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# New Research Tools for Urogenital Schistosomiasis

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**Abstract**

Approximately 200,000,000 people have schistosomiasis (schistosome infection). Among the schistosomes, *Schistosoma haematobium* is responsible for the most infections- 110 million people globally, mostly in sub-Saharan Africa. This pathogen causes an astonishing breadth of sequelae- hematuria, anemia, dysuria, stunting, uremia, bladder cancer, urosepsis, and HIV co-infection. Refined estimates of the quality of life impact of schistosomiasis suggest it rivals malaria. Despite *S. haematobium's* importance, relevant research has lagged. Here, we review advances that will deepen knowledge of *S. haematobium*. Three sets of breakthroughs will accelerate discoveries in the pathogenesis of urogenital schistosomiasis (UGS): (1) comparative genomics; (2) the development of functional genomic tools; and (3) use of animal models to explore *S. haematobium*-host interactions. Comparative genomics for *S. haematobium* is feasible, given the sequencing of multiple schistosome genomes. Features of the *S. haematobium* genome that are conserved among platyhelminth species, and others that are unique to *S. haematobium* may provide novel diagnostic and drug targets for UGS. Though there are technical hurdles, the integrated use of these approaches can elucidate host-pathogen interactions during this infection, and inform development of techniques for investigating schistosomes in their human and snail hosts and therapeutics and vaccines for the control of UGS.

## Background

Schistosomes are the etiological agents of human schistosomiasis - *Schistosoma japonicum* and *S. mansoni* cause hepatointestinal schistosomiasis in East Asia, Africa, South America and the Caribbean, whereas *S. haematobium* causes UGS throughout Africa and the Middle East and sporadically in Mediterranean Europe (1). Recalibration of health burdens have revealed that schistosomiasis causes a loss of 4.5-70 million disability adjusted life years (1), and estimates suggest that this neglected tropical disease rivals malaria in terms of socioeconomic impact. Of >110 million cases of schistosomiasis haematobia in sub-Saharan Africa, 70 million are associated with hematuria, 18 million with major bladder wall pathology, and 10 million with hydronephrosis leading to kidney damage (2). The deposition of *S. haematobium* eggs eventually leads to squamous cell carcinoma of the bladder in some chronically infected individuals. Several studies in Africa have indicated a two- to ten-fold relative risk in patients with schistosomiasis(3). Accordingly, in one study a clinical history of UGS accounted for 16% of bladder cancer cases in Egypt. Consequently, *S. haematobium* is classified as a Group 1 carcinogen by the International Agency for Research on Cancer, http://monographs.iarc.fr/ENG/Monographs/vol100B/mono100B-14.pdf. Moreover, as many as 75% of women infected with *S. haematobium* suffer from female genital schistosomiasis (FGS) (4). FGS follows from the deposition of schistosome eggs in the uterus, cervix, vagina and/or vulva, with ensuing inflammatory responses and increased susceptibility to HIV/AIDS. Research directed towards understanding this pathogen has lagged, despite its global importance. Here, we highlight recent advances in the field that will facilitate the acquisition of deeper understanding of *S. haematobium* biology, its relationship with the host, and novel genomic tools should assist in elucidating the role of targets for new interventions.

**Methodology/Principal Findings**

Recent, major advances in the field will significantly facilitate discoveries in the pathophysiology of UGS: (1) comparative genomics; (2) functional genomics; and (3) tractable animal models to explore the interactions of *S. haematobium* with host systems (Figure 1). Access to the S*. haematobium* genome and transcriptome of male and female adult worms and eggs will allow comparative -omic studies (5). Features of the *S. haematobium* genome that are conserved among members of Platyhelminthes and unique to *S. haematobium* are likely to provide new diagnostic and therapeutic targets for UGS. Moreover, RNA interference (RNAi) pathways in *S. haematobium* may be exploited to suppress key biomolecules for the viability, development and infectivity of this pathogen (6).

**Genomes – a major step forward**

Following the sequencing of the eukaryotic model organisms, including *Caenorhabditis elegans*, (7), improved, automated Sanger sequencing methods facilitated the characterization of the genomes of the first metazoan parasites, including *S. mansoni* and *S. japonicum*. These genomes provided the first, global previews of the genetics of schistosomes. In 2010, the completion of the draft genome of the giant panda using solely Illumina/Solexa-based (massively parallel) sequencing (8) led to a revolution in the sequencing of an expanding catalog of metazoans, and the *S. haematobium* genome ensued rapidly (5). In this latter project, total genomic DNA from a single pair of worms was subjected to whole-genome amplification followed by sequencing; the genome was assembled and annotated employing deep transcriptomic data from the egg and adult developmental stages (5). The availability of the draft genomes of all three main species of schistosomes of humans has paved the way for extensive

comparative (9) and functional genomics and for deep research of parasite-host interactions and pathogenesis (10).

**Toward enhanced annotation and curated databases**

The functional annotation of schistosome genes has relied on homology-based bioinformatic analyses that assign gene annotation(s) from other species. This process infers that homologous proteins have conserved functions, although many schistosome proteins do not have orthologs in other taxa for which genomic, transcriptomic and/or proteomic information are available. Thus, numerous schistosome proteins remain refractory to annotation, e.g., of the 13,073 genes predicted for *S. haematobium*, only ~ 50% are homologous to curated sequences in the SwissProt:UniProt Knowledgebase(5), or predicted to encode conserved amino acid sequence domains (InterPro) (5). Furthermore, only ~40% of genes are linked to conserved biological pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) (5) or processes inferred by gene ontology (GO) (5). The platyhelminths, including the schistosomes, are lophotrochozoans, distinct developmentally from members of the Ecdysozoa (e.g., *D. melanogaster* and *C. elegans*) and the Deuterostoma (e.g., *Homo sapiens* and *M. musculus*) whose genomes have been functionally annotated [e.g., (7)]. Therefore, the challenging functional annotation of schistosome genes and proteins relate to their evolutionary distinctiveness from other organisms for which genomes have been well characterized.

Improvements in cross-taxon annotation can be achieved by integrating genomic and other omics data (11). In schistosomes, the first step was the curation of the annotated genomes of *S. haematobium*, *S. japonicum* and *S. mansoni* within the public database, SchistoDB (12). The recent integration of data from these three species now enables informative data mining and definition of genes, GO terms, conserved domains and biological pathways. Together with databases from other key flatworm species, including GeneDB (13) and HelmDB (14), access is

now available to an abundance of genomic, transcriptomic and proteomic data to construct biological pathways and prioritize drug or vaccine candidates (15). Furthermore, the identification of parasitic flatworm orthologs in the draft genomes and transcriptomes of free-living species of flatworms that have been established as models for stem cell regeneration could facilitate molecular investigations into the biology of *S. haematobium* (16). To identify orthologs, SchistoDB and HelmDB contain protein-clustering algorithms to enhance the prediction of orthologs and paralogs. Therefore, whereas functional annotation of *S. haematobium* genes is limited, it is possible to confidently infer orthologs among the species of schistosomes. Methods used to define orthology/paralogy vary (17), but they all involve identification of orthologous genes/proteins shared by two or more species, usually based on pairwise sequence alignments (18). Based on sequence homology (BLASTp, E-value ≤ 10-5), genome-wide comparisons of the inferred proteome of *S. haematobium* revealed that 1,369, 244 and 10,880 proteins are homologous to those of *S. mansoni* alone, *S. japonicum* and both species, respectively. Overall, pairwise comparisons of shared proteins among the schistosomes showed that most annotated biological pathways are shared (5). Differences are evident among macromolecules associated with cell-cycle regulation, membrane transport and signaling, translation, and lipid/xenobiotic metabolism in *S. haematobium* and *S. mansoni* versus *S. japonicum*, and transcription in *S. haematobium* and *S. japonicum* versus *S. mansoni*. In addition, a subset of 73 predicted enzymes and other proteins was unique to *S. haematobium* (5). By using an OrthoMCL clustering approach (17), the schistosome proteomes share 6,968 orthologous clusters, of which 5,389 clusters were represented by single-copy genes. In addition, this approach identified orthologous gene clusters exclusive to *S. haematobium* and *S. mansoni* (1138 clusters; 989 single-copy genes) and *S. haematobium* and *S. japonicum* (689 clusters; 654 single-copy genes). These findings indicate that further annotation of each gene set, using similar bioinformatic tools employed to characterize the kinome (19) and G-coupled protein receptors (20) of *S. mansoni,* will likely reveal differences in physiology among the three

major species of schistosomes.

Curated genome annotations combined with advances in proteomics, e.g. through the characterization of tegumental and excretory/secretory (ES) proteins of schistosomes, can now be employed to assemble a complementary dataset of well-defined proteins for *S. haematobium*. The improved accessibility to genomic data of *S. haematobium* and other flatworms, the availability of enhanced bioinformatic tools, the standardization of annotation ontologies for genes, and the expanded functional annotation of sequences by protein clustering represent considerable advances that should expand the scope for systems biological studies of schistosomes.

**Functional genomics**

Insights into the -omics of *S. haematobium* may be confirmed and expanded by functional testing of gene candidates, particularly those exhibiting novelty or evolutionary divergence among orthologs from the schistosome species. We anticipate that the transgenesis of *S. haematobium*, in addition to other genome manipulation approaches, will accelerate discoveries relevant to UGS by querying gene function over the developmental cycle, including in cercariae, schistosomula, adult worms, eggs, and sporocysts within the intermediate host snail (21). Indeed, progress has been made in this area, and rapid advances can be predicted in development of functional genomic tools for forward and/or reverse genetic investigations of *S. haematobium* (5,22,23). However, it has been more difficult to deploy transgenesis to *S. haematobium,* in comparison with *S. mansoni* and *S. japonicum*, largely because the developmental cycle of *S. haematobium* is challenging to maintain in laboratory mice.

To overcome these hurdles, Rinaldi and coworkers (21) established protocols to culture several developmental stages of *S. haematobium* and demonstrated that square-wave electroporation

and labeled small molecule probes can be implemented to manipulate cultured developmental stages of *S. haematobium*. Cy3-siRNAs will enter eggs (Figure 2) and blood vessel stages of *S. haematobium*. In addition, firefly luciferase mRNA is translated in S*. haematobium* cells. Notably, these studies confirmed that RNA interference (RNAi) pathway is active in this schistosome; both a reporter transgene (luciferase) and an endogenous *S. haematobium* gene encoding the tegumental antigen tetraspanin-2 were post-transcriptionally silenced after specific RNA interfering molecules (siRNA and dsRNA) were introduced into the egg, schistosomular, and adult stages of this blood fluke (21).

We are now attempting to establish stable transfection employing the integration competent vector, pseudotyped murine leukemia virus retrovirus (24). Other integration-competent vectors, including the transposon *piggyBac,* that can integrate into schistosome chromosomes, will be investigated (25); this approach also shows promise for germline transgenesis in other parasitic helminths (26). *S. mansoni* transgenesis techniques, i.e., *piggyBac* and retroviruses, are facilitating the development of forward genetics (i.e. insertional mutagenesis analysis), reverse genetics, (i.e. loss-of-function approaches), gain-of-function approaches, and overexpression of reporters (e.g., genes encoding for antibiotic resistance). Thus, we anticipate that this technology will be transferable to *S. haematobium.* Table 1 presents a brief summary of the approaches and tools that have been modified and deployed for use in functional genomic studies of schistosomes. Although progress is farthest advanced in *S. mansoni*, physiological impediments are not apparent that should thwart application to *S. haematobium* to derive stable transgenic lines (27) (Figure 3). Figure 4 presents a schematic of how these approaches can be linked to propel advances in functional genomics for *S. haematobium*. Recently developed genome editing technologies that allow the introduction of site-specific mutations by employing tailor-made nucleases, such as meganucleases (MNs), zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats

(CRISPR)-associated endonucleases (e.g., Cas9), have been successfully applied to *C. elegans* to generate transgenic organisms and introduce site-specific, heritable mutations (28). It is expected that this emerging technology will be transferable to parasitic helminths, thereby expanding the nascent toolkit for functional genomic investigations of schistosomes (29).

A relevant breakthrough was the identification of stem cells in schistosomes; Newmark and colleagues (30) reported neoblast-like stem cells in adult *S. mansoni.* This population of cells, distributed throughout the soma of male and female parasites, share not only morphology with the neoblasts of planarians, but also their ability to proliferate and differentiate into derivatives of multiple germ layers (30). Based on a new cell isolation protocol for *S. mansoni* (31), it should be feasible to isolate these neoblast-like cells, target them *in vitro* with integration competent vectors such as transposons and retroviruses, and reintroduce transduced cells into larval stages as an alternative approach to derive stable, transgenic lines of schistosomes (27).

When established, these technological approaches should enable investigators to confidently address fundamental, key questions about the *S. haematobium*-mammalian host-parasite relationship, including the carcinogenic potential of *S. haematobium* eggs (particularly in tandem with Hsieh's novel mouse model of egg-induced pathogenesis (32)), host range, tropism of this species for the pelvic rather than intestinal circulation, and so forth. Some non-human primates are suitable hosts for *S. haematobium,* including patas and vervet monkeys and baboons (33–41). Application of genomics approaches (e.g. genome editing) to primate models themselves would likely be informative (42).

Technological advances in the study of UGS should have translational value. The accurate detection of infection, the central goal of diagnostics for schistosomiasis, is based on analytes that vary in form and source (i.e., protein *vs.* nucleic acid, worm *vs.* egg) across existing candidates vying for adoption. It is likely, however, that novel diagnostics using markers from

the *S. haematobium* genome will be nucleic acid-based, or based on the protein products of characterized genes. These considerations also apply to diagnostic tools designed for detection of cercariae in water and sporocysts in snails (43).

Considering the translational potential of functional genomics of *S. haematobium,* it is informative to examine the developmental stages. For instance, candidate vaccines for UGS are likely to be based on findings derived from cercariae, schistosomules, and adult stages, since the goal of such vaccines is to break the cycle of infection (44). In contrast, candidate drugs for UGS could target all of these life stages, including the egg, to prevent egg-induced pathology. Lastly, molecular characterization of the sporocyst stage may reveal new strategies for interrupting or disrupting the infection of snails by *S. haematobium.*

The potential for functional genomics of *S. haematobium* to improve diagnostics, drugs and vaccines is representative of the intersection between population-based field studies and functional genomics. Fieldwork in UGS, in the form of genetic cataloging of *S. haematobium* strains, will also be relevant to functional genomics by facilitating improved annotation of parasite gene functions and interactions. Such studies will be critical to validating observations of host-parasite interactions obtained through Hsieh's egg injection mouse models.

**The testing of hypotheses using new animal models of UGS**

Comparative and functional genomics of *S. haematobium* need to be framed in the context of host-parasite interactions. Though crucial to diagnosis and understanding of clinical disease, samples of blood and urine obtained from cases of schistosomiasis haematobia leave open important disease pathogenesis questions. Our inability to access human or parasite tissue at

sites of active infection hinders our understanding of early and ongoing events that contribute to the severe and chronic problems associated with UGS.

Our understanding of the fundamental immunology of the bladder mucosal surface is remarkably incomplete, particularly compared with other organ environments such as the gut and lung. This obstacle highlights the gaps in knowledge of immune responses in the bladder. Whether the effects of infection with *S. mansoni* on the liver and intestine are analogous to processes occurring in the bladder during UGS is not known. Thus, it is critical to identify and characterize the acute changes initiated in mammalian bladder tissue in UGS and to establish how these processes are linked to morbidity over time. This is a key area of inquiry to improve the diagnosis and therapy of complications of UGS.

Historically, the primary *in vivo* models for UGS have been *S. haematobium* cercariae-infected hamsters and non-human primates (45). Although these infections support the maturation of the parasite and and oviposition, use of these animals as research models of human disease feature challenges. Hamsters (*Mesocricetus auratus*) exhibit low rates of pelvic organ infection, the key site of human pathology, and instead develop predominantly hepato-enteric schistosomiasis. Hence, while useful, the model is not amenable for studying disease and oviposition in pelvic organs. Moreover, there are few hamster-specific reagents and tools -- primary antibodies, cytokines, transgenic strains, etc. available for mechanistic studies. Non-human primates do exhibit urogenital disease; however, use of these mammals requires substantial ethical justification, suffers from a lack of species-specific tools, and is expensive. Nevertheless, a number of non-human primates are receptive hosts for *S. haematobium,* i.e., patas (*Erythrocebus patas*) and vervet (*Chlorocebus aethiops*) monkeys and *Papio* spp. baboons (33–41). Use of parasite- and host-specific genomics techniques should be possible with these animal models.

Though laboratory mice (*Mus musculus*) are very amenable to *S. mansoni* and *S. japonicum* infections, attempts to transdermally infect mice with *S. haematobium* cercariae produce only light infections of the hepato-enteric systems and little or no oviposition in the pelvic organs (46). An inability to model this key pathogenic phase of infection in mice is particularly unfortunate, given the wide range of species-specific tools that are available for mouse research. Therefore, until recently, approaches for modeling UGS in experimental animals have been largely unsuccessful.

We developed techniques that mimic the key pathological changes induced by *S. haematobium* oviposition in bladder tissue. We reasoned that direct administration of schistosome eggs into the mouse bladder wall could reproduce pathological elements of human bladder schistosomiasis. By analogy, our understanding of pulmonary fibrosis has been greatly aided by many studies of tail vein injection of *S. mansoni* eggs that lodge in the pulmonary capillary beds. Thus, administration of *S. haematobium* eggs directly into bladder tissue has the potential to be at least as relevant for understanding the pathogenesis of disease in the bladder.

Injection of *S. haematobium* eggs into the lamina propria of the mouse bladder—the tissue layer where egg granulomata are found in infected human bladders—recapitulated key aspects of the bladder disease of UGS (32). Specifically, egg injection resulted in hematuria, increased urinary frequency, the development of persistent, fibrotic bladder granulomata, and systemic and regional type 2 immune activation (32).

A bolus injection of *S. haematobium* eggs is suitable for the controlled study of synchronized, egg-induced changes in host tissue. Microarray analysis of the whole bladder transcriptome using this model revealed differential transcription of multiple genes relevant to inflammatory fibrosis and urothelial function (47). This was the first characterization of the early transcriptional events occurring in the bladder after exposure to *S. haematobium* eggs. These data, derived

from preparations of bladders, indicated the feasibility of detailing specific molecular, cellular, and biochemical events in sub-regions of the bladder and granulomata at serial times after egg injection. Such studies could elucidate the immunopathogenesis of UGS in the bladder mucosa and identify early inflammation-related events that contribute to onset of bladder fibrosis and schistosome-associated bladder cancer.

The bladder injection model in mice lays the foundation for tractable analysis of candidate pathogenic mechanisms employed by *S. haematobium* eggs. Indeed, we have used this model to determine, for the first time, the immunologic basis of *S. haematobium*-infected host susceptibility to bacterial urinary tract co-infection (48). Through the use of transgenic IL-4 receptor-α-deficient mice and mouse-specific PCR, flow cytometry and cytokine assays, we determined that *S. haematobium* egg-induced IL-4 suppresses invariant natural killer T-cell activation-associated clearance of bacterial urinary tract co-infection. This finding emphasizes the value of a mouse-based model of UGS.

Besides transgenic mice and other mouse-specific reagents, this model is also amenable to combination with transgenic schistosomes, e.g. stably or transiently transgenic *S. haematobium* eggs or eggs subjected to exogenous RNAi could be injected, and the effects on host tissues compared with wild-type eggs. Cross-species comparisons could also be undertaken by injecting *S. mansoni* or *S. haematobium* eggs into mouse bladders, including in combination with transgenic/RNAi manipulations. Indeed, cross-species studies should facilitate the testing of hypotheses arising from comparative genomic analyses of the three human schistosomes including the tropism for pelvic versus hepato-intestinal organs. This model could also be employed to address questions derived from population-based field studies in UGS, e.g. virulence and/or pathogenicity differences among natural *S. haematobium* strains by injecting eggs from field strains into the bladder walls of mice.

Although these are new opportunities, the bladder-injection animal model has limitations. First, the approach permits study of egg-related phenomena only in host bladder tissue. Studies of the interaction between the host and cercariae, schistosomules, and adult worms will not be furthered by this technique. Regardless, much of the human pathological alterations arising from *S. haematobium* are directly related to egg deposition within bladder tissue. Thus, an egg-injection model remains valuable regarding inquiry of the pathogenesis of bladder-related disease in UGS. The injected eggs span a maturation spectrum, since they are isolated from chronically infected hamsters. This might introduce artifacts, since older or dead eggs are suddenly introduced into the bladder, rather than maturing and/or dying over time. Fortunately, it is feasible to collect eggs laid *in vitro* by the adult schistosome (49). Newly laid eggs could be introduced into the bladder wall, and may satisfactorily resemble a natural infection featuring deposition of more immature eggs. Another caveat is that stable, conditional and/or long-term transgenesis as well as gene silencing of *S. haematobium* remains to be established. Transgenic blood flukes generated *in vitro* may modulate transgene expression *in vivo*. The transgenic techniques outlined above can be expected, based on experience in model species, to improve over time and surmount these obstacles. Other limitations of this model include the fact that egg transmission and successful excretion may be difficult to examine, since egg shedding in urine after bolus injection into the bladder wall is not worm-based, unlike natural human infection. The short life span of the mouse may not be conducive to studying human carcinogenesis. Species differences in genetics, immunity, anatomy, and endocrinology are also relevant.

**Conclusions/Significance**

Comparative genomics will generate new hypotheses regarding mechanisms of pathogenesis that can be tested through genetic manipulation of *Schistosoma haematobium* in the context of wild type and transgenic mouse models. The integrated use of these new technologies by

teams of collaborating researchers can begin to elucidate how pathogen and host systems interact during this important tropical infection.

## Author Contributions

All of the authors contributed to the ideas and writing associated with this manuscript.

## Competing Financial Interests

The authors have no competing financial interests to declare.

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## Tables

**Table 1.** Functional genomics manipulations and tools modified and deployed with the human schistosomes. *Schistosoma haematobium*, Sh; *S. japonicum*, Sj; *S. mansoni*, Sm.

| Functional genomics manipulation/tool | Schistosome species | References* |
|---|---|---|
| RNA interference (RNAi) using dsRNA, siRNA | Sh, Sj, Sm | (50,51) |
| Reporter gene activity | Sh, Sj, Sm | (21,52–56) |
| Chromosomal integration by (retro)transposon | Sm | (25,57) |
| Transgene delivered long/short hairpin RNAi | Sm, Sj | (58–60) |
| Rescue from antibiotic by resistance marker (neomycin phosphotransferase) | Sm | (27) |
| Insulator activity to protect transgene (cHS4) | Sm | (29) |
| Germline transgenesis to filial generations | Sm | (27) |
| Transduction of in vitro laid eggs | Sm | (27) |

*non-inclusive list

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## Figure legends

**Figure 1. Improving understanding of urogenital schistosomiasis through comparative and functional genomics and animal models.** Data from high throughput sequencing (genomic, transcriptomic, proteomic data) are investigated by functional genomic tools in order to assign roles to novel sequences (red arrow 1). Transgenesis would also facilitate forward genetics by insertional mutagenesis that, in turn, can be analyzed by genomic approaches (blue arrow 1). Genetically modified parasites would be tested in tractable rodent models (blue arrow 2) and parasites obtained from these models studied using genomic approaches (green arrow 2) and functional tools (green arrow 1). Hypotheses derived from comparative genomic approaches would tested using functional genomic tools (red arrow 1) and animal models of urogenital schistosomiasis (red arrow 2)

**Figure 2**. **Labeled small interfering RNA (siRNA) enters *Schistosoma haematobium* eggs.** Representative micrographs of eggs three hours after soaking in Cy3-siRNA; left panel, eggs in medium containing 50 ng/µl Cy3-siRNA, bright field, right panel; eggs in medium containing 50 ng/µl Cy3-siRNA, fluorescence field. Scale bar, 50 µm. From Rinaldi et al (21).

**Figure 3**. **Cartoon depicting an approach to derive and maintain stable lines of transgenic schistosomes.** A female schistosome releasing in vitro laid eggs exposed to murine leukemia virus retroviral virions (orange particles) and cultured with or without antibiotic. Miracidia hatched from eggs would be used to infect *Bulinus truncatus* snails. Cercariae cultured ("in tandem" antibiotic selection) with or without antibiotic could be screened for the presence of the transgene, transgene copy number and expression. The transgenic line(s) could be propagated and maintained in hamsters (black arrows). When the transgenic line emerges, e.g. a line expressing an shRNA targeting a gene of interest, the eggs would be collected and analyzed *in vitro* by cellular and molecular assays and *in vivo* assays including mouse models of urogenital

schistosomiasis (red arrows) (adapted from Rinaldi et al. (27)). Image under “in vivo assays” shows bladder wall injection with *S. haematobium* eggs (as described by Hsieh and colleagues (32)). The egg bolus can be seen as a semi-opaque bleb localized to the bladder wall (blue arrow).

**Figure 4. Methods to facilitate transgenesis for *Schistosoma haematobium*.** General schema to establish transgenesis for schistosomes where 1) culture conditions of developmental stages, 2) genomic DNA transformation strategies i.e., retroviral transduction, and 3) stable expression of transgenes are linked to 4) specific selection conditions in order to specifically enrich the population of transgenic worms. Adapted from Chamberlin (61).