Korean Journal of Life Science
Vol. 13. No. 1. 128~135, 2003

Expressed Sequence Tags in Rainbow Trout (*Oncorhynchus mykiss*) Kidney and Microarray Analysis in Young and Old Kidney

Soonhag Kim*, Yong Kook Shin and In Chul Bang[1]

National Genome Research Institute, NIH, Eunpyung-Gu, Nokbun-dong, Seoul 122-701, Korea
[1]Dept. of Marine Biotechnology, Soonchunhyang University, Asan 336-745, Korea

Abstract

102 ESTs (Expressed Sequence Tags) were obtained by sequencing clones from a library of rainbow trout kidney cDNAs. Of the sequences generated, 55.8% of the ESTs were represented by 37 known genes. The 45 clones of unknown gene products potentially represent 40 novel genes. The genes involved in structural function (14.5%) and transcription/translation (11.6%) account for the major gene expression activities in the kidney. Microarray experiment was conducted to compare gene expression of the unique ESTs in young and adult rainbow trout kidneys. While mitochondrion, cytochrome b, *rho* G, spastin protein, and three unknown genes were down-regulated in the mature fish kidney, calponin 1, calcium binding protein, histone deacetylase 1, and an unknown gene were up-regulated in the mature fish kidney. This research demonstrates the feasibility and power of functional genomics in rainbow trout.

Key words – Expressed Sequence Tag, Expression. Microarray, Kidney, Rainbow Trout

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important seafood protein sources in the world. A great deal of research has been focused on the ecology, physiology, and biochemistry of rainbow trout. Nevertheless, molecular genetic studies of rainbow trout, such as Expressed Sequence Tags (EST), have not been extensive. Most characteristics of an organism are determined by the genes expressed within it[9]. Several important advances such as serial analysis of gene expression (SAGE)[9] and EST analysis[1] have provided technological possibilities for examination of gene expression at the whole genome level.

ESTs are partial cDNA sequences corresponding to mRNAs and generated from randomly selected library clones [1]. ESTs are generated by single pass sequencing of both/either the 5′ and/or the 3′ ends of cDNAs contained in selected cDNA libraries. Characterization of ESTs is a convenient and rapid method for discovery of new genes in various organisms. Additionally, it provides resources for a detailed profile of genes expressed in a tissue or cell type and development of gene chip technology. As of October 2002, database EST (dbEST) in NCBI contained 13,209,117 entries. Among the 273 species present, the most extensively studied species were the human (42.4%) and mouse (27.7%). The number of fish ESTs in dbEST, excluding for zebrafish (1.1%), was 0.13% from channel catfish, Japanese medaka, winter flounder, common carp, nile tilapia, and rainbow trout.

The most attractive aspect of functional genomics is its ability to determine gene expression changes in a global view. ESTs have been become an important biological

*To whom all correspondence should be addressed
Tel : 02-380-1531, Fax: 02-354-1078
E-mail : kimsoonhag@hotmail.com

component of functional genomics. For instance, ESTs are used in hybridization studies in which the intensity of hybridization signals varies with the quantity of messenger RNAs harvested under different physiological conditions. The simultaneous hybridization of a large number of clones permits information to be obtained on the expression of numerous genes. This is equivalent to running tens of thousands of Northern blots or equivalent to many rounds of differential display[3]. Obviously, a necessary step for functional genomics is the availability of large numbers of ESTs.

In this study, we report ESTs derived from a rainbow trout kidney cDNA library. Expression of 78 genes in immature (1 year old) and mature (2 years old) fish was compared using a gene filter approach.

Materials and Methods

Template Preparation, DNA Sequencing, and Data Analysis

The renal cDNA library, prepared in l ZapII vectors, was kindly provided by Dr. Joe Brunelli of Washington State University. The amplified library had a titer of approximately ten million plaque forming units (pfu) per microliter. About ten million pfu of this library were used for the conversion of the l libraries into plasmid libraries by using the mass *in vivo* excision procedure (Stratagene, La Jolla, CA). The XL1-blue MRF' bacteria were infected with 10 million of phage (10 cells : 1 phage), and also infected with the ExAssist helper phage at a 1:1 helper phage-to-cell ratio. Cells were incubated at 37℃ for phage absorption. The infected cells were then grown in 20 ml LB broth for 3 hours in a shaker at 37℃. During this time, phagemids were excised and secreted into medium. The cells were then heated to 70 ℃ for 20 minutes and removed by centrifugation. The excised phagemids in the supernatant were titered. SOLR cells (Stratagene, La Jolla, CA) were infected with 10^7 pfu of phagemids and incubated at 37℃ for 15 min. The cells were then plated onto LB plates containing ampicillin at 100 μg/ml. Colonies were picked and cultured in LB liquid culture for plasmid preparation.

Double-stranded plasmid DNA templates were prepared by using the Qiagen Spin Column Mini-plasmid kits (Qiagen, Valencia, CA). Because the cDNAs were directionally cloned into the l ZapII vectors, single run sequencing on the 5′ ends of each cDNA clone was performed by the Sanger dideoxy termination method [7] utilizing the BigDyeTerminator chemistry (Perkin Elmer, Foster City, CA) and a T3 primer (5′ AATTAACCCTCACTAAAGGG 3′). Sequencing products were analyzed on an ABI PrismTM310 automated DNA sequencer (Perkin Elmer, Foster City, CA). Plasmid DNAs (1μl, about 200~500 ng) were used for all sequencing reactions. The profiles for cycling were: 96℃ for 30 sec., 50℃ for 15 sec., 60℃ for 4 min. for 25 cycles. An initial two minutes of extra denaturation at 96℃ were always used.

Classification of the clones was based on their similarity to NCBI dbEST entries. In general, 400 to 450 nucleotides following the *Eco*RI adapter sequence were taken for data base comparisions with the BLASTN and BLASTX servers (NCBI, Bethesda, MD). Matches were considered to be significant only when the probability (P) was less than 0.001. Comparisons and alignments of the EST sequences were performed with the Gene Tool 1.0 program package (Edmonton, AB, Canada).

Preparation of First-Strand cDNA Probes

The kidney tissues of the immature (1 year old) and mature (2 year old) fish were harvested from freshly sacrificed fish and frozen in liquid nitrogen and stored in an 80℃ freezer. Kidney tissues were pulverized with a mortar/pestle, and then homogenized (Model 985~370, Biospec Products, Inc., Bartlesville, OK) in TRI REAGENT™ buffer (Molecular Research Center, Inc. Cincinnati, OH) following the manufacturer's protocol. Poly $(A)^+$ RNA was purified by using the Oligotex Spin

Column Kit from Qiagen according to manufacturer's instructions (Qiagen, Valencia, CA).

The probes were made by reverse transcription using MMLV reverse transcriptase (Life technologies, Inc., Bethesda, MD). A reaction mixture of 35 μl was composed of the following: 700 ng mRNA, 1X first-strand buffer (Life Technologies, Rockville, MD), 20 units of RNase Block Ribonuclease Inhibitor (Stratagene, La Jolla, CA), 500 ng of synthetic oligo $(dT)_{15}$ primer, 1.0 mM each of dATP, dGTP, dTTP; 50 μCi ^{32}P-dCTP; and 50 units of MMLV reverse transcriptase. The reaction was incubated at 37℃ for 1 hr. The probes were denatured by boiling for 5 min before hybridization reaction.

Dot Blot Analysis

After sequencing analysis, unique renal EST clones were used for dot blot analysis of gene expression. Equal amount of plasmid DNA (1μg) was used from each clone for preparation of three sets of replicate "gene filters". Beta actin was used as a control to normalize the intensity between 2 different gene filters. The dot blot analysis was conducted as previously described[2]. Briefly, the plasmid DNA was denatured by mixing with an equal volume of 1 M sodium hydroxide and kept at room temperature for 5 min. An equal volume of 2 M ammonium acetate was added and vortexed to neutralize the samples. The samples were then loaded onto a nylon membrane by using a Bio-Dot SF Microfiltration apparatus (BioRAD, Hercules, CA). One milliliter of 5X SSC was then added to each sample spot. The filter was removed and air-dried before UV crosslinking of the DNA to the membrane using a Stratalinker UV crosslinker (Stratagene, La Jolla, CA). Hybridization was conducted under standard protocols[6]. The hybridization signals were quantified by using the FluorChem v1.02e software package (AlphaInnotech, San Leandro, CA).

Results and Discussion

Sequences of the Rainbow Trout Renal cDNAs

Single-pass sequencing from the 5′ end of the cDNA was performed for 102 clones from a rainbow trout non-normalized renal library. The vector-derived sequence and ambiguous sequences were removed from the collected EST sequences prior to computer analyses. The average length of edited ESTs obtained was 420 bp. Fifty seven clones were from known genes and 45 were clones from unknown genes. Multiple clones were sequenced for 8 genes ranging from 2~12 clones per gene (Table 1). Thus, the 57 known ESTs represented gene products from 37 genes and 45 clones of unknown genes represented products of 40 genes. Comparison of the sequences of unknown clones with each other revealed that one gene was represented by two independent cDNA clones and two by a set of three, while the others are probably distinct genes. Seven of 37 gene sequences had been reported previously in rainbow trout. The high abundance of the major transcripts in the kidney tissue necessitates the construction of a subtracted cDNA library for discovery of rainbow trout genes and for cataloguing the ESTs for functional genomic studies in rainbow trout.

The Most Abundantly Expressed Genes in Rainbow Trout Kidney

We classified the 57 clones into 7 functional categories based on predicted or known functions through sequence similarity of annotated sequences (Fig. 1). Genes involved in structural functions (14.5%) accounted for the major gene expression activities in the kidney. These genes included beta actin, SH3 binding domain glutamic acid rich protein, Nodulin 28/32, and Gly-rich protein. The genes involved in transcription and translation account for 11.6%, enzymes for 7.8%, defense (genes involved in immune system function) for 4.8%,

Table 1. Rainbow trout kidney ESTs

Clone #	Accession Number	Putative Identification/Most Homologous Organism	Overlap (bp)	Probability	No. of Gene
RTK 12		Beta actin (AF012125.1)	428	0.0	12
RTK 2		28S Ribosomal RNA (U34341.1)	481	0.0	6
RTK 54	BE859110	18S ribosomal RNA (X98839.1)/*Salmo trutta*	473	0.0	3
RTK 77	BE859117	Polyubiquitin (D63782.1)/*Cricetulus sp.*	378	1e-140	2
RTK 80		Immunoglobulin heavy chain variable region (X81502.1)	363	1e-163	2
RTK 3		Mitochondrion (NC001717.1)	414	0.0	1
RTK 6	BE859097	ATP synthase (AB023582.1)/*Cyprinus carpio*	289	6e-23	1
RTK 15	BE859098	Phosphate transport protein (AF062383.1)/*choristoneura fumiferana*	79	8e-10	1
RTK 16	BE859099	Smooth muscle calponin 1 (NM001299.1)/*Homo sapiens*	38	2e-04	1
RTK 19	BE859100	Human clone 322G13 (AL096677.1)/*Homo sapiens*	25	7e-04	1
RTK 20		Cytochrome b (AF125208.1)	401	0.0	1
RTK 23	BE859101	Calcium binding protein (J05138.1)/*Oryctolagus cuniculus*	143	5e-17	1
RTK 25	BE859102	*rho* G (NM 001665.1)/*Homo sapiens*	76	2e-10	1
RTK 26		Galectin like protein (AB027452.1)	350	4e-79	1
RTK 31	BE859103	6 phosphofructo 2 kinase / fructose 2 6 biphosphatase (NM004567.1)/*Homo sapiens*	297	2e-37	1
RTK 41	BE859104	Fibrinogen like protein 2 (NM008013.1)/*Mus musculus*	80	5e-34	1
RTK 42	BE859105	Ribosomal protein L3 (NM000967.1)/*Homo sapiens*	391	7e-84	1
RTK 43	BE859106	Human clone KAT11822 (AK000562.1)/*Homo sapiens*	137	4e-11	1
RTK 44	BE859107	G protein coupled receptor 13 (NM017192.1)/*Rattus norvegicus*	57	4e-11	1
RTK 47	BE859108	Lactate dehydrogenase B (L23780.1)/*Fundulus parvipinmis*	286	2e-47	1
RTK 49	BE859109	Steroid receptor homologue (X70300.1)/*Danio rerio*	383	3e-86	1
RTK 39	BE859111	Spastin protein orthologue (AJ246002.1)/*Mus musculus*	96	7e-15	1
RTK 50	BE859112	SH3-binding domain glutamic acid rich protein (NM007341.1)/ *Homo sapiens*	30	3e-04	1
RTK 58	BE859113	Ribosomal protein L35 (NM007209.1)/*Homo sapiens*	114	1e-11	1
RTK 61	BE859114	alpha and beta globin (X97286.1)/*Salmo salar*	237	1e-121	1
RTK 63	BE859115	Nodulin 28/32 (Z68102.1)/*Vicia faba*	27	1e-04	1
RTK 65	BE859116	Histone deacetylase 1 (NM008228.1)/*Mus musculus*	65	3e-09	1
RTK 86	BE859118	Chaperonin containing TCP 1 delt (D49483.1)/*Takifugu rubripes*	149	6e-23	1
RTK 98	BE859119	Chaperonin subunit 2 (NM007636.1)/*Mus musculus*	187	1e-11	1
RTK 89		Secretory form of Ig heavy chain (X65261.1)	421	0.0	1
RTK 92	BE859120	Mitochondrial adenine nucleotide translocator (D12770.1)/*Rattus norvegicus*	168	1e-29	1
RTK 93	BE859121	Phosphoglycerate mutase B (S63233.1)/*Rattus norvegicus*	194	5e-14	1
RTK 94	BE859122	RNA polymerase III (NM007055.1)/*Homo sapiens*	242	8e-19	1
RTK 95	BE859123	T protein (D11162.1)/Gallus gallus	87	7e-07	1
RTK 100	BE859124	ATP binding cassette (NM002940.1)/*Homo sapiens*	134	1e-11	1
RTK 102	BE859125	Cytoplasmic aminopeptidase P (AF038591)/*Rattus norvegicus*	108	3e-21	1
RTK 67	BE859126	Gly-rich protein (AJ271041)/*Drosophila melanogaster*	30	6e-21	3
RTK 18	BE859136	Unknown			3
RTK 60	BE859150	Unknown			2
RTK 64	BE859152	Unknown			37
		Unknown			
Total					102

The blank in the accession number indicates that genes had been reported previously in rainbow trout in NCBI dbEST. The bold letter "Unknown"represents singleton unknown ESTs (the e-value of sequence similarity > 0.001) and were omitted from the list (Accession numbers from BE859127 to BE859166).

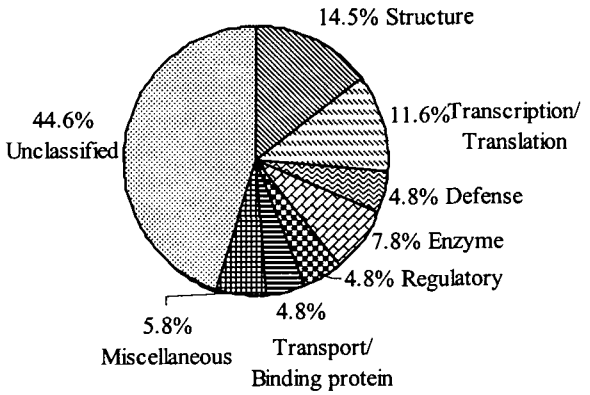

Fig 1. Gene expression profiles and predicted function in the rainbow trout kidney as revealed by expressed sequence tag (EST) analysis.

regulatory proteins for 4.8%, and transport/binding protein for 4.8%. Proteins categorized as "miscellaneous" (not included in the categories of sturcture, enzymes, regulatory, defense, transcription and translation, and binding protein) accounted for 5.8% of the major transcriptional activity. 44.6% of the ESTs identity and functions could not be determined. The total number of genes in the human is believed to be approximately 26,000 to 38,000 and that of a fruit fly is about 13,600 [4]. In any given cell type, approximately 10,000 genes are expressed from 200,000 copies to 1 copy or less per cell[5]. Here, several genes account for the major transcriptional activities of the kidney. In the categories of genes with putative functions, 5 genes were represented with more than one cDNA clone as indicated in Table 1. The most abundant genes were beta actin (11.6%) and ribosomal protein (9.7%).

The gene expression profiles in the kidney provide valuable information for strength of promoters involved in transcription of these genes although gene copy numbers are not known. Assuming that a gene is present in the genome as a single copy, identification of strong and tissue specific promoters may be useful in transgenic studies and genetic engineering.

One of the advantages of EST analysis using non-normalized libraries is the ability to produce expression profiles. The assumption is that the frequency of cDNAs in a cDNA library is a reflection of mRNA abundance in the mRNA pool. If the EST analysis is for the purpose of EST cataloging for the development of bio-reagents, repeated sequencing of highly expressed genes is not desirable. Normalized cDNA libraries[5,8] or subtracted cDNA libraries are therefore needed for characterization of large numbers of unique ESTs. Subtracted cDNA libraries of rainbow trout kidneys were developed in our laboratory using beta actin and ribosomal proteins as subtractants. Approximately 21.7% of the 3000 colonies randomly picked from the non-normalized renal cDNA library hybridized to the subtractants (data not shown). This approach has allowed as to reduce redundancy in the identification of ESTs. The observed 21.7% subtraction is very close to the predicted 20.4% for beta actin and ribosomal proteins found in renal gene expression profile in Table 1.

Gene Expression in Kidney Tissues of Different Ages

Expression of 75 transcriptomes from immature (1 year old) and mature (2 years old) fish kidneys was compared using a gene filter approach to demonstrate the feasibility of functional genomic studies in small laboratories. Plasmid DNA containing unique ESTs from rainbow trout kidney was spotted onto two sets of nylon membranes and hybridized to first-strand cDNA probes (generated from total mRNA from the kidneys of the two populations of trout). Beta actin was used as control to normalize the hybridization signal of each plasmid. To assure that the differences in radioactive signal were from the relative proportion of the probe due to its mRNA concentration in the RNA pool, but not from any errors made during the stripping process, three sets of replicate filters (six filters) were prepared and hybridized to the two probes, respectively. The consistent differences of signals were confirmed (data not shown).

As shown in Table 2, polymorphic gene expression was found for several genes. While mitochondrion (2.9×),

Table 2. Genes expressed at significantly different levels in the immature and mature rainbow trout kidneys

Gene identity	% expression (1 year)	% expression (2 year)	Change
Mitochondrion	7.7	2.7	2.9×down
Cytochrome b	2.6	1.1	2.4×down
rho G	1.4	1.1	1.5×down
Spastin protein	1.2	0.7	1.7×down
Calponin 1	0.4	0.9	2.4×up
Calcium binding protein	0.6	0.9	1.5×up
Histone deacetylase 1	0.5	0.9	1.8×up
RTK 9	0.5	0.9	1.8×up
RTK 17	1.1	0.6	1.8×down
RTK 18	1.2	0.6	2.0×down
RTK 60	1.0	0.6	1.7×down

The hybridization signal of each gene was normalized to beta actin used as control. The total filter exposure level was set at 100 percent and each individual gene was represented as a percentage of the total filter exposure.

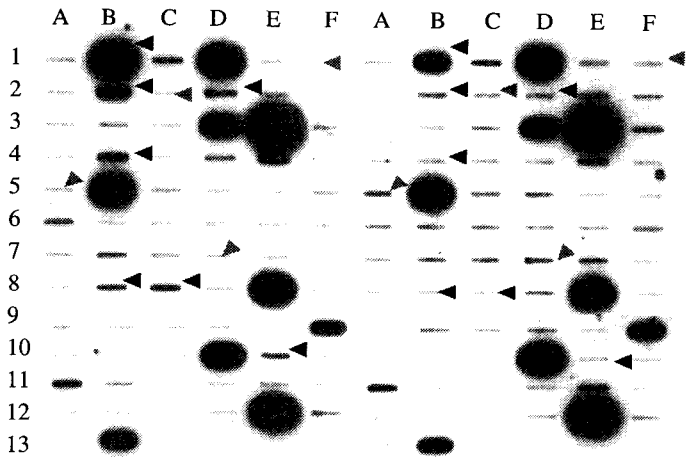

Fig 2. Dot blot analysis of gene expression in kideny tissues at 1 year old (immature fish, left) and 2 year old (mature fish, right). Plasmid DNA containing various cDNAs was immobilized onto nylon membrane and hybridized to the first-strand cDNA probes from immature fish (left) and from mature fish (right) as detailed in Materials and Methods. Genes represented by the dots are (from left to right and from top to bottom): 1A, 28S ribosomal RNA; 1B, Mitochondrion; 1C, ATP synthase; 1D, beta actin; 1E, phosphate transport protein; 1F, smooth muscle calponin 1; 2A, human clone 322G13; 2B, cytochrome b; 2C, calcium binding protein; 2D, *rho* G; 2E, galactic like protein; 2F, 6 phosphofructo 2 kinase; 3A, fibrinogen like protein 2; 3B, ribosomal protein L3; 3C, human clone KAT11822; 3D, G protein coupled receptor 13; 3E, lactate dehydrogenase B; 3F, steroid receptor homologue; 4A, RTK 84; 4B, spastin; 4C, SH3-binding domain; 4D, ribosomal protein L35; 4E, alpha and beta globin; 4F, nodulin 28/32; 5A, histone deacetylase 1; 5B, polyubiquitin; 5C, immunoglobulinheavy chain; 5D, chaperonin containing TCP 1; 5E, chaperonin subunit 2; 5F, secretory form of immunoglobulin heavy chain; 6A, mitochondrial adenine nucleotide translocase; 6B, RNA polymerase III; 6C, T protein; 6D, ATP binding cassette; 6E, cytoplasmic aminopeptidase P; 6F-13C: all are unknown genes: 6F, RTK 1; 7A, RTK 4; 7B, RTK 5; 7C, RTK 7; 7D, RTK 9; 7E, RTK 10; 7F, RTK 11; 8A, RTK 13; 8B, RTK 17; 8C, RTK 18; 8D, RTK 21; 8E, RTK 24; 8F, RTK 27; 9A, RTK 32; 9B, RTK 33; 9C, RTK 35; 9D, RTK 36; 9E, RTK 37; 9F, RTK 46; 10A, RTK 48; 10B, RTK 53; 10C, RTK 57; 10D, RTK 59; 10E, RTK 60; 10F, RTK 62; 11A, RTK 64; 11B, RTK 67; 11C, RTK 69; 11D, RTK 71; 11E, RTK 72; 11F, RTK 73; 12A, RTK 74; 12B, RTK 75; 12C, RTK 81; 12D, RTK 83; 12E, RTK 91; 12F, RTK 96; 13A, RTK99; 13B, RTK 101; 13C, RTK 104; 13D-13F, blank. Red arrows indicate genes that were expressed at significantly higher levels in the mature fish than in the immature fish; black arrows indicate genes that were expressed at significantly lower levels in the mature fish than in the immature fish.

cytochrome b (2.4×), *rho* G (1.5×), spastin protein (1.7×), and three unknown genes, RTK17 (1.8×), RTK18 (2.0×), and RTK60 (1.7×), were down-regulated in the mature fish kidney, calponin 1 (2.4×), calcium binding protein (1.5×), histone deacetylase 1 (1.8×), and an unknown gene, RTK 9 (1.8×), were up-regulated in the mature fish kidney. These "gene filter" studies demonstrate that the feasibility of gene expression analysis can be determined using a "bulk" genomics approach in a cost-effective manner. Three replicates and statistics analysis indicated that gene expression levels revealed by gene filter analysis were highly reproducible. Whole genomic signatures of gene expression, however, depend on development of great numbers of ESTs.

Summary

Seventy seven unique Expressed Sequence Tags (EST) were cloned from a library of rainbow trout kidney cDNAs. Of the sequences generated, 55.8% of the ESTs were represented by 37 known genes. The 45 clones of unknown gene products potentially represent 40 novel genes. We classified the 57 clones into 7 functional categories based on predicted or known functions through sequence similarity of annotated sequences. The genes involved in structural function (14.5%) and transcription/translation (11.6%) account for the major gene expression activities in the kidney. Microarray experiment was conducted to compare gene expression of the unique ESTs in young and adult rainbow trout kidneys. While mitochondrion, cytochrome b, *rho* G, spastin protein, and three unknown genes were down-regulated in the mature fish kidney, calponin 1, calcium binding protein, histone deacetylase 1, and an unknown gene were up-regulated in the mature fish kidney. This research demonstrates the feasibility and power of functional genomics in rainbow trout.

References

1. Adams, M. D., J. M. Kelley, J. D. Gocayne, M. Dubnick, M. H. Polymeropoulos, H. Xiao, C. R. Merril, A. Wu, B. Olde, R. F. Moreno, A. R. Kerlavage, W. R. McCombie and J. C. Venter. 1991. Complementary DNA sequencing: expressed sequence tags and human genome project. *Sci.* **252**, 1651-1656.
2. Kim S., A. Karsi, R. A. Dunham and Z. Liu. 2000. The skeletal muscle α-actin gene of channel catfish (*Ictalurus punctatus*) and its association with piscine specific SINE elements. *Gene* **252**, 173-181.
3. Liang, P. and A. B. Pardee. 1992. Differential display of eukaryotic mRNA by means of the polymerase chain reaction. *Sci.* **257**, 967-971.
4. Paabo, S. 2001. The human genome and our view of ourselves. *Sci.* **291**, 1219-1220.
5. Patanjali, S. R., S. Parimoo and S. M. Weissman. 1991. Construction of a uniform-abundance (normalized) cDNA library. *Proc. Natl. Acad. Sci. USA* **88**, 1943-1947.
6. Sambrook, J., E. F. Frisch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, New York.
7. Sanger, F., S. Nicklen and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
8. Sasaki, Y. F., D. Ayusawa and M. Oishi. 1994. Construction of a normalized cDNA library by introduction of a semi-solid mRNA-cDNA hybridization system. *Nucl. Acids Res.* **22**, 987-992.
9. Velculescu, V. E., L. Zhang, B. Vogelstein and K. W. Kinzler. 1995. Serial analysis of gene expression. *Sci.* **270**, 484-487.

(Received November 18, 2002; Accepted February 18, 2003)

초록 : 무지개송어 신장으로부터 EST 발굴 및 연령에 따른 유전자 발현 분석

김순학 · 신용국 · 방인철
(국립보건원 유전체연구소)

무지개 송어(*Oncorhynchus mykiss*) 신장조직의 유전자 발현 현황을 조사하기 위하여 무지개송어 신장 cDNA library로부터 102개의 ESTs를 조사하였다. 102개의 ESTs 중 57개의 clones 이 NCBI blast 염기서열 검색을 통해 이미 기능이 밝혀진 다른 유전자와의 유사성을 보였고, 그 결과 총 37개의 singleton으로 분류되었다. 나머지 45개의 ESTs는 기존의 밝혀진 유전자와 염기서열 유사성이 전혀 없었고, 상호 염기서열간의 유사성을 통해 40개의 유전자로 밝혀졌다. 또한, 신장조직의 유전자 구성을 기능별로 살펴보기 위하여 기능이 밝혀진 57개의 ESTs를 7개의 functional categories로 분류하였다. 그 결과, 신장조직의 구조에 관여하는 유전자가 14.5%로 가장 높았고, 그 다음으로 유전자 전이/전사에 관여하는 유전자가 11.6%로 판명되었다. 그리고 이들 77개의 유전자를 이용하여 연령에 따른 유전자 발현을 조사하기위하여 microarray 실험을 하였다. 3개의 replicate를 이용하여 p-value <0.05를 갖는 유전자중 1.5배 이상만 down- 또는 up-regulation되는 유전자만을 조사하였다. 이들 중 2년산 무지개 송어 신장에서 1.5배 이상 감소되는 유전자는 mitochondrion, cytochrome b, *rho* G, spastin protein, RTK 17, RTK 18과 RTK 60이었다. 반면에 2년산 무지개 송어 신장에서 1.5배 이상 증가되는 유전자는 calponin1, calcium binding protein, histone deacetyulase 1과 RTK 9 유전자가 유의성 있게 차이가 났다. 이상의 결과, 유전자 발현조사 및 microarray 연구가 무지개송어의 genetic improvement에 크게 영향을 미칠 수 있음을 시사하였다.