Dec 02, 2020

University of Helsinki and Natural Resources Institute Finland (Luke) protocol for DNA extraction and multiplex PCR genotyping of 16 microsatellites for whitefish (Coregonus lavaretus 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.bp73mrqn

Jarmo Koskiniemi
University of Helsinki

ABSTRACT

In this protocol we describe laboratory methods for DNA extraction and multiplex genotyping of whitefish 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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DOI

dx.doi.org/10.17504/protocols.io.bp73mrqn

PROTOCOL CITATION

Jarmo Koskiniemi, Marja-Liisa Koljonen, Tuomas Leinonen 2020. University of Helsinki and Natural Resources Institute Finland (Luke) protocol for DNA extraction and multiplex PCR genotyping of 16 microsatellites for whitefish (Coregonus lavaretus L.).. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp73mrqn

KEYWORDS

DNA extraction, multiplex PCR, genotyping, microsatellite, genetic variation, Whitefish, Coregonus lavaretus

CREATED

Nov 30, 2020

LAST MODIFIED

Dec 02, 2020

PROTOCOL INTEGER ID

45019

ATTACHMENTS

Publications_whitefish.doc
x

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 whitefish 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 16 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')
Bwf_2	MP1	CGGATACATCGGCAACCTCTG	AGACAGTCCCCAATGAGAAAA
C2_157	MP1	CTTAGATGATGGCTTGGCTCC	GGTGCAATCACTCTTACAACACC
ClaTet1	MP2	GAGCCCATCATCACTGAGAAAGA	CTGCTACCCACAAACCCCTG
ClaTet10	MP3	GCCTCACACAGTCGCTTTC	GCCGAATGGTGGACAGA
ClaTet13	MP2	TGATACATTTTTTGGCCTTTC	GGACCTGCCCTATCTGTC
ClaTet15	MP2	CCGAAATGGTCATAACTGAA	GTGGTCCTCTGTAGCCCA
ClaTet18	MP3	GATGTTTTACACCGTGGTGCC	GTGGGGGACCTGGGTT
ClaTet3	MP2	TGCTCCATCAGTCCTGCA	AAAGTGAGTCAAGCGTGAGAAGCA
ClaTet6	MP1	GAATCGGCATCTCCTGAGTCA	GCTTGGGGCATAATAACCACC
COCL_004	MP2	TGGTGTAATGGCTTTTCCTG	GGGAGCAACATTGGACTCTC
COCL_008	MP3	GCTGGAGCCACATGACATTA	ATGTTTTTCCATTGCCCAGA
COCL_010	MP1	CAGTGGAGTTAATGAGTGCC	GTGGAAATTGAATACTGCGG
COCL_018	MP3	AACAAACTAAAACATCCCAAGTC	TTAGATTGGGGCCTACCTTG
COCL_045	MP3	GAGTGACAGCAGGGAGCAG	GGCTCGGTTGAAAGTTGAGA
COCL_049	MP1	AGCCAGTTGGAGGCTATTTG	AGGGCTGCTGTTGAAGTCAT
COCL_061	MP1	CTCATGAGTAACATGATGCTTC	GATCTTTACTGTCTGATTTTGTG

Locus	Primer concentration (µM)	Dye	Orig. locus name	Reference	GenBank Accession no.
Bwf_2	0,02	6FAM	BWF2	R1	
C2_157	0,05	VIC	Cisco-157	R2	
ClaTet1	0,02	PET	ClaTet1	R3	EU311794.1

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 whitefish (Coregonus lavaretus L.).. https://dx.doi.org/10.17504/protocols.io.bp73mrqn

ClaTet10	0,03	6FAM	ClaTet10	R3	EU311803.1
ClaTet13	0,03	VIC	ClaTet13	R3	EU311806.1
ClaTet15	0,02	NED	ClaTet15	R3	EU311808.1
ClaTet18	0,03	PET	ClaTet18	R3	EU311811.1
ClaTet3	0,03	6FAM	ClaTet3	R3	EU311796.1
ClaTet6	0,03	NED	ClaTet6	R3	EU311799.1
COCL_004	0,02	6FAM	Cocl-Lav4	R4	AY453197.1
COCL_008	0,15	NED	Cocl-Lav8	R4	AY453200.1
COCL_010	0,03	PET	Cocl-Lav10	R4	AY453201.1
COCL_018	0,02	VIC	Cocl-Lav18	R4	AY453203.1
COCL_045	0,03	VIC	Cocl-Lav45	R4	AY453225.1
COCL_049	0,05	PET	Cocl-Lav49	R4	AY453212.1
COCL_061	0,10	6FAM	Cocl-Lav61	R4	AY453214.1

R1: Patton JC, Gallaway BJ, Fechhelm RG, Cronin MA (1997). Genetic variation of microsatellite and mitochondrial DNA markers in broad whitefish (Coregonus nasus) in the Colville and Sagavanirktok rivers in northern Alaska. Canadian Journal of Fisheries and Aquatic Sciences. 54:1548-1556.
R2: Turgeon J, Estoup A, Bernatchez L (1999). Species Flock in the North American Great Lakes: Molecular Ecology of Lake Nipigon Ciscoes (Teleostei: Coregonidae: Coregonus). Evolution 53:1857-1871.
R3: Winkler K, Weiss S (2008). Eighteen new tetranucleotide microsatellite DNA markers for Coregonus lavaretus cloned from an alpine lake population. Molecular Ecology Resources 8:1055-1058.
R4: Rogers S, Marchand M-H, Bernatchez L (2004). Isolation, characterization and cross-salmonid amplification of 31 microsatellite loci in the lake whitefish (Coregonus clupeaformis , Mitchill). Molecular Ecology Notes 4:89-92.

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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