MICROME SOPs		
Title: **Reaction curation in RHEA environment**		Page
SOP-02	Revision level	Effective date
Author	Primary Reviewer	

1-Overview

Chemical reactions are the essential components of metabolic pathways that allow modelling the interconversion of chemical compounds in metabolic networks. In the context of Microme project, RHEA database (http://www.ebi.ac.uk/rhea/) is used as reference resource for chemical reactions. Microme curators should be able to create RHEA reactions identified in the context of pathway curation if these are not present in the current Microme repository.

1.1-Scope

Creation of chemical reactions in RHEA database chemically balanced at ph 7.3 that will be used in the subsequent steps of pathway curation and creation by Microme curators.

1.2-Related documents

SOP01 – Compound curation in ChEBI environment

SOP03 – *De-novo* pathway creation in Microme environment

1.3-Revision history

2-Requirements

Personal account for reaction creation in RHEA database through RHEA curator tool (http://www.ebi.ac.uk/intenz/curation/rhea/login.do)

CHEBI identifiers for the compounds involved in the query reaction at correct 2D structure at ph 7.3

3-Procedure

RHEA is a database of chemical reactions from the EBI (http://www.ebi.ac.uk/rhea//home.xhtml) where all reaction participants (substrates and products) are chemical entities from ChEBI database (ChEBI compounds), which allows to provide detailed information of compound structure, formula and charge. RHEA provides reactions that are elemental and charge balanced, and provides extensive cross-references to other reaction resources like KEGG, MetaCyc, IntEnz, and to pathway resources like UniPathway. All RHEA reactions are normalized by using the major microspecie of the participant

compounds at ph 7.3. The selected ph of 7.3 corresponds to that chosen by MetaCyc, facilitating the cross-referencing between both resources.

The RHEA curator tool (http://www.ebi.ac.uk/intenz/curation/rhea/login.do) allows the creation of novel reactions in RHEA based on chemical compounds with "checked" status on ChEBI database. Three main types of reactions can be created in RHEA:

- Simple chemical transformations
- Transport reactions
- Polymerization reactions

3.1 Login to the RHEA curator tool

Once you are registered, enter your username and password

3.2 Create the reaction

3.2.1 Add ChEBI compounds

In the main page of the curator tool, click on ***"New->Simple Reaction"*** in the bar on the top of the page. Add reaction compounds through their corresponding ChEBI ID´s in the ***"Add reaction participant"*** field. By default, the option ***"disable pH normalisation"*** appears unchecked. This means that the curator tool uses the ChemAxon calculation plugging MarvinBeans (http://www.chemaxon.com) to evaluate if the 2D structure of the desired ChEBI compound is the major microspecie at ph 7.3. If is not the case, the ChEBI compound is not retrieved. If the compound is found, add it to the right or left side of the reaction by clicking on the corresponding options in the interface. Compound stoichiometry can be defined by modifying the number of molecules of each compound once added. If the curator tool returns ***"no compounds found"*** even the ChEBI ID is valid, it means that the needed compound is not present in ChEBI. In this case, the compound should be created in ChEBI (SOP01).

Once all ChEBI compounds has been added to left and right side of the reaction, the qualifiers ***"Mapped"*** and ***"Formuled"*** are automatically checked. The curator tool automatically determines if the reaction is chemically balanced or not. If not, information about the excess of mass and charge at each reaction side is provided. If the reaction is balanced, the qualifier ***"chemically balanced"*** is also checked.

For transport reactions, RHEA does not include information on concrete locations (i.e. cellular compartment, tissue, organ, species). Instead, nominal cellular compartments are specified by the tokens ***"in"*** and ***"out"*** according to the side convention of NC-IUBMB. Once this is specified for each compound of the reaction (in the ***"Location"*** field near each reaction compound), the qualifier "Transport" is automatically checked.

3.2.2 Define Status

Select the Status of the reaction from the drop-down menu “Status”:

- No Status (Default option)
- Rejected
- Pending
- Obsolete
- Preliminary
- Approved

Only chemically balanced reactions with all ChEBI compounds public can have the status ***“Approved”***. For reactions including ChEBI compounds with checked status but not present in the public database (i.e. new compounds created in ChEBI but not yet public), ***“Pending”*** status is temporarily assigned until all ChEBI compounds will be public. In this moment, the status of the reaction should be changed to ***“Approved”***. Only ***“Approved”*** reactions will be publicly available in the following database releases.

3.2.3 Define Source

Select the source of the new reaction from the drop-down menu ***“Source”*** (from which resource the new reaction comes from):

- Undefined
- NC-IUBMB
- ENZYME
- BRENDA
- Rhea
- BioCyc
- IntEnz
- UniPathway
- KEGG reaction
- EcoCyc
- MetaCyc

3.2.4 Add cross-references

If the newly created reaction is already present in other resources, RHEA curator tool allows adding cross-references to the same reaction in other related

databases. For this purpose, in the drop-down menu ***"New xref"*** select between the following 4 reference resources:

- UniPathway
- KEGG reaction
- EcoCyc
- MetaCyc

Next, specify the ID of the reaction in the selected resource in the ***"Accession"*** field of the ***"New xref"*** menu. By clicking on ***"Add"*** option, a hyperlink to the ID of the cross-reference appears in the ***"Xrefs"*** section that allows direct access to the cross-referenced reaction in its corresponding resource. Check the equivalency of reaction formulation between the new RHEA reaction and the corresponding cross-references before submit the reaction.

3.2.5 Add bibliographical references

If the newly created reaction is supported by bibliographical evidence, RHEA curator tool allows adding this information to the reaction entry. For this purpose, in the drop-down menu ***"New citation"*** select between the following 6 bibliographical resources:

- PubMed
- Chinese Abstracts
- Patent Abstracts
- CiteSeer
- Agricola
- CiteXplore

Next, specify the ID of the bibliographical reference (i.e. PMID for PubMed references) in the ***"Accession"*** field and click the ***"Add"*** button. The bibliographical reference will appear in the ***"Citation"*** field.

3.2.6 Submit reaction

Once all the previous steps has been completed and reviewed, the reaction can be submitted to the database by clicking on the ***"Submit"*** button. A new window appears with all the attributes of the new reaction and its corresponding identifier. RHEA stores each unique reaction only once assigning a unique identifier, but for each of such "master reaction" (<?> connection between left and right parts of the reaction), three derived reactions are automatically created with the same left and right parts but each of which coupled to a unique directionality:

- Left to right (LR; => connector)

- Right to left (RL; <= connector)
- Bidirectional (BD; <=> connector)

Each of these directed reactions has also its own unique identifier. By default, cross-references to other reaction resources are assigned to the master reaction. This has to be modified such that each directed reaction is cross-referenced to the corresponding directional reaction in other resources (i.e. reactions with bidirectional formulation in KEGG and directional formulation in EcoCyc/MetaCyc). For this purpose, once the new reaction has been created, make click on the ***"Edit"*** button. A new window appears which allows modifying all elements of the reaction (metabolites, cross-references, status). At the bottom of the page, 4 different tabs corresponding to the master, LR, RL, and BD reactions are displayed. Cross-references to other reaction resources should be removed from the master reaction and added to the corresponding LR, RL, or BD according with the reaction direction by the same procedure specified in section 3.2.4.

Additional comments can be added in the ***"Private comment"*** and ***"Public comment"*** text fields describing particular aspects of the reaction.

Once all the modifications have been done, click on ***"Submit"*** button to store them in the database.

4-Data management

RHEA database is updated by monthly released. Newly created reactions with ***"Approved"*** status will be publicly available in the next release of the database.

4.1-Quality control

Each group of reactions for a particular chemical transformation (Master+LR+RL+BD) has a status of curation that illustrates the level of confidence (i.e. approved, preliminary, obsolete)