Genetic Materials at the Gene Engineering Division, RIKEN BioResource Center

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Abstract: Genetic materials are one of the most important and fundamental research resources for studying biological phenomena. Scientific need for genetic materials has been increasing and will never cease. Ever since it was established as RIKEN DNA Bank in 1987, the Gene Engineering Division of RIKEN BioResource Center (BRC) has been engaged in the collection, maintenance, storage, propagation, quality control, and distribution of genetic resources developed mainly by the Japanese research community. When RIKEN BRC was inaugurated in 2001, RIKEN DNA Bank was incorporated as one of its six Divisions, the Gene Engineering Division. The Gene Engineering Division was selected as a core facility for the genetic resources of mammalian and microbe origin by the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan in 2002. With support from the scientific community, the Division now holds over 3 million clones of genetic materials for distribution. The genetic resources include cloned DNAs, gene libraries (e.g., cDNA and genomic DNA cloned into phage, cosmids, BAC, phosmid, and YAC), vectors, hosts, recombinant viruses, and ordered library sets derived from animal cells, including human and mouse cells, microorganisms, and viruses. Recently genetic materials produced by a few MEXT national research projects were transferred to the Gene Engineering Division for further dissemination. The Gene Engineering Division performs rigorous quality control of reproducibility, restriction enzyme mapping and nucleotide sequences of clones to ensure the reproducibility of in vivo and in vitro experiments. Users can easily access our genetic materials through the internet and obtain the DNA resources for a minimal fee. Not only the materials, but also information of features and technology related to the materials are provided via the web site of RIKEN BRC. Training courses are also given to transfer the technology for handling viral vectors. RIKEN BRC supports scientists around the world in the use of valuable genetic materials.

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The RIKEN DNA Bank was organized in June 1987 by the Science and Technology Agency of the Japanese government in accordance with a report by a scientific committee that called for a central repository of genetic resources that would serve scientists in Japan and Asia. In 2001, RIKEN BRC was established within RIKEN and the DNA Bank was merged into RIKEN BRC as the Gene Engineering Division. In 2002, the Gene Engineering Division was selected as a core facility for the collection, preservation and distribution of genetic resources of animal and microbe origin by the MEXT NBRP (Fig. 1). With the support of the scientific community, RIKEN BRC has collected over 3 million excellent materials such as genomic clones and libraries, expressed sequence tag (EST) and cDNA clone sets, expression clone sets, backbone vectors, recombinant adenoviruses and shuttle vectors, and genomic DNA (Fig. 2, Table 1) for the study of human diseases and gene functions. Users can easily access our genetic materials through the internet (Fig. 3) and obtain DNA resources for a minimal fee. Not only the materials, but also information of technology and features related to the materials are provided via our web site. We have focused on the following six groups of genetic resources to promote life sciences: (1) large scaled genome clones from animals and microorganisms; (2) recombinant viruses for gene transfer; (3) DNAs encompassing various promoter-reporter regions; (4) Japanese-specific genetic resources; (5) full-length cDNA sets of growth factors, receptors, transcription factors, replication factors, apoptosis, the cell cycle, signal cascades, and so on; and (6) genetic resources related to the conservation of biodiversity and reference materials for studies of biological classification.
A large amount of EST information is useful for determining the rough temporal expression pattern of genes; thus many EST cloned libraries have been established and analyzed. Individual cDNA clones of EST can be used to analyze expression patterns of genes by whole-mount in situ hybridization [19]. The EST clones of an ascidian *Ciona intestinalis* comprise ca. 452,000 clones derived from 16 developmental stages [25, 26]. Individual clones as well as expression patterns of genes are searchable using the database provided (web site of ‘*Ciona intestinalis* EST’). NIA/NIH mouse 15K and 7.4K cDNA clone sets were prepared by Dr Minoru Ko and colleagues [14, 29, 33] as results of large scale analyses of EST clones. These results were used for the construction of commercially available microarrays. The mouse 15K clone set contains about 15,000 ‘unique’ cDNA clones rearrayed from 52,374 ESTs from pre- and per-implantation embryos, E12.5 female gonad/mesonephros, and newborn ovaries. The 7.4K clone set was constructed from about 7,400 cDNA clones with no redundancy within the set and NIA Mouse 15K. Human full-CDS clones that are the products of the Genome Network Project (MEXT) are also available. There are clone sets which are used for the introduction of genes to cultured cells. The *Xenopus* EST clone set contributed to a study of gene expression in the frog. Of six clone sets of *Xenopus laevis*, two EST clone sets (*X. laevis* anterior neuroectoderm EST clones, RDB 6129; *X. laevis* Keller explants EST clones, RDB 6726) are constructed with an expression vector and they are...
useful for establishing transient expression of genes in the frog oocytes. A human HLA class I cDNA collection isolated from Japanese can be used to express histocompatibility antigen in human cells [2, 3] and is useful for immunology, especially cancer immunology [4, 5, 20]. The human SEREX (serological analysis of cancer antigens by recombinant cDNA expression cloning) clones were initially constructed for the screening of cancer antigens of Japanese patients using a phage expression system [21, 22]. Five hundred plasmid clones were converted from the phages and are available as expression vectors for use in mammalian cells. The Gene Engineering Division also provides full-CDS expression clones that were constructed by our division, and the confirmed nucleotide sequences of each clone and their expression proteins determined by western blotting.

**BAC Clones**

To compare genetic variations between evolutionally related species and the single nucleotide polymorphism (SNP) among strains of experimental animals, BAC clones have been used for the analysis of genomic DNA of various organisms. The Japanese macaque *Macaca fuscata* BAC (MSB2) consisting of ca. 200,000 clones was constructed. Although a complete genome sequence of Japanese macaques is not yet available [11], terminal sequences of the inserted genes have already been analyzed and chromosome mapping of BAC clones are accessible in a database held at the DNA Data Bank of Japan (DDBJ) of the National Institute of Genetics. The MSM/Ms mouse BAC clones have contributed to SNP analysis of Asian mouse subspecies *Mus musculus mossinus* and the C57BL/6J mouse strain by BAC-end sequence analysis [1]. BAC clones from two strains of the rat have also contributed to SNP analysis of strains...
The terminal sequence of the MSM/Ms mouse BAC and two of the rat BAC libraries have already been analyzed and individual clones of the BAC libraries can be easily accessed via the web sites of each BAC library.

The mouse is an important model organism for understanding human diseases. It always plays a key role in functional genomics [34]. Use of the BAC clone is not limited to the investigation of SNP. Recent advances in molecular biology techniques utilize BAC in functional genomic studies of the mouse. BAC clones are used for generation of transgenic mice having additional copies of genes or a genomic region to analyze gene function in the gain-of-function phenotype and in gene rescue for the loss of function mutant and knockout mice. Furthermore, techniques of modification of nucleotide sequences of BAC clones have been developed and applied to the generation of gene modified transgenic mice and gene targeting by substitution of a genomic region with marker genes.

Rats are also used as in vivo highly valuable models for hypertension, aging, infectious diseases, cancer, and neurological disorders because of their relevance to human physiology and size advantages in surgical operations over the mouse [10, 27]. However, the lack of efficient tools to manipulate the rat genome and generate transgenic rats has drastically limited the use of this research model. Recent advances in gene expression and transgenic systems have provided new possibilities for the generation of informative rat models [7]. Establishment of rat ES cells [6, 15] and iPS cells [16] may promote the generation of knockout models with BAC clones and will greatly expand the scope of present experimental limitations with new rat resources [27].

Currently, the C57BL/6N (B6N) substrain, in conjunction with B6N embryonic stem (ES) cells, is being used in worldwide knockout mouse projects and it is known as the standard inbred strain with the most uniform genetic background [17]. Although B6N ES cells are available from public resource centers such as the Mutant Mouse Regional Resource Center (MMRRC) at UC Davis and the RIKEN BioResource Center Cell Bank [30], BAC clones used for the production of transgenic and knockout mice are not available to the public. We have constructed a BAC library of the B6N substrain and the RIKEN BioResource Center will complete both 5’- and 3’-end sequencings of B6N and register the sequence data with the DDBJ in collaboration with National Institute of Genetics under the Genome Information Upgrading Program of MEXT NBRP. As a result of this program, we will be able to provide scientists a BAC library of B6N enriched with end-sequencing information, which will enable genetic engineering with high accuracy using B6N ES cells and facilitate functional genomics studies.

Promoter collection

Recently, needs of knockout animal have increased, in particular, the ability to delete a specific gene in a spatio-temporal manner. For this purpose, it is important to collect and maintain genetic materials which can express an introduced gene in tissue have and developmental stage-specific manner.

We have constructed reporter vectors harboring human and mouse promoters which are known to regulate the expression of genes in stage- and tissue-specific manners. The constructed clones were analyzed with luciferase activity using 18 lines of cultured cells, and the results have been published on the web site of RIKEN BioResource Center. Among them, promoters which have a tissue-specific [human alpha 1-antitrypsin promoter (AAT)] or ubiquitous [cytomegalovirus early promoter (CMV)] activity of gene expression were chosen to construct an expression vector expressing a nuclear Cre recombinase [13] to generate Cre-transgenic mice (Cre-Zoo) in collaboration with the BRC Experimental Animal Division. The established transgenic animals have been deposited at the BRC Experimental Animal Division [34] and are available for access by researchers (The accession numbers in the Experimental Animal Division are as follows. AAT, RBRC02153, RBRC02155, RBRC02157; CMV, RBRC01910, RBRC01963).

Recombinant adenovirus

Many researchers have been using recombinant adenovirus with great success in the research fields of cancer therapy. Its numerous advantages have made recombinant adenoviral vectors the preferred gene delivery tool for mammalian cells. However, generating a re-
combinant adenovirus may be time-consuming. In order to overcome this time constraint, we provide recombinant ready-to-use adenoviruses [31]. Researchers may perform their own experiment using our genetic materials without reconstruction of the viral vectors. We have constructed and collected more than 500 recombinant adenoviruses and shuttle vectors to generate recombinant adenoviruses which can be used for the infection of individual mice as well as cultured mammalian cells [18].

Using Cre expressing recombinant adenoviruses [13], researchers can obtain cells which have only transient Cre expression because recombinant adenoviruses do not integrate into the genome of the host cells. Thus, a recombinant adenovirus is used for abrogating the expression of a target gene in ES cells or fibroblasts from floxed mice [8, 9, 24]. Cre-expressing recombinant adenovirus is also useful for removing the neo cassette, which is often used as a marker for the knockout allele of gene of interest, in ES cells to avoid the interference and genetic ambiguity arising from the use of the selected gene. It has been reported that removal of the floxed neo gene from a knockout allele is possible by infecting morulae at the 16-cell stage with recombinant Cre adenovirus to produce mosaic mice which transmit the desired allele without the neo cassette with high frequency to their offspring [12].

We also provide a rapid method for the purification of recombinant virus and the detection of contamination of replication competent adenoviruses [28, 32]. We also hold training courses in advanced techniques for the best handing of recombinant viruses and host bacteria.

**Genetic Materials with Licensed Technology or Products**

RIKEN BRC sets the Material Transfer Agreements (MTA) for each transfer to protect the intellectual property rights of the developers of genetic materials and to define the responsibility of users. We have opened a path to the academic use of genetic materials produced by research tools owned by commercial entities. RIKEN BRC has executed a license agreement with the Life Technologies Corporation (former Invitrogen IP Holdings, Inc.) to receive, maintain, replicate and distribute Gateway® Entry clones and Expression clones. The Promega Corporation has also agreed to the deposition of the recombinant derivatives of Promega’s reporter vectors. Through the generosity of these biotechnology companies, RIKEN BRC is now able to distribute genetic materials to nonprofit organizations for not-for-profit academic research under this agreement. Thus, the core facility of the genetic materials at the NBRP is recognized as a reliable dissemination partner by these private entities.

### How to Use the Genetic Materials

Users can access our genetic materials through the websites of RIKEN BRC (Fig. 4). Users must complete an MTA for Distribution form and an order form. Sometimes permission for use is required from the Depositor using an approval form. In addition, recombinant DNA experiments in Japan must be conducted with special attention to laboratory safety and environmental conservation. Therefore, all recipients of genetic resources must submit their experiment plans and protocols to their organization’s Recombinant DNA Experiments Committee to obtain authorized approvals. RIKEN BRC starts to prepare the genetic materials upon receipt of the order documents from the users. We provide genetic materials for a minimal fee solely to reimburse the RIKEN BRC for a part of the preparation and handling costs of the requested genetic materials. This reimbursement fee is indispensable to continuing our activities of distribution. Recipients will also bear the shipping cost. The recipient of the genetic materials must expressly describe that “the genetic material was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan”, in the Materials and Methods section of all publication.

Deposition of genetic materials at RIKEN BRC frees researchers from cumbersome preservation and distribution of materials to fellow researchers. Furthermore, deposition increases the chances of collaboration and citation in a research paper. You are welcome to deposit genetic resources at the Gene Engineering Division, RIKEN BRC.
Preservation

The RIKEN Gene Engineering Division performs authentication tests and determines the stability of all its genetic materials. It also develops methods for maintenance and preservation that are appropriate for the various biological materials, using appropriate technology. These records are retained and are useful as baseline information when in-storage maintenance checks are performed and for validation after preservation and/or restocking. A scheme for the periodic assessment of all preserved materials is in place for each item stored. The Division selects methods on the basis of recommendations from the depositors and previous experience. It documents all preservation procedures to ensure that they are reproducible and all key parameters are monitored.

The genetic materials are preserved, at the minimum, as duplicate collections, namely master stocks and distribution stocks. The labels include the batch date or number and the RIKEN DNA Bank accession number (RDB No.). To ensure a minimum number of transfers or generations from the original materials, when appropriate, the Division uses the distribution stock for supplying the genetic resource. All genetic resources are stored under environmental parameters that assure the stability of their properties. The RIKEN DNA Bank provides, upon request, all methods and procedures used in validation and the results of all validations.

Distribution of genetic resources

The following conditions apply to all items for distribution. First, the original developers maintain their priority access to DNA clones. Second, recipients must state that the DNA clones or their derivatives will not be used in human experiments. Third, DNA clones and their derivatives must be used for research purposes only and must not be given or sold to a third party. Fourth, distribution of some genetic resources are subjected to the written approval of the depositor or developer. Fifth, the recipient must acknowledge the original developer and note in all publications that clones were obtained from the RIKEN Gene Engineering Division. Finally,
the developer (depositor) and recipient shall uphold the terms and conditions of the MTA.

We pack and send requested genetic materials according to current postal and quarantine regulations. Invoices are normally dispatched with the materials unless otherwise instructed. We keep records of all requests for genetic materials, dates of shipment, and names and addresses of recipients. We also record all user queries and complaints and, when possible, acknowledge such communications by return of post, fax or e-mail. We investigate complaints as rapidly as possible and implement corrective action as necessary.

Education and training

Our division holds training programs for scientists and young students to teach the application of scientific technologies. Currently these programs focus on the technology of recombinant adenoviruses.

Homepage, catalogs, newsletters, and “E-mail news”

Materials are listed on our Homepage (http://www.brc.riken.jp/lab/dna/en/) or the Gene Catalog. We also send scientists Newsletters and the E-mail News every month.

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References


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