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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 pikeperch (Sander lucioperca L.).

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1 Works for me dx.doi.org/10.17504/protocols.io.bp76mrre

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ABSTRACT

In this protocol we describe laboratory methods for DNA extraction and multiplex genotyping of pikeperch 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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PROTOCOL CITATION

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KEYWORDS

DNA extraction, multiplex PCR, genotyping, microsatellite, genetic variation, Pikeperch, Sander lucioperca

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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 pikeperch 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. 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 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.

4 17 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')
MSL_1	MP2	TGTTTGTCAGCGTCAAGAGG	TTCCGCTCCAACATATCACA
MSL_2	MP2	TTTTCACACCGTGCATGACT	ACCCTCAGCCTCTGTGTACG
MSL_3	MP2	CCGGCATCCATACACCTTAC	CACACCTGTGTCTGCCTAACA
MSL_4	MP2	TCAAGACCCCAGAACCAATC	CAGACAGCTAAGAGAACAACAGG
MSL_6	MP2	GTCGTCATCGTCAGCACAGT	ACTACACGGGACGCTGGA
MSL_7	MP2	CACACAGCAGCATGTGACAA	GGCACGGAGGTAGAATGGTA
MSL_8	MP2	AACACCTTCCTTCGTCCATC	CGTGTTTGCCTCACACAAAG
MSL_9	MP2	GCATCACTTGCGTCACTTTC	GCAGTCAGTGCTTGAAGTGG
Pfla_L3	MP1	GCCGAATGTGATTGAATG	CGCTAAAGCCAACTTAATG
Pfla_L8	MP1	GCCTTATTGTGTGACTTATCG	GGATCTTTCACTTTTTCTTTCAG
Pfla_L9	MP1	GTTAGTGTGAAAGAAGCATCTGC	TGGGAAATGTGGTCAGCGGC
Svi_18	MP1	GATCTGTAAACTCCAGCGTG	CTTAAGCTGCTCAGCATCCAGG
Svi_33	MP1	CAGGACTGCTGTGTATAGACTTG	GATATAGCTTTCTGCTGGGGTC
Svi_4	MP1	ACAAATGCGGGCTGCTGTTC	GATCGCGGCACAGATGTATTG
Svi_6	MP1	CATATTATGTAGAGTGCAGACCC	TGAGCTTCACCTCATATTCC
Svi_L7	MP1	GATGTGCATACATTTACTCC	GCTTTAATCTGCTGAGAAC
Svi_L8	MP1	GCTTATACGTCGTTCTTATG	ATGGAGAAGCAAGTTGAG

Locus	Primer concentration (µM)	Dye	Orig. locus name	Reference	GenBank Accession no.
MSL_1	0,20	VIC	MSL-1	R1	EF694018.1
MSL_2	0,10	NED	MSL-2	R1	EF694019.1
MSL_3	0,05	NED	MSL-3	R1	EF694020.1

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MSL_4	0,10	6FAM	MSL-4	R1	EF694021.1
MSL_6	0,10	PET	MSL-6	R1	EF694023.1
MSL_7	0,05	VIC	MSL-7	R1	EF694024.1
MSL_8	0,05	VIC	MSL-8	R1	EF694025.1
MSL_9	0,20	6FAM	MSL-9	R1	EF694026.1
Pfla_L3	0,40	6FAM	Pfla L3	R2	AF211828.1
Pfla_L8	1,00	PET	Pfla L8	R2	AF211833.1
Pfla_L9	0,10	NED	Pfla L9	R2	AF211834.1
Svi_18	0,20	6FAM	Svi18	R3	G36964.1
Svi_33	0,05	6FAM	Svi33	R3	G36967.1
Svi_4	0,10	NED	Svi4	R3	G36961.1
Svi_6	0,10	VIC	Svi6	R3	G36962.1
Svi_L7	0,20	VIC	Svi L7	R3	AF144740.1
Svi_L8	1,00	PET	Svi L8	R3	AF144741.1

R1: Kohlmann K, Kersten P (2008). Isolation and characterization of nine microsatellite loci from the pike-perch, Sander lucioperca (Linnaeus, 1758). Molecular Ecology Resources 8:1085-1087.
R2: Borer SO, Miller LM, Kapuscinski AR (1999). Microsatellites in walleye Stizostedion vitreum. Molecular Ecology 8:336-338.
R3: Wirth T, Saint-Laurent R, Bernatchez L (1999). Isolation and characterization of microsatellite loci in the walleye (Stizostedion vitreum), and cross-species amplification within the family Percidae. Molecular Ecology 8:1960-1962.

5 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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