Agilent DNA Fish ID solution

Discrimination of Sturgeon and Related Species by PCR-RFLP Using the 2100 Bioanalyzer System

FOOD AUTHENTICITY

A quick, robust and easy to use protocol to identify the species based on a well accepted PCR-RFLP method allows analysis from sample (meat, fin clip or roe) to result in one working day and yields good discrimination results

The global demand for seafood has grown considerably. Limitations of the resources and the potential for increased profits lead to the problem of substitution and mislabeling for a substantial part of the market. In order to monitor shipments for customs purposes as well as in supply chain management and to protect the consumer, efficient, and cost-effective tests to identify the species are needed. In addition, regulations to protect endangered species and to fight illegal, unregulated and unreported fishing drive the need for authenticity testing.

DNA based testing methods allow sensitive detection and identification from almost all but the most heavily processed food samples.

The Agilent DNA Fish ID ensemble provides all reagents and enzymes to complete a PCR-RFLP method for a *Cyt*b PCR target sequence and analysis of restriction fragment patterns on the Agilent 2100 Bioanalyzer to identify fish species based on this mitochondrial sequence. The availability of commercial screening solutions allows for more reliable and robust test results through well-matched components and facilitates testing for screening purposes by the use of mastermix formulations and streamlined protocols. The Agilent DNA Fish ID solution was evaluated for the purpose of identifying the species from fish eggs in caviar shipments. To obtain the best possible results using roe and to accommodate the high homology between sturgeon species, the protocol was modified for enhanced identification.

Species	Common name
Acipenser gueldenstaedtii	Danube sturgeon
Acipenser medirostris	Green sturgeon
Acipenser nudiventris	Fringebarbel sturgeon
Acipenser ruthenus	Sterlet sturgeon
Acipenser schrenckii	Amur sturgeon
Acipenser stellatus	Starry sturgeon
Acipenser transmontanus	White sturgeon
Huso dauricus	Kaluga
Huso huso	Beluga
Polyodon spathula	Mississippi paddlefish
Oncorhynchus keta	Chum salmon

Figure 1. Species tested in the study.

Key Benefits

- Agilent 2100 Bioanalyzer has the widest range of assays available on the market covering applications for RNA, DNA or protein
- Fast, reproducible and robust method replacing gel electrophoresis
- Agilent method using convenient mastermix format reagents will have you identifying your fish within hours, no fish standard or guesswork required.
- Flexible modular system: In-house accredited methods can replace individual building blocks of the Fish ID solution
- Fifty fish species in the Agilent library, and you can easily add your own samples to the library
- Overlay to the Expert software allows rapid identification of restriction fragments and close matches.
- Agilent method is now optimized for testing roe (caviar)
- Replacing one of the supplied enzymes leads to improved discrimination abilities for sturgeon species

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Table 2a: Grouping of Samples According to Restriction Patterns Observed. Samples Were Grouped According to the Number of Fragments Observed With Each Restriction Enzyme. Subgrouping Was Added if There Was a Clear Difference (At Least Three Standard Deviations) in the Size of At Least One Fragment

Group	Species	*Csp6* I		*Dde* I			*Hae* III			*Nla* III		
A	*Acipenser gueldenstaedtii*	325	122	462			180	148		291	193	
	Acipenser medirostris	325	122	458			180	150		293	192	
	Huso dauricus	326	122	463			180	151		294	193	
B_1	*Acipenser transmontanus*	446		455			179	149		293	192	
	Acipenser schrenckii	445		457			180	149		292	192	
B_2	*Acipenser stellatus*	489		458			180	149		291	193	
C	*Huso huso*	323	120	455			179	150	77	291	191	
D	*Polyodon spathula*	481		480			323	150		291	165	
E	*Oncorhynchus keta*	389	86	359	352	121	433			280	192	
F_1	*Acipenser nudiventris*	325	93	462			180	149		293	133	52
F_2	*Acipenser ruthenus*	349	94	455			178	149		292	134	62
G	*Salmo salar*	397	85	362	356	121	330	107	46	460		

Table 2b. Standard Deviations of Fragment Sizes. Variability of Fragment Sizing is Between 0.5% to 2% of the Average Size

Group	Species	*Csp6* I		*Dde* I			*Hae* III			*Nla* III		
A	*Acipenser gueldenstaedtii*	1.2	1.2	2.5			0.6	0.6		2.3	2.6	
	Acipenser medirostris	1.4	0.9	3.7			1.1	1.3		2.4	1.5	
	Huso dauricus	1.4	2.1	2.8			2.8	2.1		2.1	2.1	
B_1	*Acipenser transmontanus*	3.8		3.3			1.3	1.3		1.2	0.9	
	Acipenser schrenckii	2.6		3.5			1.0	0.6		0.6	0.6	
B_2	*Acipenser stellatus*	5.3		4.5			1.7	1.4		1.0	1.7	
C	*Huso huso*	2.1	1.2	5.8			1.0	0.6	0.5	2.9	2.6	
D	*Polyodon spathula*	7.7		8.9			5.4	2.2		3.2	2.4	
E	*Oncorhynchus keta*	2.2	0.6	2.6	2.6	1.0	6.9			4.1	1.7	
F_1	*Acipenser nudiventris*	2.0	0.6	3.2			0.6	0.6		2.0	0.6	0.5
F_2	*Acipenser ruthenus*	1.8	1.5	2.6			1.0	1.0		0.5	0.6	0.6
G	*Salmo salar*	2.5	1.1	2.7	2.6	1.1	2.2	0.8	1.3	4.3		

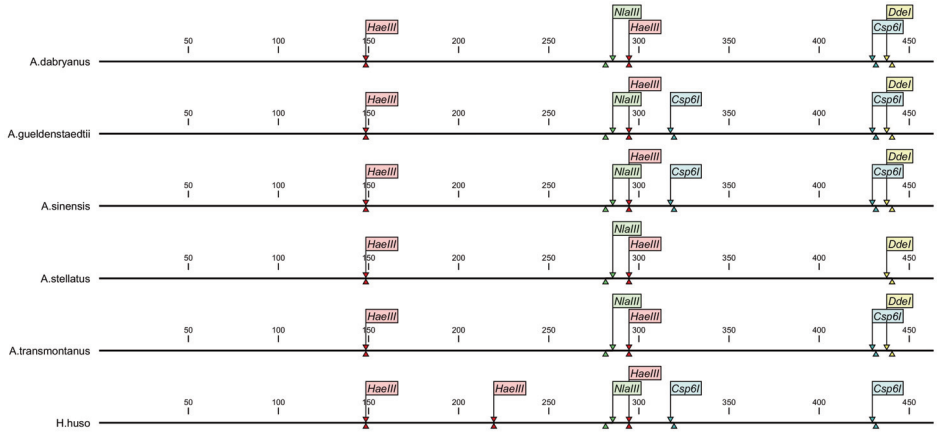

Fig. 1. Sequence analysis of Cytb target region in sturgeon species. The target region for the primers supplied with the kit was analyzed using CLC Sequence Viewer (CLC bio, Denmark). The picture shows the cutting sites for Csp6 I, Dde I, Hae III and Nla III for Acipenser dabryanus (derived from NC_005451), Acipenser gueldenstaedtii (derived from NC_012576), Acipenser sinensis (derived from NC_012646), Acipenser stellatus (derived from NC_005795), Acipenser transmontanus (derived from NC_004743) and Huso huso (derived from AY_442351).

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