Species Identification of Rainbow and Cutthroat trout

using diagnostic SNP markers

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Objective

The goal of this analysis was to estimate the species composition of seven populations of trout in Yellowstone National Park. Genetic analysis of single nucleotide polymorphisms (SNPs) was used to estimate the proportion of the genes in each population that were derived from rainbow trout (RBT), Westslope cutthroat trout (WCT), and Yellowstone cutthroat trout (YCT).

Species identification with SNP markers

A SNP is a nucleotide in a DNA sequence that is variable among individuals, populations, or species. As an example, consider a sequence fragment of cytochrome B in rainbow and cutthroat trout.

Westslope cutthroat	GATGTGGCAGACAGAGGAGAAA
Yellowstone cutthroat	GATGTGGCAGACAGAGGAGAAA
Rainbow trout	GATGTGGCAAACAGAGGAGAAA

The DNA sequences for each taxon are identical except for the G/A polymorphism that distinguishes RBT from cutthroat trout. This is a single nucleotide polymorphism (SNP). Such polymorphisms are ideal for species identification because they have a very low mutation rate and can be accurately genotyped in the laboratory. In addition, standardization among different laboratories is generally easy. In order to use SNPs for species identification, diagnostic polymorphisms must be found. A SNP is considered diagnostic if all individuals in a species have one allele and all individuals in another species have another allele.

Eleven diagnostic SNPs are currently available to differentiate between RBT, WCT, and YCT (Table 1). Five of these loci have diagnostic SNPs for RBT, three loci are diagnostic for WCT, and three are diagnostic for YCT. Diagnostic alleles were identified by screening 26 reference populations (Table 2).

Methods

Tissue samples were provided by Mike Ruhl from 200 trout collected from seven locations in Yellowstone National Park (Table 3). Eleven SNPs were genotyped for each trout (Table 1). Maximum likelihood estimates of allele frequencies were calculated using the expectation-maximization algorithm.

Results & Discussion

Six of the seven samples showed clear evidence of hybridization (Table 3, Table 4, Table 5). Each sample is discussed below.

The sample from Black Butte Creek showed high levels of hybridization between RBT and WCT. All of the fish in the sample appear to be hybrids. There was one copy of a diagnostic YCT allele at one locus (Try-III), which could indicate very low levels of hybridization with YCT. However, it is probably at least as likely that this locus is not completely diagnostic and that all of the cutthroat genes in this population are WCT.

Grayling Creek appears to be a population of WCT with a moderate amount of RBT and YCT introgression. The population is well on its way to becoming a hybrid swarm. Of the 45 individuals in the sample, only five appear to be pure WCT. Ten individuals had YCT ancestry, and evidence for YCT hybridization was present at all of three diagnostic YCT loci genotyped. Twenty one individuals in the sample contained RBT alleles.

Oxbow Creek appears to be a hybrid swarm of WCT (75%) and YCT (25%). All individuals in the sample were hybrids.

The Slough Creek (3rd Med) sample is the only sample that showed no evidence of hybridization. All individuals appear to be pure YCT. The power to detect WCT introgression, at the 1% level, was 94.5%. The power to detect RBT introgression, at the 1% level, was 97.9%.

Slough Creek Canyon (US of 1st Med) shows low levels of hybridization with RBT (6%). However, only three of the 22 individuals sampled showed RBT ancestry. The remainder of the individuals sampled appear to be pure YCT.

Slough Creek (Lower 1st Med) is a population of pure YCT that appears to have very recently been invaded by RBT. Of the 16 individuals sampled, 14 appear to be pure YCT. One looks like a pure RBT, and one looks like a RBT with a YCT mitochondrion.

The Trout Lake sample seems be composed of 31 pure YCT and four pure RBT. There are no hybrids in the sample.

The raw data for this analysis is included in Appendix 1.

Table 1. Diagnostic SNP loci used in this study.The column “Diagnostic taxon” indicates which taxon has a unique allele that is not found in the other taxa.

Locus Name	Abbreviation	Diagnostic taxon
Cytochrome b	CytB	RBT
Thymosin beta	Thymo-b	RBT
Recombination activating locus	RAG1	RBT
Vimentin	Vim	RBT
Calreticulin	Cal	RBT
Carbonyl reductase	CBR1	WCT
Proto-oncogene	P53	WCT
Transferrin exon 5 to 8	Tnsf	WCT
Prolactin II	PrL2-R1	YCT
Metallothionein B	MT1B	YCT
Trypsin III precursor	Try-III	YCT

Table 2. List of wild populations used as reference samples to identify diagnostic alleles.

Taxon	Population	State	Drains into	County	Latitude	Longitude
BCT	Bear Lake	UT/ID	Bear River	Bear Lake	41.9993	-111.327
WCT	Browns Creek	MT	Beaverhead R.	Beaverhead	45.1438	-113.2591
WCT	Bull River, East Fork	MT	Clark Fork	Sanders	48.1271	-115.7316
WCT	Cabin Creek, Middle Fork	MT	Madison River	Gallatin	44.8866	-111.3343
WCT	Canuck Creek	ID	Kootenai	Boundary	48.9317	-116.0558
WCT	Chamberlain	MT	Blackfoot River	Cascade	46.9872	-113.2515
WCT	Cottonwood Creek	MT	Beaverhead R.	Beaverhead	44.9358	-112.4298
WCT	Dirty Ike Creek	MT	Clark Fork	Missoula	46.8100	-113.7027
WCT	Garden Creek	ID	MF Salmon	Challis	44.4935	-114.2972
WCT	Graveyard Gulch	MT	Smith River	Meagher	46.9317	-110.7793
WCT	Hall Creek	MT	Crow Creek	Jefferson	46.7846	-111.7846
WCT	Henry Creek	MT				
WCT	Little Belt, North Fork	MT	Willow Creek	Cascade	47.4245	-110.6500
WCT	Main Cabin Creek	MT	Madison River	Gallatin	44.8974	-111.3141
WCT	McClure Creek (Unofficial Name)	MT	Smith River	Meagher	46.8000	-111.2945
WCT	Muskrat Creek	MT	Boulder River	Jefferson	46.3100	-112.0234
WCT	North Fork Lost	MT	Flathead	Lake	47.8863	-113.7825
WCT	Ray Creek	MT	Missouri River	Broadwater	46.3896	-111.3798
WCT	Rock Creek	MT	Clark Fork	Missoula	47.0746	-114.3710
WCT	Wallace Creek	MT	Clark Fork	Missoula	46.7816	-113.6855
WCT	Wilson Creek, West Fork	MT	Gallatin River	Gallatin	45.5278	-111.1844
YCT	Blackfoot River, ID	ID	Snake River	Caribou	42.8647	-111.589
YCT	Goose Creek	MT	Yellowstone R.	Park	45.1141	-109.914
YCT	Henry's Lake	ID	Snake River	Freemont		
YCT	McBride Lake	WY	Yellowstone R.	Park	44.9629	-110.254
YCT	Yellowstone Lake	WY	Yellowstone R.	Park	44.4708	-110.356

Table 3. Estimated genetic composition of seven populations of trout.

Location	Date	*N*	% RBT	% WCT	% YCT
Black Butte Creek	6/4/07	18	0.62	0.37	0.01
Grayling Creek	7/19/07	45	0.14	0.79	0.07
Oxbow Creek	6/14/07	40	-	0.75	0.25
Slough Creek, 3rd Med	8/10/07	24	-	-	1.00
Slough Creek Canyon, US of 1st Med	8/20/07	22	0.06	-	0.94
Slough Creek, Lower 1st Med	8/20/07	16	0.12	-	0.88
Trout Lake	7/17/07	35	0.11	-	0.89

Table 4. Estimated genetic composition of 200 individual trout. Note: Estimates of the genetic composition of individuals that have ancestry from three taxa (RBT, WCT, and YCT) are subject to substantial amounts of estimation error. Population level estimates (Table 3) are much more accurate.

	% RBT	% WCT	% YCT
Black_Butte_Creek_6_4_07_1_01	0.53	0.47	
Black_Butte_Creek_6_4_07_1_02	0.47	0.53	
Black_Butte_Creek_6_4_07_1_03	0.96	0.04	
Black_Butte_Creek_6_4_07_1_04	0.4	0.6	
Black_Butte_Creek_6_4_07_1_05	0.96	0.04	
Black_Butte_Creek_6_4_07_1_06	0.67	0.33	
Black_Butte_Creek_6_4_07_2_07	0.67	0.33	
Black_Butte_Creek_6_4_07_2_08	0.47	0.53	
Black_Butte_Creek_6_4_07_2_09	0.17	0.83	
Black_Butte_Creek_6_4_07_2_10	0.67	0.33	
Black_Butte_Creek_6_4_07_2_11	0.53	0.47	
Black_Butte_Creek_6_4_07_2_12	0.73	0.27	
Black_Butte_Creek_6_4_07_2_13	0.71	0.29	
Black_Butte_Creek_6_4_07_2_14	0.15	0.6	0.25
Black_Butte_Creek_6_4_07_2_15	0.93	0.07	
Black_Butte_Creek_6_4_07_2_16	0.67	0.33	
Black_Butte_Creek_6_4_07_2_17	0.96	0.04	
Black_Butte_Creek_6_4_07_2_18	0.53	0.47	
Grayling_Creek_7_19_07_Upper_06		0.83	0.17
Grayling_Creek_7_19_07_Upper_08	0.27	0.73	
Grayling_Creek_7_19_07_Upper_09	0.13	0.87	
Grayling_Creek_7_19_07_Upper_10	0.07	0.93	
Grayling_Creek_7_19_07_Upper_14	0.27	0.73	
Grayling_Creek_7_19_07_Upper_17		1	
Grayling_Creek_8_14_07_Upper_44	0.25	0.55	0.2
Grayling_Creek_8_14_07_Upper_45	0.13	0.87	
Grayling_Creek_8_14_07_Upper_48	0.2	0.8	
Grayling_Creek_8_14_07_Upper_50	0.2	0.8	
Grayling_Creek_8_14_07_Upper_55		1	
Grayling_Creek_8_14_07_Upper_60	0.54	0.31	0.15
Grayling_Creek_8_24_07_Upper_46	0.23	0.31	0.45
Grayling_Creek_8_24_07_Upper_47	0.15	0.6	0.25
Grayling_Creek_8_24_07_Upper_51	0.21	0.48	0.31
Grayling_Creek_8_24_07_Upper_52		0.83	0.17
Grayling_Creek_8_24_07_Upper_54	0.2	0.8	
Grayling_Creek_8_24_07_Upper_56	0.24	0.71	0.05
Grayling_Creek_8_24_07_Upper_58	0.33	0.67	
Grayling_Creek_8_24_07_Upper_62	0.27	0.73	
Grayling_Creek_8_30_07_Upper_49	0.07	0.93	

Grayling_Creek_9_20_07_Upper_01		1	
Grayling_Creek_9_20_07_Upper_02		1	
Grayling_Creek_9_20_07_Upper_03		1	
Grayling_Creek_9_20_07_Upper_04		0.83	0.17
Grayling_Creek_9_20_07_Upper_05		0.92	0.08
Grayling_Creek_9_20_07_Upper_06	0.06	0.86	0.08
Grayling_Creek_9_20_07_Upper_07		1	
Grayling_Creek_9_20_07_Upper_08		1	
Grayling_Creek_9_20_07_Upper_09	0.33	0.67	
Grayling_Creek_9_20_07_Upper_10	0.13	0.87	
Grayling_Creek_9_20_07_Upper_11		0.75	0.25
Grayling_Creek_9_20_07_Upper_12	0.2	0.8	
Grayling_Creek_9_20_07_Upper_13		0.83	0.17
Grayling_Creek_9_20_07_Upper_14		0.92	0.08
Grayling_Creek_9_20_07_Upper_15		0.92	0.08
Grayling_Creek_9_20_07_Upper_16		1	
Grayling_Creek_9_20_07_Upper_17		0.83	0.17
Grayling_Creek_9_20_07_Upper_18	0.2	0.8	
Grayling_Creek_9_20_07_Upper_19		0.92	0.08
Grayling_Creek_9_20_07_Upper_20	0.54	0.46	
Grayling_Creek_9_3_07_Upper_41	0.2	0.8	
Grayling_Creek_9_3_07_Upper_53	0.67	0.33	
Grayling_Creek_9_3_07_Upper_63	0.27	0.73	
Grayling_Creek_9_3_07_Upper_64	0.09	0.78	0.13
Oxbow_Creek_6_14_07_1_01		0.83	0.17
Oxbow_Creek_6_14_07_1_02		0.75	0.25
Oxbow_Creek_6_14_07_1_03		0.5	0.5
Oxbow_Creek_6_14_07_1_04		0.75	0.25
Oxbow_Creek_6_14_07_1_05		0.83	0.17
Oxbow_Creek_6_14_07_1_06		0.75	0.25
Oxbow_Creek_6_14_07_1_07		0.83	0.17
Oxbow_Creek_6_14_07_1_08		0.75	0.25
Oxbow_Creek_6_14_07_1_09		0.75	0.25
Oxbow_Creek_6_14_07_1_10		0.5	0.5
Oxbow_Creek_6_14_07_1_11		0.75	0.25
Oxbow_Creek_6_14_07_1_12		0.58	0.42
Oxbow_Creek_6_14_07_1_13		0.5	0.5
Oxbow_Creek_6_14_07_1_14		0.67	0.33
Oxbow_Creek_6_14_07_1_15		0.75	0.25
Oxbow_Creek_6_14_07_1_16		0.67	0.33
Oxbow_Creek_6_14_07_2_17		0.92	0.08
Oxbow_Creek_6_14_07_2_18		0.92	0.08
Oxbow_Creek_6_14_07_2_19		0.92	0.08
Oxbow_Creek_6_14_07_2_20		0.75	0.25
Oxbow_Creek_6_14_07_2_21		0.92	0.08
Oxbow_Creek_6_14_07_2_22		0.67	0.33

Oxbow_Creek_6_14_07_2_23		0.92	0.08
Oxbow_Creek_6_14_07_2_24		0.83	0.17
Oxbow_Creek_6_14_07_2_25		0.5	0.5
Oxbow_Creek_6_14_07_2_26		0.83	0.17
Oxbow_Creek_6_14_07_2_27		0.67	0.33
Oxbow_Creek_6_14_07_2_28		0.58	0.42
Oxbow_Creek_6_14_07_2_29		0.92	0.08
Oxbow_Creek_6_14_07_2_30		0.58	0.42
Oxbow_Creek_6_14_07_2_31		0.92	0.08
Oxbow_Creek_6_14_07_3_32		0.67	0.33
Oxbow_Creek_6_14_07_3_33		0.75	0.25
Oxbow_Creek_6_14_07_3_34		0.75	0.25
Oxbow_Creek_6_14_07_3_35	0.08	0.7	0.22
Oxbow_Creek_6_14_07_3_36		0.83	0.17
Oxbow_Creek_6_14_07_3_37		0.92	0.08
Oxbow_Creek_6_14_07_3_38		0.67	0.33
Oxbow_Creek_6_14_07_3_39		0.75	0.25
Oxbow_Creek_6_14_07_3_40		0.67	0.33
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Slough_Creek_3rd_Med_8_11_07_12			1
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Slough_Creek_Canyon_US_of_1st_Med_8_20_07_125			1
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Slough_Creek_Canyon_US_of_1st_Med_8_20_07_130			1
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_131			1
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Slough_Creek_Canyon_US_of_1st_Med_8_20_07_133			1
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Slough_Creek_Lower_1st_Med_7_27_07_26			1
Slough_Creek_Lower_1st_Med_7_27_07_28			1
Slough_Creek_Lower_1st_Med_7_27_07_31			1
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Trout_Lake_6_28_07_40			1
Trout_Lake_6_28_07_41			1
Trout_Lake_6_28_07_43			1
Trout_Lake_6_28_07_46			1
Trout_Lake_6_28_07_47			1
Trout_Lake_7_17_07_03			1
Trout_Lake_7_17_07_05			1

Trout_Lake_7_17_07_10		1
Trout_Lake_7_17_07_11		1
Trout_Lake_7_17_07_14		1
Trout_Lake_7_17_07_16		1
Trout_Lake_7_17_07_17		1
Trout_Lake_7_17_07_19		1
Trout_Lake_7_17_07_20		1
Trout_Lake_7_17_07_28		1
Trout_Lake_7_17_07_29		1
Trout_Lake_7_17_07_31		1
Trout_Lake_7_17_07_35		1
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Trout_Lake_7_2_07_23		1
Trout_Lake_7_2_07_24	1	
Trout_Lake_7_24_07_01		1
Trout_Lake_7_24_07_02		1
Trout_Lake_7_24_07_04		1
Trout_Lake_7_24_07_07		1
Trout_Lake_7_24_07_08	1	
Trout_Lake_7_24_07_12		1
Trout_Lake_7_24_07_13	1	
Trout_Lake_7_24_07_15		1
Trout_Lake_7_24_07_21		1
Trout_Lake_7_24_07_34		1

Appendix 1. The raw data used for this analysis.

	RBT Loci					WCT Loci			YCT Loci		
	Cytb-R2	**Thymo-β**	**RAG1**	**VIM**	**Cal**	**CBR1**	**P53**	**Tnsf**	**PrL2-R1**	**MT1B**	**Try-III**
Diagnostic alleles for **WCT**	**G**	**GG**	**CC**	**GG**	**AA**	**AA**	**TT**	**TT**	**GG**	**AA**	**GG**
Diagnostic alleles for **YCT**	**G**	**GG**	**CC**	**GG**	**AA**	**CC**	**CC**	**CC**	**AA**	**GG**	**TT**
Diagnostic alleles for **RBT**	**A**	**AA**	**TT**	**TT**	**TT**	**CC**	**CC**	**CC**	**GG**	**AA**	**GG**
Black Butte Creek											
Black_Butte_Creek_6_4_07_1_01,	A	GG	CT	TT	AT	CC	TT	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_1_02,	A	GG	CT	GT	AT	CA	CT	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_1_03,	A	GA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_1_04,	A	GG	CC	TT	AT	CA	CT	TT	GG	AA	GG
Black_Butte_Creek_6_4_07_1_05,	A	GA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_1_06,	A	GG	TT	GT	TT	CA	CC	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_07,	A	GG	CT	GT	TT	CC	CT	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_2_08,	A	GG	CT	GT	AT	CA	CT	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_09,	G	GG	CC	GG	AA	CA	TT	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_10,	A	AA	CT	GT	AA	CC	CC	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_11,	A	AA	CT	GT	AT	CA	CT	TT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_12,	A	GG	TT	GT	TT	CA	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_2_13,	A	GA	CT	GT	AT	CC	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_2_14,	G	GG	CC	GT	AA	CA	CC	TT	GG	AA	GT
Black_Butte_Creek_6_4_07_2_15,	A	AA	TT	TT	TT	CC	CC	CT	GG	AA	GG
Black_Butte_Creek_6_4_07_2_16,	A	GG	TT	GT	AT	CA	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_2_17,	A	GA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Black_Butte_Creek_6_4_07_2_18,	G	GG	CT	TT	AT	CA	CC	CT	GG	AA	GG
Grayling Creek											
Grayling_Creek_7_19_07_Upper_06,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	GT
Grayling_Creek_7_19_07_Upper_08,	A	GG	CC	GT	AT	AA	CT	TT	GG	AA	GG
Grayling_Creek_7_19_07_Upper_09,	A	GG	CC	GG	AT	AA	TT	TT	GG	AA	GG
Grayling_Creek_7_19_07_Upper_10,	G	GG	CC	GG	AT	AA	TT	TT	GG	AA	GG
Grayling_Creek_7_19_07_Upper_14,	G	GG	CT	GT	AA	CA	TT	CT	GG	AA	GG
Grayling_Creek_7_19_07_Upper_17,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_8_14_07_Upper_44,	A	GG	CT	GG	AA	AA	CT	CC	GG	AA	GT
Grayling_Creek_8_14_07_Upper_45,	G	GG	CT	GG	AT	AA	TT	TT	GG	AA	GG
Grayling_Creek_8_14_07_Upper_48,	A	GG	CC	GG	AA	CA	CT	TT	GG	AA	GG
Grayling_Creek_8_14_07_Upper_50,	G	GG	CT	GG	AA	AA	CT	CT	GG	AA	GG
Grayling_Creek_8_14_07_Upper_55,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_8_14_07_Upper_60,	A	GG	CT	GT	TT	CA	CT	CC	GG	AA	GT
Grayling_Creek_8_24_07_Upper_46,	G	GG	CC	GT	TT	AA	CT	CC	GA	GA	TT
Grayling_Creek_8_24_07_Upper_47,	G	GG	CC	GT	AA	CA	CT	CT	GG	AA	GT
Grayling_Creek_8_24_07_Upper_51,	A	GG	CT	GG	AA	CA	CT	CT	GG	GG	GG
Grayling_Creek_8_24_07_Upper_52,	G	GG	CC	GG	AA	AA	TT	CT	GG	GA	GG
Grayling_Creek_8_24_07_Upper_54,	G	GG	CT	GG	AA	CA	CT	TT	GG	AA	GG
Grayling_Creek_8_24_07_Upper_56,	G	GG	CC	GG	AT	CA	CC	TT	GG	AA	GG
Grayling_Creek_8_24_07_Upper_58,	A	GG	CT	GT	AT	CA	TT	TT	GG	AA	GG
Grayling_Creek_8_24_07_Upper_62,	G	GG	CT	GT	AT	AA	TT	CT	GG	AA	GG
Grayling_Creek_8_30_07_Upper_49,	G	GG	CC	GT	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_01,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_02,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_03,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_04,	G	GG	CC	GG	AA	AA	TT	CT	GG	GA	GG

Grayling_Creek_9_20_07_Upper_05,	G	GG	CC	GG	AA	AA	TT	TT	GG	GA	GG
Grayling_Creek_9_20_07_Upper_06,	G	GG	CC	GG	AT	AA	TT	TT	GA	AA	GG
Grayling_Creek_9_20_07_Upper_07,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_08,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_09,	A	GG	CC	GT	AA	AA	CC	CT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_10,	G	GG	CT	GG	AA	AA	TT	CT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_11,	G	GG	CC	GG	AA	CA	CT	CT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_12,	G	GG	CC	GG	AT	AA	CT	CT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_13,	G	GG	CC	GG	AA	CA	TT	TT	GG	AA	GT
Grayling_Creek_9_20_07_Upper_14,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_15,	G	GG	CC	GG	AA	AA	TT	TT	GA	AA	GG
Grayling_Creek_9_20_07_Upper_16,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_17,	G	GG	CC	GG	AA	AA	CT	TT	GG	GA	GG
Grayling_Creek_9_20_07_Upper_18,	A	GG	CC	GG	AT	AA	CT	TT	GG	AA	GG
Grayling_Creek_9_20_07_Upper_19,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GT
Grayling_Creek_9_20_07_Upper_20,	G	AA	CC	GG	TT	CA	CC		GG	AA	GG
Grayling_Creek_9_3_07_Upper_41,	G	GG	CC	GG	TT	AA	TT	CT	GG	AA	GG
Grayling_Creek_9_3_07_Upper_53,	A	GG	CT	GT	TT	CC	CC	CT	GG	AA	GG
Grayling_Creek_9_3_07_Upper_63,	G	GG	CT	GT	AA	AA	CT	CT	GG	AA	GG
Grayling_Creek_9_3_07_Upper_64,	G	GG	CT	GG	AA	AA	CT	TT	GA	AA	GG
Oxbow Creek											
Oxbow_Creek_6_14_07_1_01,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	GT
Oxbow_Creek_6_14_07_1_02,	G	GG	CC	GG	AA	AA	CT	TT	GA	AA	GT
Oxbow_Creek_6_14_07_1_03,	G	GG	CC	GG	AA	AA	CC	CT	GA	AA	TT
Oxbow_Creek_6_14_07_1_04,	G	GG	CC	GG	AA	AA	TT	TT	AA	AA	GT
Oxbow_Creek_6_14_07_1_05,	G	GG	CC	GG	AA	AA	TT	TT	GA	AA	GT
Oxbow_Creek_6_14_07_1_06,	G	GG	CC	GG	AA	AA	TT	TT	GG	GG	GT
Oxbow_Creek_6_14_07_1_07,	G	GG	CC	GG	AA	AA	CT	TT	GG	AA	GT
Oxbow_Creek_6_14_07_1_08,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	TT
Oxbow_Creek_6_14_07_1_09,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	TT
Oxbow_Creek_6_14_07_1_10,	G	GG	CC	GG	AA	AA	TT	T	AA	GG	GT
Oxbow_Creek_6_14_07_1_11,	G	GG	CC	GG	AA	AA	TT	CT	GG	AA	TT
Oxbow_Creek_6_14_07_1_12,	G	GG	CC	GG	AA	AA	CT	CT	GA	GA	GT
Oxbow_Creek_6_14_07_1_13,	G	GG	CC	GG	AA	AA	CT	CT	AA	AA	TT
Oxbow_Creek_6_14_07_1_14,	G	GG	CC	GG	AA	AA	CT	TT	AA	AA	GT
Oxbow_Creek_6_14_07_1_15,	G	GG	CC	GG	AA	AA	CT	TT	GA	GA	GG
Oxbow_Creek_6_14_07_1_16,	G	GG	CC	GG	AA	AA	CT	CT	GG	GG	GG
Oxbow_Creek_6_14_07_2_17,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GT
Oxbow_Creek_6_14_07_2_18,	G	GG	CC	GG	AA	AA	TT	TT	GG	GA	GG
Oxbow_Creek_6_14_07_2_19,	G	GG	CC	GG	AA	AA	TT	TT	GG	GA	GG
Oxbow_Creek_6_14_07_2_20,	G	GG	CC	GG	AA	AA	TT	CT	GA	AA	GT
Oxbow_Creek_6_14_07_2_21,	G	GG	CC	GG	AA	AA	TT	TT	GG	GA	GG
Oxbow_Creek_6_14_07_2_22,	G	GG	CC	GG	AA	AA	CT	CT	GG	GA	GT
Oxbow_Creek_6_14_07_2_23,	G	GG	CC	GG	AA	AA	CT	TT	GG	AA	GG
Oxbow_Creek_6_14_07_2_24,	G	GG	CC	GG	AA	AA	TT	CT	GG	GA	GG
Oxbow_Creek_6_14_07_2_25,	G	GG	CC	GG	AA	AA	CT	CT	AA	GA	GT
Oxbow_Creek_6_14_07_2_26,	G	GG	CC	GG	AA	AA	CT	TT	GA	AA	GG
Oxbow_Creek_6_14_07_2_27,	G	GG	CC	GG	AA	AA	TT	TT	GA	GA	TT
Oxbow_Creek_6_14_07_2_28,	G	GG	CC	GG	AA	AA	CT	TT	GA	GA	TT
Oxbow_Creek_6_14_07_2_29,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GT
Oxbow_Creek_6_14_07_2_30,	G	GG	CC	GG	AA	AA	CT	CT	AA	AA	GT
Oxbow_Creek_6_14_07_2_31,	G	GG	CC	GG	AA	AA	TT	TT	GG	AA	GT
Oxbow_Creek_6_14_07_3_32,	G	GG	CC	GG	AA	AA	CT	TT	GA	AA	T
Oxbow_Creek_6_14_07_3_33,	G	GG	CC	GG	AA	AA	CT	TT	GG	GA	GT
Oxbow_Creek_6_14_07_3_34,	G	GG	CC	GG	AA	AA	TT	CT	GA	AA	GT
Oxbow_Creek_6_14_07_3_35,	G	GG	CT	GG	AA	AA	TT	CT	GG	GA	GT

Oxbow_Creek_6_14_07_3_36,	G	GG	CC	GG	AA	AA	CT	CT	GG	AA	GG
Oxbow_Creek_6_14_07_3_37,	G	GG	CC	GG	AA	AA	TT	TT	GA	AA	GG
Oxbow_Creek_6_14_07_3_38,	G	GG	CC	GG	AA	AA	TT	CT	GA	AA	TT
Oxbow_Creek_6_14_07_3_39,	G	GG	CC	GG	AA	AA	TT	CT	GA	AA	GT
Oxbow_Creek_6_14_07_3_40,	G	GG	CC	GG	AA	AA	TT	CT	GA	GA	GT
Slough Creek, 3rd Med											
Slough_Creek_3rd_Med_8_10_07_04,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_10_07_06,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_10_07_20,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_10_07_21,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_10_07_22,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_01,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_02,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_03,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_05,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_07,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_08,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_09,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_10,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_11,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_12,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_13,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_14,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_15,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_16,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_17,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_19,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_25,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_27,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_3rd_Med_8_11_07_30,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough Creek Canyon, US of 1st Med											
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_101,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_102,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_103,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_104,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_105,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_106,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_107,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_109,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_112,	G	GG	CC	GG	AT	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_113,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_124,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_125,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_126,	G	GG	CT	GT	AT	CC	CC	CC	AA	GA	GT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_127,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_128,	A	AA	TT	TT	TT	CC	CC	CC	AA	GA	GG
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_129,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_130,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_131,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_132,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_133,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_134,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Canyon_US_of_1st_Med_8_20_07_135,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough Creek, Lower 1st Med											

Slough_Creek_Lower_1st_Med_7_27_07_09,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_17,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_18,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_24,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_26,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_28,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Lower_1st_Med_7_27_07_31,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_108,	G	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Slough_Creek_Upper_1st_Med_8_20_07_110,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_111,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_114,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_117,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_119,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_120,	A	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Slough_Creek_Upper_1st_Med_8_20_07_121,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Slough_Creek_Upper_1st_Med_8_20_07_123,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout Lake											
Trout_Lake_6_28_07_31,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_37,	A	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Trout_Lake_6_28_07_39,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_40,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_41,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_43,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_46,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_6_28_07_47,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_03,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_05,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_10,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_11,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_14,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_16,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_17,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_19,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_20,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_28,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_29,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_31,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_35,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_17_07_37,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_2_07_02,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_2_07_23,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_2_07_24,	A	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Trout_Lake_7_24_07_01,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_02,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_04,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_07,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_08,	A	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Trout_Lake_7_24_07_12,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_13,	A	AA	TT	TT	TT	CC	CC	CC	GG	AA	GG
Trout_Lake_7_24_07_15,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_21,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT
Trout_Lake_7_24_07_34,	G	GG	CC	GG	AA	CC	CC	CC	AA	GG	TT