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Generation of Genome-Wide Deletion Mutants and Profiling of Drug-Induced Haploinsufficiency of Fission Yeast Genome

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The sequencing of 13.8-Mb *Schizosaccharomyces pombe* genome reveals that the 4824 protein-coding genes exist in *S. pombe* genome, which is the smallest number for eukaryotes. We generated heterozygous genome-wide deletion mutants of *Schizosaccharomyces pombe* using PCR-based targeted mutagenesis. The deletion cassettes containing kanMX module as a selection marker, tag sequences as mutant identifiers and the flanking regions of target gene at 5'- and 3'-end were amplified by four round and multi-block PCR. The deletion cassettes were replaced at the target site of chromosome of diploid strain SP286 by homologous recombination. The deletions of target genes were confirmed by colony PCR of transformants. In total 2,084 heterozygous deletion mutants were generated with the deletion efficiency of 2-100%. We constructed 2,084 heterozygous diploid deletion mutants whose genes cover 43% of ORFs in *S. pombe* genome. The genes deleted in mutants are classified into 14 functional groups. They belong to metabolism (174), cell cycle control (403), transcription (416), protein synthesis (170) and modification (216), transport facilitation (132), cellular organization and Biogenesis (139) signal transduction (79), and unclassified (220).

Chemical genomics screen using chemical inhibitors that might cause conditional loss of functions of proteins has been widely used to discover and characterize biological pathways. The responses of 2083 heterozygous deletion mutants to 12 cytotoxic drugs, cerulenine, rapamycin, cisplatin, hydroxyurea, oligomycin, doxorubicin, fluphenazine, cycloheximide, hydrazinocurcumin, ketoconazole, amphotericin B, and terbinafin were examined by liquid assay with automated HTS and visualized spot assay. The collection of deletion mutants was analyzed for the response to twelve cytotoxic drugs to explore the global function of target genes and their genetic interactions in *S. pombe*. Widely different profiles of genes affecting sensitivity and resistance to drugs were observed revealing different mechanisms of cytotoxicities and effects of drugs and genetically interacting genes with target genes. In the systematic chemical genomics screening with 12 drugs, total 420 genes were identified as putative targets and genetically interacting with target genes since their deletion caused sensitivities or resistances to one of 12 drugs. Half of target genes belong to categories of cell cycle and transcription, which were known as main targets for anti-cancer and anti-fungal drugs. This

result implies that drug response screen using cytotoxic drugs is one of the simple ways to study global function of pathways and precise mechanisms involving cell division and growth regulation. Sixteen mutants were sensitive to more than 4 drugs indicating deleted genes including cam1, nda2, cka1, paa1, clr4 and uncharacterized genes seem to play important roles in growth and be critical in cell survival. Some of these genes might function as core genes in signaling pathway(s) responding to a certain range of cytotoxic drugs. They might be involved in multi-drug resistance of cells and also used as a group of genes to determine biological toxicity in the development of new drugs.

The genome-wide deletion mutant approaches offer significant advantages over traditional genetic methods since mutants display phenotypes under the condition of the screen. Now, we have 2083 deletion mutants of *S. pombe* available for genome-wide analysis, while genome-wide deletion mutants of *S. cerevisiae* have been used for a tool for since 1999. The pool of heterozygous diploid deletion mutants covering essential and non-essential genes is a valuable resource for functional annotation of genes in wide range of pathways and identification of new drug targets. Our collection of systematic deletion mutants has characteristic to examine their phenotypes simultaneously in a single culture since all mutants are labeled with two 20-mer tag sequences in each deletion strain. To identify the survived or disappeared mutants after growth under a particular condition, hybridization of oligonucleotide DNA chip with labeled tag intensities can be used to deduce the relative growth rate of each strain within the pool.

Chemical genetic approach is conditional and it is easy to target on and off by controlling drug levels. Clustering of the responding genes for one drug on the basis of their cross-sensitivities to other drugs revealed a specific core genes required for responses to a broad range of cytotoxic drugs. The simple chemical approach can find increasing application in understanding of important regulatory proteins and the cellular regulatory network. In addition, it can be used to evaluate the global effects of the therapeutic drug on cell growth and to provide valuable information regarding the potential side effects. With emerging new drug designs and screening technologies, the analysis of cytotoxicity of increasing number of drug candidates will be important at the early stage of drug development. The collection of deletion mutants will be used as a tool for cell-based drug cytotoxicity assay system observing drug response.