II. RMgmDB: A genotype and phenotype database of genetically modified rodent malaria parasites

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OVERVIEW

The RMgmDB database (Rodent Malaria parasites Genetically Modified database; www.pberghei.eu) is a manually curated web-based repository that contains information on all published (and some unpublished) genetically modified rodent malaria parasites. It provides easy and rapid access to information on the genotype and phenotype of genetically modified mutant parasites and transgenic (reporter) parasite lines. Here we describe both what is in and how to search the RMgm database. In addition we describe how to submit unpublished information to RMgmDB about mutants which do not exhibit a clear phenotype and on unsuccessful attempts to disrupt or mutate *Plasmodium* genes.

THE DATABASE

RMgmDB contains genotype and phenotype information of mutant and transgenic reporter parasites and information on gene function inferred from the mutant phenotypes (Janse et al., 2011; Khan et al., 2013). Most information has been manually extracted from published scientific literature retrieved from Medline searches. In addition, the database also contains unpublished data on mutants without a clear phenotype and on negative trials to either disrupt or mutate genes. The database is continually updated and, as of March 2013, it contained information on over 780 mutants or unsuccessful attempts to generate mutants. The database can be accessed via appropriate gene-pages in the *Plasmodium* genome resources PlasmoDB (www.plasmodb.org) and GeneDB (www.genedb.org). In PlasmoDB and GeneDB the information of a mutant reported in a rodent *Plasmodium* species is directly linked to the orthologous genes of *P. falciparum* 3D7. In RMgmDB there are direct links to GeneDB (www.genedb.org) and Medline abstracts wherever there is a gene and/or paper(s) associated with the mutant or reporter parasite. In addition to genotype and phenotype data, information is provided on the DNA-constructs used for the genetic modifications. Information on DNA constructs of mutants generated using *Plasmo*GEM vectors is linked to the information provided in the *Plasmo*GEM database (http://plasmogem.sanger.ac.uk/). The database is updated on a weekly basis and collates all the latest data from Medline searches and/or the introduction of unpublished data provided by different laboratories.

The Web Interface

The Web Interface (Figure 1) is designed to search for information on mutant parasites within the database based on searches using either (single or multiple) *Plasmodium* gene models (gene ID's) or using text terms. Moreover, mutants can be searched for using a gene ID or text term in combination with a specific genetic modification. Five different types of genetic modification have been defined: (i) mutants with *disrupted* genes (i.e. 'knock-out mutants'); (ii) mutants where the gene has been *mutated*; (iii) mutants where the gene has been *tagged*; (iv) mutants expressing *transgenes*; and (v) mutants with *'other' modifications* (Figure 1). For mutants that

express *transgenes* the regulatory regions can be further specified in the search field (i.e. the transgene and/or promoter and/or 3' UTR; Figure 1).

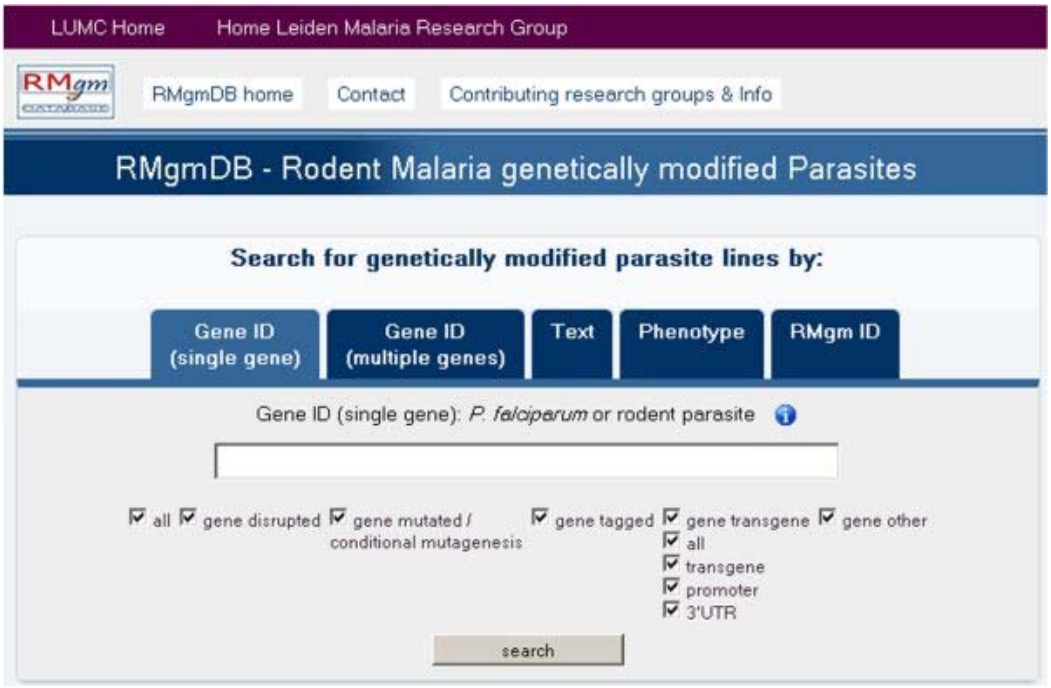

Figure 1: Searching RMgmDB for mutants using single/multiple gene ID's or text terms in combination with one or more types of genetic modification.

In addition to the different genetic modifications, the parasite life-cycle has been divided into six different stages where the phenotype of the mutant is described in comparison to the wild type parasite phenotype (see Figure 2). Specifically, (i) *asexual blood stage* (includes rings trophozoites, schizonts and merozoites); (ii) *gametocyte/gamete* (includes both male and female gametocytes/gametes; (iii) *fertilization and ookinete* (includes zygotes); (iv) *oocyst*; (v) *sporozoite* (includes midgut- and salivary gland sporozoites); and (vi) *liver stage* (includes liver-trophozoite, -schizont and -merozoite). Mutants can be searched for by specifying the life-cycle stage, with a phenotype different from wild-type phenotype, in combination with a specific genetic modification (Figure 2)

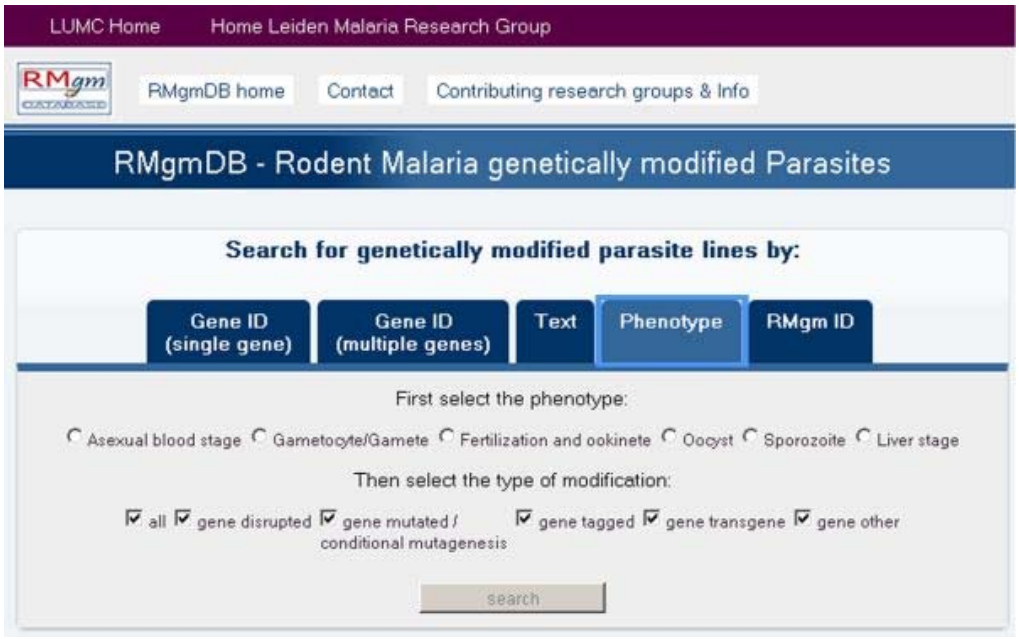

Figure 2: Searching RMgmDB for mutants with a phenotype in one or more life cycle stages which is different from wild type parasites in combination with one or more types of genetic modification.

It is **IMPORTANT** to note that RMgmDB is a database for information on mutant parasites and transgenic reporter parasites. Although it provides information on life-cycle stage mutant phenotypes it does NOT provide a systematically organized set of data on mutant (cellular/molecular) phenotypes or gene-function. The (limited) information in RMgmDB on phenotypes and gene function is provided as 'free text' using the same terminology as used in the publications describing the mutants. The lack of standardized vocabularies in *Plasmodium* for describing phenotypes and gene functions 'inferred from mutant phenotypes' limits the ability to search for mutants with a comparable phenotype or to identify genes with a similar function. For example, it is not possible for a systematic search for all mutants that have a gliding motility phenotype (see example below) or for genes that have a proven role in the motor complex etc. See also Janse et al., (Janse et al., 2011) for a more detailed discussion how the lack of standardized vocabularies for phenotypes and gene functions in *Plasmodium* limits searching RMgmDB and reduces the possibilities of integrating information on gene function and protein location from RMgmDB with gene data from other resources.

Searching the database: Search Results

Searching for mutants using a single gene ID or text terms

Searching the database for specific mutants can be performed by specifying the gene ID in combination with one or more of 5 types of genetic modifications: *disruption, mutation, tagging* and introduction of a *transgene* or *other modification* (Figure 1). Similarly, searching the database for mutants can also be performed using text-terms in combination with one or more of the 5 types of genetic modification (Figure 2; see below for limitations of RMgmDB when searching using text-terms). After submitting your search-query, the search result shows an overview of the mutants or unsuccessful attempts to disrupt or mutate genes (Figure 3; nothing is indicated where either the gene model or text term fail to retrieve any information). The search-results provides a short

summary of the mutant including the type of modification, the gene ID, the gene product and the life cycle stages with a phenotype that is different from the phenotype of wild type parasites. For *mutated* genes a short description of the mutation is given (Figure 3) and for *tagged* genes the name of the tag. For *transgenes* the following information is provided in the search-overview: the name of the transgene, the insertion/replacement locus in the genome, the promoter and the 3'UTR region. Clicking on one of the mutants in the search-results leads to the record page of the mutant. See also below for some examples of searches using a single gene ID or using text terms.

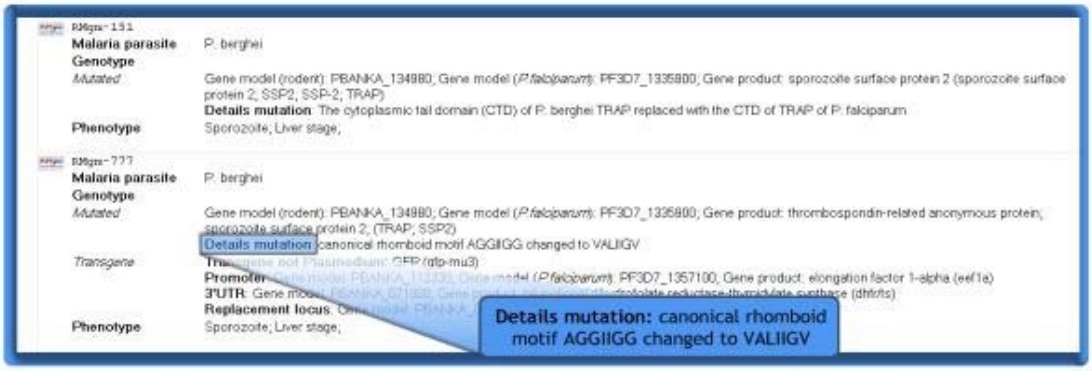

Figure 3: Search results: The search-results provides a short summary of the mutant including the type of modification, the gene ID, the gene product and the life cycle stages with a phenotype that is different from wild type parasites. For *mutated* genes a short description of the mutation is shown (see highlighted box).

Searching for mutants using multiple gene IDs

IMPORTANT: It is possible to 'batch search' for mutants using multiple gene ID's (Figure 4). Consequently, it is possible to retrieve collated information related to multiple genes at once, which not only speeds up searches but also facilitates the cross-linking and mining of data from multiple sources. The output for searches with multiple gene ID's (in table format) include: the gene IDs (rodent and *P. falciparum*); the RMgmDB IDs, the types of modification (*disrupted, mutated, tagged, other modification*); if the modifications were (un)successful; life cycle stages with a phenotype if the phenotype is different from wild type parasites. The search-results from a search using multiple gene IDs is displayed in a table on the website (Figure 5) or it can be downloaded as an Excel/spreadsheet compatible format, i.e. as a CSV file (Figure 4). Clicking on the RMgmDB ID in the table (and spreadsheet) leads directly to the mutant record page.

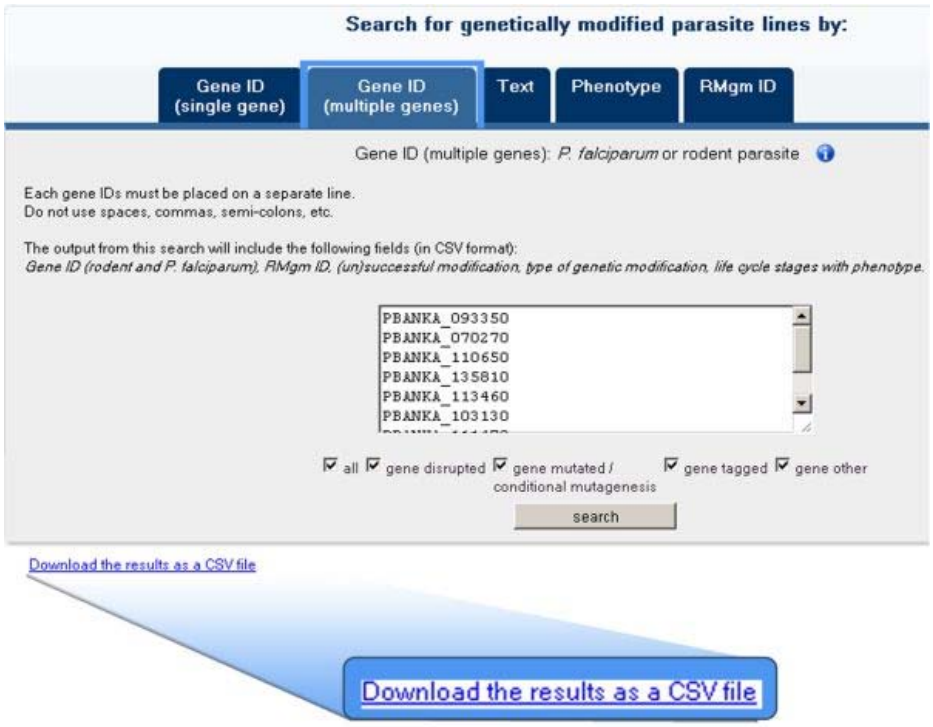

Figure 4: Batch-searching using multiple genes (each gene ID must be placed on a separate line; do not use spaces, commas, semi-colons, etc). If you wish to download the search-results as an Excel/spreadsheet compatible format (i.e. CSV file), click the text indicated otherwise they will be displayed as a table on the website (see Figure 5).

Legends phenotype	
X	Phenotype different from wild type
nd	Phenotype not different from wild type
nt	Not tested

					Modification type				Phenotype					
Searched ID	RMgmDB	Gene ID rodent	Gene ID falciparum	Successful modification	Disrupted	Mutated	Tagged	Other	Asexual blood stage	Gametocyte, Gamete	Fertilization and ookinete	Oocyst	Sporozoite	Liver stage
PBANKA_093350	RMgm-176	PBANKA_093350	PF3D7_1114100	yes	X				X	nd	X	X	X	X
PBANKA_093350	RMgm-177	PBANKA_093350	PF3D7_1114100	yes	X				nd	nd	nd	nd	nd	X
PBANKA_093350	RMgm-761	PBANKA_093350	PF3D7_1114100	yes	X				nd	nd	nd	nd	nd	X
PBANKA_070270	RMgm-178	PBANKA_070270	PF3D7_0828000	yes	X				nd	nd	nd	X	X	nt
PBANKA_070270	RMgm-763	PBANKA_070270	PF3D7_0828000	yes			X		nd	X	X	X	nt	nt
PBANKA_110650	RMgm-107	PBANKA_110650	PF3D7_0506900	no	X									
PBANKA_110650	RMgm-764	PBANKA_110650	PF3D7_0506900	yes			X		X	X	nt	nt	nt	nt
PBANKA_135810	RMgm-758	PBANKA_135810	PF3D7_1345200	no	X									
PBANKA_113460	RMgm-759	PBANKA_113460	PF3D7_1358300	no	X									
PBANKA_103130	RMgm-760	PBANKA_103130	PF3D7_1411200	no	X									
PBANKA_111470	RMgm-762	PBANKA_111470	PF3D7_0515100	yes	X				nd	nd	nd	nd	nd	nd
PBANKA_111780	RMgm-179	PBANKA_111780	PF3D7_0618600	yes	X				nd	nd	nd	nd	nd	nd

Figure 5: Example of a search results when using multiple gene IDs, displayed in a table on the website.

Searching with multiple gene ID's will, for example, allow the user to (i) rapidly identify genes that have been targeted for disruption but did not result in selection of mutants, indicating an essential role for these genes in blood stage development; (ii) identify genes that give a phenotype at multiple life cycle stages, such as asexual blood stages, gametocytes/gametes, oocysts, liver stages etc.; (iii) identify genes that have been tagged (for example with fluorescent markers); and (iv) identify genes for which no information based on mutant phenotypes currently exists. See also below for some examples of searches using multiple gene ID's.

Searching for mutants with a phenotype in (a) specific life cycle stage(s)

The database can be searched for mutants with a phenotype in one or more life cycle stage if the phenotype is different from wild type parasites (Figure 2). The following life cycle stages can be specified in combination with one or more of the 5 types of modification: *asexual blood stage, gametocyte/gamete, fertilization and ookinete,* oocyst, *sporozoite* and *liver stag*e. See below for an example of a search for mutants with a phenotype at (a) specific life cycle stage(s).

The mutant Record Page

Clicking on one of the mutants in the search-overview, as described above, leads to the record page of the mutant. The information within the database for each mutant parasite has been subdivided into three sections (Figure 6).

The first section provides general information on the generation of the mutant, which includes links to the relevant Medline publications describing the mutant and information on the researchers and research group who generated the mutant.

The second section provides information on the procedures used to generate and select the mutants, and information on the genotype of the mutants. Five different types of genetic modification have been defined: *disruption*, *mutation*, *tagging, transgene expression* and '*other modifications*'. A single mutant parasite may contain multiple different genetic modifications. For each modification, details are provided of the targeted gene, such as gene ID which is linked to GeneDB and the 'gene product name' as provided by GeneDB.

- Mutants with altered genes designed to conditionally regulate gene expression fall in the category *mutated genes*. For example, mutants with 'flirted' genes used to conditionally remove/silence genes using the FLP/FRT recombinase system (Lacroix et al., 2011), mutants with modified/substituted promoters (i.e. 'promoter swap mutants') used to silence gene expression at specific stages of development, e.g. gametocytes or ookinetes (Laurentino et al., 2011; Siden-Kiamos et al., 2011; Sebastian et al., 2012), or mutants with modified genes that contain a tet-inducible promoter for down-regulation of expression by tetracycline derivatives (Pino et al., 2012).
- Genes that have been replaced with an ortholog from another *Plasmodium* species or from another organism are classified as *mutated genes*.
- However, if orthologs are introduced as an additional copy either as episomal plasmids or integrated into a 'silent' locus of the genome, the orthologs are classified as *transgenes*.
- Genes encoding reporter proteins such as fluorescent and luminescent proteins are classified as *transgenes*.
- The database contains a large number of mutants containing epitope-tagged proteins, tagged with for example fluorescent proteins or c-Myc.

- The category '*other modifications*' include for example mutants containing a transposase-mediated *piggyBac* insertion in their genome (Fonager et al., 2011), mutants containing circular or linear '*Plasmodium* artificial chromosomes' (C-PAC; L-PAC) (Iwanaga et al., 2010; Iwanaga et al., 2012a; Iwanaga et al., 2012b)or GIMO-transfection reference mutants that contains the *hdhfr::yfcu* positive-negative selection marker in the silent *230p* locus (Lin et al., 2011).
- For all *mutated genes* a detailed description of the mutation exists and, in addition, a short description of what type of mutation has been engineered is reported in the overview of search-results (Figure 3).

Guidelines for generation, genotyping and describing/reporting of rodent malaria parasite mutants have been provided and discussed in Janse *et al.* and Khan *et al.* (Janse et al., 2011; Khan et al., 2013).

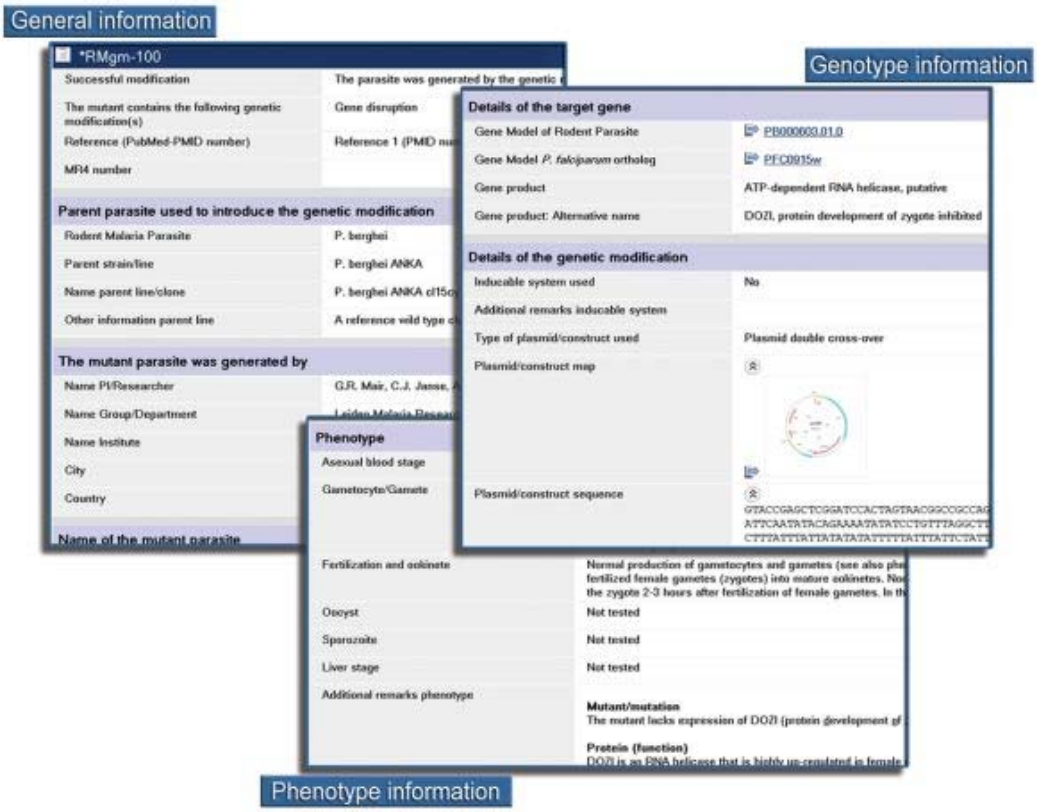

Figure 6: The record page of all mutants: General information and details on genotype and phenotype are provided for each mutant parasite (Janse et al., 2011)

The third section of the mutant information consists of phenotype descriptions of the mutants as well as some additional information on the (function of) gene/protein that has been targeted by the genetic modification. The phenotype description is subdivided according to different parasite life-cycle stages. The six different life cycle stages are: *asexual blood stage, gametocyte/gamete*, *fertilization and ookinete, oocyst, sporozoite* and *liver stage*. Together with the phenotype description, information is provided on the gene function that is inferred from the phenotype analyses and from additional assays described in the publications.

RMgmDB also contains information on unsuccessful attempts to disrupt or mutate genes, and therefore these genes may be refractory to mutation or the mutation may be lethal for asexual blood stage growth. For these unsuccessful trials, we provide information on the DNA constructs used in modifying the genes, the selection procedure and the number of independent transfections attempted.

Examples of searches

1) Search the database for mutants with a disrupted or mutated gene encoding TRAP (thrombospondin-related anonymous protein; gene ID PF3D7_1335900).

- Select 'gene ID (single gene)' tab (Figure 1)
- Type/paste in the search box the gene ID, PF3D7_1335900 (**IMPORTANT**: using the *P. falciparum* Gene ID will retrieve corresponding mutants in all rodent *Plasmodium* species, however if you search with the *P. berghei* gene ID, the corresponding *P. yoelii* mutants will NOT appear in the search-overview)
- Specify the type of modification by selecting 'gene disrupted' and 'gene mutated/conditional mutagenesis' fields (Figure 1)

This search (as of March 2013) results in 18 different mutants, 16 of which contain a mutated *trap* gene**. IMPORTANT**: In the search-overview a short description is given of the type of mutation; for example; 'the canonical rhomboid motif AGGIIGG changed to FFFIIGG' (highlighted in Figure 3). Clicking on one of the mutants will show the record page with the genotype and phenotype of this specific mutant.

2) Search the database for all mutants for the term 'gliding motility'

- Select 'Text' tab (Figure 1)
- Type/paste in the box the text term 'gliding motility'
- Specify all 5 types of modification by selecting 'all' (Figure 1)

This search (as of March 2013) retrieves and displays 87 mutants. **IMPORTANT**: Since text terms search the complete database, and the 'gliding motility' phenotype has not been defined as a specific feature, this search does **NOT** provide a list of only the mutants with a gliding motility phenotype that is different from wild type. For example, mutants appear in this list where in the phenotype description is mentioned that '*the disruption/mutation/tagging has no effect on gliding motility*'. As mentioned above the design of RMgmDB and 'free text' searching, combined with the lack of defined vocabularies for *Plasmodium* phenotypes or gene function, limits the use of text terms searches to identify mutants with defined cellular or molecular phenotypes or to identify genes with a defined function that can be inferred from mutant phenotypes.

3) Search the database for disrupted or mutated gene mutants that have a sporozoite phenotype that is different from wild type sporozoites

- Select 'Phenotype' tab (Figure 2)
- Select 'Sporozoite' field
- Specify the type of modification by selecting gene 'disrupted' and 'gene mutated/conditional mutagenesis' (Figure 2)

This search (as of March 2013) retrieves 140 mutants. **IMPORTANT**: This search will also include mutants that have an 'oocyst phenotype' resulting in reduced or absent production of sporozoites. Therefore, when searching mutants with a specific 'life cycle phenotype' as defined in RMgmDB it cannot be concluded that all mutants in the search-overview have a molecular/cellular function only in that specific life-cycle stage.

4) Search the database for all mutants with disrupted or mutated *Plasmodium* protein kinase genes

- Select 'Gene ID (multiple genes)' tab (Figure 4)
- Type/paste in the box all gene ID's of *P. falciparum* or rodent *Plasmodium* protein kinases (each gene ID must be placed on a separate line; do not use spaces, commas, semi-colons, etc.). **IMPORTANT**: using the *P. falciparum* Gene IDs will retrieve mutants made in all rodent *Plasmodium* species; however if the *P. berghei* gene IDs are used the *P. yoelii* mutants will not appear in the search-overview
- Specify the type of modification by selecting 'gene mutated/conditional mutagenesis' and 'gene tagged' (Figure 4)

The output from this search is displayed in a table on the website (Figure 5) or it can be downloaded as an Excel/spreadsheet compatible format (i.e. as a CSV file; Figure 4). The table and excel sheet include the following fields: Gene ID (rodent and *P. falciparum)*, RMgmDB ID, successful modification, type of genetic modification, life cycle stages with a phenotype that is different from wild type parasites.
This search (as of March 2013) retrieves a search-overview of more than 50 kinase genes that were refractory to disruption/mutation; 18 genes that were amenable to disruption/mutation and the mutants exhibit phenotypes at various points of the life-cycle, of these 5 have a phenotype during fertilisation/zygote stage.

5) Search the database for all mutants which express a tagged *Plasmodium* rhomboid protease

- Select 'Gene ID (multiple genes)' tab (Figure 4)
- Type/paste in the box all gene IDs of *P. falciparum* or rodent *Plasmodium* of the 8 rhomboid protease encoding genes (each gene ID must be placed on a separate line; do not use spaces, commas, semi-colons, etc.)
- Specify the type of modification by selecting 'all' (Figure 4).

This search (as of March 2013) retrieves in a search-overview all mutants that contain mutated, disrupted or tagged rhomboid encoding genes (Figure 5), including 2 rhomboids tagged with GFP or mCherry (ROM3, PBANKA_070270, RMgmDB-763 and ROM4, PBANKA_110650, RMgmDB-764). Note: Selecting 'all' generates a table with all mutants that contain genetically modified rhomboid genes including the tagged rhomboids (Figure 5). Selecting 'tagged' will only show the mutants where the *Plasmodium* rhomboid protein has been tagged.

Submitting information on unpublished mutants without a clear phenotype or negative attempts to disrupt/mutate genes to RMgmDB

RMgmDB is intended to be a dynamic and responsive resource, and researchers are encouraged to update the information on phenotypes of the mutants they have generated. Importantly, much unpublished data exist on *Plasmodium* mutants that were generated but did not show a clear phenotype or where researchers attempted, on multiple occasions, to disrupt a parasite gene but were unsuccessful. This information, while difficult to publish, is of significant value and can also prevent an unnecessary duplication of effort. Moreover, the existence of null-mutants without a distinct phenotype might provide information about the functional redundancy of the target gene. Similarly, the lack of an observable phenotype might also be the result of assays that are currently inadequate or, as yet, too insensitive to reveal a phenotypic effect of the genetic modification. Further analysis of such mutants in improved phenotype assays might

reveal novel aspects of gene function. Below is shown the template for submitting to RMgmDB the information on unpublished mutants and negative trials to disrupt or mutate genes.

Submission of information on mutants already available in RMgmDB

For all mutants a 'comment box' is available on the mutant record page where additional information can be submitted: corrections, comments, or suggestions for improving the description of the mutants.

Submitting information to RMgmDB on unpublished mutants without a clear phenotype

Information on new mutants without a clear phenotype can be submitted in spread sheet (Excel) format. This must include a minimum set of information on the constructs used and the mutant, see Table 1 (left-hand column).

Submitting information to RMgmDB on negative attempts to disrupt or mutate genes

Information on negative attempts to disrupt or mutate genes can be submitted in spread sheet (Excel) format. This must include a minimum set of information on the constructs used and number of attempts to disrupt the gene of interest, see Table 1 (right-hand column).

Table 1
Information required by RMgmDB to report unpublished mutants without a clear phenotype (left-hand column) or unsuccessful attempts to disrupt/ mutate genes (right-hand column). Adapted from (Khan et al., 2013).

Mutants without a clear phenotype	**Unsuccessful attempts to disrupt/mutate genes**
- Rodent parasite species - Parent strain/isolate/line - Name PI/Researcher - Name Group/Department - Name Institute	- Rodent parasite species - Parent strain/isolate/line - Name PI/Researcher - Name Group/Department - Name Institute
- Gene Model of Rodent Parasite (as cited in GeneDB) - Type of modification: disruption/mutation - Name of mutant - Plasmid/construct - double or single cross-over - Partial or complete disruption ORF - Other details of mutation, if applicable - Selection method (drug-treatment, FACS, etc.) - Selectable marker - Promoter selectable marker - Drug used to select	- Gene Model of Rodent Parasite (as cited in GeneDB) - Type of modification: disruption/mutation - Not applicable - Plasmid/construct - double or single cross-over - Partial or complete disruption ORF - Other details of mutation, if applicable - Selection method (drug-treatment, FACS, etc.) - Selectable marker - Promoter selectable marker - Drug used to select
- Primer information (= sequence and name) *Primers for disruption* primer sequence target region 5' forward primer sequence target region 5' reverse primer sequence target region 3' forward primer sequence target region 3' reverse *Other primers used for gene mutation*	- Primer information (= sequence and name) *Primers for disruption* primer sequence target region 5' forward primer sequence target region 5' reverse primer sequence target region 3' forward primer sequence target region 3' reverse *Other primers used for gene mutation*
- Mutant cloned? (yes or no) - Not applicable.	- Not applicable - Number of transfection attempts

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