Expressed sequence tags (ESTs) analysis of Acanthamoeba healyi

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Abstract: Randomly selected 435 clones from *Acanthamoeba healyi* cDNA library were sequenced and a total of 387 expressed sequence tags (ESTs) had been generated. Based on the results of BLAST search, 130 clones (34.4%) were identified as the genes enconding surface proteins, enzymes for DNA, energy production or other metabolism, kinases and phosphatases, protease, proteins for signal transduction, structural and cytoskeletal proteins, cell cycle related proteins, transcription factors, transcription and translational machineries, and transporter proteins. Most of the genes (88.5%) are newly identified in the genus *Acanthamoeba*. Although 15 clones matched the genes of *Acanthamoeba* located in the public databases, twelve clones were actin gene which was the most frequently expressed gene in this study. These ESTs of *Acanthamoeba* would give valuable information to study the organism as a model system for biological investigations such as cytoskeleton or cell movement, signal transduction, transcriptional and translational regulations. These results would also provide clues to elucidate factors for pathogenesis in human granulomatous amoebic encephalitis or keratitis by *Acanthamoeba*.

Key words: Acanthamoeba healyi, expressed sequence tags, novel genes

INTRODUCTION

The genus, Acanthamoeba, a human pathogen causing granulomatous amoebic encephalitis (GAE) and amebic keratitis (Sisson et al., 1994), has been known to be a vector for pathogenic microbes such as Mycobacterium spp., Listeria spp., and Legionella spp. (Jadin,

1973; Ly & Muller, 1990; Field, 1991). In addition to these medical importances, it is also well known that *Acanthamoeba* is a good model system to study eukaryotic cell biology due to its relatively large size, rapid growth in culture, active motility, and well developed cytoskeleton (Byers et al., 1990).

Although the ploidy and the total DNA content of the genus Acanthamoeba are unclear at the present, Byers et al. (1990) speculated that the genome size of the amoeba would be $\sim 1 \times 10^8$ bp of which the size could express more than 5,000 transcripts; however, only a few genes and proteins have been reported. Most of the genes identified are 18s rDNA sequences for the taxonomic purpose (Gast et al., 1996; Stothard et al., 1998), actin, myosins and actin binding proteins to study cytoskeleton (Nellen & Gallwitz, 1982; Cooper

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et al., 1986; Jung et al., 1989; Pollard & Rimm, 1991; Kelleher et al., 1995; Lee et al., 1999), and mitochondrial genome (Burger et al., 1995). Therefore most genes of *Acanthamoeba* have yet to be uncovered.

Characterization of the transcribed genes in a certain organism by expressed sequence tag (EST) analysis, single pass sequencing of randomly selected cDNA clones has been applied to many organisms including parasitic protozoa such as *Plasmodium falciparum* (Chakrabarti et al, 1994), *Trypanosoma brucei rhodesiense* (El-Sayed et al., 1995), *Toxoplasma gondii* (Wan et al., 1996; Manger et al., 1998), and *Entamoeba histolytica* (Azam et al., 1996; Tanaka et al., 1997). In addition to the candidate genes for pathogenic factors, many novel genes for stage specific, cell cycle regulatory, or related to cell signaling were identified in these protozoa by EST analysis.

ESTs analysis of *Acanthamoeba* could characterize the expression pattern of the genes, providing invaluable information to understand the genetics and the identification of many novel genes in this genus.

In the present study, we report the results of ESTs analysis of *A. healyi* OC-3A strain isolated from the brain of a GAE patient.

MATERIALS AND METHODS

Amoeba culture

An isolate of *Acanthamoeba* from the brain of a GAE patient, *A. healyi* OC-3A, was obtained from ATCC and cultured in Proteose peptone-Yeast extract-Glucose medium at 25°C.

RNA preparation

Trophozoites of *A. healyi*, washed with phosphate- buffered saline (PBS), were homogenized with β -mercaptoethanol and RNA denaturation solution (Stratagene, San Diego, CA, USA). One milliliter of 3 M sodium acetate (pH 4.0) was added to the solution and mixed by inversion. Water saturated phenol was added and mixed well with the solution. The mixture was shaken vigorously after adding chloroform/isoamyl alcohol (24:1), and then was incubated on ice for 15 min. The supernatant of the mixture after centrifugation

was moved to a new tube and incubated with 1 volume of isopropanol at -20°C for 1 hr for precipitation of the RNA. The RNA pellet was dissolved with the RNA denaturation solution and reprecipitated with equal volume of isopropanol. DEPC treated Q-water was added to the ethanol washed and vacuum dried RNA pellet. mRNA was purified from total RNA sample using Poly (A) Quick mRNA isolation kit (Stratagene, San Diego, CA, USA).

Construction of cDNA library

A unidirectional oligo (dT)-primed EcoRI/ Xho I cDNA library was constructed in UNI-ZAPTM (Stratagene, San Diego, CA, USA). Briefly, first strands, synthesized by reverse transcription of mRNA of A. healyi, were used to make second strands by the action of RNase H and DNA polymerase I. cDNA with blunted termini were ligated with Eco RI adapters and digested with Xho I. After size fractionation, cDNA over the size 400 bp were packaged into UNI-ZAPTM XR vector arms by ligation. Packaged cDNAs were incubated with the host cell of XL1-blue MRF strain on agarose LB medium. More than 5 millions clones of this library were amplified and the aliquots of the library were stored at 4°C until use.

Sequencing of randomly selected cDNA clones

cDNA library mixture were incubated with XL1-Blue MRF cell to allow in vivo excision using ExAssist helper phage, transfected into Escherichia coli SOLR strain, and plated on LB-ampicillin agar plates. Each randomly selected colony from the plates was inoculated into LB-ampicillin broth and incubated at 37°C overnight. Plasmid with the cDNA insert was extracted with plasmid DNA purification system (Wizard® Plus Minipreps, Promega, USA). The size of cDNA inserts obtained by digestion of the plasmid DNA with EcoR I and Xho I were estimated by electrophoresis on agarose gel with Hind III digested λ phage DNA, a DNA size standard. Alkaline denaturation of the plasmid DNA and the dideoxy chain termination method using DNA sequencing kit (T7 Sequenase version 2.0, Amersham, USA) and 35S dATP were applied to elucidate the sequence data of the randomly selected cDNA clones. After electrophoresis of the reaction samples for sequencing, the vacuum dried acrylamide gel was exposed on X-ray film. Sequence data were edited to remove vector and ambiguous sequences, and less than 100 bases were also rejected.

Basic Local Alignment Search Tool (BLAST) search

The sequence data of cDNA clones by random partial sequencing were subjected to examine similarities in the nucleic acid and protein databases using the BLAST on the National Center for Biotechnology Information (NCBI). The cDNA sequences were compared against nucleotide data by the program BLASTN, and the conceptual translation products of query sequences against translated nucleic acid and protein databases by BLASTX. Matches were considered to be significant only when the probability (P) was less than 0.0001 and scores were >160 for BLASTN and >80 for BLASTX.

RESULTS

Among 435 cDNA clones sequenced, the sequence data of 378 clones were submitted for blast search (Table 1). It was 130 clones (34.4%) identified by high homology with the DNA sequence of *Acanthamoeba* or other organisms in the public data base. Although 15 clones were matched with *Acanthamoeba*

Table 1. Acanthamoeba healyi cDNA library sequencing

EST category	No. of clones
Total clones sequenced	435
ESTs submitted for BLAST search	378
ESTs identified by homology	130
Unique ESTs identified	94
ESTs with homology to Acanthamoeba	15
genes	
Redundant ESTs	20
Common ESTs	
actin	12
60S acidic ribosomal protein PO	4
elongation factor 1 alpha	3
major vault protein	3

genes already studied, they were just 3 kinds of genes and twelve of them were the actin gene, the most commonly found gene in this study. The other 115 clones (88.5%, 94 different genes) are reported in the present paper for the first time in this genus.

Based on the results of BLAST search, ESTs with predicted or known functions were classified into putative cellular roles (Table 2). They were 4 clones for surface protein, 2 clones for DNA metabolism, 12 clones for energy metabolism, 10 clones for kinase and phosphatase, 17 clones for other metabolism, 1 clone for protease, 10 clones for signal transduction, 18 clones for structure and cytoskeleton, 3 clones for cell cycle related proteins, 5 clones for transcription factor, 32 clones for transcription and translational machinery, 4 clones for transporters, and 12 clones for not classified.

DISCUSSION

It is the first time to analyze ESTs of Acanthamoeba known as an human pathogen and a good model system for biological studies. The most frequently presented gene was actin gene which appeared 12 times. This was as expected because Acanthamoeba has well developed cytoskeleton and move very actively (Pollard, 1982). The biggest class among identified clones was genes for protein synthesis with the number of 32 from 130 clones. Similar results were reported in the EST analysis of Entamoeba histolytica, Typanosoma cruzi, and E. dispar (Tanaka et al., 1997; Verdun et al., 1998; Sharma et al., 1999).

Among four clones of genes for surface protein, two were identified as non-integrin type laminin binding protein. Adhesion would be the very first and important step to infect host by tissue invading parasites. Laminin binding proteins has been reported from several parasitic organisms including Trichomonas vaginalis (Silva Filho et al., 1988), Trypanosoma brucei rhodesiense (gene bank, W99296), Leishmania donovani (Ghosh et al., 1996) and Echinococcus granulosus (Zhang et al., 1997). The ability to recognize extracellular matrix proteins such as laminin or fibronectin

Table 2. Significant matches of Acanthamoeba healyi ESTs with database sequences from other organisms

Clone				Data	Accession	·	
No.	Length	Identification	Organism	base	No.	Score	Probability
Surface protein	protein						
Ah161	808	coatomer delta subunit	Oryza sativa	${ m sp}^{a)}$	P49661	182	3.80E-17
Ah217	275	laminin receptor like protein (P40)	Daucus carota	${ m dbj^{b)}}$	AB012702	214	3.10E-21
Ah477	256	laminin receptor like protein (P40)	D. carota	dbj	AB012702	202	1.20E-19
Ah489	202	70 kD peroxisomal membrane protein	Mus musculus	ds	P55096	145	4.30E-12
DNA metabolism	tabolism						
Ah468	506	methyl transferase	Saccharomyces cerevisiae	$^{\mathrm{ds}}$	P25087	226	2.60E-23
Ah534	308	double-strand break repair protein	Caenorhabditis elegans	${f g}{f b}^{ m c)}$	U40029	236	3.50E-24
Energy n	Energy metabolism	E					
Ah015	153	acyl-CoA dehydrogenase	C. elegans	$^{ m emb^{d)}}$	AL032621	102	1.40E-06
Ah029	227	ATP synthase gamma chain	Arabidopsis thaliana	ds	Q01908	88	0.00034
Ah035	180	3-hydroxyisobutyrate dehydrogenase	Rabbit	$^{\mathrm{ds}}$	P32185	107	5.50E-07
Ah089	262	adenylosuccinate synthetase precursor	A. thaliana	$^{\mathrm{ds}}$	Q96529	217	1.00E-21
Ah110	261	NADP-isocitrate dehydrogenase	Glycine max	qg	AF095445	586	3.20E-31
Ah335	256	NADP-isocitrate dehydrogenase	Pig	gp	M86719	523	1.80E-33
Ah452	193	6-phosphogluconate dehydrogenase	Cunninghamella elegans	emp	Y17297	140	1.90E-11
Ah475	181	fatty acid synthase, subunit alpha	Candida albicans	ds	P43098	506	1.20E-20
Ah532	364	NADH-ubiquinone oxidoreductase	Homo sapiens	$^{\mathrm{ds}}$	Q16795	277	1.00E-29
Ah542	281	enolase	Spongilla sp.	qg	U85829	198	5.00E-19
Ah577	254	fatty acid synthase, subunit alpha	C. albicans	$^{\mathrm{ds}}$	P43098	135	5.40E-23
Ah595	137	transketolase	Spinacia oleracea	qg	L76554	127	5.50E-10
Immunology	logy						
Ah342	228	immunoglobulin heavy chain V-D-J region	Oryctolagus cuniculus	qg	AF058603	77	0.015
Ah464	244	Leukotriene A-4 hydrolase	Cavia porcellus	ds	P19602	110	4.90E-07
Kinase a	Kinase and phosphatase	hatase					
Ah042	225	cytohesin 2	H. sapiens	emp	Z94160	100	1.00E-05
Ah066	271	mevalonate kinase	Rat	$^{\mathrm{ds}}$	P17256	100	2.10E-06
Ah119	243	c2 domain	H. sapiens	qg	AC005278	177	2.90E-16
Ah172	569	3-phosphoglycerate kinase	Thermotoga maritime	emp	X75437	254	8.90E-27
Ah177	160	myosin heavy chain kinase B	Dictyostelium discoideum	ds	P90648	91	1.30E-07
Ah233	232	nucleoside diphosphate kinase 3	A. thaliana	qg	AF044265	227	9.30E-23
Ah401	119	S-phase kinase associated protein like	D. discoideum	qg	U73686	06	9.50E-05
Ah436	207	Skb1Hs	H. sapiens	qg	AF015913	167	4.40E-15
Ah463	223	phosphatases pleiotropic regulator	A. thaliana	$^{\mathrm{ds}}$	Q39190	280	1.40E-30

Table 2. Continued

Ab1824 242 phosphoenolpyruvate carboxykinase Chlorobium limicola sp Q08862 Ab1834 272 protein kinase Chlorobium limicola sp Q082245 Ab103 208 ADP-ribosylgycohydrolase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomutase Cchlosylamomomomutase Cchlosylamomomomutase Cchlosylamomomomomomomomomomomomomomomomomomomo	Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
No P-ribosylgycohydrolase Methanococcus jannaschii pire hosphomannomutase C. elegans C. elegans binguitin horedoxin peroxidase garden pea garden pea garden pea garden pea garden pea pir hioredoxin peroxidase Pig M. musculus sporotesion degradation protein 1 Drosophiia melanogaster sporotesoome subunit DD5 D. discoideum sporotesoome subunit DD5 D. discoideum sporotesoome subunit DD5 D. melanogaster sporotesoome subunit DD5 D. discoideum A. thaliana C.I.AV protein (RNA metabolism) D. discoideum sporotein (TabB4) D. discoideum sporotei	Ah482	242	phosphoenolpyruvate carboxykinase	Chlorobium limicola	ds	Q08262	116	7.30E-08
OPP-ribosylglycohydrolase Methanococcus jannaschii pire) hosphomannomutase C. elegans emb higuitin Acanthamoeba castellnii pir hioredoxin peroxidase D. discoideum garden pea pir fillydrolipoamide dehydrogenase Pig sp protein disulfide isomerase Pig sp hopquitin fusion degradation protein 1 M. musculus sp protein disulfide isomerase Pig sp hopquitin fusion degradation protein 1 M. musculus sp protein disulfide isomerase D. discoideum sp protein decarboxylase D. discoideum sp A. thaliana A. thaliana pp diptuamate decarboxylase D. discoideum sp phiquitin D. discoideum sp phiquitin D. discoideum sp phiazole	Ah584	272	protein kinase	Schizosaccharomyces pombe	emb	AL022245	80	8.90E-03
Methanococcus jamaschii pire) hosphomannomutase hisophomannomutase hisophomannomutase hisophomannomutase hisophomannomutase hisophomannomutase hisophomannomutase hisophomannomutase hisordedum hisordedoxin peroxidase hisordedoxin peroxidase hisordedoxin peroxidase hisordedoxin peroxidase hisordedoxin peroxidase hisordeoxin subunit DD5 hisordeoxin subunit DD5 hisordeoxin hisordeoxin hisordeoxin subunit DD5 hisordeoxin hisordeoxin subunit DD5 hisordeoxin hisor	Other me	etabolism						
biquitin hioredoxin peroxidase hioredoxin more decide binding protein hioredoxin peroxin RABA hioredoxin peroxin R	Ah030	808	ADP-ribosylglycohydrolase	Methanococcus jannaschii	pire	C64448	83	0.0017
hioredoxin peroxidase brotein disulfide isomerase linydrolipoamide dehydrogenase linydrolipoa	Ah039	237	phosphomannomutase	C. elegans	emp	AL021481	84	2.00E-03
hioredoxin peroxidase protein disulfide isomerase protein disulfide isomerase protein disulfide isomerase play and protein disulfide isomerase play and protein degradation protein 1 Pig protein Pig protein (Pig protein Pig protein (Pig protein Pig protein (Pig Pig protein (Pig Pig protein (Pig Pig Pig Pig Pig Pig Pig Pig Pig Pig	Ah043	190	ubiquitin	Acanthamoeba castellnii	pir	S45304	252	6.30E-27
big displayed by d	Ah090	217	thioredoxin peroxidase	Trypanosoma brucei rhodesiense		Q26695	213	2.30E-21
lihydrolipoamide dehydrogenase Pig parden pea phir hioredoxin peroxidase Pig	Ah111	293	protein disulfide isomerase	D. discoideum	qg	AF019112	245	1.80E-25
hioredoxin peroxidase biquitin fusion degradation protein 1 brosophila melanogaster brotein disulfide isomerase rotein disulfide isomerase brotein disulfide isomerase Sus scrofa Drosophila melanogaster Sus scrofa A. thaliana A. thaliana Biquitin A. castellnii B. discoideum Broteosome subunit PAC1 D. melanogaster B. discoideum Broteosome subunit DD5 D. discoideum Broteosome subunit DD5 D. discoideum Broteosome subunit DD5 D. discoideum Brotein (RNA metabolism) B. discoideum Brotein (RNA metabolism) B. discoideum B.	Ah162	214	dihydrolipoamide dehydrogenase	garden pea	pir	A42494	66	1.10E-05
biquitin fusion degradation protein 1 Drosophila melanogaster sp Sus scrofa	Ah171	897	thioredoxin peroxidase	Pig	ďs	P52552	117	4.00E-09
brotein disulfide isomerase Drosophila melanogaster Sus scrofa Sus scrofa Dr. discoideum Subunit DD5 Dr. discoideum Subunit PAC1 Ar. thaliana Ar. castellnii Subunit DD5 Dr. melanogaster Ar. castellnii Subunit DD5 Dr. discoideum Ar. thaliana Dr. discoideum Ar. thaliana Dr. melanogaster Subunit DB5 Dr. discoideum Ar. thaliana Dr. melanogaster Subunit DB5 Dr. discoideum Subunit DB5 Dr. discoideum Ar. thaliana Dr. melanogaster Subunit DB5 Dr. discoideum Subunit DB5 Dr. discoideum Ar. thaliana Dr. melanogaster Subunit DB5 Dr. discoideum Sub	Ah173	224	ubiquitin fusion degradation protein 1	M. musculus	ds	P70362	112	5.70E-09
Sus scrofa D. discoideum D. discoideum D. discoideum A. thaliana D. melanogaster A. castellnii D. melanogaster A. castellnii D. discoideum D. melanogaster A. castellnii D. discoideum D. discoideum Sp D. melanogaster Sp Hiazole mono Thairanle protein (RNA metabolism) Thairanle mono Thairanle mo	Ah174	185	protein disulfide isomerase	Drosophila melanogaster	ds	P54399	168	3.30E-15
oroteosome subunit DD5 O. discoideum A. thaliana O. melanogaster A. castellnii D. discoideum A. castellnii D. discoideum D. discoideum D. discoideum D. discoideum Sp A. castellnii D. discoideum Sp D. melanogaster Sp D. melanogaster Sp D. melanogaster Sp Escherichia coli A. thaliana M. thaliana A. thaliana A. thaliana PA. dapiens Sp Chlamydomonas reinhardtii Sp Granine like protein (rab2) Granine nucleotide binding protein B. melanogaster Sp Hydra vulgaris Sp Hydra v	Ah179	275	uricase	Sus scrofa	ds	P16164	61	1.20E-06
20S proteosome subunit PAC1 D. melanogaster plitamate decarboxylase bliquitin proteosome subunit DD5 D. discoideum D. discoideum D. discoideum D. melanogaster Sp Escherichia coli D. melanogaster Sp Escherichia coli D. melanogaster Sp Escherichia coli Sp Escherichia	Ah346	236	proteosome subunit DD5	D. discoideum	ds	P34120	522	3.50E-36
bliquitin broteosome subunit DD5 b. discoideum broteosome subunit DD5 castellnii broteosome subunit DD5 castellnii broteosome subunit DD5 castellnii broteosome subunit DD5 castellnii cast	Ah381	260	20S proteosome subunit PAC1	A. thaliana	qg	AF043521	335	5.50E-38
b. discoideum b. discoideum b. discoideum b. discoideum b. melanogaster hiazole mono thiazole thiazo	Ah421	151	glutamate decarboxylase	D. melanogaster	ds	P20228	117	1.20E-08
b. discoideum TAV protein (RNA metabolism) D. melanogaster Escherichia coli D. melanogaster P. melanogaster P. melanogaster P. thaliana A. thaliana A. thaliana A. thaliana A. thaliana A. thaliana B. discoideum P. disc	Ah458	150	ubiquitin	A. castellnii	ds	P49634	148	6.60E-13
Hiazole mono Mininopeptidase-like protein (RNA metabolism) Mininopeptidase-like protein The binding protein (rab2) The sapiens The binding protein (rab2) The sapiens The sa	Ah461	229	proteosome subunit DD5	D. discoideum	ds	P34120	310	1.10E-34
minopeptidase-like protein minopeptidase-like protein The binding protein (rab2) The sapiens The	Ah581	228	ELAV protein (RNA metabolism)	D. melanogaster	ds	P16914	102	5.50E-06
emb chain opeptidase-like protein (rab2) The binding protein (rab2) The binding protein (rab2) The binding protein (rab2) The saleted protein RAB4 The saleted protein Ra	Ah582	190	thiazole mono	Escherichia coli	ds	Q46948	97	2.30E-07
emb chain opeptidase-like protein (rab2) The binding protein (rab2) The saction of the protein RAB4 The saction of the protein RAB4 The saction of the protein and the pro	Protease							
chamydomonas reinhardtii sp D. discoideum sp D. discoideum sp D. discoideum sp distrin like protein 3 (VILIP-3) H. sapiens Hydra vulgaris sp Garanine nucleotide binding protein ac GTP binding protein Rab-7 H. sapiens Sp D. melanogaster sp Sp	Ah068	195	aminopeptidase-like protein	A. thaliana	emp	80266Z	104	1.80E-06
260GTP binding protein (rab2)Chlamydomonas reinhardtiisp250ras related protein RAB4D. discoideumsp284visinin like protein 3 (VILIP-3)H. sapienssp182Guanine nucleotide binding proteinHydra vulgarissp18514-3-3 proteinD. melanogastersp17014-3-3 proteinD. melanogastersp283rac GTP binding protein Rab-7A. thalianagb246small GTP binding protein Rab-7H. sapiensgb307GTP binding nuclear protein SPI1S. pombesp251G protein beta subuit likeM. musculuspir255G protein beta subuit likeM. musculuspir	Signal tr.	ansduction	uo					
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284visinin like protein 3 (VILIP-3)H. sapienssp182Guanine nucleotide binding proteinHydra vulgarissp18514-3-3 proteinD. melanogastersp17014-3-3 proteinD. melanogastersp283rac GTP binding protein Arac10A. thalianagb246small GTP binding protein Rab-7H. sapiensgb307GTP binding nuclear protein SPI1S. pombesp251G protein beta subuit likeM. musculuspir255G protein beta subuit likeM. musculuspir	Ah155	250	ras related protein RAB4	D. discoideum	ds	P36410	386	4.60E-45
182Guanine nucleotide binding proteinHydra vulgarissp18514-3-3 proteinD. melanogastersp17014-3-3 proteinD. melanogastersp283rac GTP binding protein Arac10A. thalianagb246small GTP binding protein Rab-7H. sapiensgb307GTP binding nuclear protein SPI1S. pombesp251G protein beta subuit likeM. musculuspir255G protein beta subuit likeM. musculuspir	Ah184	284	visinin like protein 3 (VILIP-3)	H. sapiens	ds	P37235	29	2.00E-05
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283 rac GTP binding protein Arac10 A. thaliana gb 246 small GTP binding protein Rab-7 H. sapiens gb 307 GTP binding nuclear protein SPI1 S. pombe 251 G protein beta subuit like M. musculus pir 255 G protein beta subuit like M. musculus pir	Ah501	170	14-3-3 protein	D. melanogaster	ds	P92177	183	1.20E-16
246small GTP binding protein Rab-7H. sapiensgb307GTP binding nuclear protein SPI1S. pombesp251G protein beta subuit likeM. musculuspir255G protein beta subuit likeM. musculuspir	Ah503	283	rac GTP binding protein Arac10	A. thaliana	дg	AF079485	275	1.20E-29
307GTP binding nuclear protein SPI1S. pombesp251G protein beta subuit likeM. musculuspir255G protein beta subuit likeM. musculuspir	Ah578	246	small GTP binding protein Rab-7	H. sapiens	дg	U44104	188	8.90E-18
251 G protein beta subuit like M. musculus pir 255 G protein beta subuit like M. musculus pir	Ah603	307	GTP binding nuclear protein SPI1	S. pombe	ds	P28748	386	7.00E-45
255 G protein beta subuit like M. musculus pir	Ah622	251	G protein beta subuit like	M. musculus	pir	149700	288	3.80E-31
	Ah627	255		M. musculus	pir	149700	588	5.40E-33

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real and cytoskeletal Urganism base real and cytoskeletal Organism base 125 actin capping protein bets subunit A castellanii sp 245 actin bundling protein A castellanii sp 246 actin actin A castellanii sp 286 actin A castellanii sp 192 gelation factor (Arp2) A castellanii sp 271 actin A castellanii sp 272 actin A castellanii sp 273 actin A castellanii sp 274 actin A castellanii sp 275 actin A castellanii sp 270 actin A castellanii sp 287 actin A castellanii sp 287 actin A castellanii sp 287 actin A castellanii sp 288 actin A castellanii sp 281	Clone	1 7			Data	Accession	7	-
apping protein bets subunit A. castellanii sp A.	No.	Lengtn	Identification	Organism	base	No.	Score	Probability
A. castellanii sp A. castellanii sp Physarum polycephalum pir A. castellanii sp A. castellanii sp A. castellanii sp O. sativa A. castellanii sp O. sativa A. castellanii sp	Structura	al and cy	toskeletal					
A. castellanii sp Physarum polycephalum pir A. castellanii sp A. castellanii sp A. castellanii sp O. sativa A. castellanii sp A. castellan	Ah016	225		D. discoideum	ds	P13021	220	2.80E-22
rotein A. castellanii A. castellanii B. D. discoideum B. D. discoideum B. D. melanogaster B	Ah031	195	actin	A. castellanii	ds	P02578	280	9.90E-31
A. castellanii sp A. castellanii sp A. castellanii sp D. discoideum A. castellanii sp O. sativa A. castellanii sp A. cas	Ah072	245	actin-bundling protein	Physarum polycephalum	pir	S32566	93	2.50E-12
A. castellanii sp A. castellanii sp D. discoideum A. castellanii sp O. sativa A. castellanii sp A. cas	Ah145	217	actin	A. castellanii	ds	P02578	337	1.80E-38
lein 2 (Arp2) A. castellanii A. castellanii B. discoideum A. castellanii A. castellanii B. A. castellanii A. castellanii B. A. castellanii C. castellanii B. A. castellanii C. castellanii C. celegans B. discoideum C. celegans C. celegans D. melanogaster	Ah215	586	actin	A. castellanii	$^{\mathrm{ds}}$	P02578	425	2.60E-50
D. discoideum A. castellanii O. sativa A. castellanii A. castellanii A. castellanii Sp A. castellanii A. castellanii A. castellanii A. castellanii Sp A. castellanii Sp A. castellanii A. castellanii Sp A. castellanii A. castellanii Sp A. castellanii Sp A. castellanii B. A. castellanii A. castellanii Sp A. castellanii	Ah219	173	actin-related protein 2 (Arp2)	A. castellanii	ďs	P53487	197	1.10E-19
A. castellanii sp O. sativa A. castellanii sp A.	Ah220	192	gelation factor	D. discoideum	ds	P13466	162	3.30E-14
A. castellanii sp A. castellan	Ah221	897	actin	A. castellanii	ds	P02578	384	1.40E-44
A. castellanii sp A. castellan	Ah231	271	tubulin alpha 1	O. sativa	ds	P28752	297	1.00E-32
A. castellanii sp A. castellan	Ah341	275	actin	A. castellanii	ds	P02578	596	4.50E-38
A. castellanii sp B. discoideum B. melanogaster amb C. chinery C	Ah344	197	actin	A. castellanii	$^{\mathrm{ds}}$	P02578	227	2.90E-30
A. castellanii sp B. discoideum B. melanogaster cemb Cyprinus carpio dbj B. melanogaster cemb D. melanogaster cemb D. melanogaster cemb D. melanogaster cemb C. elegans A. castellanii sp B. castellanii cemb C. elegans B. C. ele	Ah354	159	actin	A. castellanii	ds	P02578	233	1.20E-24
A. castellanii sp Cycridanii sp B. discoideum sp B. H. sapiens sp Cyprinus carpio dbj B. melanogaster emb D. melanogaster emb D. melanogaster emb C. elegans C. elegans D. C. elegans D. H. sapiens D. H. sapiens D. H. sapiens D. Fritillaria agrestis Sp	Ah361	287	actin	A. castellanii	ds	P02578	461	2.80E-55
A. castellanii sp A. castellanii sp d. castellanii sp A. castellanii sp ding protein) D. discoideum sp Toxocara canis gb H. sapiens Gyprinus carpio gb Phaseolus vulgaris gb D. melanogaster emb D. melanogaster sp D. melanogaster emb D. melanogaster sp D. melanogaster sp D. melanogaster emb D. melanogaster sp	Ah388	270	actin	A. castellanii	ds	P02578	469	2.00E-56
A. castellanii sp A. castellanii sp ding protein) D. discoideum D. discoideum Sp H. sapiens H. sapiens By	Ah402	167	actin	A. castellanii	ds	P02578	277	1.00E-30
A. castellanii sp nding protein) D. discoideum Toxocara canis H. sapiens Pr-binding factor 2 Cyprinus carpio Phaseolus vulgaris D. melanogaster emb Chinery D. melanogaster emb D. melanogaster emb D. melanogaster emb C. elegans 2 C. elegans pir H. sapiens sp	Ah499	295	actin	A. castellanii	ds	P02578	498	2.30E-60
rding protein) D. discoideum Toxocara canis H. sapiens H. sapiens B. melanogaster Cyprinus carpio Phaseolus vulgaris B. melanogaster emb D. melanogaster emb D. melanogaster emb D. melanogaster emb Chinery rat L.38 C. elegans pir H. sapiens sp	Ah599	164	actin	A. castellanii	$^{\mathrm{ds}}$	P02578	260	2.30E-28
Toxocara canis gb H. sapiens Er-binding factor 2 D. melanogaster chinding factor 2 Cyprinus carpio D. melanogaster D. melanogaster emb D. melanogaster emb D. melanogaster emb D. melanogaster emb D. celegans Tat emb C. elegans Trat H. sapiens Eritillaria agrestis Sp	Ah614	211	coronin (actin binding protein)	D. discoideum	ds	P27133	164	1.20E-14
Toxocara canis gb H. sapiens F. Pinding factor 2 Cyprinus carpio O. Melanogaster D. melanogaster D. melanogaster D. melanogaster Ethinery O. melanogaster Chinery Tat Chinery Tat Celegans Tat Celegans Thillaria agrestis Sp Trillaria agrestis Sp	Cell cycle	e related						
H. sapiens r-binding factor 2 D. melanogaster chinding factor 2 Cyprinus carpio Phaseolus vulgaris D. melanogaster D. melanogaster D. melanogaster chinery rat 2 C. elegans 1 gamma T. H. sapiens pritillaria agrestis pripara gb dbj gb gb chinery Chinery Tat Emb C. elegans Pritillaria agrestis pripara pritillaria agrestis pripara prip	Ah114	193	prohibitin	Toxocara canis	qg	U97204	100	5.90E-06
r-binding factor 2 D. melanogaster cryptinus carpio (Pv42p) (Pv42p) D. melanogaster D. melanogaster chinery 1.38 C. elegans 1 gamma 1 gamma C. elegans D. melanogaster emb C. elegans Pritilaria agrestis Sr Fritilaria agrestis Sr Fritilaria agrestis Sr	Ah151	234	diaphanous 1	H. sapiens	qg	AF051782	131	6.00E-10
r-binding factor 2 D. melanogaster emb r-binding factor 2 Cyprinus carpio D. melanogaster emb D. melanogaster emb D. melanogaster emb T.1.38 C. elegans T.2 C. elegans D. melanogaster emb T.38 C. elegans T.4 H. sapiens D. melanogaster emb T.7 H. sapiens Sp	Transcri	otion fact	tors					
r-binding factor 2 Cyprinus carpio dbj (Pv42p) Phaseolus vulgaris gb D. melanogaster emb D. melanogaster emb r L38 rat 2 C. elegans 1 gamma C. elegans T. H. sapiens Phaseolus vulgaris gb cmb C. elegans T. H. sapiens pir Fritillaria agrestis sp	Ah147	263	myocyte-enhancer-binding factor 2	D. melanogaster	emp	X83527	114	4.40E-13
r (Pv42p) Phaseolus vulgaris gb D. melanogaster emb D. melanogaste	Ah163	230	myocyte-enhancer-binding factor 2	Cyprinus carpio	dbj	AB012884	82	1.10E-10
Chinery D. melanogaster emb chinery D. melanogaster emb n L38 rat emb 2 C. elegans sp 1 gamma C. elegans sp n L7 H. saplens pir Otein S16 Fritillaria agrestis sp	Ah235	271	SNF4-like protein (Pv42p)	Phaseolus vulgaris	qg	U40713	06	3.60E-04
chinery D. melanogaster emb n L38 rat emb 2 C. elegans sp 1 gamma C. elegans sp n L7 H. saplens pir otein S16 Fritillaria agrestis sp	Ah356	150	dribble protein	D. melanogaster	emp	Z96931	151	2.50E-13
chinery rat emb 1.238 C. elegans sp 2 C. elegans sp 1 gamma C. elegans sp n.L.7 H. saplens pir otein S16 Fritillaria agrestis sp	Ah396	202	dribble protein	D. melanogaster	emp	Z96931	280	1.10E-30
289 ribosomal protein L38 cale page 249 elongation factor 2 C. elegans Sp 266 elongation factor 1 gamma C. elegans Sp 228 ribosomal protein L7 H. sapiens Sp 237 40S ribosomal protein S16 Fritillaria agrestis Sp 237 40S ribosomal protein S16 Fritillaria agrestis Sp 240 Fritillaria agrestis Sp 251 40S ribosomal protein S16 Fritillaria agressis Sp 251 40S ribosomal protein S16 40S	Transcrip	otion and	translational machinery					
249 elongation factor 2 C. elegans sp 266 elongation factor 1 gamma C. elegans sp 228 ribosomal protein L7 H. sapiens pir 5237 40S ribosomal protein S16 Fritillaria agrestis sp	Ah006	588	ribosomal protein L38	rat	emp	X57007	215	8.90E-08
266 elongation factor 1 gamma C. elegans sp 228 ribosomal protein L7 H. sapiens pir 237 40S ribosomal protein S16 Fritillaria agrestis sp	Ah082	249		C. elegans	ds	P29691	106	1.80E-14
228 ribosomal protein L7 H. sapiens pir 237 40S ribosomal protein S16 Fritillaria agrestis sp	Ah085	566	elongation factor 1 gamma	C. elegans	ds	P54412	108	4.00E-07
237 40S ribosomal protein S16 Fritillaria agrestis sp	Ah116	228	ribosomal protein L7	H. sapiens	pir	S30212	114	2.60E-07
Je and the second secon	Ah146	237	40S ribosomal protein S16	Fritillaria agrestis	ds	022647	215	1.50E-21

Table 2. Continued

Ah14B 2.83 RAMe L Inhibition Mm seculins gp U90446 181 8.0E-17 Ah15G 2.84 ATP-dependent Textor 1-alpha Blasnosystis hominis sp 190446 184 2.5.0E-28 Ah15G 2.84 ATP-dependent Textor 1-alpha Blasnosystis hominis sp 174768 187 1.10E-20 Ah18T 2.75 Gongation factor 18 Rank hilling protein funtiliple splicing H. saplens sp 19851 1.10E-20 Ah23B 2.75 GoNs acidic ribosomal protein SQ H. saplens sp P19951 1.10E-20 Ah33B 2.57 GoNs acidic ribosomal protein SQ H. saplens sp P25520 1.10E-20 Ah33G 2.57 Gongation factor 1-alpha B. mort D. Sobolo 1.1 1.10E-20 Ah36B 2.81 Gongation factor 1 alpha D. Mouse sp P25520 2.1 2.0E-21 Ah38G 2.82 H. Sopomal protein ST A. Topic SI A. Topic SI A. Topic SI Ah38G 2.82 </th <th>Clone No.</th> <th>Length</th> <th>Identification</th> <th>Organism</th> <th>Data base</th> <th>Accession No.</th> <th>Score</th> <th>Probability</th>	Clone No.	Length	Identification	Organism	Data base	Accession No.	Score	Probability
44 Elemegation factor 1-alpha Biastocystis hominis sp P54959 335 70 Elemegation factor 1-alpha Biastocystis hominis sp P54959 335 70 Elemegation factor 1B gamma 0. sathers 0. sathers 0. sathers app 198871 151 77 GOS actific ribosomal protein PO Bos taurus sp Q95140 185 87 Elemegation factor 2 alpha Trypanosoma cruzi dbj D58066 161 86 elongation factor 1 alpha Bonbyx mori sp P2530 241 86 clongation factor 1 alpha Bombyx mori sp P2530 241 86 clongation factor 1 alpha B. mori sp P25520 241 86 clongation factor 1 beta D. melanogaster sp P25520 241 87 clongation factor 1 beta D. melanogaster sp P25520 241 88 clossomal protein PO RAnsteam sp P25520 241 89 clossomal protein PO Pleasmodium falciparum sp P468154 136 80 clos ribosomal protein PO	Ah148	258	RNAse L inhibitor	M. musculus	qg	U90446	181	8.90E-17
88 APP-dependent RNA helicase H. sapiens emb Y14768 198 70 60S acidic ribosomal protein PO 0.0 sativa sp pp 19951 1 114 71 60S acidic ribosomal protein PO H. sapiens sp P19951 1 114 72 60S acidic ribosomal protein S14 H. sapiens sp P19951 1 114 73 6 clongation factor 2 B. taurus sp P19951 1 114 74 60S acidic ribosomal protein PO B. taurus sp P29520 241 185 80 clongation factor 1-alpha Bombyx mori sp P29520 241 185 81 clossmal protein S16 Mouse Bombyx mori sp P29520 241 82 closs ribosomal protein L4 R. fragilis pp P14131 1 160 84 ribosomal protein L4 C. maltosa sp P404660 91 85 clossmal protein L4 S. cerevisiae sp P403186 91 86 closs ribosomal protein PO P. faigidparum sp P403660 91 87 clossomal protein S2 P. faiciparum sp P40213 <td>Ah156</td> <td>264</td> <td>elongation factor 1-alpha</td> <td>Blastocystis hominis</td> <td>ds</td> <td>P54959</td> <td>335</td> <td>5.50E-38</td>	Ah156	264	elongation factor 1-alpha	Blastocystis hominis	ds	P54959	335	5.50E-38
00 Consideration for on the part of th	Ah181	288	ATP-dependent RNA helicase	H. sapiens	emp	Y14768	198	1.10E-20
71 GOS actidic ribosomal protein PO Bos taurus sp Q95140 185 72 ROA actidic ribosomal protein Cutlippe splicing) H. sapiens sp C95140 185 73 elongation factor 2 Trypanosoma cruzi dbj D84107 111 74 GOS calder ribosomal protein S14 Trypanosoma cruzi dbj D550806 161 75 GOS actide ribosomal protein S16 B. taurus sp P259200 241 86 ribosomal protein S16 B. mori sp P259200 241 74 ribosomal protein L41 K. fragilis sp P259200 415 75 closyal protein D4 K. fragilis sp P293200 415 76 clos ribosomal protein L41 K. fragilis sp P293200 415 76 clos ribosomal protein D4 P. cmaltosa sp P40213 294 8 dos ridosomal protein D5 P. falciparum sp P40213 294 9 clos ribosomal protein D5 R. sapiens sp P40213 294 10 dos ribosomal protein D5 P. falciparum sp <td>Ah187</td> <td>270</td> <td>elongation factor 1B gamma</td> <td>O. sativa</td> <td>dbj</td> <td>D89802</td> <td>151</td> <td>1.40E-12</td>	Ah187	270	elongation factor 1B gamma	O. sativa	dbj	D89802	151	1.40E-12
5.5 RANA binding protein (multiple splicing) H. sapiens dbj D984107 114 5.7 40Sr blosomal protein S14 Maize sp P19951 149 5.7 40S ribosomal protein S14 Typanosoma cruzi dbj D50806 161 5.7 60S acidic ribosomal protein PO B. taurus sp P29520 241 5.9 elongation factor 1 alpha B. mori sp P14131 160 5.7 elongation factor 1 alpha B. mori sp P29520 241 5.0 elongation factor 1 beta D. melanogaster sp P29520 415 6.0 fibosomal protein L4 B. mori sp P29520 415 6.0 fibosomal protein L4 B. mori sp P40213 36 6.0 fibosomal protein L4 C. maltosa sp P40213 36 6.0 fibosomal protein L4 H. sapiens sp P40213 36 8 40S ribosomal protein R516 H. sapiens sp<	Ah193	247	60S acidic ribosomal protein PO	Bos taurus	ds	Q95140	185	2.30E-17
YY 40S ribosomal protein S14 Maize sp P19951 149 55 elongation factor 2 Trypanosoma cruzi dbj D50806 161 99 elongation factor 1-alpha Bombyx mori sp P29520 241 80 ribosomal protein S16 Mouse sp P14131 160 80 ribosomal protein S16 Bombyx mori sp P29520 241 80 ribosomal protein L41 R. fragilis gb M62394 154 81 ribosomal protein SPLI C. maltosa sp P40218 154 80 tRNA splicing protein L41B S. cerevisiae sp P40218 154 81 ribosomal protein SPLI C. maltosa sp P40218 154 80 60S ribosomal protein R516 S. cerevisiae sp P40218 146 81 40S ribosomal protein R516 S. cerevisiae sp P40218 149 82 60S acidic ribosomal protein R516 H. sapiens sp	Ah208	275	RNA binding protein (multiple splicing)	H. sapiens	dbj	D84107	114	1.90E-07
Fig. 20 Fig. 20 Fig. 20 Fig. 20 Fig. 20	Ah232	167	40S ribosomal protein S14	Maize	ds	P19951	149	4.70E-13
57 6058 actidic ribosomal protein PO B. taurus sp Q95140 185 99 elongation factor 1-alpha Mounbyx mori sp P29520 241 87 elongation factor 1 alpha Moune sp P29520 241 19 elongation factor 1 alpha B. mori sp P29520 415 41 ribosomal protein SDL C. melanogaster emb AL031863 86 20 elongation factor 1 alpha D. melanogaster emb AL031863 86 21 ribosomal protein SDL C. merevisiae sp P38754 136 22 60S ribosomal protein PO Plasmodium falciparum sp P48154 136 23 60S acidic ribosomal protein PO P. falciparum sp P48154 136 24 40S ribosomal protein SSA H. sapiens sp P48154 135 25 60S acidic ribosomal protein PO P. falciparum sp P48154 135 26 60S acidic ribosomal protein PO	Ah339	255	elongation factor 2	Trypanosoma cruzi	dbj	D50806	161	5.20E-14
99 elongation factor 1-alpha Bombyx mori sp P29520 241 86 ribosomal protein S16 Mouse sp P14131 160 86 ribosomal protein S16 B. mort sp P29520 415 99 elongation factor 1 alpha D. melanogaster emb AL031863 86 41 ribosomal protein L41 C. maltosa sp P87187 146 90 tRNA splicing protein SPLI C. maltosa sp P87187 146 90 etos ribosomal protein PO Plasmedium falciparum sp P40213 294 10 40S ribosomal protein RS16 S. cerevisiae sp P40813 294 11 40S ribosomal protein PO P. fatciparum sp P40813 294 12 40S ribosomal protein RS16 S. cerevisiae sp P40813 145 13 40S ribosomal protein PO P. fatciparum sp Q94660 145 14 phenylalany-tRNA synthetase C. albicans	Ah345	257	60S acidic ribosomal protein PO	B. taurus	ds	Q95140	185	2.60E-17
56 ribosomal protein S16 Mouse sp P14131 160 77 elongation factor 1 alpha B. mori sp P29520 415 87 elongation factor 1 alpha D. melanogaster emb P29520 415 84 ribosomal protein L41 K. fragilis sp P87187 146 90 GSC scilor chosomal protein D14EB S. cerevisiae sp P38754 136 90 GOS acidic ribosomal protein PO Plasmodium faciparum sp P40213 294 90 GOS acidic ribosomal protein PO P. falciparum sp P40213 294 4 OS ribosomal protein SA H. sapiens sp AF088519 149 4 OS ribosomal protein PO P. falciparum sp AF088519 149 6 OS acidic ribosomal protein PO P. falciparum sp AF088519 149 11 6OS acidic ribosomal protein L14 (CAG-ISL 7) H. sapiens sp P50914 163 11 6OS acidic ribosomal protein L14 (CAG-ISL 7) H. sapiens<	Ah347	509	elongation factor 1-alpha	Bombyx mori	ds	P29520	241	2.90E-25
97 elongation factor 1 alpha B. mori sp P29520 415 19 elongation factor 1 beta D. melanogaster emb AL031863 86 19 ribosomal protein L14 C. matlosa sp P87187 154 20 tRNA splicing protein SPL1 C. matlosa sp P87187 164 22 60S ribosomal protein L14EB S. cerevisiae sp P48154 136 30 60S acidic ribosomal protein RS16 H. sapication sp P48154 136 40S ribosomal protein RS4 Avicennia marina sp Q94660 145 4 GS ribosomal protein RS7 Avicennia marina sp Q94660 145 5 GS acidic ribosomal protein PO P. falciparum sp Q94660 145 6 GS acidic ribosomal protein L14 (CAC-ISL 7) H. sapiens sp Q94660 145 11 dosomal protein L13c H. sapiens sp Q08170 95 22 splicing factor H. sapiens sp P29520 315	Ah365	236	ribosomal protein S16	Mouse	$^{\mathrm{ds}}$	P14131	160	5.90E-14
19 elongation factor 1 beta D. melanogaster emb AL031863 86 44 ribosomal protein L41 K. fragilis gb M62394 154 50 tRNA splicing protein SPL1 C. maltosa sp P87187 146 50 60S acidic ribosomal protein L14EB S. cerevisiae sp P40213 294 50 60S acidic ribosomal protein R516 S. cerevisiae sp P40213 294 51 40S ribosomal protein R516 H. sapiens sp P40213 294 51 40S ribosomal protein R516 H. sapiens sp P40213 294 54 40S ribosomal protein R51 P. falciparum sp AF098519 149 54 40S ribosomal protein R57 P. falciparum sp AF038519 149 54 phenylalanyl-tRNA synthetase C. albicans sp AF038519 149 54 phenylalanyl-tRNA synthetase C. albicans sp P50914 163 55 splicing factor	Ah390	287	elongation factor 1 alpha	B. mori	ds	P29520	415	6.20E-49
94 ribosomal protein L41 K. fragilis gb M62394 154 90 tRNA splicing protein SPL1 C. maltosa sp P87187 146 22 60S ribosomal protein L14EB S. cerevisiae sp Q94660 91 90 60S acidic ribosomal protein RS16 H. sapiens sp P40213 294 14 40S ribosomal protein RS16 H. sapiens sp P48154 135 24 40S ribosomal protein RS16 H. sapiens sp AF098519 149 25 60S acidic ribosomal protein RS1 H. sapiens sp AF098519 149 26 elongation factor 1 beta C. albicans emb AL031863 86 27 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 28 phenylalanyl-tRNA synthetase C. albicans pp 174 38 phenylalanyl-tRNA synthetase C. albicans pp 174 40 ribosomal protein L13 (CAG-ISL 7) H. sapiens pp	Ah399	219	elongation factor 1 beta	D. melanogaster	emp	AL031863	98	7.80E-04
00 tRNA splicing protein SPL1 C. maltosa sp P87187 146 22 60S ribosomal protein L14EB S. cerevisiae sp P38754 136 90 60S actidic ribosomal protein R316 S. cerevisiae sp P40213 294 18 40S ribosomal protein R316 H. sapiens sp P48154 136 19 40S ribosomal protein R34 H. sapiens sp P48154 136 19 40S ribosomal protein R34 H. sapiens sp Q94660 145 19 40S ribosomal protein R5A P. falciparum sp Q94660 145 19 elongation factor 1 beta C. albicans emb AL031863 86 11 60S acidic ribosomal protein L13C H. sapiens sp Q94660 145 11 60S acidic ribosomal protein L13C H. sapiens sp Q94660 145 12 60S ribosomal protein L13C H. sapiens sp Q94660 145 12 60S ribosomal protein L14 (CAG-ISL 7)	Ah420	134	ribosomal protein L41	K. fragilis	ф	M62394	154	7.90E-07
22 60S ribosomal protein L14EB S. cerevisiae sp P38754 136 90 60S acidic ribosomal protein PO Plasmodium falciparum sp Q94660 91 11 40S ribosomal protein RS16 S. cerevisiae sp P44213 294 18 40S ribosomal protein S3A H. sapiens sp P48154 135 18 40S ribosomal protein PO P. falciparum sp Q94660 149 19 40S ribosomal protein PO P. falciparum emb AL031863 86 24 40S ribosomal protein PO P. falciparum emb AL031863 86 24 phenylalanyl-tRNA synthetase C. albicans sp P50914 163 24 phenylalanyl-tRNA synthetase C. albicans sp P50914 163 25 phenylalanyl-tRNA synthetase H. sapiens sp Q08170 95 26 splicing factor H. sapiens sp Q08170 95 28 elongation factor 1 alpha H	Ah448	200	tRNA splicing protein SPL1	C. maltosa	ds	P87187	146	3.10E-12
50 60S actidic ribosomal protein PO Plasmodium falciparum sp Q94660 91 11 40S ribosomal protein RS16 S. cerevisiae sp P40213 294 18 40S ribosomal protein S3A H. sapiens sp P408559 149 19 40S ribosomal protein S3A P. falciparum sp Q94660 145 19 60S actidor ribosomal protein PO P. falciparum sp Q94660 145 11 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens sp P50914 163 11 60S ribosomal protein L13 H. sapiens sp Q08170 95 10 ribosomal protein L14 (CAG-ISL 7) H. sapiens sp Q08170 95 11 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens sp Q08170 95 12 splicing factor H. sapiens sp Q08170 95 12 splicing factor H. sapiens sp Q08170 95 13 mitochondrial carrier protein DIF-1<	Ah454	222	60S ribosomal protein L14EB	S. cerevisiae	$^{\mathrm{ds}}$	P38754	136	3.30E-11
11 40S ribosomal protein RS16 S. cerevisiae sp P40213 294 18 40S ribosomal protein S3A H. sapiens sp P48154 135 14 40S ribosomal protein S3A H. sapiens sp AF008519 149 17 60S acidic ribosomal protein PO P. falciparum sp Q94660 145 18 elongation factor 1 beta C. albicans emb AL033503 120 11 elongation factor 1 beta C. albicans emb AL033503 120 11 elongation factor 1 beta H. sapiens sp Q08170 95 11 elos ribosomal protein L14 (CAG-ISL 7) H. sapiens sp Q08170 95 12 ribosomal protein L13 H. sapiens sp Q08170 95 13 elongation factor 1 alpha B. mori C. elegans pir S55056 119 14 protein transport protein SEC13 H. sapiens sp Q06053 14 14 protein transport pro	Ah460	160	60S acidic ribosomal protein PO	Plasmodium falciparum	$^{\mathrm{ds}}$	Q94660	91	4.70E-05
18 40S ribosomal protein S3A H. sapiens sp P48154 135 34 40S ribosomal protein S7 Avicennia marina gb AF098519 149 37 60S acidic ribosomal protein PO P. falciparum sp Q94660 145 19 elongation factor 1 beta C. albicans emb AL033503 120 34 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 41 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens gb L05772 174 55 splicing factor H. sapiens sp Q08170 95 55 splicing factor B. mori sp P29520 315 53 elongation factor 1 alpha H. sapiens sp P55735 87 71 mitochondrial carrier protein DIF-1 C. elegans print 160247 141 77 protein transport protein SEC23 M. musculus sp Q06053 142 8 hypothetical 69.8 kD protein	Ah493	301	40S ribosomal protein RS16	S. cerevisiae	$^{\mathrm{ds}}$	P40213	294	3.30E-32
94 4OS ribosomal protein S7 Avicennia marina gb AF098519 149 74 60S acidic ribosomal protein PO P. falciparum sp Q94660 145 19 elongation factor 1 beta D. melanogaster emb AL031863 86 24 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 24 phenylalanyl-tRNA synthetase C. albicans sp P50914 163 24 phenylalanyl-tRNA synthetase C. albicans gb L05772 174 25 splicing factor H. sapiens sp Q08170 95 25 splicing factor B. mori sp P29520 315 23 elongation factor 1 alpha B. mori Sp P55735 87 24 mitochondrial carrier protein DIF-1 C. elegans sp P55735 87 25 protein transport protein SEC23 M. musculus pir I60247 141 25 hypothetical 69.8 kD protein S. cer	Ah500	218	40S ribosomal protein S3A		$^{\mathrm{ds}}$	P48154	135	1.40E-10
7 60S acidic ribosomal protein PO P. falciparum sp Q94660 145 19 elongation factor 1 beta D. melanogaster emb AL031863 86 24 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 24 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 25 ribosomal protein L14 (CAG-ISL 7) H. sapiens gb L05772 174 25 splicing factor H. sapiens sp Q08170 95 23 elongation factor 1 alpha B. mori sp P29520 315 24 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 27 protein transport protein SEC13 H. sapiens sp P55735 87 28 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 29 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142	Ah511	204	40S ribosomal protein S7	Avicennia marina	qg	AF098519	149	1.20E-12
19 elongation factor 1 beta D. melanogaster emb AL031863 86 34 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 11 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens sp P50914 163 10 ribosomal protein L32 Kluveromyces lactis gb L05772 174 55 splicing factor H. sapiens sp Q08170 95 53 elongation factor 1 alpha B. mori sp P29520 315 73 protein transport protein DIF-1 C. elegans pir S55056 119 74 protein transport protein SEC23 H. sapiens sp P55735 87 81 protein transport protein SEC23 M. musculus pir I60247 141 85 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 86 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142	Ah514	207	60S acidic ribosomal protein PO	P. falciparum	$^{\mathrm{ds}}$	Q94660	145	4.80E-12
34 phenylalanyl-tRNA synthetase C. albicans emb AL033503 120 11 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens sp P50914 163 10 ribosomal protein L14 (CAG-ISL 7) Kluveromyces lactis gb L05772 174 15 splicing factor H. sapiens sp Q08170 95 23 elongation factor 1 alpha B. mori sp P29520 315 17 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 17 protein transport protein SEC13 H. sapiens sp AJ003197 266 18 protein transport protein SEC23 M. musculus pir I60247 141 1 protein transport protein S. cerevisiae sp Q06053 142 15 hypothetical 69.8 kD protein S. cerevisiae emb Z72510 119	Ah575	219	elongation factor 1 beta	D. melanogaster	emp	AL031863	98	7.90E-04
11 60S ribosomal protein L14 (CAG-ISL 7) H. sapiens sp P50914 163 10 ribosomal protein L32 Kluveromyces lactis gb L05772 174 55 splicing factor H. sapiens sp Q08170 95 23 elongation factor 1 alpha B. mori sp P29520 315 71 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 77 protein transport protein SEC13 H. sapiens sp AJ003197 266 81 protein transport protein SEC23 M. musculus pir I60247 141 1 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah579	134	phenylalanyl-tRNA synthetase	C. albicans	emp	AL033503	120	4.90E-09
10 ribosomal protein L32 Kluveromyces lactis gb L05772 174 55 splicing factor H. sapiens sp Q08170 95 23 elongation factor 1 alpha B. mori sp P29520 315 71 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 77 protein transport protein SEC13 H. sapiens ppir AJ003197 266 81 protein transport protein SEC23 M. musculus pir I60247 141 15 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 15 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah615	211	60S ribosomal protein L14 (CAG-ISL 7)	H. sapiens	$^{\mathrm{ds}}$	P50914	163	1.60E-14
55 splicing factor H. sapiens sp Q08170 95 23 elongation factor 1 alpha B. mori sp P29520 315 71 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 77 protein transport protein SEC13 H. sapiens emb AJ003197 266 81 protein transport protein SEC23 M. musculus pir I60247 141 1 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 15 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah616	240	ribosomal protein L32	Kluveromyces lactis	ф	L05772	174	1.80E-04
23 elongation factor 1 alpha B. mori sp P29520 315 71 mitochondrial carrier protein DIF-1 C. elegans pir S55056 119 87 77 protein transport protein SEC13 H. sapiens pp P55735 87 266 81 adenine nucleotide translocator Lupinus albus pir I60247 141 1 protein transport protein SEC23 M. musculus pir I60247 141 1 hypothetical 69.8 kD protein S. cerevisiae sp Q066053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah621	255	splicing factor	H. sapiens	$^{\mathrm{ds}}$	Q08170	92	6.50E-05
mitochondrial carrier protein DIF-1 C. elegans Protein transport protein SEC13 H. sapiens H. sapiens H. sapiens H. sapiens Eupinus albus M. musculus M. musculus M. musculus Pir R60247 141 S. cerevisiae S. cerevisiae emb AJ003197 266 141 142 143 144 S. cerevisiae P55735 87 141 141 S. cerevisiae P55735 87 141 141 141 S. cerevisiae P55735 P7560 P72510 P772510 P78 P78 P78 P78 P78 P78 P78 P7	Ah623	223		B. mori	$^{\mathrm{ds}}$	P29520	315	2.10E-35
1 mitochondrial carrier protein DIF-1 C. elegans pir \$55056 119 7 protein transport protein SEC13 H. saplens sp P55735 87 1 adenine nucleotide translocator Lupinus albus emb AJ003197 266 1 protein transport protein SEC23 M. musculus pir I60247 141 5 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Transpor	rters						
7 protein transport protein SEC13 H. sapiens sp P55735 87 1 adenine nucleotide translocator Lupinus albus emb AJ003197 266 1 protein transport protein SEC23 M. musculus pir I60247 141 5 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah037	171	mitochondrial carrier protein DIF-1	C. elegans	pir	S55056	119	8.50E-10
1adenine nucleotide translocatorLupinus albusembAJ0031972661protein transport protein SEC23M. musculuspirI602471415hypothetical 69.8 kD proteinS. cerevisiaespQ060531421yeast UTR3 proteinS. cerevisiaeembZ72510119	Ah074	177	protein transport protein SEC13	H. sapiens	ds	P55735	87	4.10E-05
1 protein transport protein SEC23 M. musculus pir I60247 141 5 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah528	241	adenine nucleotide translocator	Lupinus albus	emp	AJ003197	566	1.40E-28
5 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 1 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah572	181		M. musculus	pir	160247	141	1.10E-11
215 hypothetical 69.8 kD protein S. cerevisiae sp Q06053 142 311 yeast UTR3 protein S. cerevisiae emb Z72510 119	Not class	ified						
311 yeast UTR3 protein S. cerevisiae emb Z72510 119	Ah008	215		S. cerevisiae	$^{\mathrm{ds}}$	Q06053	142	1.40E-11
	Ah013	311	yeast UTR3 protein	S. cerevisiae	emp	Z72510	119	4.90E-08

Table 2. Continued

Clone	Longth	Idomtification	Onconiem	Data	Accession	Coons	Drobobility
No.	rengui		Organism	base	No.	Score	Frobability
Ah087	222	hypothetical 36.8 kD protein	S. pombe	ds	Q10169	93	8.70E-05
Ah335	214	Major vault protein alpha	D. discoideum	ds	P34118	198	3.30E-25
Ah384	196	hypothetical protein YER007c-a	S. cerevisiae	pir	S53543	66	2.50E-09
Ah446	169	hypothetical protein YER007c-a	S. cerevisiae	pir	S53543	101	3.40E-06
Ah457	240	SGT protein	Rattus norvegicus	emp	AJ222724	90	3.10E-05
Ah478	185	Nbr1	M. musculus	qg	U73039	102	2.90E-06
Ah486	291	Down syndrome critical region protein A	H. sapiens	ds	014972	215	2.40E-21
Ah492	248	EST yk400g3.3	C. elegans	emp	Z81592	98	1.10E-03
Ah543	218	Major vault protein alpha	D. discoideum	ds	P34118	165	1.00E-14
Ah574	175	Major vault protein beta	D. discoideum	ds	P54659	137	2.10E-11
rRNA							
Ah197	256	large subunit ribosomal RNA	Plumbago auriculata	qg	AF036492	746	5.60E-71
Database	abbrevia	Database abbreviation: ^{a)} sp, swissprot; ^{b)} dbj, Data Base of Japa	^{b)} dbj, Data Base of Japan; ^{c)} gb, GenBank; ^{d)} emb, EMBL; ^{e)} pir, Protein Informatio Resource.	e)pir, Protein	Informatio Resou	ırce.	

has been known to correlate with invasiveness (Silva Filho et al., 1988; Ghosh et al., 1999). Further characterization of laminin binding protein in *A. healyi* would help to discover the mechanisms of the invasion by the amoeba.

A lot of genes for proteins involved in various metabolism were found in *Acanthamoeba* for the first time in this study except for the ubiquitin (Ub) gene (Hu & Henney, 1997). Six clones were identified to be associated with Ub-proteasome protein destruction system. Ub-proteasome pathway of intracellular proteolysis has been shown to be involved in various biologically important processes, such as the cell cycle, cellular metabolism, apoptosis, signal transduction, immune response, and protein quality control (Hilt and Wolf, 1996; Ciechanover, 1998; Tanaka, 1998).

Little information has been reported for signal transduction in genus Acanthamoeba. In this study, many kinds of cell signaling molecules, including Rabs, 14-3-3 protein and rac, were identified. Rabs regulate the flux through individual steps of the intracellular membrane trafficking pathway. The small GTPase Rab2 is a resident of pre-Golgi intermediates and required for protein transport from the endoplasmic reticulum to the Golgi complex (Tisdale et al., 1992). The GTP binding motif, GDTGVGKS, was conserved in the sequence of the clone Ahc040 identified as Rab2. A Rab protein isolated by EST analysis had been characterized in Trypanosoma brucei by Field et al. (1999).

In addition, gene for prohibitin known to negatively regulate cell proliferation in mammals was identified in *Acanthamoeba*. Further studies on this gene would give information in the regulation of *Acanthamoeba* proliferation and development. Three clones were identified as gene for vault protein showing high homology with that of *Dictyostelium discoideum*. Although vault proteins are found in nearly all eukaryotic cells, the function of the protein has yet to be elucidated.

Lots of the genetic information of *Acanthamoeba* obtained in this study would be very helpful to figure out pathogenetic mechanisms of GAE or keratitis by *Acanthamoeba* and to develop therapeutic

reagents specific to the amoeba.

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