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Plant DNA Resources in RIKEN BRC to Bridge the Gap between Gene Function and Phenotype

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National Bioresource Project started in 2002 by the support of Ministry of Education, Culture, Science, Sports and Technology (MEXT) in Japan. The important purpose of this project is to collect, preserve, and distribute bioresources (such as experimental animals, plants and microbes etc.) that are essential experimental materials for life sciences research. Developing fundamental technologies to improving the value of each bioresources, and enriching genome information are also important target of this project. At the same period, the Experimental Plant Division of RIKEN BioResource Center (RIKEN BRC: http://epd.brc.riken.jp/en/) was established in the RIKEN Tsukuba Institute in 2001 to promote resource activities of *Arabidopsis* plant (Kobayashi, 2011). *Arabidopsis* is one of the most well-analyzed experimental model plants. In the end of 20th century, the entire genome sequence of Arabidopsis has been completely characterised (The Arabidopsis Genome Initiative, 2000). Since then, *Arabidopsis* genome sequence data create massive amount of useful and desirable information by several omics-based analyses. Huge quantities of genome resources are also developed by international *Arabidopsis* research community. In RIKEN BRC, we collect seeds of RIKEN Arabidopsis transposon-tagged mutants, activation-tagged lines, FOX (full-length cDNA overexpressor gene) hunting system, and natural accessions, as well as Arabidopsis suspension cell culture T87 cells. We also collect DNA resources such as RIKEN Arabidopsis full-length cDNA clones (RAFL clones). Here, we introduce outline of our full-length cDNA resources and give detailed description about our approach to bridge the gap between gene function and phenotype.

RIKEN Arabidopsis Full-length cDNA (RAFL) Clones

Unlike in the case of partial cDNA libraries, developing full-length cDNA libraries are very useful for further analyses of gene function, and also large-scale gene discovery projects, or other -omics research (Seki *et al*., 2009). Several techniques have been established to prepare enriched full-length cDNA libraries (Seki *et al*., 1998). The usefulness of full-length cDNAs has been confirmed in humans, mice, and in various plants such as Arabidopsis (Seki *et al*., 2002). RIKEN Arabidopsis full-length cDNA (RAFL) have been developed by RIKEN Genome Sciences Center. RAFL clones have been utilised as standard clones in *Arabidopsis* community. Currently, more than 200,000 clones are available from RIKEN BRC.

Other Full-length cDNA Clones in RIKEN BRC

Full-length cDNA projects were performed in many plant species in Japan (Umezawa *et al.,* 2008, Aoki *et al*., 2010, Abe *et al*., 2011). In RIKEN BRC, we collect full-length cDNAs of model plant species. Recently we started the distribution of approx. 40,000 clones of *Brachypodium distachyon* full-length cDNAs. Approximately 150,000 clones of *Physcomitrella patens* full-length cDNAs, 20.000 clones of Poplar full-length cDNAs, 20,000 clones of *Manihot esculenta* full-length cDNAs, 40,000 clones of *Striga hermonthica* full-length cDNAs are also available from RIKEN BRC. In all case, we perform the end sequencing of every clones to confirm the absence of contamination or mishandling before shipping.

SABRE Database to Cross-Search Plant Genetic Resources through Publicly Available *Arabidopsis* Information

After genome sequencing project, international *Arabidopsis* research community started '*Arabidopsis* 2010 Project' (Ausubel, 2002) to investigate the function of about 27,000 entire genes of *Arabidopsis*. As a result, huge amount of information about each *Arabidopsis* gene function have been obtained. To utilise this useful information about *Arabidopsis* gene function for other plant species, RIKEN BRC have developed SABRE (Systematic Consolidation of *Arabidopsis* and other Botanical REsources) database (Fukami-Kobayashi *et al*., 2013). In SABRE (http://sabre.epd.brc.riken.jp/

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SABRE2. html), plant cDNA clones are linked to TAIR (The *Arabidoposis* Information Resource) gene models and their annotations through sequence homology. Approximately 1.5 million plant cDNA information from the National BioResource Project (NBRP) are integrated in SABRE database, and these data are composed of cDNA information from 14 plant model plant species (*Arabidoposis*, barley, cassava, Chinese cabbage, lotus, morning glory, poplar, *Physcomitrella patens, Striga hermonthica*, soybean, *Thellungiella halophila*, tobacco, tomato and wheat).

Fox Hunting System to Bridge Between Gene Function and Phenotype

FOX hunting system (Full-length cDNA Over-eXpressing gene hunting system) have been developed in RIKEN Plant Science Center to create gain-of-function mutant lines caused by ectopic expression of full-length cDNAs (Ichikawa *et al*., 2006). Approximately 10,000 *Arabidopsis* full-length clones were over-expressed in plants under the control of cauliflower mosaic virus 35S-promoter and created more than 10,000 transgenic lines. These lines were deposited in the RIKEN BRC and available for phenotypic screening. We also collect and distribute FOX hunting lines, overexpressing rice full-length cDNA in *Arabidopsis* plants.

Conclusion

We recently collected and started the distribution of *Arabidopsis* TAC (transformation-competent artificial chromosome) clones (Shibata *et al*., 2000). TAC clones can accept large genomic DNA fragment in *Agrobacterium tumefaciens* as well as *Escherichia coli*. Therefore, TAC clone can be directly used for *Agrobacterium* mediated *Arabidopsis* transformation, and accelerate genetic complementation to understand *Arabidopsis* gene function. We also started distribution of the RIKEN *Arabidopsis* Transcription Factor (RARTF) ORF clones. The cDNAs encoding transcription factor are distinctly important DNA resources. However, all of these cDNA set were found in RAFL clones. Accordingly, RARTF clones were PCR amplified using *Arabidopsis* genome information (Iida *et al*., 2006). Fujita *et al*. (2007) have performed the mini scale fox hunting system, over-expressing stress inducible Arabidopsis transcription factor genes. They successfully isolated the stress tolerance related transcription factor genes. Strategic development of DNA Resources would enable us to bridge the gap between gene function and phenotype.

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