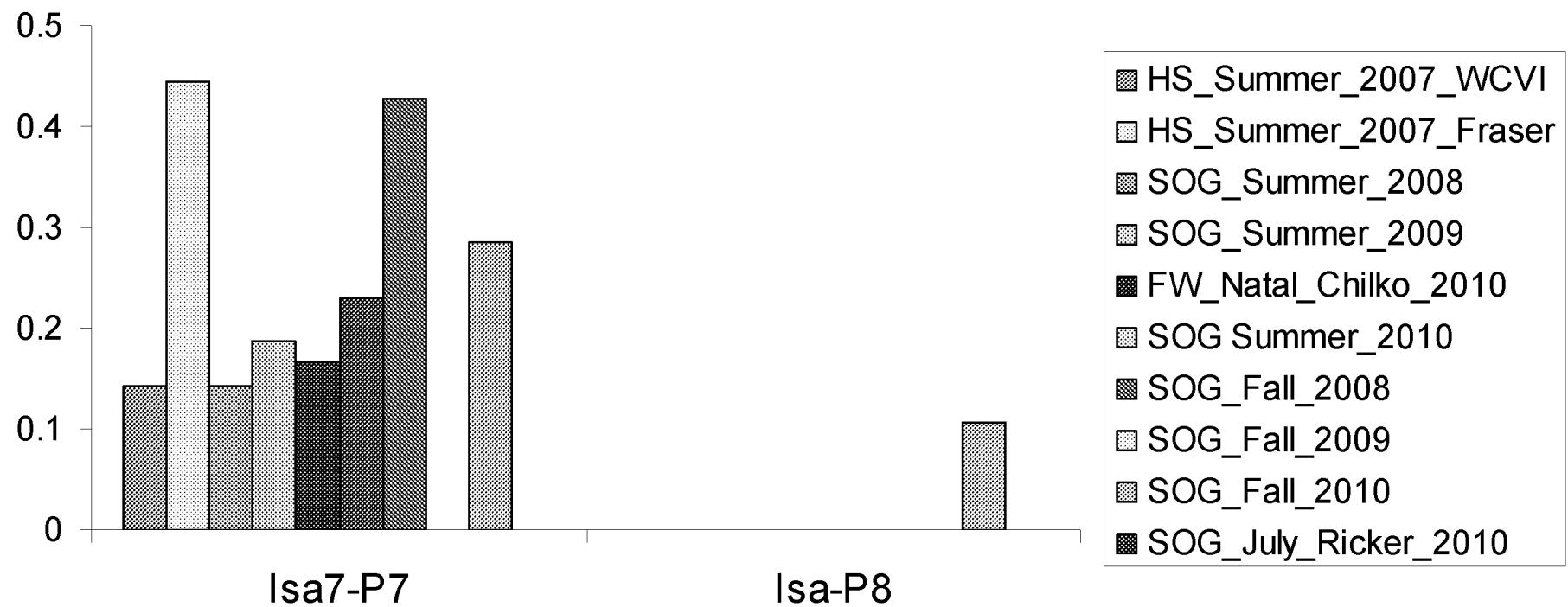


Alignment of ISA7 showing three fixed differences and highlighting that the two North American strains also show a fixed difference that differentiates them both from the sockeye isolate and European strains

63567	1	TGGGATCATGTGTTCTGCTACCCGG-G-CGACGAACCTGACGAGGGGCCATGTGAACTTGCATCTGAGAACATGGATTTC	81	
HQ011268	224-G.-A.....T.....	304	E-Ch
GU830909	203-G.-A.....T.....	283	E-Ch
GU830901	203-G.-A.....T.....	283	E-Ch
EU118821	203-G.-A.....T.....	283	E-Nhpr0
DQ785272	203-G.-A.....T.....	283	E-N
DQ785271	203-G.-A.....T.....	283	E-N
DQ785270	203-G.-A.....T.....	283	E-N
DQ785267	203-G.-A.....T.....	283	E-N
DQ785264	203-G.-A.....T.....	283	E-N
DQ785260	203-G.-A.....T.....	283	E-N
DQ785259	203-G.-A.....T.....	283	E-N
EF432566	203-G.-A.....T.....	283	E-Ca
DQ673254	203-G.-A.....T.....	283	E-N
AY044132	224-G.-A.....T.....	304	Bremnes
HQ259677	224-G.-A.....T.....C.....	304	N-Glesv
EF523763	203-TG.-A.....T.....	283	E-UK
DQ785269	203-G.-A.....T.....C.....	283	E-N
DQ785268	203-G.-A.....T.....C.....	283	E-N
DQ785266	203-A.A.....T.....	283	E-N
DQ785265	203-G.-A.....C.....T.....	283	E-N
DQ785263	203-G.-A.....T.....C.....	283	E-N
DQ785262	203-G.-A.....T.....C.....	283	E-N
DQ785261	203-G.-A.....T.....C.....	283	E-N
AF429990	93-G.-A.....T.....C.....	173	E-N
AF401083	203-G.-A.....T.....C.....	283	N-H
AF401086	71-G.-A.....T.....C.....	151	E-UK
AF401083	18-G.-A.....T.....C.....	98	N-H
AY151802	18-TG.-A.....T.....	98	E-UK
AY151801	18-G.-A.....T.....C.....	98	E-N
AJ306488	203-G.-A.....T.....C.....	283	N-Glesv
AJ306487	222-TG.-A.....T.....	302	E-UK

Screened 171 Livers over Four Years
Overall Prevalence 21% Isa7, 2% Isa8

ISA Prevalence in Livers of Sockeye smolts



Screened 414 Gills over Four Years
Overall Prevalence 10% Isa7, 3% Isa8

