13 Activation Tagging Systems in Rice

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13.1 Introduction

Sequencing of the 389-Mb rice genome (Oryza sativa L.) is nearly complete and map-based, finished quality sequence now covers 95% of the genome. Determining function of the 37,544 predicted genes in the rice genome, however, remains a formidable challenge that will require multiple,
complementary approaches to be achieved. As with the dicotyledonous model species, *Arabidopsis thaliana*, rice genetic resources have been heavily invested toward the generation of random loss-of-function mutants, or knockouts, involving the use of mutagens such as γ-rays or, more frequently, transferred DNA (T-DNA) and transposon-based systems such as Ac/Ds, En/Spm, and Tos17 (see Hirochika et al. 2004 for review of rice mutant resources). To date, roughly 300,000 mutants have been generated using these strategies, providing invaluable genomic tools for gene mining in the model monocotyledonous species. In addition, gene targeting techniques have recently emerged that allow for specific rice loci to be disrupted (Terada et al. 2002; Cotsaftis and Guidersoni 2005), yet optimization is still required before these techniques can be used to generate knockouts on order.

Despite widespread application, the traditional knockout approach is limited in its ability to fully saturate the rice genome with mutations. Genes with lethal or deleterious knockout phenotypes (particularly at the embryonic stage of development) are not amenable to the loss-of-function approach, and the investigation of large gene families is often hampered by the redundant activity of one gene member compensating for the loss of another. This is particularly relevant to the rice genome, in which 29% of predicted genes have been amplified at least once to form tandem repeats, with some tandem repeats stretching up to 134 members (International Rice Genome Sequencing Project 2005). To address this significant obstacle and maximize the usefulness of knockout collections, gene, promoter, and enhancer traps have often been included in T-DNA and transposon-based insertion systems to enable reporter visualization of native gene activity when other phenotypes are not necessarily present (Jeon et al. 2000; Ito et al. 2004; Peng et al. 2005). Trapped patterns report on spatial and developmental activity of native rice genes, although the identification of genomic elements responsible for those patterns can be laborious and not always apparent (Peng et al. 2005). RNA silencing is a well documented phenomenon in plants (Baulcombe 2004), with the clear advantage over gene knockouts of simultaneously silencing multiple members of a particular gene family. The extent to which RNA silencing can be used to suppress gene targets in the rice genome remains to be seen, with a recent study of the OsRac gene family reporting a maximum of three gene members efficiently suppressed using inverted repeat constructs (Miki et al. 2005). Continued refinements to RNA silencing technology, such as the development of artificial microRNAs with greater targeting control than traditional hairpin constructs (Schwab et al. 2006), promise to increase the efficiency and accuracy of RNA silencing in plants.

While interruption or silencing of a particular coding sequence may not lead to a detectable phenotype, for a variety of reasons, dominant mutant phenotypes are more likely to result from upregulation, or activation