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University of Helsinki and Natural Resources Institute Finland (Luke) protocol for DNA extraction and multiplex PCR genotyping of 16 microsatellites for brown trout (Salmo trutta L.).

Jarmo Koskiniemi[1], Marja-Liisa Koljonen[2], Tuomas Leinonen[2]

[1]University of Helsinki; [2]Natural Resources Institute Finland (Luke)

1 Works for me dx.doi.org/10.17504/protocols.io.bp7ymrpw

Jarmo Koskiniemi
University of Helsinki

ABSTRACT

In this protocol we describe laboratory methods for DNA extraction and multiplex genotyping of brown trout 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

DNA extraction, multiplex PCR, genotyping, microsatellite, genetic variation, Brown trout, Salmo trutta

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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 brown trout 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 16 microsatellite loci are analyzed in two 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')
BS131	MP1	CACATCATGTTACTGCTCC	CAGCCTAATTCTGAATGAG
OneU9	MP2	CTCTCTTTGGCTCGGGGAATGTT	GCATGTTCTGACAGCCTACAGCT
SSA197	MP2	GGGTTGAGTAGGGAGGCTTG	TGGCAGGGATTTGACATAAC
SSA289	MP2	CTTTACAAATAGACAGACT	TCATACAGTCACTATCATC
SSA407	MP1	TGTGTAGGCAGGTGTGGAC	CACTGCTGTTACTTTGGTGATTC
SSA85	MP1	AGGTGGGTCCTCCAAGCTAC	ACCCGCTCCTCACTTAATC
SSOSL311	MP1	TAGATAATGGAGGAACTGCATTCT	CATGCTTCATAAGAAAAAGATTGT
SSOSL417	MP1	TTGTTCAGTGTATATGTGTCCCAT	GATCTTCACTGCCACCTTATGACC
SSOSL438	MP2	GACAACACACAACCAAGGCAC	TTATGCTAGGTCTTTATGCATTGT
SSsp1605	MP2	CGCAATGGAAGTCAGTGGACTGG	CTGATTTAGCTTTTTAGTGCCCAATGC
SSsp2201	MP2	TTTAGATGGTGGGATACTGGGAGGC	CGGGAGCCCCATAACCCTACTAATAAC
Str15INRA	MP1	TGCAGGCAGACGGATCAGGC	AATCCTCTACGTAAGGGATTTGC
Str60INRA	MP1	CGGTGTGCTTGTCAGGTTTC	GTCAAGTCAGCAAGCCTCAC
Str73INRA	MP2	CCTGGAGATCCTCCAGCAGGA	CTATTCTGCTTGTAACTAGACCTA
Str85INRA	MP2	GGAAGGAAGGGAGAAAGGT	GGAAAATCAATACTAACAA
Strutta58	MP1	AACAATGACTTTCTCTGAC	AAGGACTTGAAGGACGAC

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 16 microsatellites for brown trout (Salmo trutta L.).. https://dx.doi.org/10.17504/protocols.io.bp7ymrpw

Locus	Primer concentration (µM)	Dye	Orig. locus name	Reference	GenBank Accession no.
BS131	0,10	VIC	BS131	R1	
OneU9	0,06	VIC	Oneæ9	R2	U56709.1
SSA197	0,02	NED	Ssa197	R3	U43694.1
SSA289	0,50	PET	SSa289	R4	
SSA407	0,15	NED	Ssa407UOS	R5	AJ402724.1
SSA85	0,02	VIC	Ssa85	R3	U43692.1
SSOSL311	0,15	NED	SSOSL311	R6	Z48597.1
SSOSL417	0,08	PET	SSOSL417	R6	Z48598.1
SSOSL438	0,15	VIC	SSOSL438	R7	Z49134.1
SSsp1605	0,04	NED	SSsp1605	R8	AY081812.1
SSsp2201	0,03	6FAM	SSsp2201	R8	AY081807.1
Str15INRA	0,10	6FAM	15	R9	AB001058.1
Str60INRA	0,08	PET	60	R9	AB001057.1
Str73INRA	0,08	VIC	73	R9	AB001056.1
Str85INRA	0,60	6FAM	MST-85	R10	AB001059.1
Strutta58	0,10	6FAM	Strutta-58	R11	U60223.1

R1: Estoup A, Rousset F, Michalakis Y, Cornuet J-M, Adriamanga M, Guyomard R (1998). Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (Salmo trutta). Molecular Ecology 7:339-353.
R2: Scribner KT, Gust J, Fields RL (1996). Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences. 53:833-841.
R3: 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.
R4: 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.
R5: Cairney M, Taggart JB, H›yheim 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.
R6: Slettan A, Olsaker I, Lie O (1995). Atlantic salmon, Salmo salar, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Animal Genetics 26:277-285.
R7: 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.
R8: 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.
R9: Estoup A, Presa P, Krieg F, Vaiman D, Guyomard R (1993). (CT) and (GT) microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). Heredity 71:488-496.
R10: Presa P, Guyomard R (1996). Conservation of microsatellites in three species of salmonids. Journal of Fish Biology 49:1326-1329.
R11: Poteaux C, Bonhomme F, Berrebi P (1999). Microsatellite polymorphism and genetic impact of restocking in Mediterranean brown trout (Salmo trutta L.). Heredity 82:645-653.

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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