Molecular Characterization of Megalocytiviruses from Diseased Fishes in Korean Aquatic Farms from 2013 to 2017

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2013년~2017년 국내 양식장 어류에서 검출된 megalocytiviruses의 분자생물학적 특성

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Abstract

We identified structural and non-structural gene regions encoding major capsid protein (MCP) and DNA polymerase (DPOL) of megalocytiviruses collected from infected cultured fishes in RBIVD outbreak farms in 2013-2017 in Korea. With the two PCRs using 1-F/1-R and 4-F/4-R primer sets of the Manual of Diagnosis Tests for Aquatic Animals of the World Organization for Animal Health (OIE), amplicons were generated from the spleen and kidney tissue from approxmately ~30 fishes, including rock bream (Oplegnathus fasciatus), red sea bream (Pagrus major), and rock fish (Sebastes schlegeli), from 15 outbreak regions in the aquatic farms of the South Sea and Jeju Island. In phylogenetic analysis, complete MCP and partial DPOL genes belonged to RSIV type-subgroup2. Interestingly, these genes formed a cluster indicating closer relatedness to GSIV-K1, RIE12-1, and RBIV-C1, which were previously isolated from Japan and China, than with RBIV-KOR-TY1 isolated from Korea. However, the nucleotide sequence identities of the MCP and DPOL genes of these viruses were high, at >99.8% and >99.7%, respectively, compared with RBIV-KOR-TY1. Comparisons of nucleotide and amino acid sequences showed minimal differences between the obtained strains in the MCP gene, however, one or two nucleotide sequences substitutions of the DPOL gene were detected in nine strains, including a silent mutation detected in five strains. These findings suggest a slow rate of evolution of megalocytiviruses in this region, but the potential for mutations and new pathogenic strains warrants continuous surveillance.

Key words : Megalocytivirus, RSIV, RBIV, MCP, DNA polymerase, Phylogenetic tree

	I. Introduction						egalo	cytivirus	are	icosał	nedral	viruses	that	con	tain
						a	doub	ole-stran	led	DNA	genon	ne (Rin	nmer	et	al.,
Members	of	the	family	Iridoviridae	genus	20	016; \$	Subrama	niam	et al.	, 2012	; Whitt	ington	et	al.,

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2010). This genus is the major agent causing disease in >50 freshwater and marine fish species, resulting in significant economic losses to the aquaculture industry in East and Southeast Asia (Song et al., 2008; Jung et al., 2017a; Inouye et al., 1992; Shi et al., 2004).

The Iridoviridae comprises the following five genera: Megalocytivirus, Ranavirus, Lymophocystivirus, Iridovirus, and Choloriridovirus (Chinchar et al., 2005). Megalocytiviruses have been divided into three types based on phylogenetic analysis of the major capsid protein (MCP) gene: the red sea bream iridovirus (RSIV) type cluster, including RSIV subgroup1 and rock bream iridovirus (RBIV) subgroup2, reported as the major epizootic viral strains affecting aquaculture worldwide (Kim et al. 2018, Jung and Oh, 2000; Zhang et al., 2012. 2013); infectious spleen and kidney necrosis virus (ISKNV) type that generally causes diseases in freshwater fish species (He et al. 2000); and turbot reddish body iridovirus (TRBIV) type (Shi et al., 2004), which infects flatfish species.

Megalocytiviruses mainly occur in summer when the water temperature reaches 23°C and above (Jung et al., 2015, 2016, 2017a, 2017b; Jun et al., 2009). Diseased fish show signs of lethargy, exhibit severe anemia and petechiae in the gills, and have enlarged spleens. Histopathology is characterized by development of enlarged cells with basophilic inclusion bodies in the spleen (Do et al., 2004; Do al., 2005; OIE 2018). In et Korea, megalocytiviruses have caused massive damage to aquacultures of various fish species such as rock bream (Oplegnathus fasciatus), rockfish (Sebastes schlegelii), red sea bream (Pagrus major), and olive flounder (Paralichthys olivaceus) (Jeong et al., 2003, 2006; Jin et al., 2018; Won et al., 2013; Kim et al., 2018).

Megalocytiviruses are notable for their variability in infecting a broad host range. Genomic analysis of RSIVs can be useful in identifying the introduction of exotic viruses due to the importation of live fish or natural fish migration from the sea of other countries. Thus. gaining a better understanding of the genetic diversity of megalocytiviruses would provide critical information of their biology and evolution to guide strategies for controlling these diseases. To contribute such information, in this study, we investigated the genetic relationships of the MCP and DNA polymerase (DPOL) genes of megalocytiviruses, especially RSIV type-subgroup2, isolated from diseased aquacultured fish in Korea from 2013 to 2017 and compared these findings with previous reports.

II. Materials and Methods

1. Samples

Moribund or dead fishes showing typical clinical symptoms of iridoviral disease were collected from 15 domestic aquatic farms by surveillance, disease identification, or an investigation into damage of the National Institute of Fisheries Science (NIFS) institutions specializing in the disease and identification of aquatic organism diseases from 2013 to 2017. The aquatic disease control division of the NIFS confirmed the RBIVD outbreak by verification with additional experiments. Specifically, rock bream was sampled in Tongyeong (four farms), Yeosu (five farms), Wando (one farm), Sacheon (one farm), Namhae (one farm), and Seogwipo of Jeju Island (one farm) and red sea bream and rock fish were sampled from one farm each at Tongyeong <Table 1>. Pooled samples

including spleen and kidney tissue from approximately ~ 30 fishes collected at each farm were used for virus detection.

2. Total nucleic acid extraction and megalocytivirus identification

Total nucleic acids (50 μ L) were isolated from 200 μ L of the tissue homogenate in phosphate buffered saline (pH 7.4, 1:10 w/v) using a Viral NA Extraction Kit on a SPRI-TETM Nucleic Acid Extractor (Beckman Coulter Inc., USA) according to the manufacturer's instructions. Polymerase chain reactions (PCRs) were performed in a final volume of 25 μ L with 2 μ L of DNA template using Takara EX TaqTM (Takara) in a Mastercycler (Eppendorf, Germany). The primer sets 1-F/1-R and 4-F/4-R (Kurita et al. 1998) were used for megalocytivirus detection, and reaction conditions were determined according to the Manual of Diagnostic Tests for Aquatic Animals of the World Organization for Animal Health (OIE, 2018). The PCR conditions for sequence analysis were as follows: pre-denaturation at 94°C for 5 min; 30 cycles at 94°C for 30 s, 58°C for 60s, and 72°C for 60 s; followed by an extension period at 72°C for 5 min. The amplified PCR products were subjected to QIAxcel Advanced System (Qiagen). For identification of megalocytivirus, nucleotide sequences were determined by the ABI 3500x1 genetic analyzer (Thermo Fisher Scientific, UK)using the PCR products purified from a PCR Purification Kit (GeneAll, Korea).

<Table 1>. Samples infected with megalocytivirus used in this study

Viral strains	Year of occurence	Geographic origin	Host species
ADC-RSIV2013-24	2013	Yeosu	Rock bream Oplegnathus fasciatus
ADC-RSIV2014-35	2014	Seogwipo	Rock bream Oplegnathus fasciatus
ADC-RSIV2014-44	2014	Tongyeong	Rock fish Sebastes schlegelii
ADC-RSIV2014-46	2014	Tongyeong	Rock bream Oplegnathus fasciatus
ADC-RSIV2015-24	2015	Yeosu	Rock bream Oplegnathus fasciatus
ADC-RSIV2016-2	2016	Yeosu	Rock bream Oplegnathus fasciatus
ADC-RSIV2016-29	2016	Wando	Rock bream Oplegnathus fasciatus
ADC-RSIV2016-38	2016	Yeosu	Rock bream Oplegnathus fasciatus
ADC-RSIV2016-41	2016	Tongyeong	Rock bream Oplegnathus fasciatus
ADC-RSIV2016-56	2016	Tongyeong	Rock bream Oplegnathus fasciatus
ADC-RSIV2017-18	2017	Namhae	Rock bream Oplegnathus fasciatus
ADC-RSIV2017-22	2017	Sacheon	Rock bream Oplegnathus fasciatus
ADC-RSIV2017-27	2017	Tongyeong	Red sea bream Pagrus major
ADC-RSIV2017-49	2017	Tongyeong	Rock bream Oplegnathus fasciatus
ADC-RSIV2017-68	2017	Yeosu	Rock bream Oplegnathus fasciatus

<table< th=""><th>2>.</th><th>Primers</th><th>used</th><th>in</th><th>this</th><th>study</th></table<>	2>.	Primers	used	in	this	study

Primer	Sequence (5' to 3')	Expected size (object)	Reference
1-F	CTCAAACACTCTGGCTCATC	570 bp (Detection)	Kurita et al. 1998
1-R	GCACCAACACATCTCCTATC	-	
4-F	CGGGGGCAATGACGACTACA	568 bp (Detection)	Kurita et al. 1998
4-R	CCGCCTGTGCCTTTTCTGGA	-	
M1F	GAGAGACCCCAACACGAC	1828 bp (MCP gene analysis)	He et al. 2001
M1R	ACCTGGTGGCTCCAGTGC		
DPO-F	CTTCAGCTTCAGGTTCACGCATGC	1264 bp (DPOL gene analysis)	Our previous study
DPO-R	GTGATGAGTCTGCCATTGTTTTTATATAGCGGG		

3. Cloning and sequencing of MCP and DPOL genes

For sequence analysis of MCP and DPOL genes, PCR was performed with the M1F/M1R (He et al., 2001) and DPO-F/DPO-R primer sets targeting the full-length sequence of the MCP gene and the partial sequence of the DPOL gene using extracted DNA samples as the template <Table 2>. The PCR conditions for sequence analysis were pre-denaturation at 95°C for 5 min; 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 90 s; followed by an extension period at 72°C for 7 min. The amplified PCR products were cloned to a TA vector (Invitrogen) and the nucleotide sequences were determined using the ABI 3500xl genetic analyzer (Thermo Fisher Scientific, UK).

Multiple alignment and phylogenetic analysis of the MCP and DPOL genes

The sequences of both viral genome segments were compared by gene alignment using BioEdit software (ver. 7.0.6 Department of Microbiology, North Carolina State University, Raleigh, NC, USA) to determine the identity of the genotype sequences.

A phylogenetic tree was constructed using ClustalW software (http://www.ebi.ac.uk/clustalW) based on neighbor-joining analysis with MEGA4 (ver. 4.1) (Center for Evolutionary Functional



[Fig. 1]. PCR detection test for megalocytiviruses from diseased fishes of Korean aquatic farms from 2013 to 2017 based on the QIAxcel capillary electrophoresis system. Electrophoresis after PCR using 1-F/1-R (A) and 4-F/4-R primer sets (B). Lane 1, ADC-RSIV2016-2; lane 2, ADC-RSIV2017-18; lane 3, ADC-RSIV2017-22; lane 4, ADC-RSIV2013-24; lane 5, ADC-RSIV2015-24; lane 6, ADC-RSIV2017-27; lane 7, ADC-RSIV2016-29; lane 8, ADC-RSIV2014-35; lane 9. ADC-RSIV2016-38; lane 10. ADC-RSIV2016-41; lane 11, ADC-RSIV2014-44; lane 12. ADC-RSIV2014-46: lane 13, ADC-RSIV2017-49; lane 14, ADC-RSIV2017-68; 15. lane ADC-RSIV2016-56; N, negative control; P, positive control; M, 15 bp \sim 3 kb marker.

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Nucleotide

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)
	(1) ADC-RSIV2016-2		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(2) ADC-RSIV2017-18	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(3) ADC-RSIV2017-22	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(4) ADC-RSIV2013-24	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(5) ADC-RSIV2015-24	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(6) ADC-BSIV2017-27	100.0	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
	(7) ADC-BSIV2016-29	100.0	100.0	100.0	100 0	100.0	100.0		100 0	100.0	100 0	100 0	100.0	100.0	100.0	99.9	100.0	100 0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
~	(8) ADC-BSIV2016-41	100.0	100.0	100.0	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
4	(9) ADC-BSIV2014-44	100.0	100.0	100.0	100 0	100.0	100.0	100 0	100 0		100 0	100 0	100.0	100.0	100.0	99.9	100.0	100 0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
₫.	(10) ADC-BSIV2014-46	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100 0	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
ă	(11) ADC-BSIV2017-68	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	00.0	100.0	100.0	100.0	100.0	00.5	07 Q	07 Q	95.1	95.1	94.6
0	(12) ADC-BSIV2016-38	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100 0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	100.0	99.5	97.9	97.9	95.1	95.1	94.6
2	(12) ADC-RSIV2017-40	00.8	00.8	00.8	00.8	00.8	00.8	00.8	00.8	00.8	00.8	00.8	00.8	100.0	00.8	00.8	00.0	00.0	00.0	00.0	00.0	07.8	07.0	05.0	05.0	04.5
Ξ.	(14) ADC_PSIV2016_56	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	33.0	00.0	100.0	100.0	100.0	100.0	00.5	07.0	07.0	05.1	05.1	04.6
	(15) ADC-RSIV2014-35	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	00.0	100.0	100.0	100.0	100.0	00.5	07.0	07.0	05.1	05.1	04.6
	(16) AD017456	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	00.8	100.0	100.0	100.0	100.0	00.5	07.0	07.0	05.1	05.1	04.6
	(10) AT017450 (17) KT804738	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	00.0	100.0	100.0	100.0	100.0	00.5	07.0	07.0	05.1	05.1	04.6
	(17) K1004730	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.0 00.0	100.0	100.0	100.0	100.0	99.5	97.9	97.9	90.1	90.1	94.0
	(10) NG244102 (10) AV904242	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.0 00.0	100.0	100.0	100.0	100.0	99.5	97.9	97.9	90.1	90.1	94.0
	(19) A1094343	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.8	100.0	100.0	100.0	~ ~	99.5	97.9	97.9	90.1	90.1	94.6
	(20) AY532606	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.0	99.3	99.6	99.6	99.6	99.6		97.5	97.6	94.9	94.9	94.2
	(21) AB104413	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.3	99.6	99.6	99.6	99.6	99.1	400.0	99.9	94.2	94.2	94.2
	(22) AY//9031	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.3	99.6	99.6	99.6	99.6	99.1	100.0		94.3	94.3	94.3
	(23) K1/81098	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.5	98.7	98.7	98.7	98.7	98.2	98.2	98.0		100.0	94.0
	(24) AF371960	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.7	98.5	98.7	98.7	98.7	98.7	98.2	98.2	98.0	100.0		94.0
	(25) GQ273492	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	98.0	97.8	98.0	98.0	98.0	98.0	97.6	97.6	97.6	98.0	98.0	
	101																									
	(D)								1	Nuc	cle	otic	le													
	(<u>b</u>)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	1 (8)	(9)	(10)	otio	le (12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)
	(D) (1) ADC-BSIV2016-2	(1)	(2)	(3)	(4)	(5)	(6)	(7)	1 (8) 0.00	99 9	(10)	otic (11) 99.9	1e (12) 99.9	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)
	(1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18	(1)	(2) 100.0	(3) 100.0	(4) 100.0	(5)	(6) 100.0	(7) 100.0	1 (8) 9.99	99.9	(10) 99.9	0tic (11) 99.9	1e (12) 99.9	(13) 99.9	(14) 99.8 99.8	(15) 99.8	(16) 100.0	(17) 100.0	(18) 100.0	(19) 100.0	(20) 99.7	(21) 98.0 98.0	(22) 98.0	(23) 94.8	(24) 94.8 94.8	(25) 92.9 92.9
	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22	(1) 100.0	(2) 100.0	(3) 100.0 100.0	(4) 100.0 100.0	(5) 100.0 100.0	(6) 100.0 100.0	(7) 100.0 100.0	1 (8) 9.99 9.99 9.99	NU((9) 99.9 99.9	(10) 99.9 99.9	otic (11) 99.9 99.9 99.9	1e (12) 99.9 99.9	(13) 99.9 99.9 99.9	(14) 99.8 99.8 99.8	(15) 99.8 99.8 99.8	(16) 100.0 100.0	(17) 100.0 100.0	(18) 100.0 100.0	(19) 100.0 100.0	(20) 99.7 99.7 99.7	(21) 98.0 98.0 98.0	(22) 98.0 98.0 98.0	(23) 94.8 94.8 94.8	(24) 94.8 94.8 94.8	(25) 92.9 92.9 92.9
	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22	(1) 100.0 100.0	(2) 100.0 100.0	(3) 100.0 100.0	(4) 100.0 100.0	(5) 100.0 100.0 100.0	(6) 100.0 100.0 100.0	(7) 100.0 100.0 100.0	1 (8) 9.99 9.99 9.99 9.90	Nuc (9) 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9	0tic (11) 99.9 99.9 99.9 99.9	1e (12) 99.9 99.9 99.9 99.9	(13) 99.9 99.9 99.9	(14) 99.8 99.8 99.8	(15) 99.8 99.8 99.8	(16) 100.0 100.0 100.0	(17) 100.0 100.0 100.0	(18) 100.0 100.0 100.0	(19) 100.0 100.0 100.0	(20) 99.7 99.7 99.7 99.7	(21) 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9
	(1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2013-24	(1) 100.0 100.0 100.0	(2) 100.0 100.0 100.0	(3) 100.0 100.0 100.0	(4) 100.0 100.0 100.0	(5) 100.0 100.0 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 	(7) 100.0 100.0 100.0 100.0	1 (8) 9.9 9.9 9.9 9.9 9.9	99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9	0tic (11) 99.9 99.9 99.9 99.9 99.9	1e (12) 99.9 99.9 99.9 99.9	(13) 99.9 99.9 99.9 99.9 99.9	(14) 99.8 99.8 99.8 99.8 99.8	(15) 99.8 99.8 99.8 99.8 99.8	(16) 100.0 100.0 100.0 100.0	(17) 100.0 100.0 100.0 100.0	(18) 100.0 100.0 100.0 100.0	(19) 100.0 100.0 100.0 100.0	(20) 99.7 99.7 99.7 99.7 99.7	(21) 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9 92.9 92.9
	(1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (5) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2017-27	(1) 100.0 100.0 100.0 100.0	(2) 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0	(4) 100.0 100.0 100.0	(5) 100.0 100.0 100.0	(6) 100.0 100.0 100.0 100.0	(7) 100.0 100.0 100.0 100.0 100.0	1 (8) 99.9 99.9 99.9 99.9 99.9	99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9	0tic (11) 99.9 99.9 99.9 99.9 99.9 99.9	1e (12) 99.9 99.9 99.9 99.9 99.9	(13) 99.9 99.9 99.9 99.9 99.9	(14) 99.8 99.8 99.8 99.8 99.8	(15) 99.8 99.8 99.8 99.8 99.8 99.8	(16) 100.0 100.0 100.0 100.0 100.0	(17) 100.0 100.0 100.0 100.0 100.0	(18) 100.0 100.0 100.0 100.0 100.0	(19) 100.0 100.0 100.0 100.0 100.0	(20) 99.7 99.7 99.7 99.7 99.7	(21) 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9
	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2015-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2017-27 (7) ADC-RSIV2016-29	(1) 100.0 100.0 100.0 100.0 100.0	(2) 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0	(4) 100.0 100.0 100.0 100.0	(5) 100.0 100.0 100.0 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 	(7) 100.0 100.0 100.0 100.0 100.0	1 (8) 99.9 99.9 99.9 99.9 99.9 99.9	99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	0tic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	1e (12) 99.9 99.9 99.9 99.9 99.9 99.9	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(14) 99.8 99.8 99.8 99.8 99.8 99.8	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8	(16) 100.0 100.0 100.0 100.0 100.0 100.0	(17) 100.0 100.0 100.0 100.0 100.0 100.0	(18) 100.0 100.0 100.0 100.0 100.0 100.0	(19) 100.0 100.0 100.0 100.0 100.0 100.0	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 92.
	(1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-41	(1) 100.0 100.0 100.0 100.0 100.0 100.0	(2) 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0	 (4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	(5) 100.0 100.0 100.0 100.0 100.0 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 	 (7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	1 (8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	VU((9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 100.0 100.0	(17) 100.0 100.0 100.0 100.0 100.0 100.0	(18) 100.0 100.0 100.0 100.0 100.0 100.0 20.0	(19) 100.0 100.0 100.0 100.0 100.0 100.0	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 92.
Ar	(L) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2013-24 (6) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (7) ADC-RSIV2016-41 (9) ADC-RSIV2016-41	 (1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0	 (4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	VU((9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 90.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.8	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 92.
Ami	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2017-27 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46	 (1) 100.0 	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (4) 100.0 	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	VUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	Dtic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7	 (25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0
Aming	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-64 (11) ADC-RSIV2014-64	 (1) 100.0 	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (4) 100.0 	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (6) 100.0 	 (7) 100.0 	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	VUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	clei (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 100.0	Dtic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0
Amino	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-28 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2015-27 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2014-38 (12) ADC-RSIV2014-38	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 20.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 00.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 00.0 0 00.0	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 0 0.0 5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0	 (6) 100.0 00.0 00.5 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 00.0 5	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 100.0 100.0 100.0	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	1e (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 96.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0
Amino ac	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2016-38 (12) ADC-RSIV2016-38	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 90.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 90.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 90.5	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5	 (6) 100.0 99.5 90.5 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 90.5	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 100.0 100.0 100.0 99.5 00.5	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	Dtic (11) 99.9 99.8 99.8 99.8 99.5 99.5	1e (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0
Amino acio	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2015-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2017-48 (12) ADC-RSIV2017-48 (13) ADC-RSIV2017-49 (14) ADC-RSIV2017-45 (14) ADC-RSIV2017-55 (14) ADC-RSIV2017-55 (14) ADC-RSIV2017-55 (14) ADC-RSIV2017-55 (14) ADC-RSIV2017-55 (14) ADC-RSIV2017-55 (15) A	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 100.0 100.0 100.0 99.5 99.5	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 9	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2017-68 (12) ADC-RSIV2017-68 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (14) ADC-RSIV2016-38 (15) ADC RSIV2016-41 (15) ADC RSIV2016-45	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 00.6	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 90.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 00.5 	 (7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 00.5 	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 100.0 100.0 99.5 99.5 100.0 00.5	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.8 99.5 99.5 100.0 95.5	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.0 99.0	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.7 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2017-27 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2014-46 (11) ADC-RSIV2014-46 (12) ADC-RSIV2017-68 (13) ADC-RSIV2017-69 (14) ADC-RSIV2016-56 (15) ADC-RSIV2014-35 (16) AD024265	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 	 (7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.5 100.0 99.5	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.0 99.0 99.0	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.8 100.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.7 00.6 	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.6 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2013-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-41 (11) ADC-RSIV2016-44 (11) ADC-RSIV2016-38 (12) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-36 (15) ADC-RSIV2016-35 (16) AP017456 (15) ADC-RSIV2014-35 (16) AP017456 (15) ADC-RSIV2014-35 (16) AP017456 (15) ADC-RSIV2014-35 (16) AP017456 (15) ADC-RSIV2014-35 (16) AP017456 (17) VT2004720 (17) VT2004720	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	 (4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 	 (7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 	(8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 90.5 100.0 99.5 100.0	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.7 99.8 99.7 99.8 99.7 	(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-28 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2014-46 (12) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-36 (15) ADC-RSIV2016-35 (16) AP017456 (17) KT804738 (18) V2044192	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	Image: Constraint of the second sec	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.5 100.0 99.5 100.0 100.0	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.7 99.8 99.7 99.8 99.7 99.8 99.7 	(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-28 (4) ADC-RSIV2017-27 (5) ADC-RSIV2015-24 (6) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-41 (11) ADC-RSIV2016-48 (12) ADC-RSIV2016-48 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-56 (15) ADC-RSIV2016-56 (15) ADC-RSIV2016-56 (15) ADC-RSIV2016-35 (16) AP017456 (17) KT804738 (18) KC244182 (19) AVC0225	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 90.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 90.5	<u>Г</u> (8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	NUC (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	(10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.5 90.0 99.5 100.0 100.0 100.0 100.0	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.0 99.0 99.0 99.0 99.8 </th <th>(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0</th> <th>(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th> (15) 99.8 99.8<th>(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6</th><th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.8 94.8 94.8 94.8</th><th>(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93</th></th>	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.8<th>(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6</th><th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.8 94.8 94.8 94.8</th><th>(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93</th>	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.8 94.8 94.8 94.8	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2015-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2014-44 (10) ADC-RSIV2014-46 (11) ADC-RSIV2014-46 (12) ADC-RSIV2014-46 (13) ADC-RSIV2016-58 (14) ADC-RSIV2016-58 (15) ADC-RSIV2016-56 (17) KT804738 (18) KC244182 (19) AY894343 (19) AY894343	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 10	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0 100.0	<u>Г</u> (8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	c) Lee (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.5 90.0 99.5 100.0 100.0 100.0 100.0	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.0 99.0 99.0 99.0 99.8 </th <th>(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99</th> <th>(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th> (15) 99.8 99.8<th>(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.</th><th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.1 92.9 92.9 92.9 92.9 92.9</th></th>	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.8<th>(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.</th><th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.1 92.9 92.9 92.9 92.9 92.9</th>	(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.1 92.9 92.9 92.9 92.9 92.9
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2017-27 (7) ADC-RSIV2017-27 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-46 (11) ADC-RSIV2017-68 (12) ADC-RSIV2017-68 (13) ADC-RSIV2017-68 (13) ADC-RSIV2017-69 (14) ADC-RSIV2016-56 (15) ADC-RSIV2014-35 (16) AP017456 (17) K1804738 (18) KC244182 (19) AY894343 (20) AY532606 (21) ADU-125	(1) 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 0 100.0	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 0 90.5	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0	<u>Γ</u> (8) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	Clee (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 100.0 99.5 100.0 100.0 100.0 100.0	le (12) 99.9 99.8 99.8 99.0 99.0 99.0 99.0 99.0 99.8 </th <th>(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99</th> <th>(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.</th> <th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93</th>	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.7 99.	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2017-27 (7) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2014-46 (11) ADC-RSIV2014-46 (12) ADC-RSIV2017-68 (13) ADC-RSIV2017-68 (13) ADC-RSIV2017-69 (14) ADC-RSIV2017-65 (15) ADC-RSIV2016-56 (15) ADC-RSIV2016-56 (15) ADC-RSIV2016-56 (15) ADC-RSIV2016-56 (17) KT804738 (18) KC244182 (19) AY894343 (20) AY532606 (21) AB104413 (20) AY532606	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 99.5	 (6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 98.7 	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0	<u>Г</u> (8) 99.9 99.9 99.9 99.9 99.9 99.9 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	CLCC (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 100.0 99.5 100.0 100.0 100.0 98.7	le (12) 99.9 99.8 99.8 99.8 99.0 99.0 99.0 99.0 99.8 </th <th>(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0</th> <th>(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6</th> <th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 92.9 92.9 92.9 92.9 92.9 92.9 93.0 92.9</th>	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 92.9 92.9 92.9 92.9 92.9 92.9 93.0 92.9
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-22 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2016-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-48 (11) ADC-RSIV2016-48 (12) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (14) ADC-RSIV2016-36 (15) ADC-RSIV2016-36 (15) ADC-RSIV2016-36 (16) AP017456 (17) KT804738 (18) KC244182 (19) AY894343 (20) AY532606 (21) AB104413 (22) AY779031 (22) M779031	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 1	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 98.7 98.7	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 1	(5) 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 99.5 1 100.0 99.5 1 100.0 1 00.0 1 00.0 1 00.0 1 00.0 98.7 98.7	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5	<u>Г</u> (8) 99.9 99.9 99.9 99.9 99.9 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 98.7 98.7	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	CLCC (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99	otic (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 90.0 90.0 100.0 100.0 100.0 100.0 98.7 98.7 98.7 98.7 98.7 98.7 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.5 100.0 100.0 98.7 98.7	le (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.0 99.0 99.8 99.0 99.8 </th <th>(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0</th> <th>(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.</th> <th>(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th> <th>(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6</th> <th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th> <th>(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th> <th>(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.1 92.9 93.1 92.9</th>	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 92.9 92.9 92.9 92.9 92.9 92.9 92.9 93.1 92.9 93.1 92.9
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-28 (4) ADC-RSIV2017-22 (4) ADC-RSIV2013-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-48 (11) ADC-RSIV2016-48 (12) ADC-RSIV2016-38 (13) ADC-RSIV2017-49 (14) ADC-RSIV2016-56 (15) ADC-RSIV2017-49 (14) ADC-RSIV2016-56 (15) ADC-RSIV2017-49 (14) ADC-RSIV2016-56 (17) KT804738 (18) KC244182 (19) AY894343 (20) AY532606 (21) AB104413 (22) AY779031 (23) KT781098	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 98.7 98.7 96.7	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 98.7 98.7 98.7	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 98.7 98.7 96.7	(5) 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 1 100.0 9 9.5 1 100.0 9 9.5 1 100.0 1 100.0 1 100.0 1 100.0 9 9.5 1 100.0 1 100.0 9 9.5 1 100.0 9 8.7 9 6.7 9 6.7	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 98.7 98.7 96.7	I (8) 99.9 90.0 100.0 100.0 98.7 96.7	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	Clei (10) 99.9 99.8 90.0 90.0 100.0 100.0 98.7 96.7	Diff (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 90.0 90.5 100.0 100.0 100.0 100.0 98.7 96.7	de (12) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.8 99.0 99.0	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.7 100.0 100.0 98.7 98.7 96.7	(15) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	(16) 100.0 100.0 100.0 100.0 99.9 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.7 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.0 93
Amino acid	(D) (1) ADC-RSIV2016-2 (2) ADC-RSIV2017-18 (3) ADC-RSIV2017-28 (4) ADC-RSIV2017-22 (4) ADC-RSIV2015-24 (5) ADC-RSIV2015-24 (6) ADC-RSIV2016-29 (8) ADC-RSIV2016-29 (8) ADC-RSIV2016-41 (9) ADC-RSIV2016-41 (10) ADC-RSIV2016-41 (11) ADC-RSIV2016-48 (12) ADC-RSIV2014-44 (11) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (13) ADC-RSIV2016-38 (14) ADC-RSIV2016-36 (17) KT804738 (18) KC244182 (19) AY89433 (20) AY532606 (21) AB104413 (22) AY779031 (23) KT781098 (24) AF371960	(1) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 98.7 96.7 96.7 96.7 96.7	(2) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 98.7 98.7 96.7 96.7	(3) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 1	(4) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 98.7 98.7 96.7 96.7	(5) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 100.0 99.5 100.0 99.5 100.0 99.5 90.5	(6) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 99.5 100.0 100.0 100.0 100.0 99.5 100.0 98.7 96.7 96.7 96.7 96.7	(7) 100.0 100.0 100.0 100.0 100.0 100.0 100.0 100.0 99.5 100.0 99.5 100.0 99.5 100.0 100.0 100.0 100.0 100.0 98.7 98.7 96.7 96.7	I (8) 99.9 90.0 100.0 100.0 100.0 98.7 96.7 96.7	Nuc (9) 99.9 99.9 99.9 99.9 99.9 99.9 99.9	Clea (10) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 100.0 99.5 100.0 100.0 100.0 100.0 98.7 96.7 96.7 96.7	Diff (11) 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.5 90.0 90.7 96.7 96.7	1 e (12) 39.9 39.9 39.9 39.9 39.9 39.9 39.9 39.	(13) 99.9 99.9 99.9 99.9 99.9 99.9 99.8 99.8 99.8 99.8 99.8 99.8 99.0 99.0	(14) 99.8 99.8 99.8 99.8 99.8 99.8 99.8 99.	 (15) 99.8 99.8<th>(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9</th><th>(19) 100.0 100.0 100.0 100.0 99.9 99.9 99.9</th><th>(20) 99.7 99.7 99.7 99.7 99.7 99.6 99.6 99.6</th><th>(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0</th><th>(23) 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7</th><th>(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.1 92.9 93.1 92.9 92.9 92.9 92.9 92.9 92.9 93.0 92.9 93.0 92.9 92.9 92.9 92.9 92.9 92.9 92.9 92</th>	(16) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(17) 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(18) 100.0 100.0 100.0 100.0 100.0 100.0 99.9 99.9	(19) 100.0 100.0 100.0 100.0 99.9 99.9 99.9	(20) 99.7 99.7 99.7 99.7 99.7 99.6 99.6 99.6	(21) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(22) 98.0 98.0 98.0 98.0 98.0 98.0 98.0 98.0	(23) 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(24) 94.8 94.8 94.8 94.8 94.8 94.7 94.7 94.7 94.7 94.7 94.7 94.7 94.7	(25) 92.9 92.9 92.9 92.9 92.9 93.0 93.0 93.0 93.0 93.0 93.0 93.1 92.9 93.1 92.9 92.9 92.9 92.9 92.9 92.9 93.0 92.9 93.0 92.9 92.9 92.9 92.9 92.9 92.9 92.9 92

[Fig. 2]. Degree of identity (%) of the nucleotide and deduced amino acid sequence of complete major capsid protein (MCP) (A) and partial DNA polymerase (DPOL) (B) gene in megalocytiviruses of this study to those of other strains.

Genomics, The Biodesign Institute, Tempe, AZ, analysis with 1,000 replicates. The complete USA), and nodes were confirmed from bootstrap sequences of MCP genes and the partial sequences

of DPOL genes from megalocytiviruses in the GenBank database (http://www.ncbi.nlm.nih.gov/ GenBank) [Fig. 3] were used to construct the phylogenetic tree for comparison.

III. Results

1. Detection of megalocytiviruses from fish tissue samples

We carried out PCR with various primer sets for known fish viruses, including megalocytivirus. However, all 15 samples produced the expected 570 bp and 568 bp amplicons only when running the PCR using both primer sets for megalocytivirus detection, 1-F/1-R and 4-F/4-R, respectively [Fig. 1]. The BlastN analysis (http://blast.ncbi.nlm.nih.gov/blast.cgi) of all sequences from the PCR products obtained using the two primer sets showed more than 99.8% nucleotide sequence identities compared with other known megalocytiviruses (GenBank accession no. AP017456, KT804738, KC244182, and AY894343) belonging to RSIV type-subgroup2.

2. Sequence identity of megalocytivirus MCP and DPOL genes

The identities of nucleotide sequence between the obtained virus genes ranged from 99.8 to 100% (amino acid sequence identity: 99.8 to 100%) for the MCP gene and from 99.7 to 100% (amino acid sequence identity: 99.5 to 100%) for the DPOL gene [Fig. 2]. However, comparison of obtained genes with the other virus subgroups ISKNV and TRBIV showed lower sequence identities ranging from 94.5 to 95.1% (amino acid sequence identity: 97.8 to 98.7%) for the MCP gene and from 92.9% to 94.8% (amino acid sequence identity: 95.7 to 97.0%) for the DPOL gene. All of the virus

sequences obtained in this study have been uploaded to the NCBI Entrez database (GenBank accession number MK025667 ~ MK025696).

3. Phylogenetic analysis of megalocytiviruses

The phylogenetic tree showed that the nucleotide sequences of both the MCP and DPOL genes comprised three subtypes: RSIV type, ISKNV type, and TRBIV type. The 15 viral strains obtained in this study formed a single cluster of RSIV type-subgroup2 [Fig. 3]. Phylogenetic trees of both the MCP and DPOL genes showed closer clustering of the 15 viral sequences with RIE12-1 and GSIV-K1 isolated in Japan and China than with RBIV-KOR-TY1 isolated in Korea. In addition, the nucleotide sequence divergence from other isolates belonging to RSIV type-subgroup2 was low.

4. Multiple alignments of MCP and DPOL gene sequences in megalocytiviruses

Sequence alignment of the MCP gene among viral strains showed that the majority of the deduced amino acid sequences were identical with only one strain (ADC-RSIV2017-49) showing a single amino acid substitution (N->D at position 107) compared with the others [Fig. 4]. By contrast, for the DPOL gene, three strains contained an amino acid substitution: ADC-RSIV2017-49 (C->R at position 661), ADC-RSIV2016-38 (C->R at position 661), and ADC-RSIV2014-35 (M->T at position 944). Moreover, the DPOL gene of five strains. ADC-RSIV2017-22 867), ADC-(aa RSIV2017-27 (aa 871), ADC-RSIV2015-24 (aa 804), ADC-RSIV2014-44 (aa 909), and ADC-RSIV2014-35 (aa 945), showed a silent mutation in a single region, in which there was no amino acid substitution despite the nucleotide substitution.



[Fig. 3]. Molecular phylogenetic tree by neighbor-joining analysis constructed based on the complete sequences of megalocytiviruses MCP and DPOL genes. The trees were constituted to compare the phylogenetic relationship of MCP gene (A) and DNA polymerase gene (B) with other megalocytivirus genes from Entrez Database. Bootstrap values were obtained from 1,000 replicates and these values less than 90 were hidden in the tree. The scale bar represents 0.01 nucleotide substitution per site. We marked analyzed sequences of this study (◆).

Molecular characterization of megalocytiviruses from diseased fishes in Korean aquatic farms from 2013 to 2017

(A)

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ADC-RS1V2016-2	MSAISGAW/1	SOFIDISAFD	AVETHLYGGD	NAVTYFARET	VRSSHYSILP	VILSKOTCHA	NEGGEFSVTV	ARGEDYL INV	WLRW(IPSIT	SSIENSYIRM	CONLINEVE	EVSVSFNOLV	AGTL TSEFLD	FINICMPGS	KOSCYNKMIG	150	
ADC-RS1V2017-18																150	
ADC-DC11/2017-22																150	
ADG-R5142017-22	*********			*********		*********	********	********		********	*********			********	********	130	
ADC-RS1V2013-24	********			********	*********		*********	*********	********	********	*********	*********		********		150	
ADC-RS1V2015-24							*********			********					********	150	
ADC-RS1V2017-27																150	
ADC-RS1V2016-29																150	
ADG ROLLING LO		********			*********			********					*********			150	
ADC-R5142014-35	*********	*********	*********	********			*********	*********	*********	********	*********	********	*********	********	********	150	
ADC-RS1V2016-38					*********		********		********	********		********		********	********	150	
ADC-RS1V2016-41											*********		********		*********	150	
ADC-RS1V2014-44																150	
ADC-DE1V2014-46																150	
ADC-R5142014-46	*********		*********	********	*********	*********	*********	*********	*********	*********	*********	********	*********	*********		130	
ADC-RS1V2017-68	********	********	*********	********	********	*********	*********	*********	********	********	********	********	*********	********	*********	150	
ADC-RS1V2016-56									second and							150	
ADC-RS1V2017-49											D					150	
AY532606 RRIV-KOR-TY1																150	
ADDITAGE DIF12-1																150	
7F017436_K1E12-1	*******	********	*********					*********		********	*********		*********		********	150	
KT804738_GS1V-K1	********	*********	********	********	********	*********	********	********	********	********	*******	********	*********	********	********	150	
AY894343_OSG1V																150	
KC244182 RB1V-C1																150	
ABIOAA13 Ebime-1																150	
AV770021 LC1H																150	
AT/79031_LCTV	*******	********	********	*******	********	********	*********	*********	********	********	*******	*******	********	********	********	150	
GG273492_TRBTV	******			*******			********			*******	********				********	150	
KT781098_RS1V-Ku																150	
AF371960_1SKNV																150	
ADC-RS1V2016-2	MRSDLVGG11	NGCTMPAAYL	NPIPLFTR	DTGLALPTVS	LPYNEVRIHE	KLARMEDILLI	SOSTCADMAI	STVTLANION	VAPALTINVSV	MGTYAVLITSE	EREVVACSSR	SML/EQCOVA	PRVPVTPVDN	SLWLOLRFS	HPVKALFFAV	300	
ADC-RS1V2017-18	1101-1100-0		Section and	Normal Sont	inter desite	ALL STATES	Ionina anna	000000-000	STREET PROPERTY			- Anna - Contra	in the second	Treasure and		300	
ADC R5112017 10								*********								200	
AUC-R51V2017-22		*********	********		*********		*********	*********	*********	********	*********	*********	********	********	*******	300	
ADC-RS1V2013-24	*******	********	********		*********		********	*********	********	********		*********		********		300	
ADC-RS1V2015-24																300	
ADC-RS1V2017-27																300	
ADC-PS1V2016-29																300	
100 00110014 25					*********										*******	200	
ADC-R5192014-35	********	********			*********	********	*********	********		********	*********	********		********		300	
ADC-RS1V2016-38																300	
ADC-RS1V2016-41	*********								*********					*********	*********	300	
ADC-RS1V2014-44																300	
ADC-RS1V2014-46																300	
ADC-PS1V2017-68																300	
ADC-R3142017-06	******							*********					*********	*********	*********	300	
ADC-RS1V2016-56		*********														000	
													•••••			300	
ADC-RS1V2017-49													•••••	•••••		300 300	
ADC-RS1V2017-49 AY532606_RB1V-K0R-TY1										C.G			·····			300 300 300	
ADC-RS1V2017-49 AY532606_RB1V-KOR-TY1 AP017456_R1E12-1										c.c					•••••	300 300 300 300	
ADC-RS1V2017-49 AY532606_RB1V-K0R-TY1 AP017456_R1E12-1 KTR04738_CS1V-K1	······									c.c				······	••••••	300 300 300 300 300	
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ADC-RS1V2017-49 AY532606_R81V-K0R-TY1 AP017456_R1E12-1 KT804738_GS1V-K1 AY894343_0SG1V										c.c				······		300 300 300 300 300 300	
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ADC-R51V2017-49 AY532606_R81V-K0R-TY1 AP017456_R1E12-1 KT804738_G51V-K1 AY894343_0SG1V KC244182_R81V-C1 AB104413_Ehime-1								v		C.G						300 300 300 300 300 300 300 300	
ADC-RS1V2017-49 AY532606_RB1V-K0R-TY1 AP017456_R1E12-1 KT804738_GS1V-K1 AY894343_OSG1V KC244182_RB1V-C1 AB104413_Ehime-1 AY779031_LC1V																300 300 300 300 300 300 300 300 300	
ACC-RS1V2017-49 AY532606_R81V-K0R-TY1 AY017456_R1E12-1 K1804738_GS1V-K1 AY894343_GSG1V KC244182_R81V-C1 AB104413_Ehime-1 AY779031_LC1V C0273402_R81V																300 300 300 300 300 300 300 300 300 300	
A0C-RSIV2017-49 AY532606_R8IV-K0R-TY1 AY532606_R8IV-K0R-TY1 KT804738_CSIV-K1 AY894343_0SGIV KC244182_R8IV-C1 A8104413_Ehime=1 AY779031_LC1V C0273492_TR8IV								V								300 300 300 300 300 300 300 300 300 300	
ACC-RS1V2017-49 AV532606_R814-K0R-TY1 AP017456_R1E12-1 KT804738_CS1V-K1 AY894343_CSG1V KC244182_R81V-C1 A8104413_Ehime-1 AY779031_LC1V G2273492_R81V KT781098_RS1V-Ku								V								300 300 300 300 300 300 300 300 300 300	
ADC-RS1V2017-49 APC1785(26)(17-49) AP017456[R1E12-1 K1804738[CS1V-K1 AP84343_CS1V-K1 AP84343_CS1V-K1 AP84343[CS1V-K1 AB104413_Ehime-1 AP179031_LC1V G2273492_TR81V K17781088[RS1V-Ku AF371960_1S0W	G. S. A.												A. A.			300 300 300 300 300 300 300 300 300 300	
ADC-RS1V2017-49 ANS2360, R81V+K0R-TY1 AP017456, R1E12-1 K1804738, CS1V-K1 AY894343, CSS1V-K1 AY894343, CSS1V-K1 AB104413, EN1W-C1 AB104413, EN1W-C1 AB104413, EN1W-C1 AV779031, LC1V C0273492, TR81V K17781098, RS1V-Ku AF371960, ISOW	G.S. A.									C.G						300 300 300 300 300 300 300 300 300 300	
ADC-RS1V2017-49 ADC352606_R81V-K0R-TY1 AP017456_R1E12-1 KTB04738_CS1V-K1 AY894343_CSG1V KC244182_R81V-C1 AB104413_Ehime-1 AY779031_LC1V C0273492_R81V-Ku AF371960_1S0NV ADC-RS1V2016-2			NKWLPLLA	TNPLSEVSLI	YENTFRUKOM	GVDYFTSVDP		V		TNYCRLSNVT	LSC/VSDIVK	TTAAGGGGNG			MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454
ADC-RSIV2017-49 APC1785266,R8IV+K0R-TY1 AP017456,RIE12-1 K1804738,CSIV-K1 AY894343,OSGIV KC244182,R8IV-C1 AB104413,Ehime-1 A7779031,LC1V G0273492,TR8IV G02734,TR8IV G0273492,TR8IV G02734,TR8IV G0274,TR8IV G0274,TR8IV G0274,TR8IV G02	GSA AA KONTI-FENNOS		NKONLELA	TNPLSEVSLI	YENTFRLIOM	GVD/FTS/DP		V	. T	INVERLSINT	LSOKVSDNWK	TTAACCOORG		LVVIAVNNI	WINDGAR	300 300 300 300 300 300 300 300 300 300	454
ADC-RS1V2017-49 ADC-RS1V2017-45 AP017456_RE14-2-1 KT804738_CS1V-K1 AY894343_CSG1V KC244182_R81V-C1 A8104413_ENime-1 AY779031_LC1V G0273492_R81V KT781098_RS1V-Ku AF371960_ISK0W ADC-RS1V2016-2 ADC-RS1V2017-28	G. SAAAAA	HT. V. V.	NKINEPLA	TIPLSEVSLI	YENTFRLHOM	GVDYFTSVDP		V	.T T DACNINFACS	THYCELSINT	LSOXVSDNWK	TTAKGGO2NG		LVVIANHNI	WKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454
ADC-RSIV2017-49 APC17851/2017-49 AP017456_R1E12-1 K1804738_CSIV-K1 AY894343_OSCIV KC244182_R81V-C1 AB104413_Ehime-1 A779031_LC1V C0273492_TRB1V KT781098_RSIV-Ku A7371960_1S0W ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2013-24		HT. V. V.	NKSNLPLLA	THPLSEVSLI	YENTFRUKOM	GVD#FTSVDP				C.G	LSOKVSDNWK	TTAAGGOOMG		LVVIAVNHNI	w.i/ACG/ACF	300 300 300 300 300 300 300 300 300 300	454 454 454
ADC-RS1V2017-49 ADC-RS1V2017-45 AP017456_R1E12-1 KT804738_CS1V-K1 AY894343_OSG1V KC244182_R81V-C1 A8104413_ENime-1 AY779031_LC1V G273432_TR61V KT781038_RS1V-Ku AF371960_ISGW ADC-RS1V2016-2 ADC-RS1V2017-18 ADC-RS1V2017-12 ADC-RS1V2017-22 ADC-RS1V2017-24 ADC-RS1V2017-24 ADC-RS1V2017-24 ADC-RS1V2017-24 ADC-RS1V2017-24 ADC-RS1V2017-24			NKINLPLA	TIPLSEVSLI	YENTFRUKOM	GVDMFTSVDP	N	V	. T	TIVYCPLSIWT	LSOXVSDWK	TTAKGGOPK		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454
ADC-RSIV2017-49 APC1785102017-49 AP017456_R1E12-1 K1804738_CSIV-K1 AP017456_R1E12-1 K1804738_CSIV-K1 AP84343_CSGIV KC24182_R8IV-C1 AB10413_Ehime-1 A779031_LC1V C0273492_TR8IV KT781098_RSIV-Ku A7371960_IS0W ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24			NKONEPLLA	TMPLSEVSLI	YENTFRUOM	GVD#TSVDP	N	VS.		TRYGELSINT	LSOXVSENW	TTAKGGSING		LVVIAVNENI	windgavæ	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-45 AP017456_RIE12-1 K1804738_CSIV-K1 AV894343_DSGIV KC244182_R8IV-C1 AB104413_Ehime-1 AV779031_EC1V G2273492_TR81V KT781038_RSIV-Ku AF371960_ISGW ADC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-24 ADC-RSIV2017-27			NKINLPLA	THPLSEVSLI	YENTFRUKOM	GVDHFTSVDP		V		, C. G	LSDKVSDNVK	TTAAGGGEAG		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-KI AP017456_RIE12-1 K1804738_CSIV-KI AP894343_OSGIV KC244182_R8IV-CI AB104413_Ehime-1 AV779031_LCIV G273492_TR8IV KT781098_RSIV-Ku A7371960_ISGW ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2015-24 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27			NKSNLPLLA	THPLSEVSLI	YENTFRUHOM	GVD/FTS/DP	NN	V		THYCFLSNVT	LSC/VSDNWK	TTACCCOM		LVVIANENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454
ADC-RSIV2017-49 APD17456_RIE12-1 XF32366_R8IV+K0R-TY1 AP017456_RIE12-1 KT804738_CSIV-K1 AY894343_OSGIV KC244182_R8IV-C1 AB104413_Ehime-1 AY779031_LC1V G0273492_TR8IV G0273492_TR8IV AC7371960_ISoW ADC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27		HTVV.	NKONEPELA	TNPLSEVSLI	YENTFRLHOM	GVDHFTSVDP	VIEW CONTRACTOR C			INVGRLSINT	LSDXVSDNWK	TTAKGGODMG		LVVIAVINI	WINDGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP894343_OSGIV KC244182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-K1 AP779031_LC1V G2273492_TR8IV ACC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-29 ADC-RSIV2016-38			NKORPLA	TIMESEVSLI	YENTFRUOM	GVDMFTSVDP	NN. N.YHFAPSMPEM	V	.T T	Thyorismu	LSD(VSDNW	TTAKGGGM	A. A. A. TOYTWORFE	LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP894343_OSGIV KC244182_R81V-C1 AB104413_Ehime-1 AP179031_LC1V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G02-RSIV2016-2 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-29 ADC-RSIV2016-28 ADC-RSIV2016-28 ADC-RSIV2016-38 ADC-RSIV2016-41		HT V. V. NYTA/SPVV	NKONEPELA	THPLSEVSLI	YENTFRUKOM	GVD#FTSVDP			. T	Thirder Shart	LSOXVSDNAK	TTAACGGOAG		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP894343_OSGIV KC244182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 ADC-RSIV2016-1 ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-23 ADC-RSIV2017-24 ADC-RSIV2017-27 ADC-RSIV2016-29 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2016-41			NKORPLA	TNPLSEVSLI	YENTFRIJON	GVD#FTS/DP	N.N.N.YHFAPSMPEM			INVGRISIWT	LSDKVSDNWK	TTAKGGON	A. A. A. A. TOYTWORFE	LVVIAVNINI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	54 54 54 54 54 54 54 54 54 54 54 54 54 5
ADC-RSIV2017-49 APC17851/2017-49 APO17456_R1E12-1 K1804738_CSIV-K1 AP017456_R1E12-1 K1804738_CSIV-K1 AP894343_OSCIV KC244182_R81V-C1 AB104413_Ehime-1 A7779031_LC1V G2273482_TR81V G22744444444444444444444444444444444444			NKINLPLA	THPLSEVSLI	YENTFRUKOM	GVDHFTSVDP	NN	V	.T	The contract of the contract o	LSD/VSDNWK	TTAKGGGAK		LVVIAVNENI	MKIADGAACF	300 300 300 300 300 300 300 300 300 300	25 25 25 25 25 25 25 25 25 25 25 25 25 2
ADC-RSIV2017-49 ADC-RSIV2017-45 AP017456_RIE12-1 KT804738_CSIV-K1 AP017456_RIE12-1 KT804738_CSIV-K1 AP894343_DSIV-C1 AB104413_Ehime-1 AV779031_LC1V G2273432_TR61V KT781038_RSIV-Ku AF371960_1SQW ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2016-28 ADC-RSIV2016-28 ADC-RSIV2016-28 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-41			NHONEPELA	THELSEVSLI	YENTFRIJKOM	GVDVFTSVDP	N. N. YHTAPSMPDM		T. T. T. T. DADNINPAGS	INVERLISING	LSDXVSDNWK	TTAKGGGOM	A. A. A. TOTWOFE	LVVIANENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-45 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP84343_CSIV-K1 AP84343_CSIV-C1 AB104413_Ehime-1 AV779031_LCIV C0273492_TRBIV K1781098_RSIV-Ku A7791960_IS0W ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2017-27 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-29 ADC-RSIV2016-29 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-46 ADC-RSIV2017-68		HTVV.	NKONLPLA	TIMESEVSLI	YENTFRUOM	GVDHFTSVDP		V	.TT	C.G	LSDAVEDWAK	TTAKGGGENG		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-45 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP04413_Ehime-1 AP179031_LCIV G0273492_TR81V ADC-RSIV2017-12 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-24 ADC-RSIV2017-24 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2016-28 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2014-44 ADC-RSIV2014-46 ADC-RSIV2014-68 ADC-RSIV2016-56			NNONEPELA	THPLSEVSLI	YENTFRUKOM	GVDIFTSVDP	NN.	UNIT YOUR STREET	TT.	Thirderson	LSD://SDN/K	TTAKGGG2MG		LVVIAWENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP894343_OSGIV KC244182_R81V-C1 AB104413_Ehime-1 AT779031_LC1V C0273492_TR81V KT781098_RSIV-Ku A773903_LC1V C0273492_TR81V ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-29 ADC-RSIV2016-29 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2016-46 ADC-RSIV2017-468		HTVV.	NKONEPLA	TIMESEVSLI	YENTRUOM	GVDMFTSVDP	N			Thyorismy	LSOCVEDNIK	TTAKGGGAN		LVVIAVNENI	WKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 APC17456_R1212-1 APO17456_R1212-1 K1804738_C5IV-K1 AP017456_R1212-1 K1804738_C5IV-K1 AP04413_Ehime-1 AP179031_LC1V G0273492_TR81V-K0 AF371960_150W ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2017-24 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2016-28 ADC-RSIV2016-28 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-34 ADC-RSIV2016-44 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56			NKONEPLLA	THPLSEVSLI	YENTFRUKEN	GVDAFTSVDP	NN. YHFAPSMEDM	U	.TT	THYGELSNYT	LSOVVEDNAK	TTAKSSEN		LVVIAWENI	M <iadgaagf< th=""><th>300 300 300 300 300 300 300 300 300 300</th><th>45 45 45 45 45 45 45 45 45 45 45 45 45 4</th></iadgaagf<>	300 300 300 300 300 300 300 300 300 300	45 45 45 45 45 45 45 45 45 45 45 45 45 4
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-KI AP017456_RIE12-1 K1804738_CSIV-KI AP894343_OSGIV KC24182_R8IV-CI AB10413_Ehime-1 AV779031_LCIV C0273492_TR8IV KT781098_RSIV-Ku A779031_LCIV C0273492_TR8IV KT781098_RSIV-Ku A779031_LCIV C0273492_TR8IV ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-44 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-46 ADC-RSIV2017-49 AV532606_R8IV+X0R-TY1		HTVV.	NHONEPELA	TNPLSEVSLI	YENTFRUOM	GVDHFTSVDP	N	VS. DOWTYCYTL		INVGRLSNVT	LSOXYSDNW	TTAKGGOOK		LVVIAVNENI	WUNDGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 APC-RSIV2017-45 APO17456_RIE12-1 KT804738_CSIV-KI AP017456_RIE12-1 KT804738_CSIV-KI AP894343_OSGIV KC244182_R81V-C1 AB104413_Ehime-1 APT79031_LC1V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2017-27 ADC-RSIV2017-27 ADC-RSIV2016-29 ADC-RSIV2016-29 ADC-RSIV2016-29 ADC-RSIV2016-38 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56 ADC-RSIV2016-56			NKONEPLLA	THPLSEVS.I	YENTFRIJEN	GVDrFTSVDP		UNATIVOTE		THYOFILSING	LSD/VSDNWK	TTAACGG276	A	LVVIAWHNI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-KI X7894343_OSGIV KC244182_R8IV-C1 AB104413_Ehime=1 A7779031_LC1V C0273492_TR8IV KT781098_RSIV-Ku A7731960_ISW ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-44 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2017-49 AVS28066_R8IV+K0R-TY1 AP017455_RIE12-1 KT804738_CSIV-K1		HTVV.	NKONEPLLA	TNPLSEVSLI	YENTRUOM	GVDHFTSVDP	N	V		C.G	LSDXVSDNWK	TTAKGSOOM		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-K1 AP017456_RIE12-1 K1804738_CSIV-K1 AP04413_Ehime-1 AP179031_LC1V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G0273492_TR81V G02-RSIV2016-2 ADC-RSIV2017-27 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-29 ADC-RSIV2016-29 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2016-41 ADC-RSIV2017-68 ADC-RSIV2017-68 ADC-RSIV2017-68 ADC-RSIV2017-49 AV532606_RSIV-46 ADC-RSIV2017-49 AV532606_RSIV-46 ADC-RSIV2017-49 AV532606_RSIV-40R-TY1 AP017456_RIE12-1 K1804738_CSIV+K1			NKONEPLLA	THPLSEVS.1	YENTFRIJEN	GVDNFTSVDP		U V V V V V V V V V V V V V V V V V V V		THYGHLSINT	LSD/VSDNVK	TTACCCOR	A	LVVIANHNI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-KI AP017456_RIE12-1 K1804738_CSIV-KI AP044182_R8IV-C1 AB1044182_R8IV-C1 AB1044182_R8IV-C1 AB104413_Ehime-1 AT779031_LCIV G2273492_TR8IV KT781088_RSIV-Ku ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2017-22 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2015-24 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-56 ADC-RSIV2017-49 AV523606_R8IV+K0R-TV1 AP017456_RE1E12-1 K1804738_CSIV-K1 AV524182_R8IV-C1			NKORPLA	THPLSEVSLI	YENTFRUKOM	GVD/FTS/DP	N. N. YHFAPSMPEM	V	.TT	INVERUSING	LSDCVSDNWC	TTAK660296		LVVIANHNI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-45 AP017456_RIE12-1 K1804738_CSIV-KI AP017456_RIE12-1 K1804738_CSIV-KI AP04413_Ehime-1 AP179031_CIV G0273492_TR61V G0274412_00000000000000000000000000000000000			NKONEPLLA	THPLSEVS.1	YENTFRIJKEN	GVDHFTSVDP	VIENE	V	.TT	THYGELSING	LSD(VSDNWK	TTACCCOM		LVVIAVNENI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	454 454 454 454 454 454 454 454 454 454
ADC-RSIV2017-49 ADC-RSIV2017-49 AP017456_RIE12-1 K1804738_CSIV-KI AP017456_RIE12-1 K1804738_CSIV-KI AP044182_R8IV-CI AB1044182_R8IV-CI AB1044182_R8IV-CI AB1044182_R8IV-KI AP779031_LCIV G2273492_TR8IV ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2016-2 ADC-RSIV2017-18 ADC-RSIV2016-2 ADC-RSIV2017-22 ADC-RSIV2017-22 ADC-RSIV2016-28 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-38 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-48 ADC-RSIV2016-58 ADC-RSIV2017-68 ADC-RSIV20			NHONEPELA	THELSEVSLI	YENTFREHOM	GVDYFTSVDP	N. N. YHTAPSMPDM	V	.TT	THYCRUSHYT	LSDXVSDNWK	TTAKG532MG		LVVIANHNI	MKIADGAAGF	300 300 300 300 300 300 300 300 300 300	55 45 45 45 45 45 45 45 45 45 45 45 45 4
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ADC-RS1V2016-29		150
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ADC-PSIV2016-29	R	150
ADC-R3112010-36		150
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AY532606_RB1V-KOR-TY1	a	150
AB104413_Ehime-1		150
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ADC-DC192016-29		300
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AF371960_1SKNV	NT	
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ADC-RS1V2017-18	396	
ADC-RS1V2017-22		
ADC-RS1V2013-24	396	
ADC-PS1V2015-24	202	
100-10112013-24		
ADC-RS172017-27		
ADC-RS1V2016-29		
ADC-RS1V2014-35		
ADC-RS1V2016-38	396	
ADC-RS1V2016-41	396	
AUC-RS1V2014-44		
ADC-RS1V2014-46		
ADC-RS1V2017-68		
ADC-RS1V2016-56	395	
ADC-RS1V2017-49	302	
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AP017456_RIE12~1		
KT804738_GS1V-K1	396	
KC244182_RB1V-C1		
AY894343 OSCIV	396	
AV522606 001V-VOD TV1	1	
A1332000_RB1V-K0K-1Y1	350	
AB104413_Ehime-1		
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[Fig. 4]. Comparison of the deduced amino acid sequences of complete MCP (A) and partial DPOL (B) genes of megalocytiviruses of this study with known strains, each representing 3 subtypes of Genus *Megalocytivirus*. Amino acid sequences written in alphabet, instead of dots which are represented identical amino acid residues, refer to sequence substitution compared with genes of representative ADC-RSIV2016-2.

Molecular characterization of megalocytiviruses from diseased fishes in Korean aquatic farms from 2013 to 2017

W. Discussion

RSVID, one of the major pathogenic agents in Korean marine aquaculture, is listed as a notifiable viral disease by the OIE. RSIVD, including RBIVD, is listed as a third-class communicable disease, requiring movement control and disinfection in Korea. In this study, we cloned the MCP and DPOL genes of megalocytiviruses obtained from tissues of infected fish and performed phylogenetic analysis and comparisons of genetic variation to investigate the evolution of megalocytiviruses in Korea from 2013 to 2017. We detected megalocytiviruses in moribund or dead fishes showing typical clinical symptoms of iridoviral disease with PCR using two different primer sets of the OIE Manual of Diagnostic Tests for Aquatic Animals [Fig. 1] : the 1-F/1-R of PCR primer set can amplify partial gene of RSIV and ISKNV type, whereas 4-F/4-R can amplify only the RSIV type. In addition to RSIVD, TRBIVD has also been reported in Korea (Do et al., 2005; Won et al., 2013), and there is no specific detection method for TRBIVD in Manual of Diagnostic Tests for Aquatic Animals of OIE. Therefore, to determine the genetic subtype of megalocytivirus that caused the iridoviral diseases in our sample, we are now developing a duplex PCR method that can detect both the RSIV and TRBIV types so as to be able to quickly prevent spread of the disease through rapid diagnosis.

The MCP and DPOL genes represent structural and non-structural genomic regions of megalocytiviruses, respectively, that are highly conserved, and thus serve as useful targets for evolutionary investigations. Megalocytivirus was previously divided into three subtypes, including RSIV, ISKNV, and TRBIV, based on the MCP gene (Do et al., 2004; Do et al., 2005; Song et al., 2008; Jeong et al., 2006). RSIV type-subgroup2 was shown to be widely distributed in various fish species worldwide, especially in Korea, Japan, China, and other Asian countries (Song et al., 2008; Wang et al., 2009; Zhang et al., 2012; Inouye et al., 1992). Consistently, our phylogenetic analysis demonstrated that megalocytiviruses can be divided into three clusters represented by RSIV, ISKNV, and TRBIV [Fig. 3]. Moreover, even though the MCP and DPOL genes of these viruses that have been present in Korea for the last 5 years clustered more closely with RIE12-1 and OSGIV from Japan and China than with RBIV-KOR-TY1 from Korea [Fig. 3], all of these virus genes still showed high identities (>99.8%) RBIV-KOR-TY1 [Fig. with 2]. This pattern suggests that RSIV type-subgroup2 in Korea have evolved in relation to other RSIV strains existing in the marine environment throughout East Asia.

Because a large number of fishes are transported into domestic aquaculture for commercial and ornamental use, gaining an understanding of the viral host range is important for the control of introducing exotic types from other countries and for disease prevention. Although megalocytiviruses were detected in Korean rockfish as well as rock bream as the main hosts, and were even found over a broad area, including Jeju Island <Table 1>, their gene sequences were still found to be nearly identical. Thus, the clustering pattern of the viral genes of RSIV-subgroup2 is not dependent on the host species, geographical location, or isolation year.

Multiple alignment of the MCP and DPOL nucleotide sequences also demonstrated a lower rate of genetic variation compared with the other strains [Fig. 4]. Our findings support that DNA viruses can evolve slowly to develop genetic variations with distinct amino acid substitutions (Kurita and

Nakajima, 2012; Huang et al., 2011). However, the greater variation of nucleotide sequences implies that evolution of megalocytiviruses is nevertheless underway, and thus mutant strains may emerge, including novel pathogenic types. Therefore, it is necessary to conduct functional analysis of the amino acid substitutions and their potential relation to pathogenicity.

Taken together, the present results based on phylogenetic analysis and genetic comparisons suggest that megalocytivirus isolates from different belong marine fish in Korea to RSIV with minimal type-subgroup2 genetic variation compared with genes of reported strains. This study could provide valuable insight and novel genetic information of recently isolated megalocytiviruses in Korea, laying a foundation for the development of effective vaccines for preventing the spread of megalocytivirus disease in the aquaculture industry.

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