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# Web application for genetic modification flux with database to estimate metabolic fluxes of genetic mutants

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**Computational analysis of metabolic fluxes is essential in understanding the structure and function of a metabolic network and in rationally designing genetically modified mutants for an engineering purpose. We had presented the genetic modification flux (GMF) that predicts the flux distribution of a broad range of genetically modified mutants. To enhance the feasibility and usability of GMF, we have developed a web application with a metabolic network database to predict a flux distribution of genetically modified mutants. One hundred and twelve data sets of *Escherichia coli*, *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, and Chinese hamster ovary were registered as standard models.**


[**Key words:** Systems biology; Metabolic flux analysis; Elementary mode; Metabolic flux estimation; Genetic mutant database; Metabolic network data; Flux mutant prediction; Genetic modification]

Metabolic flux analysis (MFA) is widely used to investigate the metabolic fluxes of a variety of cells (1,2). MFA is based on the stoichiometric matrix of metabolic reactions and their thermodynamic constraints. The matrix is derived from a metabolic network map, where the rows and columns represent metabolites, chemical/transport reactions, respectively. MFA is very effective in understanding the mechanism of how metabolic networks generate a variety of cellular functions and in rationally planning a gene deletion/amplification strategy for strain improvements. Computational modeling is a challenge to predict the metabolic flux distribution of a broad range of genetically modified mutants.

Flux balance analysis (FBA) (3) is used to predict the steady-state flux distribution of genetically modified cells under different culture conditions. Minimization of metabolic adjustment (MOMA) was developed to predict the flux distributions of gene deletion mutants (4). FBA and MOMA often lead to incorrect predictions in situations where the constraints associated with regulation of gene expression or activity of the gene products are dominant, because they apply the Boolean logics or its related simple logics to gene regulations and enzyme activities (5). On the other hand, network-based pathway analyses, elementary modes (EMs) (6) and extreme pathways (7) emerge as alternative ways for constructing a mathematical model of metabolic networks with gene regulations. EM analysis was suggested to be convenient for integrating an enzyme activity profile into the flux distribution. Enzyme control fluxes (ECFs) (8) uses the relative enzyme activity profile of a mutant to wild type to predict its flux distribution. Genetic modification flux (GMF) was presented to predict flux distribution of genetically modified mutants with under-expressed/over-expressed genes (9–11).

Despite the usefulness of ECF and GMF, there have been no user-friendly application programs. Use of them had required handling computer programs, which often hampers the general and broad use. Thus, we have developed a user-friendly web application together with the database of metabolic networks that helps users accessing metabolic network data with a variety of experimental data (12).

## MATERIALS AND METHODS

**Systems overview** Fig. 1 shows a workflow of the web application of GMF. Metabolic reaction network files written in the Microsoft Excel format are registered in the database attached to the GMF web application. These files can be freely downloaded. Users either select a registered or uploaded user's own data file. The application reads the selected or uploaded file and generates its associated stoichiometric matrix with the format available for the efmtool (13). Users can select one algorithm out of the three: GMF, mCEF or ECF to predict the flux distribution of genetic mutants. To perform GMF and ECF, they select one of the four objective functions and specify a ratio type of gene or enzyme. The calculated result is displayed and can be downloaded. In addition, we have developed the stand-alone version of the GMF web application that functions on the MATLAB (The MathWorks). The main workflow of the GMF stand-alone version is the same as the web.

**Database** The web application is equipped with a database. All data files are written in Microsoft Excel format, with three sheets; sheet 1, experimental condition; sheet 2, reactions; and sheet 3, metabolites. Sheet 1 (experimental condition) contains the experiment information, including the author and title of original publication from where the data were extracted. Sheet 2 (reactions) provides the metabolic reactions and their associated flux distributions, gene expressions, and

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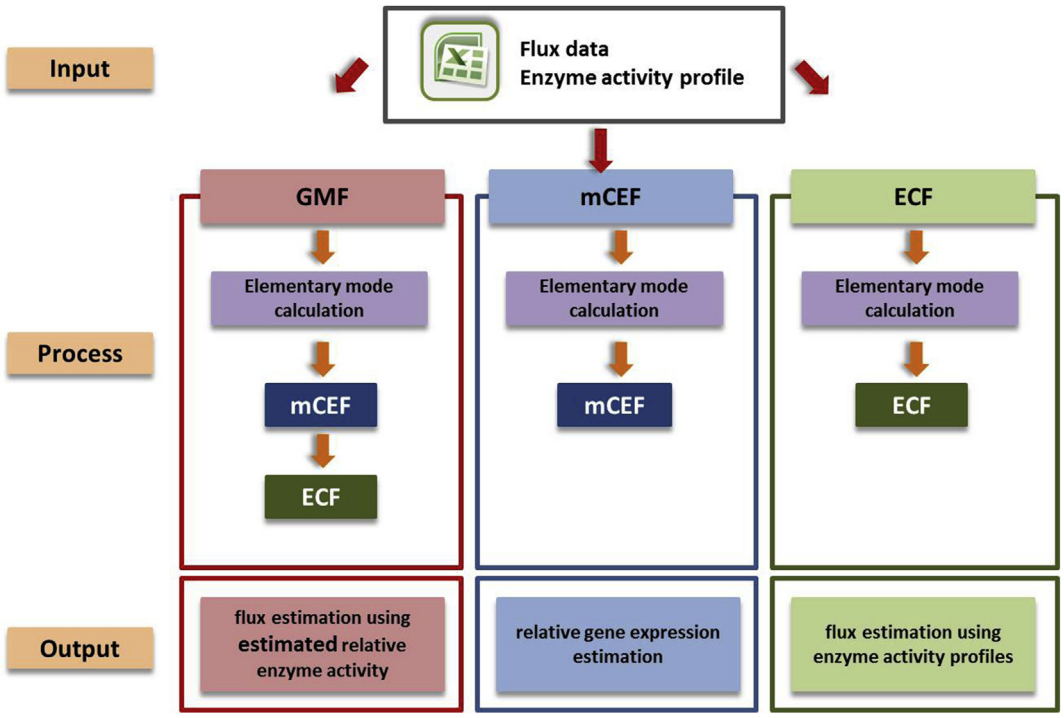


FIG. 1. A workflow of the web application of GMF. A metabolic network file written in the Microsoft Excel format is put in the application. To perform GMF and ECF, users need to select an objective function out of MEP, MeanLP, LP and QP.

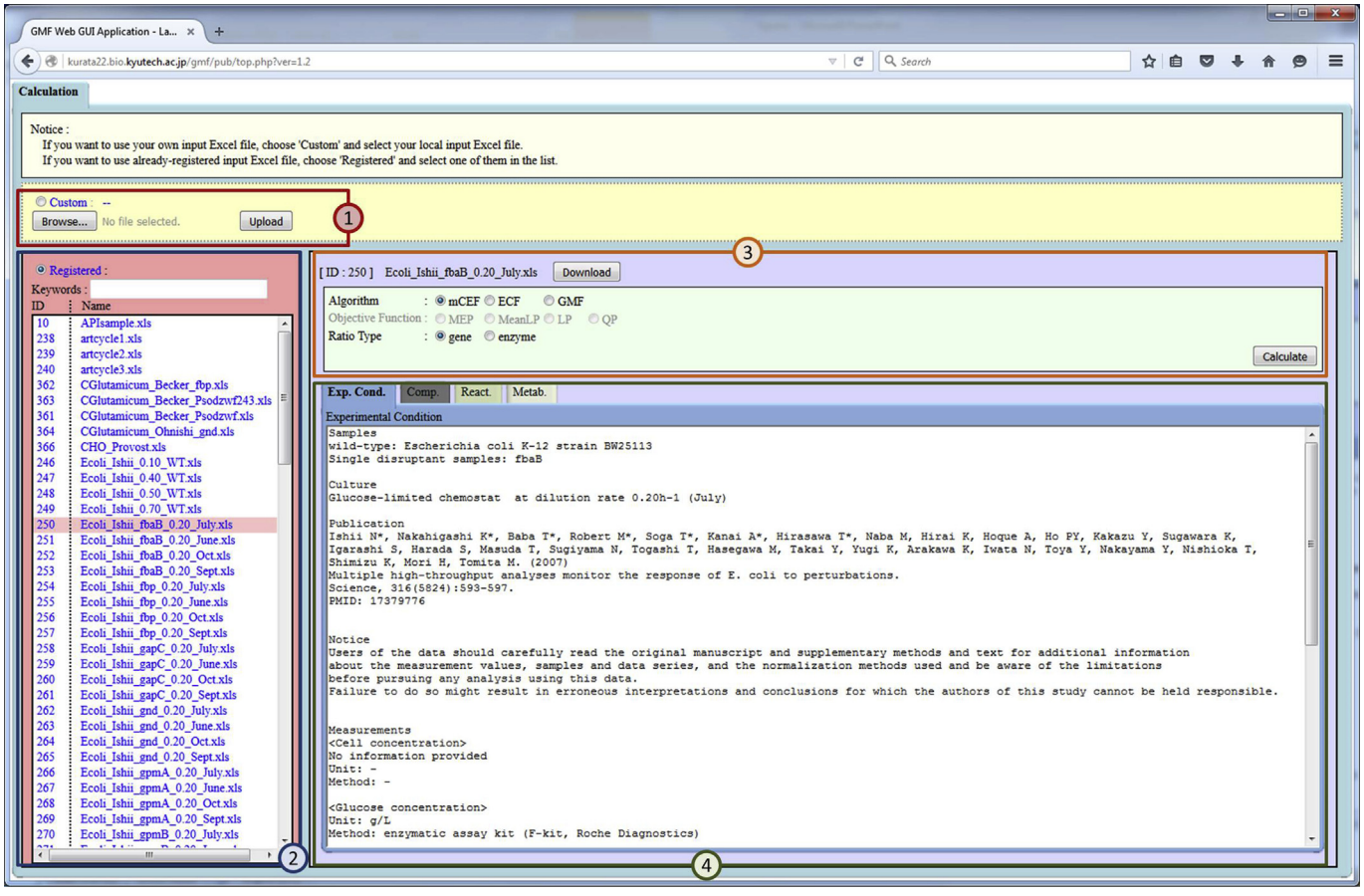


FIG. 2. The main page of GMF web application. Users can (i) upload their own file or (ii) select a file out of the registered files. (iii) Users select an algorithm to estimate a flux distribution or gene expression profile, a ratio type of gene expression or enzyme activity profiles, and an objective function. (iv) Once an input file is selected, details in the metabolic network are displayed.