

## Summary of PCR Primers for Salmonid Genetic Studies

Wally Buchholz  
Steve J. Miller  
and  
William J. Spearman



Fish Genetics Laboratory  
U.S. Fish and Wildlife Service  
1011 East Tudor Road  
Anchorage, Alaska 99503

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# Summary of PCR Primers for Salmonid Genetic Studies

**Wally Buchholz, Steve J. Miller, and William J. Spearman**

*Fish Genetics Laboratory, U.S. Fish and Wildlife Service,  
1011 East Tudor Road, Anchorage, Alaska 99503*

Population genetic studies conducted by the U.S. Fish and Wildlife Service's Fish Genetics Laboratory, have led to the testing and development of genetic markers for salmonid fishes. This report summarizes preliminary results from our ongoing work on microsatellites.

We are applying primers developed in other laboratories as well as those developed in our laboratory. Primer sets are being selected that meet the following criteria: polymorphic, predictable inheritance, interpretable results, and reliable PCR performance. Efforts were made to minimize the number of PCR protocols for the primer sets to support high throughput of samples and enhance quality control. A single PCR thermocycle regimen is being used where, for most reactions, only the annealing temperature is varied for each primer set and species. Allele identities are being assigned by comparison to allele ladders, two-base ladders, and commercial size standards. Work is in-progress to determine true allele sizes. Inheritance testing is completed for some loci and either in-progress or being planned for other loci. Primer sequences and laboratory protocols are presented for each of the following species and loci:

## chum salmon (*Oncorhynchus keta*)

**Oke3** (Buchholz, Fish Genetics Laboratory)

**Oke4** (Buchholz, Fish Genetics Laboratory)

**Oke8** (Buchholz, Fish Genetics Laboratory)

**Oki1** (Smith et al. 1998)

**Ots2.1** (Banks et al. 1999)

**Ots3.1** (Banks et al. 1999)

## chinook salmon (*O. tshawytscha*)

**Oke1** (Buchholz, Fish Genetics Laboratory)

**Oke2** (Buchholz, Fish Genetics Laboratory)

**Oke3** (Buchholz, Fish Genetics Laboratory)

**Oke4** (Buchholz, Fish Genetics Laboratory)

**Oki10** (Smith et al. 1998)

**Oki11** (Smith et al. 1998)

**One13** (Scribner et al. 1996)

**Ots3.1** (Banks et al. 1999)

## coho salmon (*O. kisutch*)

**Oke1** (Buchholz, Fish Genetics Laboratory)

**Oke2** (Buchholz, Fish Genetics Laboratory)

**Oke3** (Buchholz, Fish Genetics Laboratory)

**Oke4** (Buchholz, Fish Genetics Laboratory)

**Oki1** (Smith et al. 1998)

**Oki3** (Smith et al. 1998)

**Oki11** (Smith et al. 1998)

**Oki13.1** (Smith et al. 1998)

**Oki24.1** (Smith et al. 1998)

**One3** (Scribner et al. 1996)

**Ots3.1** (Banks et al. 1999)

**Ots105** (Nelson 1998)

For more information contact the authors.

**chum salmon**  
*Oncorhynchus keta*



**Oke3** 5' --ACC CTG AGA GCA ATC AAC-- 3'

Buchholz, Fish Genetics Lab

microsat 5' --TCA GGG ATA TGC AGT AAA TAG TA-- 3'

Buchholz, Fish Genetics Lab

Repeat [in species of origin]: (TCCCTCTCGTCTC)8

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

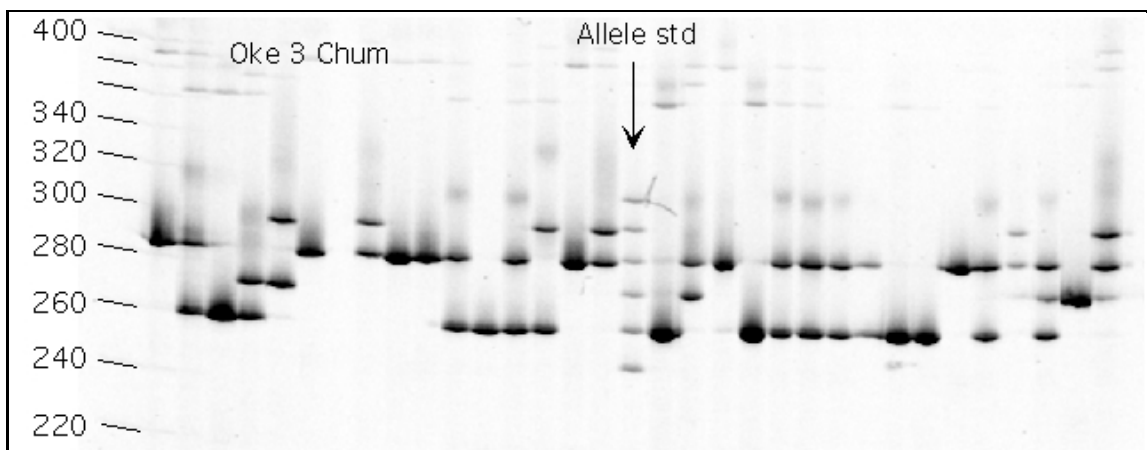
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



The faint bands seen high in each lane are artifacts caused by imperfect duplication of the true alleles.

For more information contact:

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**chum salmon**  
*Oncorhynchus keta*



**Oke4** 5' --AGG CCC AAA GTC TGT AGT GAA GG-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --GAT GAA TCG AGA GAA TAG GGA CTG AAT-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (CA)4A(CA)9

Mix Name: Microsat 1 Total vol. (ul): **25.00**

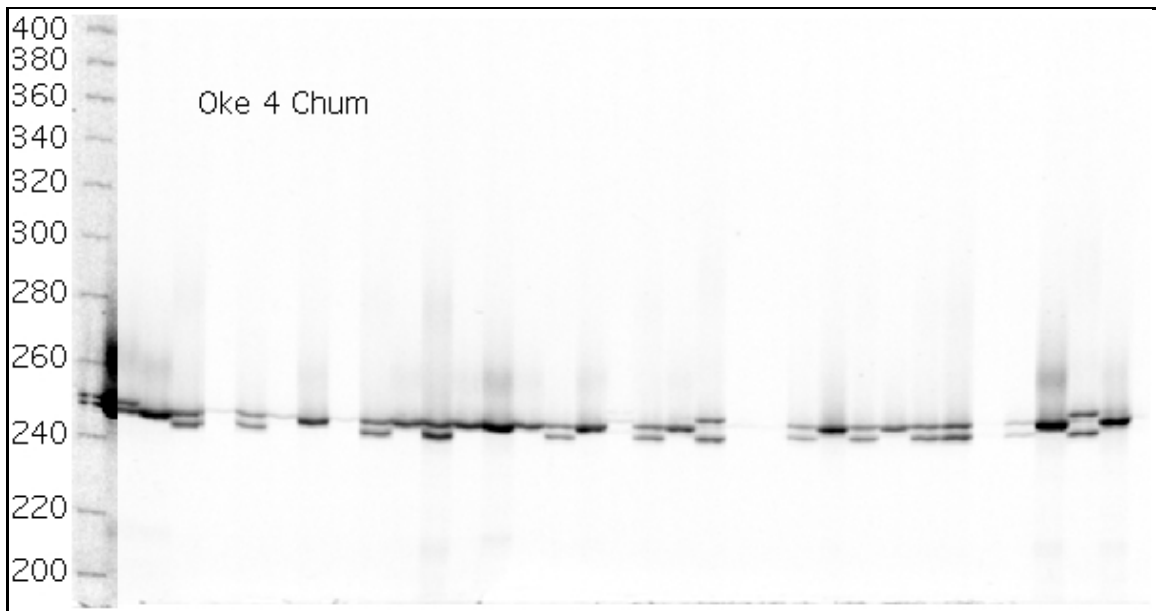
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro58a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>58</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



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**chum salmon**  
*Oncorhynchus keta*



**Oke8** 5' --CAT CTG GGT TCC TCT GTC TCC AA--3' Buchholz, Fish Genetics Lab  
 microsat 5' --CGC CTA CGA CTT CAT CAT GAC ACT AC--3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (CA)<sub>2</sub>GA(CA)<sub>7</sub>(GACA)<sub>2</sub>GA(GACA)<sub>2</sub>

Mix Name: Microsat 1 Total vol. (ul): **25.00**

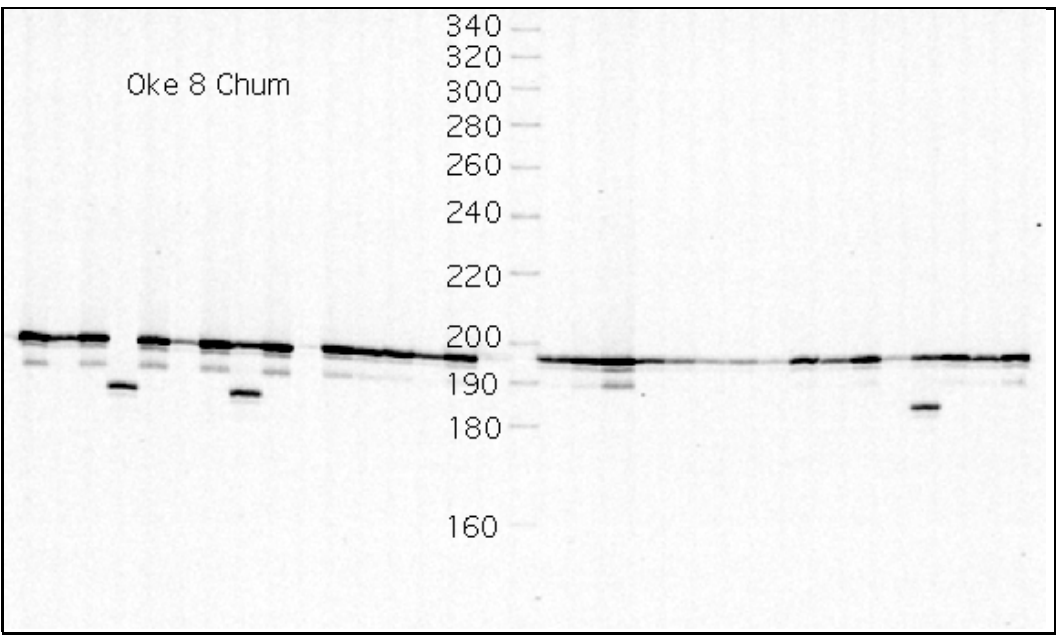
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



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**chum salmon**  
*Oncorhynchus keta*



**Oki1** 5' --AGG ATG GCA GAG CAC CAC T--3'  
microsat 5' --CAC CCA TAA TCA CAT ATT CAG A--3'  
Repeat [in species of origin]: (CTGT)<sub>16</sub>

Smith et al. 1998

Smith et al. 1998

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro56a

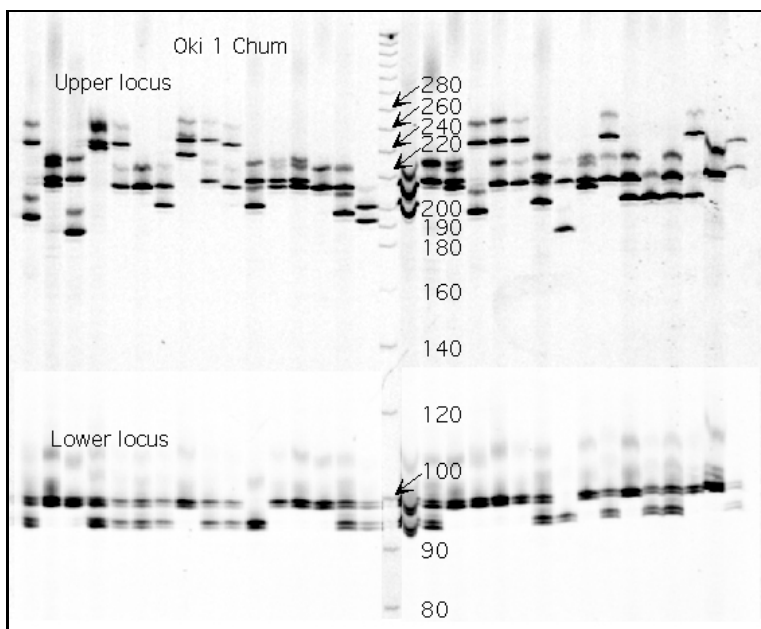
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>56</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



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**chum salmon**  
*Oncorhynchus keta*



**Ots2.1** 5' --ATG TAA ATG TCA AGT GGG AAC A--3' Banks et al. 1999  
 microsat 5' --ATC CTG GTT TAA TAT CCT TCA CAC--3' Banks et al. 1999  
 Repeat [in species of origin]: (CA)<sub>16</sub>  
 Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.

Mix Name: Microsat 1 Total vol. (ul): **25.00**

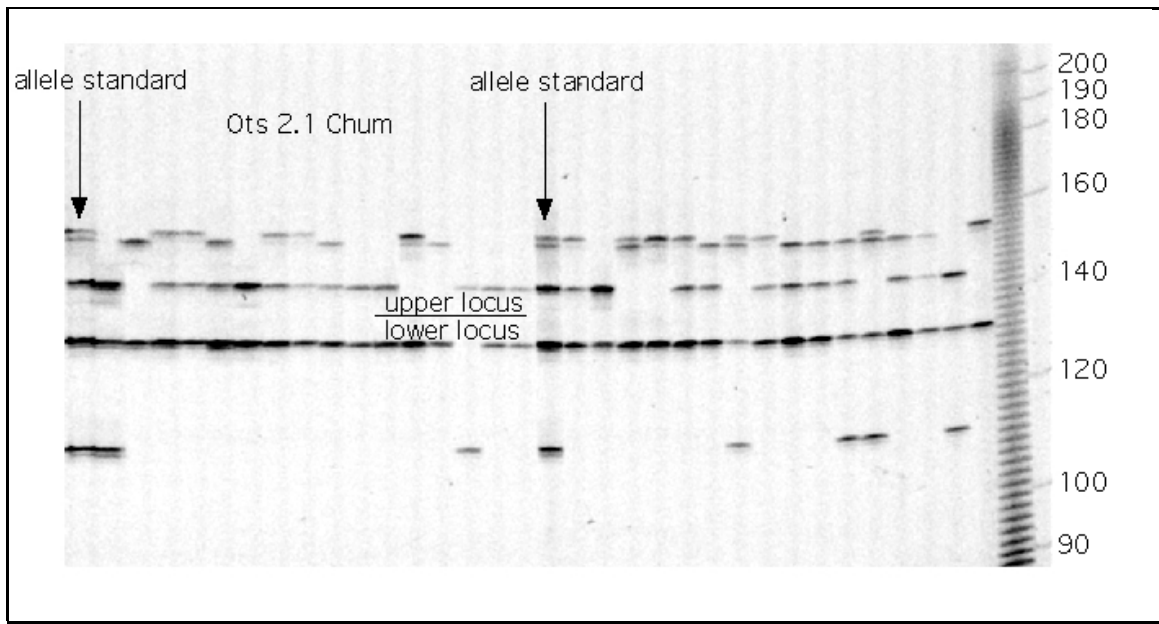
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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# chum salmon

*Oncorhynchus keta*



**Ots3.1** 5' --CAG CCC ATC TGT CAC TCA CAC T-- 3' Banks et al. 1999  
 microsat 5' --GGT GGA GAG AGT TTG AGA ATC ACA-- 3' Banks et al. 1999

Repeat [in species of origin]: (CT)<sup>17</sup>  
 Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.

Mix Name: Microsat 1 Total vol. (ul): **25.00**

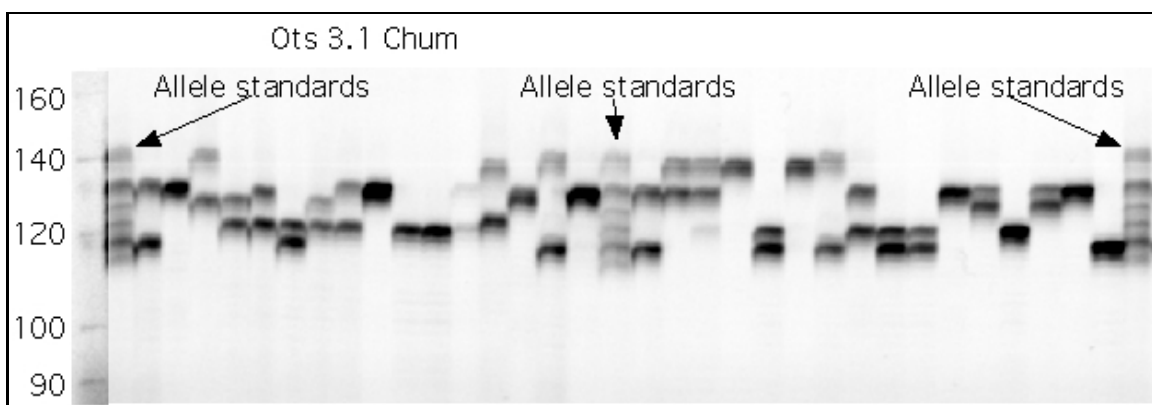
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro56a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>56</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



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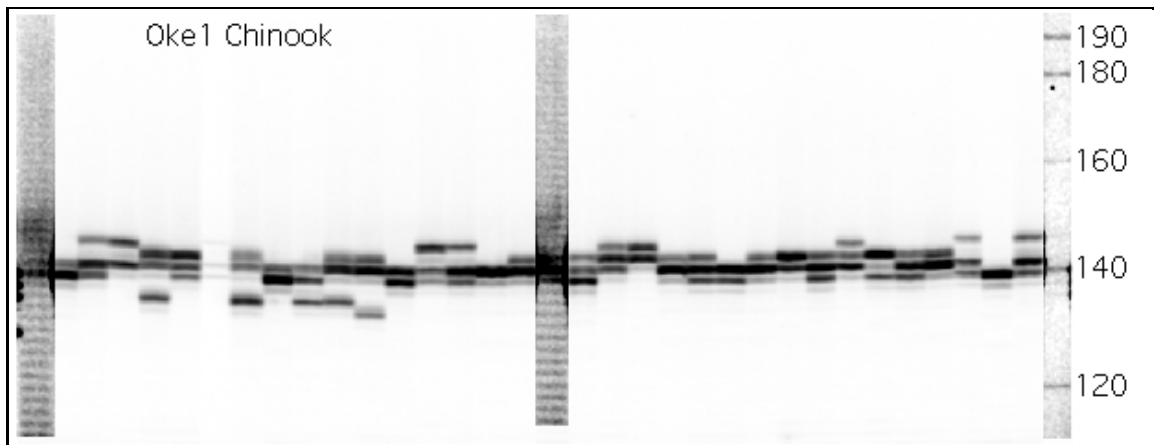
# chinook salmon

*Oncorhynchus tshawytscha*



<b>Oke1</b>	5' --TCA GTA ATC TCT CTT CAG GTG ACC CT-- 3'	Buchholz, Fish Genetics Lab
microsat	5' --CAT TAT CAG TAT TCA TGT CGG TTT CC-- 3'	Buchholz, Fish Genetics Lab
Repeat [in species of origin]: (TG) <sup>31</sup>		
Isolocus model.		

Mix Name: Microsat 1 Total vol. (ul): <b>25.00</b>				Cyclename: micro50a		
	<b>Stock Conc</b>	<b>Reaction Conc</b>	<b>Reaction Volume</b>		<u>Temp (C)</u>	<u>Time (min, sec)</u>
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul	Denature	95	1, 0
Buffer	10X	1X	2.500 ul PerkinElmer	<b>Anneal</b>	<b>50</b>	1, 0
dATP	8.0mM	0.20 mM	0.625 ul	Extend	70	1, 0
dCTP	8.0 mM	0.20 mM	0.625 ul	<hr/>		
dGTP	8.0 mM	0.20 mM	0.625 ul	Autoextend: 0	Cycles: 30	
dTTP	8.0 mM	0.20 mM	0.625 ul	Notes: Initial cycle of 3 min, 95C		
Primer 1	10.0 uM	0.20 uM	0.500 ul	Final cycle of 5 min, 70C		
Primer 2	10.0 uM	0.20 uM	0.500 ul			
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677	<b>Gel Conditions</b>		
H <sub>2</sub> O			15.375 ul	6.00 % denaturing vert polyacrylamide		
DNA	50 ng/ul	100 ng	2.000 ul			
Betaine	M	M	ul			



For more information contact:

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# chinook salmon

*Oncorhynchus tshawytscha*



**Oke2** 5' --AGG GCC AGA GAA AAG TCT CAC TAT-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --GTC AGT CCT GCC CTC TGT GTC CTA-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (GT)44

Mix Name: Microsat 1 Total vol. (ul): **25.00**

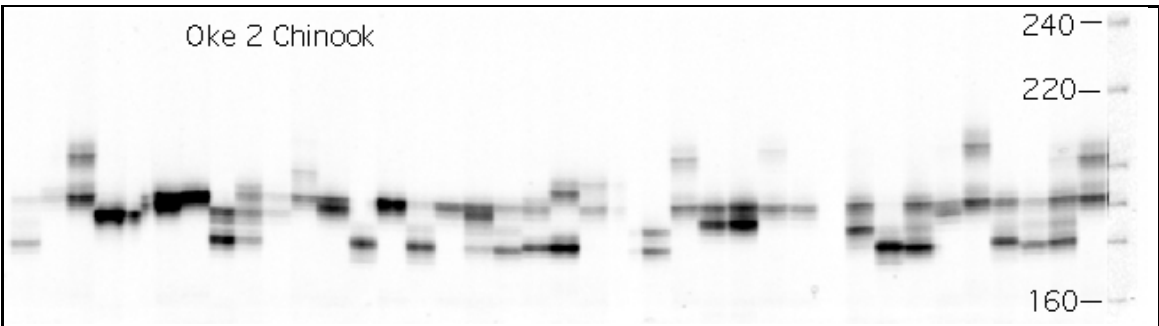
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro68a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>68</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



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**chinook salmon**  
*Oncorhynchus tshawytscha*

**Oke3** 5' --ACC CTG AGA GCA ATC AAC-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --TCA GGG ATA TGC AGT AAA TAG TA-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (TCCCTCTCGTCTC)8

Mix Name: Microsat 1 Total vol. (ul): **25.00**

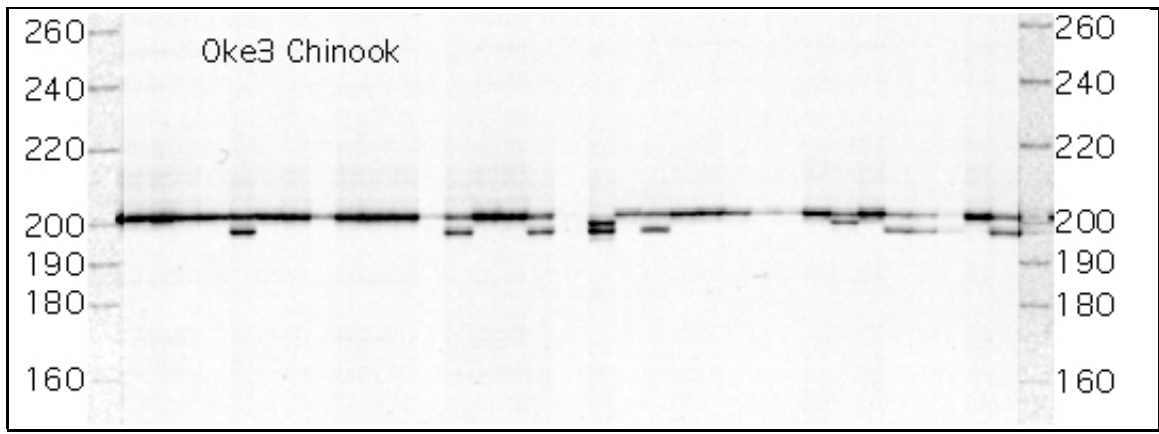
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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# chinook salmon

*Oncorhynchus tshawytscha*



**Oke4** 5' --AGG CCC AAA GTC TGT AGT GAA GG-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --GAT GAA TCG AGA GAA TAG GGA CTG AAT-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (CA)4A(CA)9

Mix Name: Microsat 1 Total vol. (ul): **25.00**

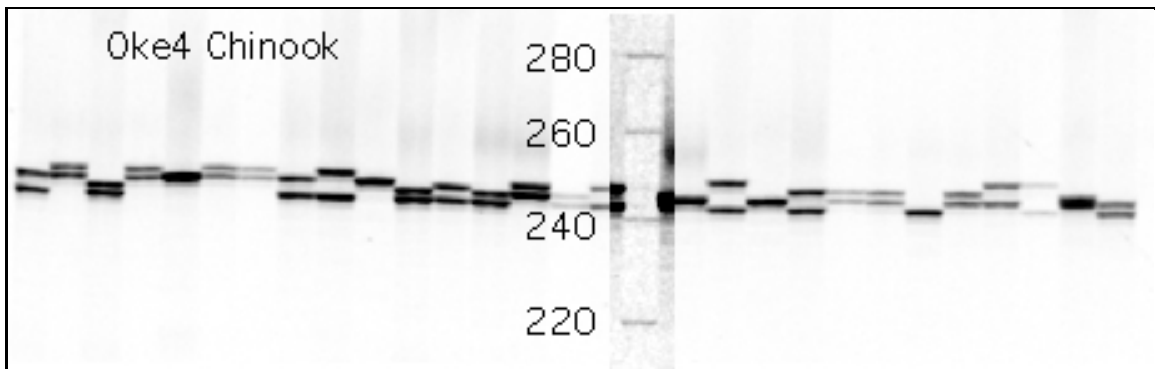
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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# chinook salmon

*Oncorhynchus tshawytscha*



**Oki10** 5' --GGA GTG CTG GAC AGA TTG G--3'  
 microsat 5' --CAG CTT TTT ACA AAT CCT CCT G--3'  
 Repeat [in species of origin]: (CTGT)22

Smith et al. 1998

Smith et al. 1998

Mix Name: Microsat 1 Total vol. (ul): **25.00**

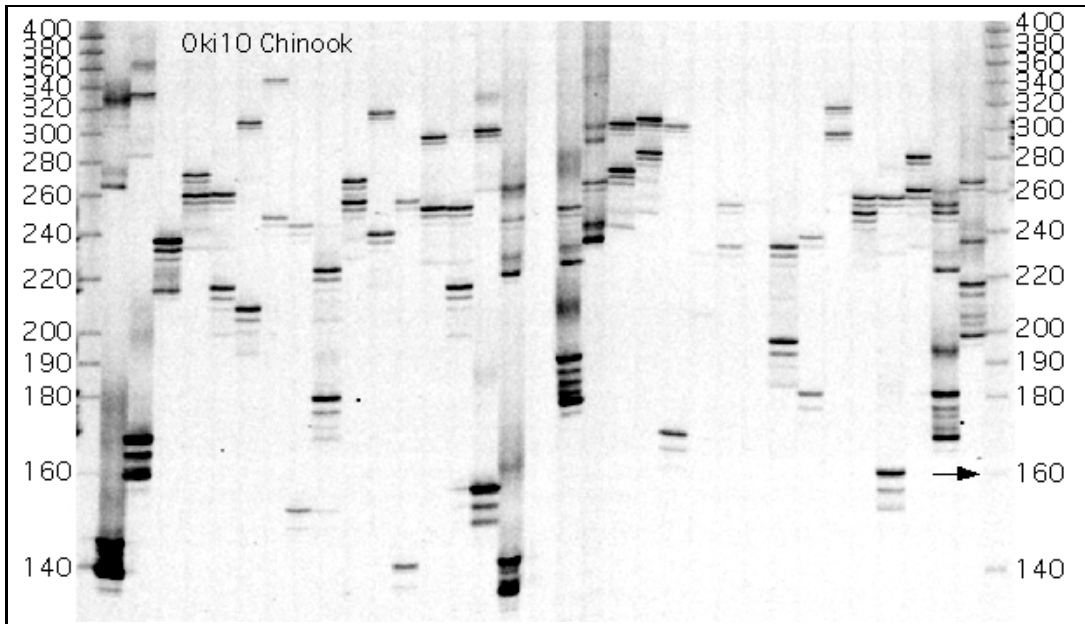
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro60a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>60</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**chinook salmon**  
*Oncorhynchus tshawytscha*

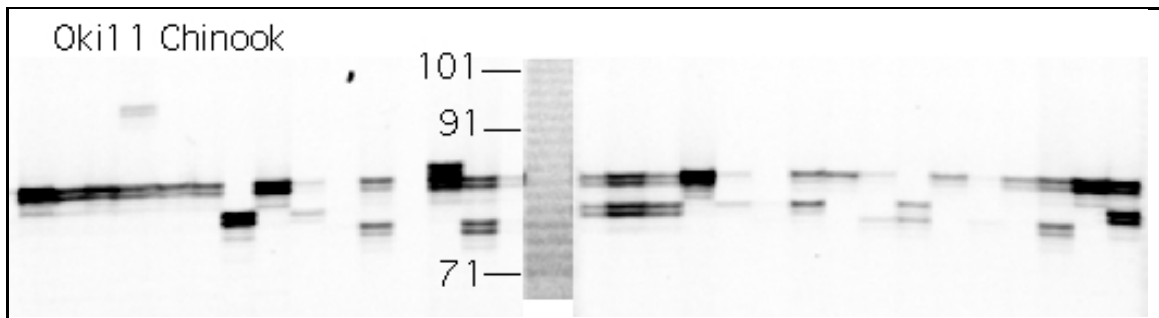


<b>Oki11</b>	5' --TCT GAG ACA GGC AAA TGC AC--3'	Smith et al. 1998
microsat	5' --GTT TTA AAC CTC ACC ATT GAG T--3'	Smith et al. 1998
Repeat [in species of origin]: (GT)6N8(GT)3N2(GT)4		

Mix Name: Microsat 1 Total vol. (ul): <b>25.00</b>			
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro54a		
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>54</b>	1, 0
Extend	70	1, 0
Autoextend: 0 Cycles: 30		
Notes: Initial cycle of 3 min, 95C Final cycle of 5 min, 70C		

Gel Conditions  
6.00 % denaturing vert polyacrylamide



For more information contact:

Fish Genetics Laboratory, U.S. Fish & Wildlife Service, 1011 East Tudor Road, Anchorage, AK 99503, (907)786-3617

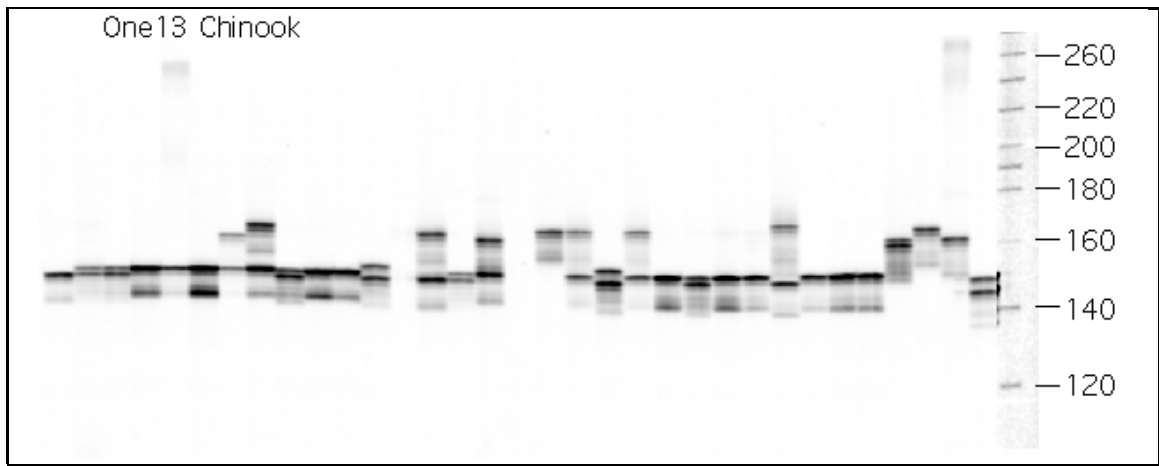
# chinook salmon

*Oncorhynchus tshawytscha*



**One13** 5' --TCA TAC CCC ATG CCT CTT CTG TT-- 3' Scribner et al. 1996  
 microsat 5' --GAT GAG TGA AAG AGA GGG AGC GA-- 3' Scribner et al. 1996  
 Repeat [in species of origin]: (GA)<sub>20</sub>  
 Presence of additional alleles >220 bases is currently being investigated.

Mix Name: Microsat 1 Total vol. (ul): <b>25.00</b>				Cyclename: micro52a		
	<b>Stock Conc</b>	<b>Reaction Conc</b>	<b>Reaction Volume</b>	<b>Temp (C)</b>	<b>Time (min, sec)</b>	
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul	Denature	95	1, 0
Buffer	10X	1X	2.500 ul PerkinElmer	<b>Anneal</b>	<b>52</b>	1, 0
dATP	8.0 mM	0.20 mM	0.625 ul	Extend	70	1, 0
dCTP	8.0 mM	0.20 mM	0.625 ul			
dGTP	8.0 mM	0.20 mM	0.625 ul	Autoextend: 0	Cycles: 30	
dTTP	8.0 mM	0.20 mM	0.625 ul	Notes: Initial cycle of 3 min, 95C		
Primer 1	10.0 uM	0.20 uM	0.500 ul	Final cycle of 5 min, 70C		
Primer 2	10.0 uM	0.20 uM	0.500 ul			
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677	<b>Gel Conditions</b>		
H <sub>2</sub> O			15.375 ul	6.00 % denaturing vert polyacrylamide		
DNA	50 ng/ul	100 ng	2.000 ul			
Betaine	M	M	ul			



For more information contact:

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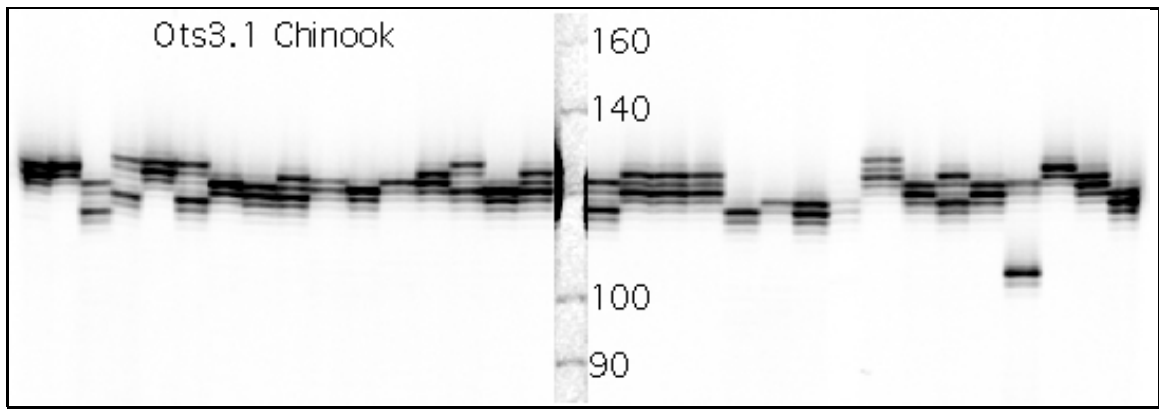
# chinook salmon

*Oncorhynchus tshawytscha*



**Ots3.1** 5' --CAG CCC ATC TGT CAC TCA CAC T-- 3' Banks et al. 1999  
 microsat 5' --GGT GGA GAG AGT TTG AGA ATC ACA-- 3' Banks et al. 1999  
 Repeat [in species of origin]: (CT)<sup>17</sup>  
 Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.

Mix Name: Microsat 1 Total vol. (ul): <b>25.00</b>				Cyclename: micro56a	
	<b>Stock Conc</b>	<b>Reaction Conc</b>	<b>Reaction Volume</b>	<u>Temp (C)</u>	<u>Time (min, sec)</u>
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul	Denature 95	1, 0
Buffer	10X	1X	2.500 ul PerkinElmer	<b>Anneal 56</b>	1, 0
dATP	8.0mM	0.20 mM	0.625 ul	Extend 70	1, 0
dCTP	8.0 mM	0.20 mM	0.625 ul		
dGTP	8.0 mM	0.20 mM	0.625 ul	Autoextend: 0	Cycles: 30
dTTP	8.0 mM	0.20 mM	0.625 ul	Notes: Initial cycle of 3 min, 95C	
Primer 1	10.0 uM	0.20 uM	0.500 ul	Final cycle of 5 min, 70C	
Primer 2	10.0 uM	0.20 uM	0.500 ul		
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677		
H <sub>2</sub> O			15.375 ul		
DNA	50 ng/ul	100 ng	2.000 ul	Gel Conditions	
Betaine	M	M	ul	6.00 % denaturing vert polyacrylamide	



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oke1** 5' --TCA GTA ATC TCT CTT CAG GTG ACC CT-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --CAT TAT CAG TAT TCA TGT CGG TTT CC-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (TG)<sub>31</sub>  
 Isolocus model.

Mix Name: Microsat 2 Total vol. (ul): **25.00**

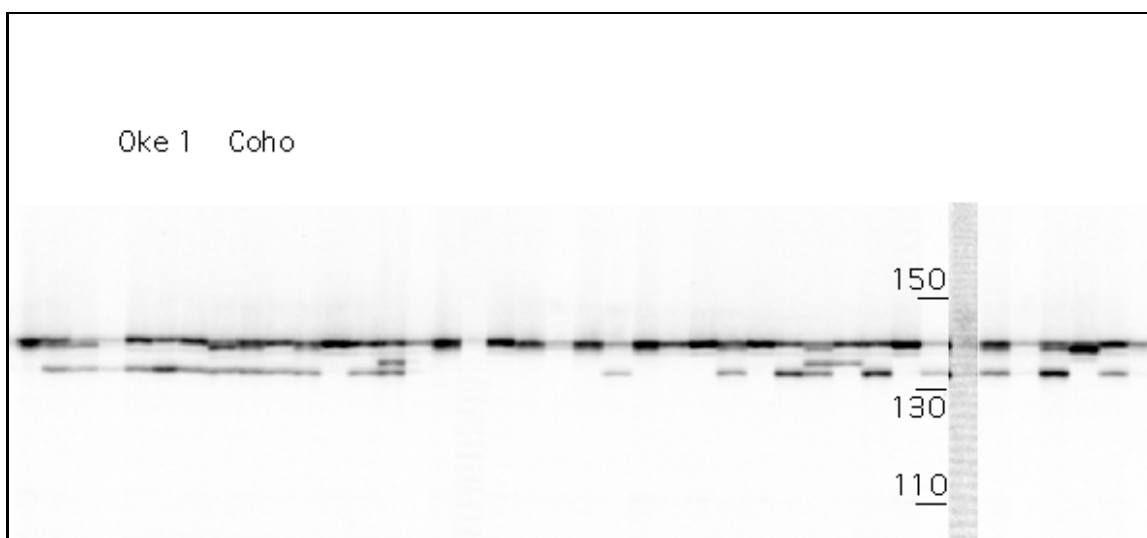
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	2.50 mM	2.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			14.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oke2** 5' --AGG GCC AGA GAA AAG TCT CAC TAT-- 3'

Buchholz, Fish Genetics Lab

microsat 5' --GTC AGT CCT GCC CTC TGT GTC CTA-- 3'

Buchholz, Fish Genetics Lab

Repeat [in species of origin]: (GT)44

Mix Name: Microsat 2 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	2.50 mM	2.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			14.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro62a

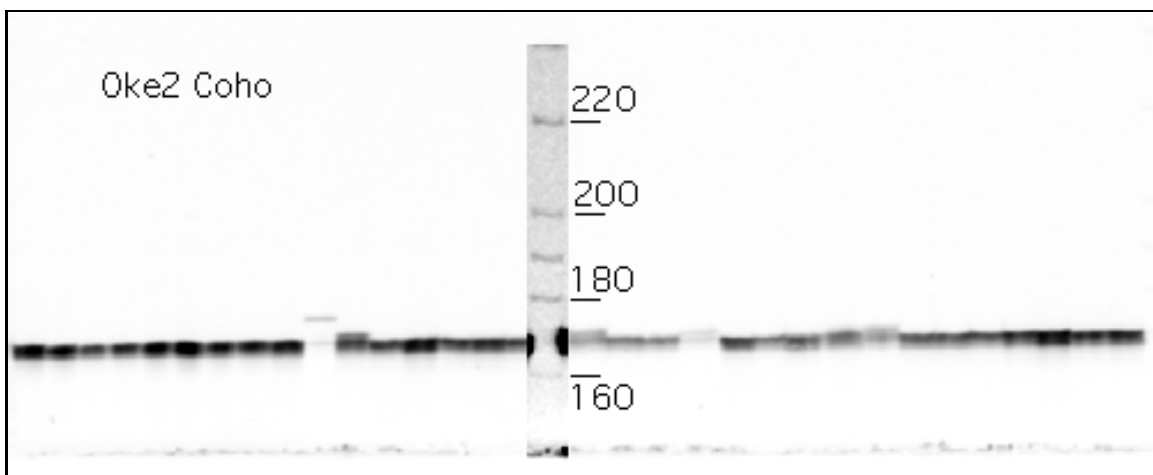
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>62</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oke3** 5' --ACC CTG AGA GCA ATC AAC-- 3'

Buchholz, Fish Genetics Lab

microsat 5' --TCA GGG ATA TGC AGT AAA TAG TA-- 3'

Buchholz, Fish Genetics Lab

Repeat [in species of origin]: (TCCCTCTCGTCTC)8

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro46a

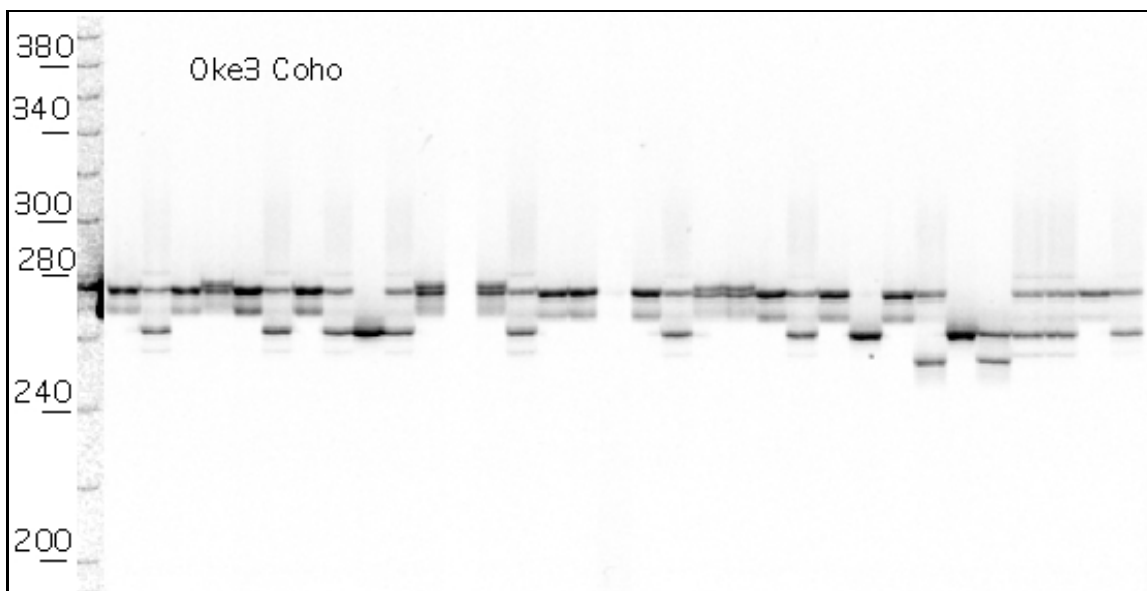
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>46</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oke4** 5' --AGG CCC AAA GTC TGT AGT GAA GG-- 3' Buchholz, Fish Genetics Lab  
 microsat 5' --GAT GAA TCG AGA GAA TAG GGA CTG AAT-- 3' Buchholz, Fish Genetics Lab  
 Repeat [in species of origin]: (CA)4A(CA)9

Mix Name: Microsat 2 Total vol. (ul): **25.00**

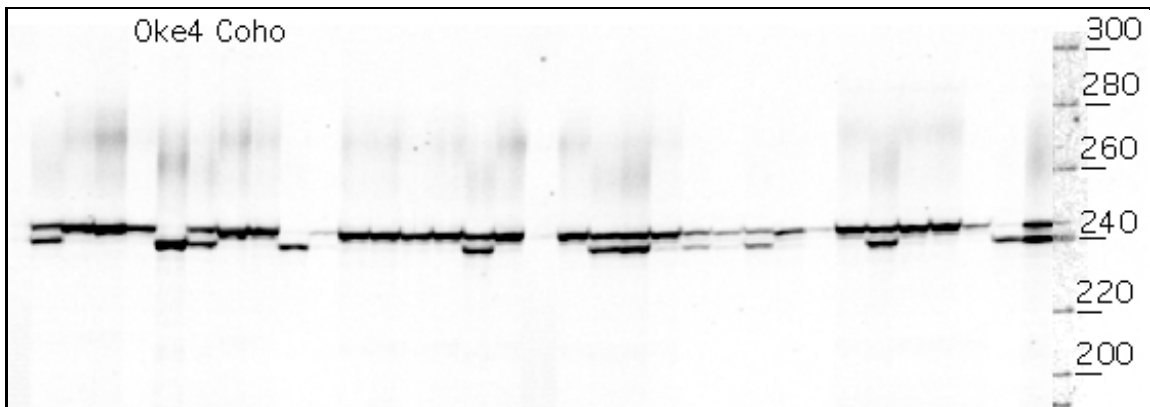
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	2.50 mM	2.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			14.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oki1** 5' --AGG ATG GCA GAG CAC CAC T--3'

Smith et al. 1998

microsat 5' --CAC CCA TAA TCA CAT ATT CAG A--3'

Smith et al. 1998

Repeat [in species of origin]: (CTGT)<sub>16</sub>

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro56a

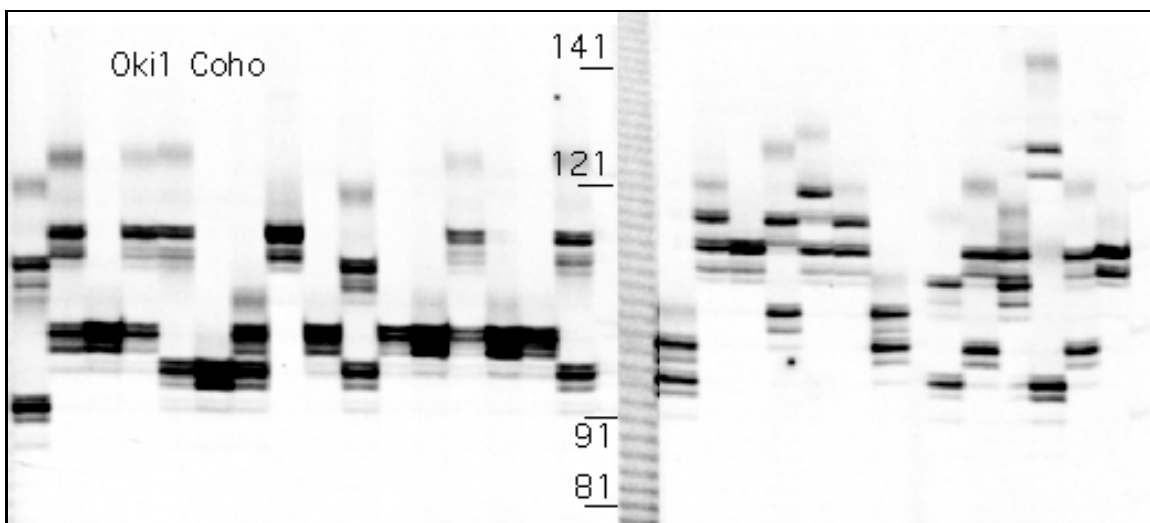
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>56</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oki3** 5' --GGA GCC CCT TAT TGG AAG G-- 3'  
microsat 5' --CTT CCA GCA GAG TGT CCC AG-- 3'  
Repeat [in species of origin]: (CCA)<sub>6</sub>

Smith et al. 1998

Smith et al. 1998

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro58a

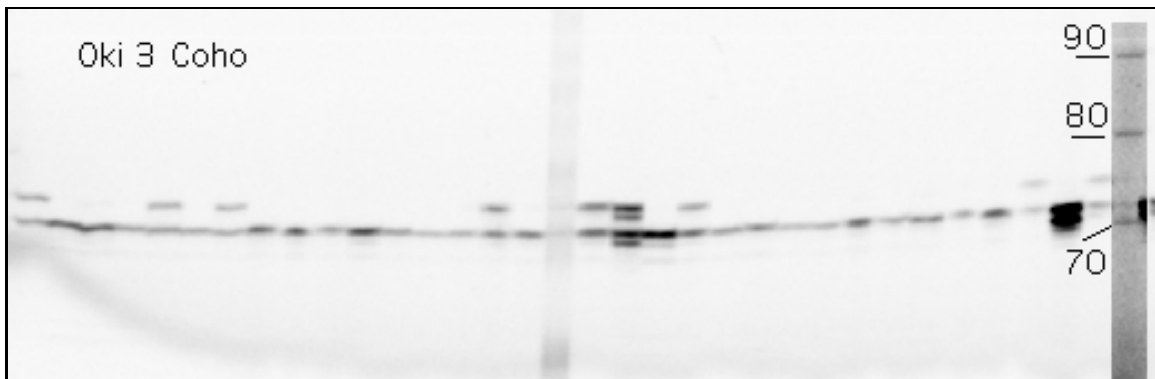
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>58</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oki11** 5' --TCT GAG ACA GGC AAA TGC AC--3' Smith et al. 1998  
 microsat 5' --GTT TTA AAC CTC ACC ATT GAG T--3' Smith et al. 1998  
 Repeat [in species of origin]: (GT)6N8(GT)3N2(GT)4

Mix Name: Microsat 1 Total vol. (ul): **25.00**

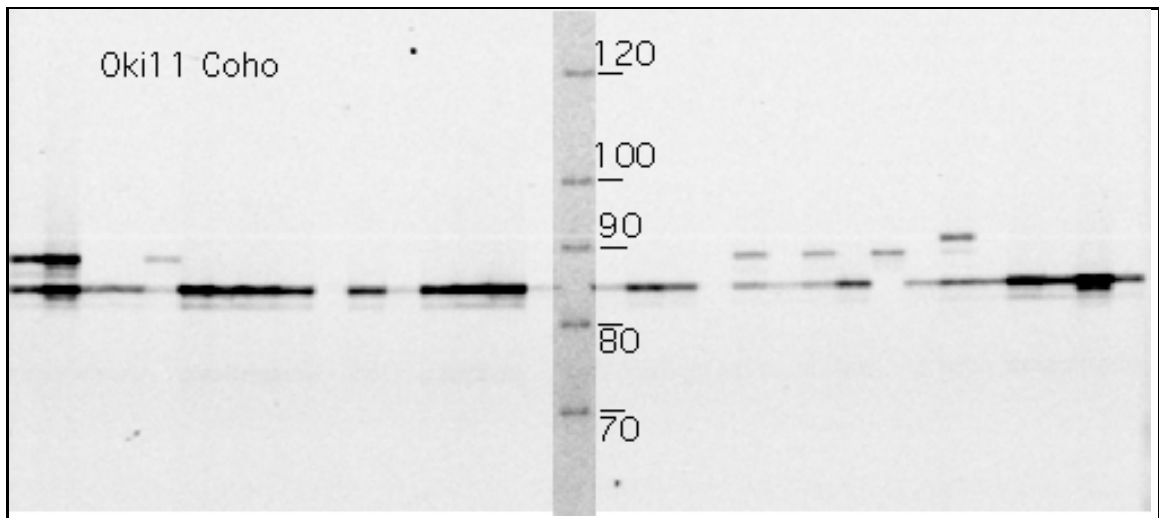
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oki13.1** 5' -- **CCA GAG GAA GTA CGG AGA AGC** --3' Smith et al. 1998  
 microsat 5' -- **GGG ACC TCT ATC TGG TGT CAG G** --3' Smith et al. 1998

Repeat [in species of origin]: (GA)<sub>9</sub>  
 Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.

Mix Name: Microsat 1 Total vol. (ul): **25.00**

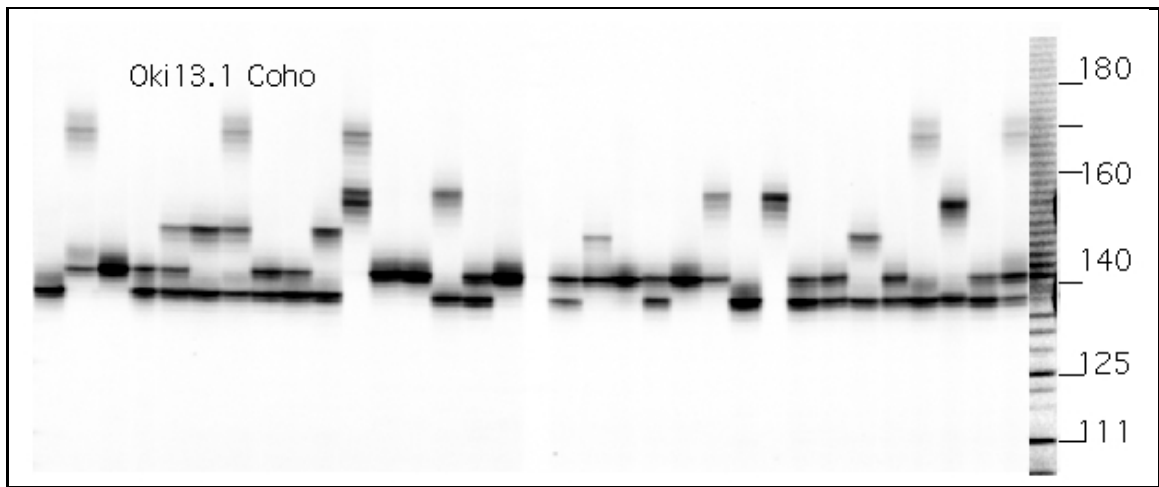
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro55a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>55</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Oki24.1** 5' --AGA GCG AGG GCC AGA GAG ATA ATG-- 3' Smith et al. 1998  
 microsat 5' --CGT GGG CAC TAG GCA CTG ACA T-- 3' Smith et al. 1998

Repeat [in species of origin]: (GT)6GC(GT)4GC(GT)7(N)6(GT)4GC(GT)11  
 Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.

Mix Name: Microsat 1 Total vol. (ul): **25.00**

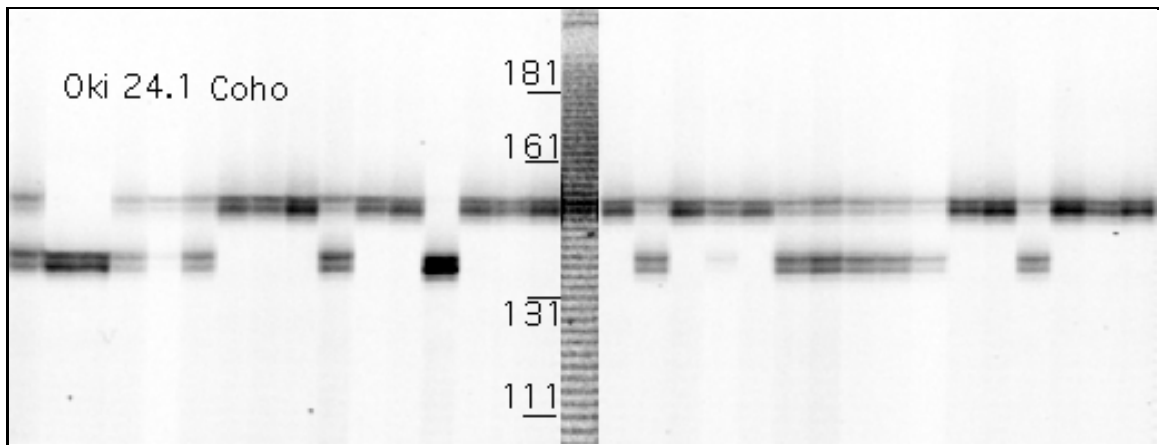
	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro52a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>52</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**One3** 5' --TCT CCT TGG TCT CTC TGT CCC TT-- 3'  
 microsat 5' --CTA TCA GCC AAT CGC ATC AGG AC-- 3'  
 Repeat [in species of origin]: (GA)18

Scribner et al. 1996

Scribner et al. 1996

Mix Name: Microsat 1 Total vol. (ul): **25.00**

	Stock Conc	Reaction Conc	Reaction Volume
MgCl2	25.0 mM	1.50 mM	1.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H2O			15.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro56a

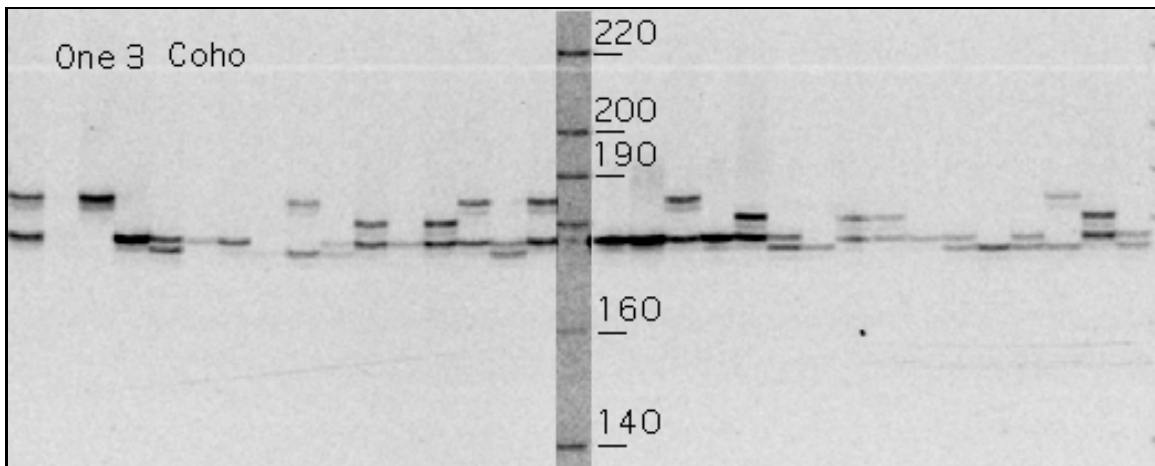
	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>56</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30

Notes: Initial cycle of 3 min, 95C  
Final cycle of 5 min, 70C

Gel Conditions

6.00 % denaturing vert polyacrylamide



For more information contact:

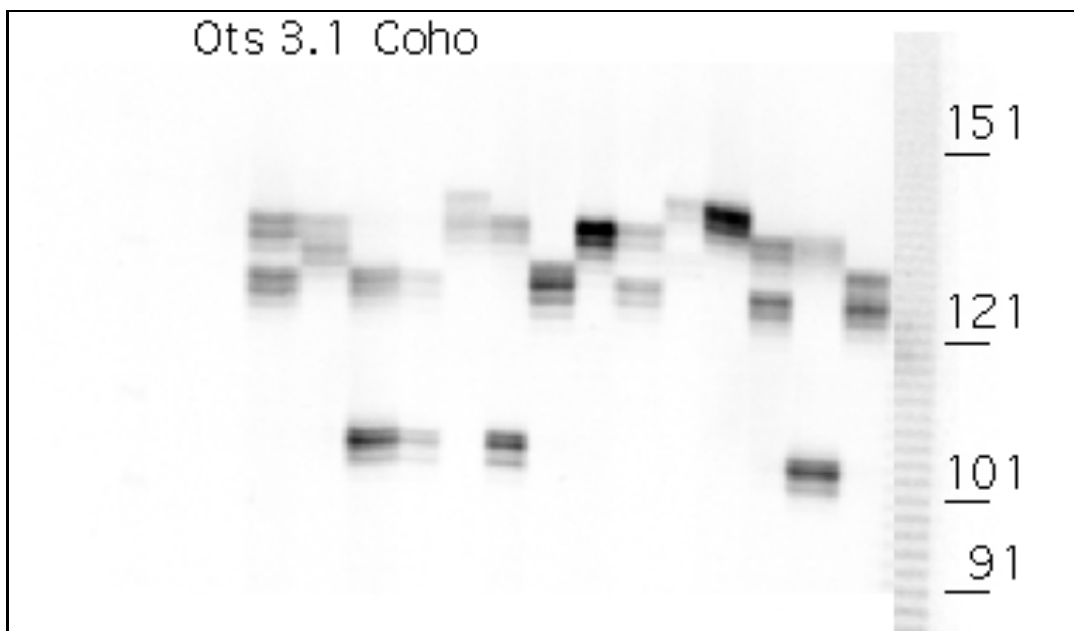
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**coho salmon**  
*Oncorhynchus kisutch*



<b>Ots3.1</b>	5' --CAG CCC ATC TGT CAC TCA CAC T-- 3'	Banks et al. 1999
microsat	5' --GGT GGA GAG AGT TTG AGA ATC ACA-- 3'	Banks et al. 1999
Repeat [in species of origin]: (CT) <sup>17</sup>		
Primer sequences redesigned by Buchholz, Fish Genetics Laboratory.		

Mix Name: Microsat 4 Total vol. (ul): <b>25.00</b>				Cyclename: micro56a		
	<b>Stock Conc</b>	<b>Reaction Conc</b>	<b>Reaction Volume</b>		<u>Temp (C)</u>	<u>Time (min, sec)</u>
MgCl <sub>2</sub>	25.0 mM	2.50 mM	2.500 ul	Denature	95	1, 0
Buffer	10X	1X	2.500 ul PerkinElmer	<b>Anneal</b>	<b>56</b>	1, 0
dATP	8.0mM	0.20 mM	0.625 ul	Extend	70	1, 0
dCTP	8.0 mM	0.20 mM	0.625 ul	<hr/>		
dGTP	8.0 mM	0.20 mM	0.625 ul	Autoextend: 0	Cycles: 30	
dTTP	8.0 mM	0.20 mM	0.625 ul	Notes: Initial cycle of 3 min, 95C		
Primer 1	10.0 uM	0.20 uM	0.500 ul	Final cycle of 5 min, 70C		
Primer 2	10.0 uM	0.20 uM	0.500 ul			
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677	Gel Conditions		
H <sub>2</sub> O			9.375 ul	6.00 % denaturing vert polyacrylamide		
DNA	50 ng/ul	100 ng	2.000 ul			
Betaine	5.0 M	1.00 M	5.000 ul			



For more information contact:

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**coho salmon**  
*Oncorhynchus kisutch*



**Ots105** 5' --GAG GAT CTA TCA ACA TTA TC--3' Nelson 1997  
 microsat 5' --GCA GCA CCA GCT TCC C--3' Nelson 1997  
 Repeat [in species of origin]: (TCTG)<sub>5</sub>

Mix Name: Microsat 2 Total vol. (ul): **25.00**

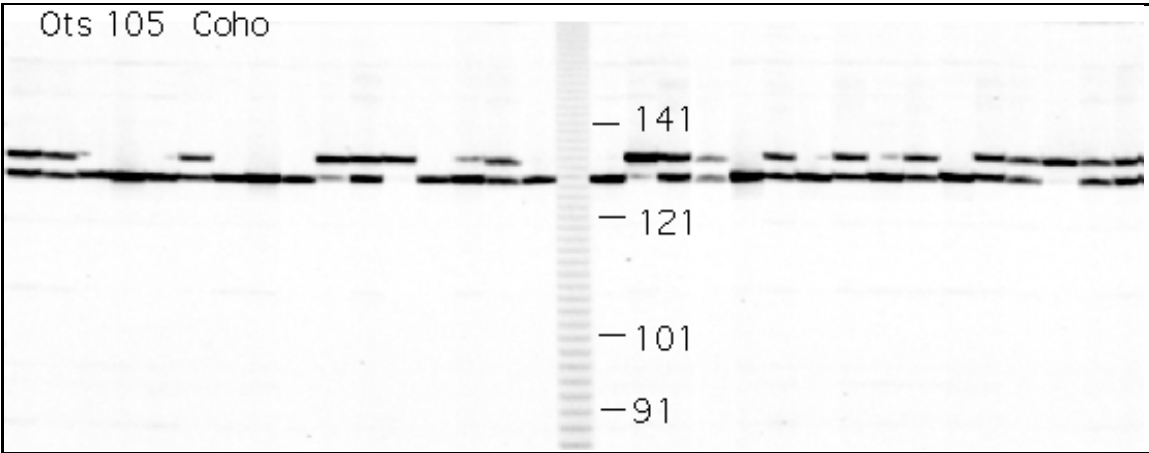
	Stock Conc	Reaction Conc	Reaction Volume
MgCl <sub>2</sub>	25.0 mM	2.50 mM	2.500 ul
Buffer	10X	1X	2.500 ul PerkinElmer
dATP	8.0mM	0.20 mM	0.625 ul
dCTP	8.0 mM	0.20 mM	0.625 ul
dGTP	8.0 mM	0.20 mM	0.625 ul
dTTP	8.0 mM	0.20 mM	0.625 ul
Primer 1	10.0 uM	0.20 uM	0.500 ul
Primer 2	10.0 uM	0.20 uM	0.500 ul
Taq	5.0 U/ul	0.625 U	0.125 ul Sigma #D6677
H <sub>2</sub> O			14.375 ul
DNA	50 ng/ul	100 ng	2.000 ul
Betaine	M	M	ul

Cyclename: micro50a

	Temp (C)	Time (min, sec)
Denature	95	1, 0
<b>Anneal</b>	<b>50</b>	1, 0
Extend	70	1, 0

Autoextend: 0 Cycles: 30  
 Notes: Initial cycle of 3 min, 95C  
 Final cycle of 5 min, 70C

Gel Conditions  
 6.00 % denaturing vert polyacrylamide



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The mission of the Fish Genetics Laboratory is to provide the necessary genetics expertise and support to permit sound stewardship of Alaska fishery resources, including conservation of the natural diversity of wild fish populations and aquatic ecosystems. Responsibilities include providing U.S. Fish and Wildlife Service leadership for conservation of genetic resources, particularly Pacific salmon stocks; development and implementation of genetic stock identification studies to delineate stocks for use in fisheries management and allocation decisions; evaluations of genetic impacts resulting from stock introductions, exploitation, and other activities; monitoring stocks for genetic change; coordination of genetic issues within and outside the Service; and conducting outreach activities that promote the importance of maintaining genetic diversity.

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