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University of Helsinki and Natural Resources Institute Finland (Luke) protocol for DNA extraction and multiplex PCR genotyping of 17 microsatellites for Atlantic salmon (Salmo salar L.).

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1 Works for me dx.doi.org/10.17504/protocols.io.bp7umrnw

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ABSTRACT

In this protocol we describe laboratory methods for DNA extraction and multiplex genotyping of Atlantic salmon with microsatellite markers. The protocol has been used in several studies at the University of Helsinki and the Natural Resources Institute Finland (Luke). Publications from these studies are listed in the attachment.

ATTACHMENTS

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KEYWORDS

Atlantic salmon, Salmo salar, DNA extraction, multiplex PCR, genotyping, microsatellite, genetic variation

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MATERIALS TEXT

DNeasy Blood & Tissue Kit (250), QiagenCatalog #69506
DNeasy 96 Blood & Tissue Kit (12), QiagenCatalog #69582
Type-it Microsatellite PCR Kit (200), QiagenCatalog #206243
Type-it Microsatellite PCR Kit (2000), QiagenCatalog #206246

ABSTRACT

In this protocol we describe laboratory methods for DNA extraction and multiplex genotyping of Atlantic salmon with microsatellite markers. The protocol has been used in several studies at the University of Helsinki and the Natural Resources Institute Finland (Luke). Publications from these studies are listed in the attachment.

1 DNA is extracted from dried scales or from fins or other tissues preserved in alcohol, frozen or fresh, or from eggs. The extractions are done using Qiagen DNeasy or DNAeasy 96 Blood & Tissue Kits with the kit manual's 'Animal Tissues' protocols with a few modifications for the egg samples.

2 Usually only 1 scale, or if they are very small, 2-3 scales are used. From the tissue samples, a small piece (max. 10 mg) is cut and the pieces from samples in alcohol are kept overnight in open tubes to let the alcohol evaporate.

3 The eggs are used as whole. When needed, the volume of the ATL-buffer and proteinase K mixture is increased from the volume suggested in the kit manual so that the volume of the mixture is always at least 4 times the volume of the egg. The eggs are punctured with sharp tweezers. If the volumes of the ATL-buffer and proteinase K are increased, also the volume of AL-buffer and alcohol mixture is also increased so, that it is 2.05 times the volume of the ATL-buffer and proteinase K mixture.

4 The PCRs are done using Qiagen Type-it Microsatellite Kit. The kit manual's 'Optimized cycling protocol for multiplex PCR amplification of microsatellites' is used with the annealing temperature of 56°C, but with modifications on the reaction volumes. When the samples are fresh, or have been kept frozen or in alcohol for max. 1 year, 10 ul reactions are used. For max. 1 year old dried scales, 15 ul reactions are used. If samples are kept frozen or in alcohol for more than 1 year or if the dried scales are older than 1 year, 25 ul reactions are used. The extracted DNA is usually used without dilution. When the samples are very old, the extracted DNA is concentrated to 1/10 of the original volume by keeping the DNA in open tubes at room temperature. For the 10 ul reaction, 5 ul of kit's master mix and 3 ul of extracted DNA are used. For the 15 ul reaction, these volumes are multiplied by 1.5, and for 25 ul reactions by 2.5.

5 17 microsatellite loci are analyzed in three multiplex-reactions. The multiplexes, primer sequences, primer concentrations, dyes, loci names in references and GenBank, references for each locus and GenBank accession numbers are:

Locus	Multiplex	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
SSa14	MP1	CCTTTTGACAGATTTAGGATTTC	CAAACCAAACATACCTAAAGCC
SSA171	MP2	GAGGTCGCTGGGGTTTACTAT	TTATTATCCAAAGGGGTCAAAA
SSA197	MP2	GGGTTGAGTAGGGAGGCTTG	TGGCAGGGATTTGACATAAC
SSA202	MP2	CTTGGAATATCTAGAATATGGC	TTCATGTGTTAATGTTGCGTG
SSA289	MP2	CTTTACAAATAGACAGACT	TCATACAGTCACTATCATC
SSA407	MP1	TGTGTAGGCAGGTGTGGAC	CACTGCTGTTACTTTGGTGATTC
SSA85	MP2	AGGTGGGTCCTCCAAGCTAC	ACCCGCTCCTCACTTAATC
SSaD157	MP1	ATCGAAATGGAACTTTTGAATG	GCTTAGGGCTGAGAGAGGAATAC
SSOSL417	MP2	TTGTTCAGTGTATATGTGTCCCAT	GATCTTCACTGCCACCTTATGACC
SSOSL438	MP2	GACAACACACAACCAAGGCAC	TTATGCTAGGTCTTTATGCATTGT
SSOSL85	MP2	TGTGGATTTTTGTATTATGTTA	ATACATTTCCTCCTCATTCAGT
SSsp1605	MP1	CGCAATGGAAGTCAGTGGACTGG	CTGATTTAGCTTTTTAGTGCCCAATGC
SSsp2201	MP1	TTTAGATGGTGGGATACTGGGAGGC	CGGGAGCCCCATAACCCTACTAATAAC
SSsp2210	MP3	AAGTATTCATGCACACACATTCACTGC	CAAGACCCTTTTTCCAATGGGATTC
SSsp2216	MP1	GGCCCAGACAGATAAACAAACACGC	GCCAACAGCAGCATCTACACCCAG

Citation: Jarmo Koskiniemi, Marja-Liisa Koljonen, Tuomas Leinonen (12/02/2020). University of Helsinki and Natural Resources Institute Finland (Luke) protocol for DNA extraction and multiplex PCR genotyping of 17 microsatellites for Atlantic salmon (Salmo salar L.).. https://dx.doi.org/10.17504/protocols.io.bp7umrnw

SSsp3016	MP1	GACAGGGCTAAGTCAGGTCA	GATTCTTATATACTCTTATCCCCAT
SSspG7	MP3	CTTGGTCCCGTTCTTACGACAACC	TGCACGCTGCTTGGTCCTTG

Locus	Primer concentration (µM)	Dye	Orig. locus name	Reference	GenBank Accession no.
SSa14	0,20	PET	SSa14	R1	
SSA171	0,05	NED	Ssa171	R2	U43693.1
SSA197	0,03	VIC	Ssa197	R2	U43694.1
SSA202	0,10	PET	Ssa202	R2	U43695.1
SSA289	0,35	VIC	SSa289	R1	
SSA407	0,25	VIC	Ssa407UOS	R3	AJ402724.1
SSA85	0,06	NED	Ssa85	R2	U43692.1
SSaD157	0,20	6FAM	SSaD157	R4	AF525204.1
SSOSL417	0,10	PET	SSOSL417	R5	Z48598.1
SSOSL438	0,15	6FAM	SSOSL438	R6	Z49134.1
SSOSL85	0,15	6FAM	SSOSL85	R5	Z48596.1
SSsp1605	0,07	PET	SSsp1605	R7	AY081812.1
SSsp2201	0,07	NED	SSsp2201	R7	AY081807.1
SSsp2210	0,05	6FAM	SSsp2210	R7	AY081808.1
SSsp2216	0,04	6FAM	SSsp2216	R7	AY081811.1
SSsp3016	0,10	NED	SSsp3016	R8	AY372820.1
SSspG7	0,20	VIC	SSspG7	R7	AY081813.2

R1: McConnell SK, O'reilly P, Hamilton L, Wright JM, Bentzen P (1995). Polymorphic microsatellite loci from Atlantic salmon (Salmo salar): genetic differentiation of North American and European populations. Canadian Journal of Fisheries and Aquatic Sciences 52:1863-1872.
R2: O'reilly P, Hamilton LC, McConnell SK, Wright JM (1996). Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences. 53:2292-2298. R3: Cairney M, Taggart JB, Høyheim B (2000). Characterization of microsatellite and minisatellite loci in Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. Molecular Ecology 9:2175-2178.
R4: King TL, Eackles MS, Letcher BH (2005). Microsatellite DNA markers for the study of Atlantic salmon (Salmo salar) kinship, population structure, and mixed-fishery analyses. Mol. Ecol. Notes 5:130-132.
R5: Slettan A, Olsaker I, Lie O (1995). Atlantic salmon, Salmo salar, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Animal Genetics 26:277-285.
R6: Slettan A, Olsaker I, Lie O (1996). Polymorphic Atlantic salmon, Salmo salar L., microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. Animal Genetics 27:57-64.
R7: Paterson S, Piertney SB, Knox D, Gilbey J, Verspoor E (2004). Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (Salmo salar L.) microsatellites. Molecular Ecology Notes 4:160-162.
R8: Patterson S, Verspoor E, Knox D (2003). Unpublished.

6 Microsatellite genotypes are detected with an Applied Biosystems ABI 3130 automated DNA sequencer, and analysed with GeneMapper analysis software v5.0, with the size standard of Applied Biosystems GeneScan 500LIZ. Automatic outputs are checked for errors and corrected manually.

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