

# The DT40 web site: sampling and connecting the genes of a B cell line

Jean-Marie Buerstedde\*, Hiroshi Arakawa, Akira Watahiki<sup>1</sup>, Piere Piero Carninci<sup>1</sup>, Y. Yoshihide Hayashizaki<sup>1</sup>, Bernhard Korn<sup>2</sup> and Jiri Plachy<sup>3</sup>

Department of Cellular Immunology, Heinrich-Pette-Institute, Martinistrasse 52, 20251 Hamburg, Germany, <sup>1</sup>Laboratory for Genome Exploration Research Project, Genomic Sciences Center and Genome Science Laboratory, RIKEN Tsukuba Institute, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan, Genome Exploration Research Group, RIKEN Genomic Sciences Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan, <sup>2</sup>RZPD—Ressourcenzentrum für Genomforschung, INF506, 69120 Heidelberg, Germany and <sup>3</sup>Academy of Sciences of the Czech Republic, Institut of Molecular Genetics, Flemingovo nam. 2, 16637 Praha 6, Czech Republic

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## ABSTRACT

Thousands of new vertebrate genes have been discovered and genetic systems are needed to address their functions at the cellular level. The chicken B cell line DT40 allows efficient gene disruptions due to its high homologous recombination activity. However, cloning the gene of interest is often cumbersome, since relatively few chicken cDNA sequences are present in the public databases. In addition, the accumulation of multiple mutations within the same cell clone is limited by the consumption of one drug-resistance marker for each transfection. Here, we present the DT40 web site (<http://genetics.hpi.uni-hamburg.de/dt40.html>), which includes a comprehensive database of chicken bursal ESTs to identify disruption candidate genes and recyclable marker cassettes based on the loxP system. These freely available resources greatly facilitate the analysis of genes and genetic networks.

## INTRODUCTION

The precise modification of chromosomal sequences by targeted integration of artificial constructs provides a powerful approach to determine the function of genes and regulatory elements within a living cell. In vertebrates, heterozygous mutations are most often introduced into murine embryonic stem cells which are then used to breed homozygous knock-out strains of mice (1).

If the mutant phenotype is measurable in cell culture, the production of homozygous mutant cell clones by step-wise disruption of both alleles can be a valid alternative to experiments with whole animals. The chicken B cell line DT40 has been popular in the study of cell autonomous (2–4) and B cell specific processes (5,6) due to high ratios of targeted to random integration of transfected constructs (7).

Although the identification of targeted integration events is straightforward, a common inconvenience of the DT40 system used to be the necessity to isolate the chicken cDNA of the target gene either by cross-hybridization or by inverse PCR. Another shortcoming has been the need of a new selectable marker for each transfection. While this is not a concern for the disruption of a single gene, the flexibility to freely accumulate multiple mutations is highly desirable for the analysis of genetic pathways.

## THE BURSAL EST DATABASE

To allow the electronic retrieval of chicken cDNA sequences of interest, a large database of ESTs from bursal lymphocytes is imbedded into the DT40 web site (<http://genetics.hpi.uni-hamburg.de/dt40Est.html>). The first version of the database included about 7000 sequences derived from a bursal cDNA library named dkfz426 (8). In the meantime another 3000 sequences from this library have been added to the database. The dkfz426 library seems to faithfully reflect the gene expression profile of chicken bursal lymphocytes and has yielded sequences of thousands of new chicken orthologs of mammalian genes. However, dkfz426 was not normalized and contained only a low percentage of full-length gene coding inserts.

Another bursal B cell cDNA library was therefore synthesized using the biotinylated cap trapper method (9,10). This new normalized library named riken1 is of excellent quality with a high percentage of full-length gene inserts. More than 5000 ESTs of riken1 have been incorporated to the Bursal EST database and further sequences are added a rate of about 300 sequences per week.

All bursal ESTs are automatically annotated based on the results of BLAST searches against the public databases. In addition to the sequences and the BLAST reports, the PHRED scores as well as information about possible sequence cluster and polymorphisms are accessible at the web site. The software developed for the creation of the database and the Internet

\*To whom correspondence should be addressed. Tel: +49 40 48051 290; Fax: +49 40 48051 296; Email: buersted@genetics.hpi.uni-hamburg.de

presentation is freely available under the open source license (<http://genetics.hpi.uni-hamburg.de/FOUNTAIN.html>).

It is hoped that the Bursal EST database will eventually comprise all genes expressed in bursal B cells and the DT40 cell line. Based on the searches for members of DNA repair pathways it seems that the database already represents orthologs of most of the housekeeping repair genes known from mammalian species. Likewise most orthologs of the known B cell specific transcription factors can be found. Since a high percentage of the ESTs from the riken1 library include the 5' end, it is possible to determine the full-length reading frame of these ESTs by simple sequence extension.

## RECYCLABLE MARKER CASSETTES

To allow recycling of the resistance markers, new marker cassettes have been designed in which the drug resistance genes are flanked by mutant LoxP sites (11). These new marker cassettes can be efficiently excised by the CRE recombinase (12) after targeted integration into the chromosome. Genomic instability after marker excision should be minimal, as the new LoxP sites resulting from the excision will be resistant to further Cre mediated cleavage. The new marker cassettes are freely distributed as part of the DT40 web site (<http://genetics.hpi.uni-hamburg.de/dt40Reagents.html>).

Drug resistance markers for knock-out experiments in DT40 were previously distributed as *Bam*HI fragments and it was recommended that the cassettes are cloned into *Bam*HI or *Bgl*II sites between the upstream and downstream arms of the target gene. Since the new marker cassettes are also available as *Bam*HI fragments, it is possible to convert previously designed knock-out constructs by a simple cloning step. All knock-out constructs based on this principle can be combined with each other in the same cell, if the transfection is followed by the excision of the marker.

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