

Materials List for:

Improved Polymerase Chain Reaction-restriction Fragment Length Polymorphism Genotyping of Toxic Pufferfish by Liquid Chromatography/Mass Spectrometry

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Materials

Name	Company	Catalog Number	Comments
DNeasy Blood & Tissue Kit (50)	Qiagen	69504	DNA extraction kit
Proteinase K	Qiagen	19131	
Ethanol (99.5+ vol%)	Wako	054-07225	For dilution of wash buffers
TE buffer (pH 8.0)	Wako	310-90023	For dilution of DNA sample
PCR primer	Fasmac	NA	Purified with reverse-phase cartridge column by the supplier
Template DNA	Eurofins Genomics	NA	Purified by HPLC by the supplier
Ultrapurified water	NA	NA	Generated with a Milli-Q Direct water purification system (Merck Millipore), used for sample preparation
Detergent Free 5x Phusion HF Buffer	Thermo Fisher	F-520L	Use instead of the provided buffer of DNA polymerase
Pfu-X DNA polymerase	Jena Bioscience	PCR-207S	
dNTP mix (20 mM each)	Jena Bioscience	NA	Supplied with the DNA polymerase
10× Universal buffer M	Takara Bio	NA	Containing 100 mM Tris-HCl (pH 7.5), 100 mM MgCl ₂ , 10 mM dithiothreitol and 500 mM NaCl
Dra I	Thermo Fisher	FD0224	Restriction enzyme
Msp I	Thermo Fisher	FD0544	Restriction enzyme
1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)	Fluka	42060-50ML	Eluent additive for LC-MS grade.
Triethylamine (TEA)	Fluka	65897-50ML	Eluent additive for LC-MS grade.
Trifluoroacetic acid	Sigma-Aldrich	T6508	For preparation of calibrant.
Sodium hydroxide (1.0 M)	Fluka	72082	Dilute to 10 mM with ultrapurified water and use for titration of trifluoroacetic acid.
Acetonitrile	Fluka	34967	For preparation of calibrant, LC/MS grade.
Methanol	Kanto	25185-76	For mobile phase, LC/MS grade.
Water	Thermo Fisher	W6-1	For mobile phase, LC/MS grade.
Microcentrifuge tube (0.5 ml)	Eppendorf	0030123301	PCR clean grade
Microcentrifuge tube (1.5 ml)	Eppendorf	0030123328	PCR clean grade
UVette	Eppendorf	0030106300	Disposable UV cuvette.
Gel Green	Biotium	41004	Fluorescent DNA stain
Cosmospin filter G (0.2 μm)	Nakalai Tesque	06549-44	Made of hydrophilic polytetrafluoroethylene (PTFE)

			membrane. Any centrifugal filter unit (pore size, 0.2–0.5 µm) made of hydrophilic PTFE or another low-binding membrane is applicable.
300 µl PP screw vial	American Chromatography Supplies	V0309P-1232	
Preassembled screw cap and septa	Finneran	5395-09R	
Monobis C18 analytical column (2.0×50 mm, mesopore size = 30 nm)	Kyoto Monotech	2050H30ODS	Outside Japan, available for purchase from GL Sciences via its distributors. (http://www.glsciences.com/distributors/)
Monobis C18 guard column	Kyoto Monotech	GCSET-ODS3210	Holder included.
Cadenza CW-C18 (3.0×250 mm)	Imtakt	CW036	C ₁₈ -bonded particulate silica column
ProSwift RP-4H (1.0×250 mm)	Thermo Fisher	066640	Poly(styrene-divinylbenzene) monolith column
Themo Mixer C	Eppendorf	5382 000.023	For digestion of fish tissues.
Spectrophotometer	Shimadzu	UV-3150	Quantification of DNA concentration.
Thermal cycler	Bio-Rad	T100	
Portable refrigerator	Twinbird	D-CUBE	For aqueous mobile phase to avoid evaporation of HFIP. This can be replaced by an ice box.
Ultimate 3000 liquid chromatograph	Thermo Fisher	NA	
Q Exactive mass spectrometer	Thermo Fisher	NA	Fourier transform mass spectrometer equipped with the modified Kingdon trap analyzer.
Protein deconvolution 3.0	Thermo Fisher	NA	Use version 3.0 or higher having an isotopic patten model of nucleotide.