Chapter 36

Formalizing Phenotypes of Regeneration

Daniel Lobo

Abstract

Regeneration experiments can produce complex phenotypes including morphological outcomes and gene expression patterns that are crucial for the understanding of the mechanisms of regeneration. However, due to their inherent complexity, variability between individuals, and heterogeneous data spreading across the literature, extracting mechanistic knowledge from them is a current challenge. Toward this goal, here we present protocols to unambiguously formalize the phenotypes of regeneration and their experimental procedures using precise mathematical morphological descriptions and standardized gene expression patterns. We illustrate the application of the methodology with step-by-step protocols for planaria and limb regeneration phenotypes. The curated datasets with these methods are not only helpful for human scientists, but they represent a key formalized resource that can be easily integrated into downstream reverse engineering methodologies for the automatic extraction of mechanistic knowledge. This approach can pave the way for discovering comprehensive systems-level models of regeneration.

Key words Formalization, Modeling, Inference, Regeneration

1 Introduction

The resultant phenotypes of regeneration experiments can include complex morphologies, spatial patterns, anatomical manipulations, and temporal dynamics—an extraordinary rich dataset to aid in the understanding of the mechanisms of regeneration [1–3]. Organisms such as planarian worms can regenerate any body part after almost any amputation, including new heads, eyes, brain, etc., and produce aberrant morphologies after genetic perturbations [4–7]. Amphibians and insects can regenerate their amputated limbs and appendages by re-growing them from the rest of their bodies, and perturbations can result in ectopic segments [8–10]. In addition, grafting experiments surgically transplanting tissue or appendages to different locations can highlight the system response to ectopic signals and result in the regeneration of a completely new anterior–posterior axis, such as with an inverted graft in planaria [11], or the regeneration of supernumerary limbs, as in the case of graft rotations in axolotl [12] and cockroach [13]. These experiments are

Simon Blanchoud and Brigitte Galliot (eds.), *Whole-Body Regeneration: Methods and Protocols*,
Methods in Molecular Biology, vol. 2450, https://doi.org/10.1007/978-1-0716-2172-1_36, © The Author(s) 2022

essential to discern the spatial localizations of the signals dictating the tissue organization during regeneration.

However, the experimental perturbations and the anatomical features of the regenerated morphologies are usually characterized in the literature with microscopy images containing much variability between similar phenotypes [14] and described in natural language, which can be ambiguous and miss crucial details [15, 16]. As a result, these rich and large datasets cannot be readily analyzed by neither human scientists nor computational methods for the extraction of mechanistic knowledge. Instead, a first step formalizing the experimental procedures and the phenotypes of regeneration is needed to encode both their qualitative and quantitative features in a standardized way.

For the unambiguous formalization of regeneration phenotypes, we have developed mathematical formalisms that can precisely describe anatomical structures, organ locations, and overall morphological shapes. We have developed such formalisms for encoding the regeneration phenotypes of planaria whole-body morphology [17], and salamander, frog, crustacean, insect, and arachnid limb and appendages [18]. These formalisms are based on mathematical graphs, where the nodes of the graph represent morphological regions or organs, such as the head or tail of a planarian worm, and the edges represent the connections and topological information between these regions, such as the segments forming a limb. Importantly, the topological information of the graphs can be extended with quantitative data regarding the overall shapes and sizes of the morphological structures, their angles of interconnection, and the localization vectors and rotations of the organs, a process that removes the unimportant differences between individual organisms. These formalizations can also include unambiguous descriptions of the experiments that produce the resultant phenotypes. A mathematical tree can define how different amputations, graftings, and irradiations are performed in a particular experiment. In this way, these formal descriptions based on mathematical structures can encode unambiguously both the experimental procedures and the resultant phenotypes of regeneration.

To facilitate working with these formalizations, we have developed user-friendly tools that allows any user to easily encode the phenotypes of regeneration. Planform [19] is a software tool for the formalization of regeneration experiments and phenotypes of planarian worms. Any user without special training can easily create the mathematical formalisms with the provided drag-and-drop interface for the specification of the different types of worm regions, sizes, and overall shapes. Similarly, the interface allows the definition of precise surgical manipulations, the grafting of pieces between worms, and the total or partial irradiation of regions of the worm body. For the formalization of limb regeneration experiments, we have developed Limbform [20], a similar software tool with a user-friendly interface. Limbform can be used to define experiments and phenotypes for salamander, frog, crustacean, insect, and arachnid. The number and position of each anatomical structure, such as digits and ectopic limbs, can be easily defined. Importantly, these tools automatically produce from the graph encodings the cartoon diagrams of the created reference morphologies, which can guide the user during the formalization of complex morphologies. The morphologies, experiments, and expression patterns formalized with these tools are stored in a centralized local database file. Using this protocol, we have curated a database of planarian regeneration with more than 1500 different experiments from the literature (*see* **Note 1**). In addition, we have curated a database of limb regeneration experiments with more than 800 different experiments (*see* **Note 2**). Both databases are freely available resources for the community, and the tools include a search functionality to find particular regeneration phenotypes resulting from any manipulation, gene, and drug of interest included in these large databases.

Gene expression patterns represent another fundamental phenotype in regeneration experiments. In situ hybridization [21, 22] and immunohistochemistry [23–25] assays can reveal the spatial gene expression pattern of a specific gene at the level of the whole organism. Gene expression patterns through space and time can reveal the genetic regulation of the key elements controlling regeneration. We have developed PlanGexQ [26] as a user-friendly software tool to formalize planarian gene expression patterns in standard reference morphologies. The spatial localization of gene expression patterns can be easily input into a standard 2D worm morphology defined with a similar mathematical graph formalism as in Planform. Predefined expression patterns for organs are also available for the user to select, which transfers directly to the current standard morphology. In addition, the tool automatically assigns gene ontology terms from the Planarian Anatomy Ontology [27] by scanning the spatial locations of the gene expression in the reference morphology as well as searching for keywords in the entered captions and descriptions.

Crucially, the mathematical nature of these formalized datasets can be integrated in a reverse-engineering methodology for the inference of mechanistic models or regeneration directly from experimental phenotypes [28] (*see* **Note 3**). Figure 1 illustrates the main steps of this iterative methodology and its integration with the formalization of phenotypes. First, functional experiments are performed at the bench to obtain morphological phenotypes from surgical, pharmacological, and genetic perturbations. These experiments and phenotypes are then formalized with the methodology presented in this chapter. Mathematical models based on ordinary or partial differential equations are built to include the

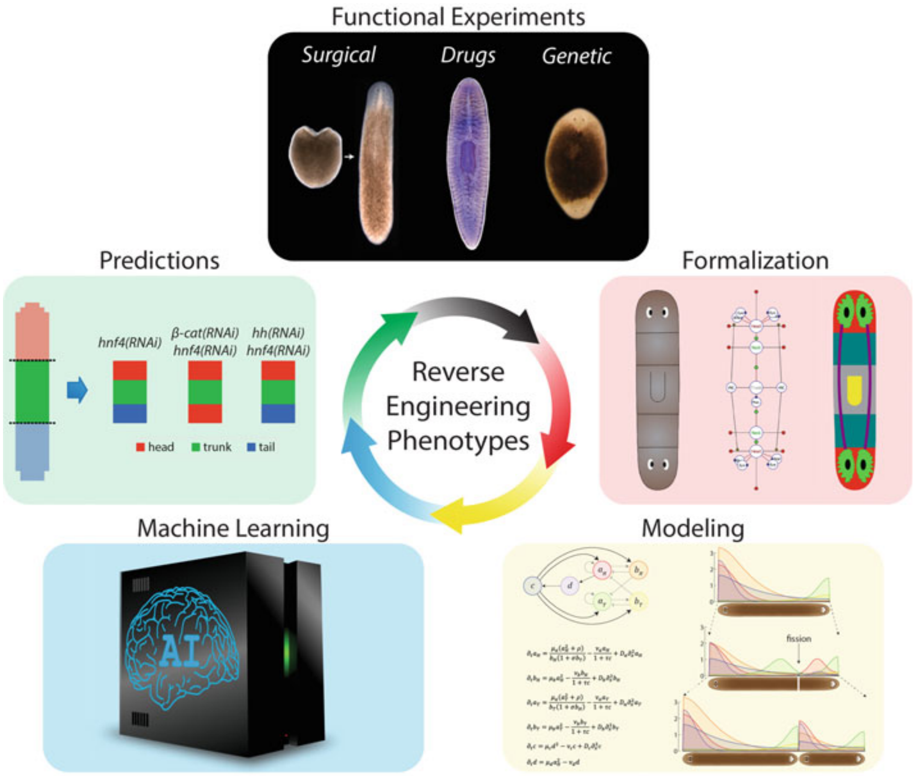


Fig. 1 The formalization protocol presented in this chapter can be integrated as a key component in an iterative methodology for the reverse engineering of the phenotypes of regeneration. Functional experiments at the bench provide the input datasets, which need to be formalized before knowledge can be computationally extracted. Mathematical models based on dynamical systems are designed to include the relevant regulatory networks and biophysical interactions. The model parameters, regulatory interactions, and particular equations can be discovered by machine learning to infer a mechanistic hypothesis explaining the input experimental dataset. Predictions are made with the model, which are then tested with new experiments, closing the methodology loop

main components of the system, their regulations, and the relevant cell and tissue biophysical forces [29–31]. Features of the model such as the parameters and the particular interactions in the regulatory network can be automatically inferred from the experimental datasets with a machine learning approach [32–34]. Finally, the inferred mechanistic model can be employed to formulate testable predictions in terms of novel phenotypes, genes, and perturbations that can be validated with further experiments at the bench [35–37], closing the reverse engineering cycle. In this way, the phenotypes of regeneration formalized with the presented protocols can be computationally analyzed toward the inference of mechanistic knowledge directly extracted from them.

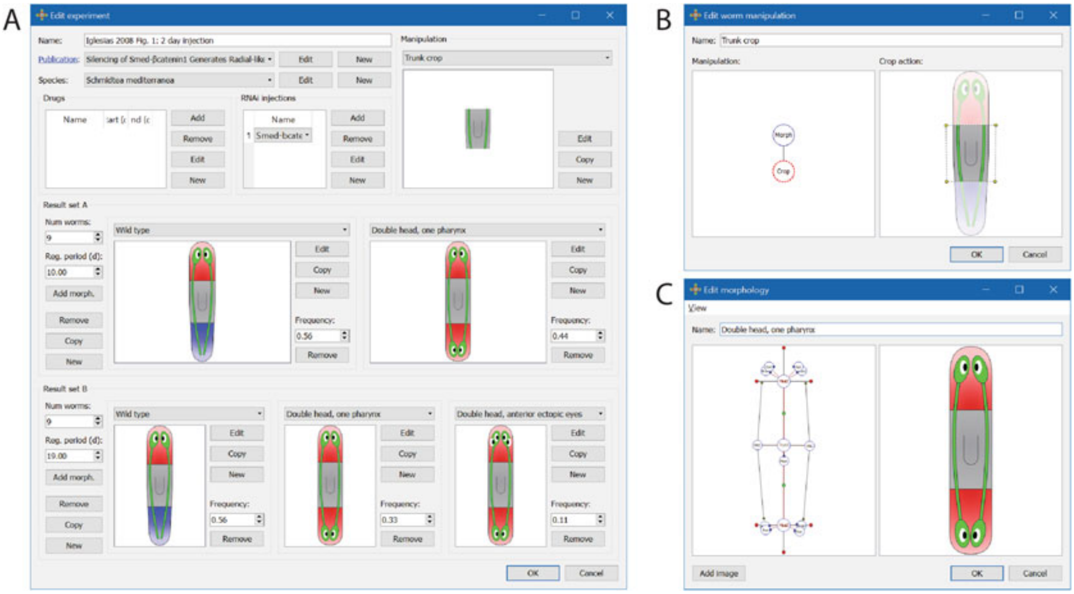
This chapter presents detailed protocols for the formalization of regeneration experiments and phenotypes using user-friendly interactive tools (*see* **Note 4**). The first protocol aims to formalize planarian regeneration. The curation process includes the definition of experimental details, detailed spatial manipulation procedures, and morphological outcomes—body configurations, organ positions, and geometric properties. The second protocol can be used to formalize limb regeneration experiments for a variety of model organisms. Complex amputation and grafting procedures can be unambiguously encoded together with the precise limb and appendage configurations during regeneration. Finally, the last protocol details a procedure to encode planarian gene expression patterns using reference morphologies. This methodology facilitates the curation of a formal dataset of standardized and ontologically annotated gene patterns that can streamline their comparative analysis and downstream mechanistic inference. Practical notes are included for clarifying key steps and implementation details.

2 Materials

1. Personal computer with Windows, Mac OS X, or Linux operating systems.
2. PlanForm software tool installed in the computer. Planform is freely available at https://lobolab.umbc.edu/planform/. The user-friendly program is compatible with Windows, Mac OS X, and Linux operating systems.
3. LimbForm software tool installed in the computer. Limbform is freely available at https://lobolab.umbc.edu/limbform/. The user-friendly program is compatible with Windows, Mac OS X, and Linux operating systems.
4. PlanGexQ software tool installed in the computer. PlanGexQ is freely available at https://lobolab.umbc.edu/plangexq/. The user-friendly program is compatible with Windows, Mac OS X, and Linux operating systems.
5. Microscopy images of planarian or limb regeneration phenotypes and the experimental and manipulation details used to obtain them.
6. Planarian gene expression pattern phenotypes from in situ microscopy images and the experimental and manipulation details used to obtain them.

3 Methods

This protocol includes detailed instructions to formalize a planarian experimental phenotype with the Planform software tool. Start by executing the program and continue with the following steps.



Name: Iglesias 2008 Fig. 1: 2 day injection
Publication: Silencing of Smed-βcatenin1 Generates Radial-lik
Species: Schmidtea mediterranea
Drugs: Name | tart (c | nd (c
RNAi injections: Name
1 Smed-bcate
Manipulation: Trunk crop
Result set A
Num worms: 9
Reg. period (d): 10.00
Wild type — Frequency: 0.56
Double head, one pharynx — Frequency: 0.44
Result set B
Num worms: 9
Reg. period (d): 19.00
Wild type — Frequency: 0.56
Double head, one pharynx — Frequency: 0.33
Double head, anterior ectopic eyes — Frequency: 0.11

Name: Trunk crop
Manipulation:
Crop action:

Name: Double head, one pharynx

Fig. 2 Screenshots of the user-friendly software tool Planform for the curation and formalization of experimental procedures and resultant phenotypes of planarian regeneration. (**a**) An experiment formalization includes procedure details, manipulations, and the resultant morphologies. (**b**) Manipulations are unambiguously defined as a hierarchy of simpler actions, such as amputations and graftings. (**c**) Worm morphologies are defined with a mathematical graph, where the nodes represent worm regions and organs

3.1 Formalizing Planarian Regeneration Phenotypes with Planform

1. Create a new database by clicking *File* and then *New database*. Input a new file name in any computer folder when prompted (*see* **Note 5**). Alternatively, open any existing database already created or downloaded with *Open database* (*see* **Note 6**).
2. Within the *Experiments* tab in Planform main window, click *New*. A form appears to enter the experimental conditions and resultant regeneration phenotypes (Fig. 2a).
3. Type the name of the experiment (*see* **Note 7**).
4. Select from the drop-down list, or create new, the publication (*see* **Note 8**), and planarian species used for the experiment.
5. Indicate the drugs, if any, used in the experiment and the time period of its application relative to the worm manipulation time (considered day 0). Use negative numbers to indicate days pre-manipulation. Multiple drugs and time periods can be specified clicking *Add*. The database stores a list of drugs used in the experiments, which can be modified with the *Edit*, and *New* buttons.
6. Indicate the genes that were knockdown, if any, with RNA interference (RNAi) treatments during the experiment. Similar to drugs, multiple knockdown genes can be specified in a single experiment.

7. Specify a new manipulation clicking *New* or copy or edit an existing one from the drop-down menu by clicking *Edit* or *Copy* (*see* **Notes 9** and **10**). If the manipulation is already created in the database, select it from the dropdown menu and continue with **step 21**.
8. Give a descriptive name to the new manipulation.
9. A manipulation is defined as a hierarchical combination (represented as a mathematical tree) of simpler actions and base worm morphologies (Fig. 2b). First, create a new base morphology for the manipulation. If the appropriate morphology is already present in the database, select it from the drop-down menu and continue with **step 18**.
10. A new morphology can be created starting from an empty morphology (clicking *New*) or from an existing one by directly editing it (clicking *Edit*) or by copying into a new morphology (clicking *Copy*) (*see* **Note 11**).
11. A morphology is defined as a network of connected worm regions (represented as a mathematical graph), their organs, and parameters to define their shape and size (Fig. 2c).
12. Type a descriptive name for the new morphology.
13. Optionally, a worm microscopy image of the corresponding morphology can be added clicking *Add image* and then selecting an image file from the local computer. This image will be also stored in the database.
14. Add new region and organ nodes by right clicking an existing region node and selecting the region or organ type to connect. Place the region or organ in the correct location by dragging the node (*see* **Note 12**).
15. Define the relative positions between the regions by dragging the corresponding nodes (green circles) (*see* **Note 13**).
16. Define the size and orientation parameters of regions and organs by dragging the corresponding parameter nodes (small red and blue circles) (*see* **Note 14**).
17. The cartoon diagram shows in real-time a representation of the defined morphology to aid in the formalization (*see* **Note 15**). To save the resultant morphology, click *OK* (*see* **Note 16**).
18. Select the appropriate manipulation actions performed in the experiment using the right-click menu (*see* **Note 17**). The actions available are surgical manipulations, such as crops, removals, and joins (grafts), and complete or partial irradiations (*see* **Note 18**).
19. Specify the parameters of the manipulation action with the drag-and-drop interface within the cartoon visualization. The areas of surgical and irradiation manipulations are defined with

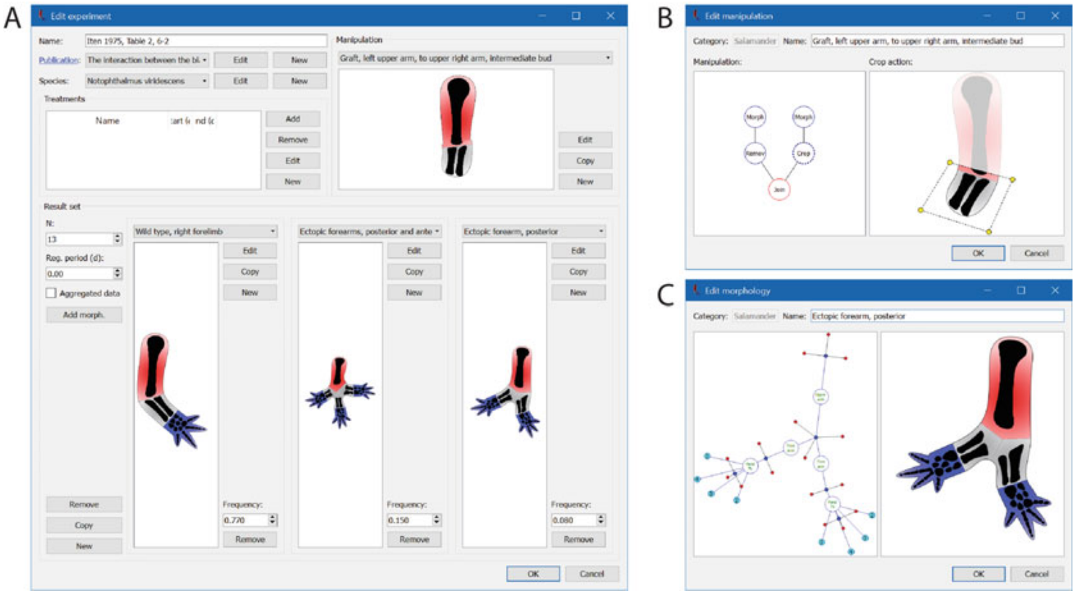
polygons with an arbitrary number of points (vertices), which can be edited with the right-click menu. The position and orientation of grafting manipulations can be defined as well with the drag-and-drop cartoon interface. Clicking and dragging a grafted piece translates it with respect the host morphology, while clicking and dragging on empty space rotates the graft.

20. Repeat **steps 18** and **19** until all the manipulation actions in the experiment are defined. Then click *OK*.
21. Specify the set of resultant morphologies obtained in the experiment. Multiple time points can be defined to formalize a time series of morphological outcomes. For each time point to formalize in the experiment, click *New* and then define the number of worms in the experiment (*N*) as well as the regeneration period as days after the manipulation.
22. For each regeneration period, define a set of morphological outcomes and their frequency. To add a new resultant morphology, click *Add morph.* and indicate the ratio of worms (frequency) that resulted in that regeneration phenotype (*see* **Note 19**).
23. For each resultant morphology, specify the phenotype by selecting it from the drop-down menu. If the morphology is not already created in the database, create a new one following **steps 10–17**.
24. Repeat **steps 21–23** for each resultant phenotype obtained in the experiment. Then click *OK* to save the formalized experiment in the database (*see* **Note 20**).

3.2 Formalizing Limb Regeneration Phenotypes with Limbform

Limbform follows the same general design than Planform, but for the formalization of regeneration phenotypes of salamander, frog, crustacean, insect, and arachnid limb and appendages. To formalize a new phenotype, follow the same procedure as with Planform except for the following steps.

1. Define all treatments, such as drug injections, using a unified list and include for each of them the time period of its application relative to the limb manipulation time (considered day 0). Use negative numbers to indicate days pre-manipulation (Fig. 3a).
2. The list of actions that define a manipulation (Fig. 3b) are extended with relocation and reverse actions. Use a relocation action to define together an amputation of a limb section and its graft in an ectopic location. Use a reverse action to define similarly an amputation of a limb section and its graft in an ectopic location, but where the graft is 180° rotated around the proximal–distal axis.



Edit experiment

Name: Iten 1975, Table 2, 6-2

Publication: The interaction between the bl

Species: Notophthalmus viridescens

Manipulation: Graft, left upper arm, to upper right arm, intermediate bud

N: 13

Reg. period (d): 0.00

□ Aggregated data

Wild type, right forelimb — Frequency: 0.770

Ectopic forearms, posterior and ante — Frequency: 0.150

Ectopic forearm, posterior — Frequency: 0.080

Edit manipulation

Category: Salamander Name: Graft, left upper arm, to upper right arm, intermediate bud

Edit morphology

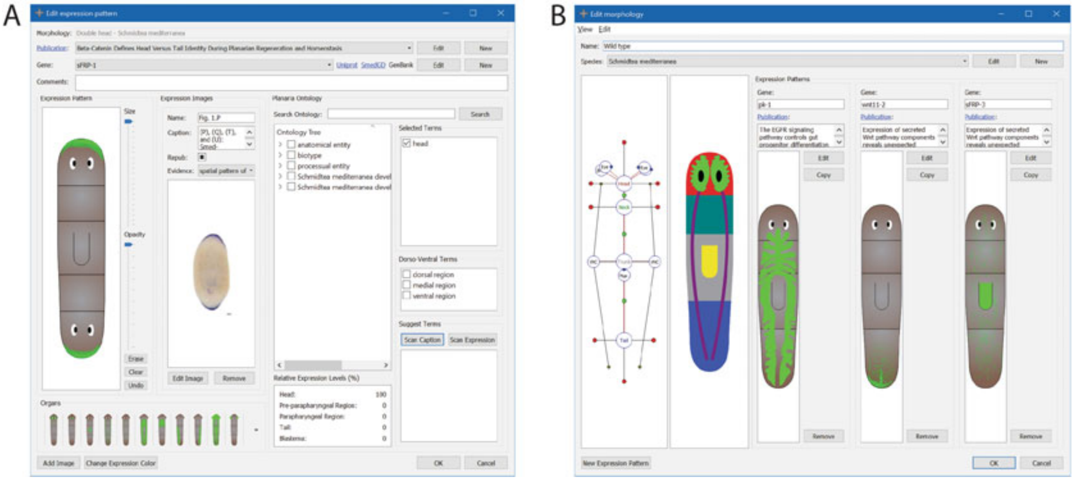
Category: Salamander Name: Ectopic forearm, posterior

Fig. 3 Screenshots of the software tool Limbform for the curation and formalization of salamander, frog, crustacean, insect, and arachnid limb and appendage regeneration experiments and morphologies. (**a**) Example of a formalized grafting experiment with salamander resulting in multiple ectopic forearms. (**b**) User-friendly interface for the formalization of a grafting experiment from the left to the right arm. (**c**) Limb phenotype with ectopic forearms, including details such as the number of bones in each limb segment and digit

3. Formalize limb morphologies by defining with the interactive interface limb segments as the edges of the graph and their joints as the vertices of the graph (Fig. 3c) (*see* **Note 21**).
4. Define the type of each segment (which is particular to the animal species) and the body side it developed or regenerated (left or right) by right clicking on each node. In a similar way, define for each segment whether they are reversed (180° rotation around the proximal–distal axis) or inverted (swap of the proximal–distal segment ends) (*see* **Note 22**).
5. For salamander and frog limbs, define the number of bones for each segment as well as for their digits. These options are also available in the right-click menu. The selected number of bones and digits are also shown in the cartoon diagram (Fig. 3c).
6. Finally, for salamander and frog limbs, optionally define nerves as a segmented line through the limb morphology. Use the right-click menu to add and remove points in a nerve.

3.3 Formalizing Planarian Gene Expression Patterns with PlanGexQ

PlanGexQ is a software tool for the formalization of planarian gene expression patterns into standard morphologies. Start by executing the program and continue with the following steps.



Morphology: Double head - Schmidtea mediterranea

Publication: Beta-Catenin Defines Head Versus Tail Identity During Planarian Regeneration and Homeostasis

Gene: sFRP-1

Comments:

Name: Fig. 1.P

Caption: (P), (Q), (T), and (U): Smed-

Repub: ■

Evidence: spatial pattern of

Search Ontology:

- ☐ anatomical entity
- ☐ biotype
- ☐ processual entity
- ☐ Schmidtea mediterranea devel
- ☐ Schmidtea mediterranea devel

Selected Terms: ☑ head

Dorso-Ventral Terms: ☐ dorsal region ☐ medial region ☐ ventral region

Relative Expression Levels (%)

Head:	100
Pre-parapharyngeal Region:	0
Parapharyngeal Region:	0
Tail:	0
Blastema:	0

Name: Wild type

Species: Schmidtea mediterranea

Gene: pk-1 — Publication: The EGFR signaling pathway controls gut progenitor differentiation

Gene: wnt11-2 — Publication: Expression of secreted Wnt pathway components reveals unexpected

Gene: sFRP-3 — Publication: Expression of secreted Wnt pathway components reveals unexpected

Fig. 4 Screenshots of the software tool PlanGexQ for the registration and ontological annotation of gene expression patterns in planarian worms. (**a**) Interface for the curation of a gene expression pattern in a double head regeneration phenotype, including the original microscopy image and ontology terms. (**b**) Formalized standard morphology together with curated gene expression patterns. Registering expression patterns in standard morphologies facilitates their downstream analysis with machine learning tools

1. Create a new database by clicking *File* and then *New database*. Input a new file name in any computer folder when prompted (*see* **Note 23**). Alternatively, open any existing database already created or downloaded with *Open database*.
2. Before curating a new gene expression pattern, make sure that the corresponding worm morphology exists in the *Morphologies* tab. To create a new standard morphology, select the *Morphologies* tab, and click *New*. The procedure to create new worm morphologies is similar than for Planform (*see* **steps 11–17** in Subheading 3.1).
3. Within the *Expression Patterns* tab in PlanGexQ main window, click *New*. A new form appears to select the standard morphology of the worm to formalize among those stored in the database. After selecting a morphology, a new form appears to enter the gene expression pattern details (Fig. 4a).
4. Select from the drop-down list, or create new, the publication where the gene expression pattern is being curated from, if any.
5. Select from the drop-down list, or create new, the gene reported in the expression pattern. For new genes, additionally input their accession number from the Uniprot [38], SmedGD [39], and GenBank [40] databases (*see* **Note 24**).
6. Define the gene expression pattern in the cartoon reference morphology with the interactive tool. An automated registration method to map the gene expression patterns in the reference morphology directly from microscopy images is available

starting from v.3.0 of the tool. If the expression pattern includes specific organs, click on their diagram to directly include their expression pattern. Optionally, select a different expression color by clicking *Change Expression Color.*

7. Add one or more microscopy images of the gene expression pattern clicking *Add Image*. For each image, input a name and a caption. Optionally, select from the drop-down menu the type of evidence as a term from the Evidence and Conclusion Ontology [41]. In particular, gene expression patterns obtained with in situ hybridization assays can be annotated with the term ECO_0000047, *spatial pattern of transcript expression evidence*.
8. Scan the caption and expression pattern to automatically assign ontology terms from the Planarian Anatomy Ontology [27] to the gene expression pattern by clicking *Scan Caption* and *Scan Expression* (*see* **Note 25**).
9. If needed, assign additional ontology terms by clicking them on the complete ontology tree hierarchy shown in the interface. The tree can be easily searched typing keywords in the *Search Ontology* box (*see* **Note 26**).
10. When finished, click *OK* to save the gene expression pattern information in the database.

4 Notes

1. A curated database of planarian regeneration experiments is freely available at https://lobolab.umbc.edu/planform/download/ and can be opened and explored with the latest version of Planform in any of the compatible operating systems.
2. A curated database of limb regeneration experiments is freely available at https://lobolab.umbc.edu/limbform/download/ and can be opened and explored with the latest version of Limbform in any of the compatible operating systems.
3. A simulation software including reverse-engineered models of planarian regeneration is freely available at https://lobolab.umbc.edu/planarianmodels/. These models were automatically inferred directly from regeneration phenotypes formalized following the first protocol presented in this chapter. In addition, a methodology for inferring dynamic gene regulatory network models from spatial expression patterns (including an open source software implementation) is available at [34].
4. The formalization tools used in this chapter (Planform, Limbform, and PlanGexQ) were developed in C++ using the Qt libraries (The Qt Company; https://www.qt.io/). This approach allows the implementation of highly customized

native tools that are multiplatform, as the same code can be compiled for Windows, Mac OS X, and Linux operating systems. The Qt libraries are especially useful for implementing the user interface—including forms, widgets, menus, and any other graphical element—and abstracting the access to the database file. Importantly, Qt also provides a Graphics View Framework, which was used for the visualization and interaction with the morphology and manipulation graphs as well as for the worm and limb cartoon diagrams. This framework provides a surface for displaying interactive custom-made 2D graphical items and support zooming and rotation. In this way, the nodes and lines of a morphological graph can be shown as interactive objects for the user to easily formalize a worm, limb, or expression pattern. Furthermore, the framework supports the creation of custom shapes using Bézier curves, which was used to implement the dynamic real-time generation of worm and limb cartoon diagrams directly from the corresponding graph formalization.

5. The file extension for Planform databases is *edb* and for Limbform databases is *ldb*. During the installation of the tools, file types and extension associations are automatically created in the operating system so that double clicking database files automatically open them in their respective tools. To guarantee compatibility with external tools, these database files are locally stored in the SQLite version 3 (public domain) format, the most used database engine in the world.
6. A database is stored in a single file containing all the data in a centralized fashion. This approach facilitates the sharing of information between researchers. In addition, Planform, Limbform, and PlanGexQ include functionality to import the data from one database into another. For this, open the target database and select the *Import* command in the *File* menu. Using the file selection window that popups, select the database file with the source data. The program then compares all the data from both databases and shows all the elements that are modified, new, or deleted. The user can then select the specific data to import.
7. The experiment name must be unique within a database. The name convention in the public curated Planform database is the first author last name, year of publication, and the figure panel in the paper where the resultant phenotype is reported.
8. A publication includes the title, year, and URL that links to the full manuscript text. To maximize long-term accessibility, we recommend using the DOI link when possible.
9. To create a complex manipulation, it is recommended to copy an existing one already in the database and change the steps

that differ, rather than starting completely from a new empty manipulation.

10. When editing an element in the database, such as a manipulation or morphology, the new changes will be reflected in all experiments containing such element. If that behavior is not desired, copy the element instead, and assign a new name.
11. It is recommended to create a new morphology by first copying an existing one already in the database and change the region and organs that differ. Intact wild-type morphologies are automatically created in a new database.
12. A link can be added between two existent regions by holding the shift key and selecting both nodes followed by right click. Links can be deleted between regions by right clicking on the link and selecting the appropriate menu option.
13. The spatial location of each region is defined relative to the other regions, and hence, a morphology is invariant under translations or rotations. The spatial location and orientation of organs are defined relative to the region they are linked to. In this way, region translations do not affect the relative location of the organs within the region.
14. For more precise control and placement of nodes, a user can zoom in and out the graph and cartoon representations in both morphologies and manipulations by holding the Ctrl key (key on a Mac) and moving the mouse wheel. If any part of the morphology or graph is not visible in the current view, scroll bars appear to translate the visualization. In addition, vertical scrolling can be achieved by holding the Shift key and moving the mouse wheel. Horizontal scrolling can be achieved by holding the Alt key (Option key on a Mac) and moving the mouse wheel.
15. Cartoon diagrams and graph representations can be exported as image files by right-clicking on any empty space and then selecting *Save image*.
16. Clicking *OK* on any form in the tool saves the changes made in that form into the local database file without the need of any further command in the *File* menu. To save and continue using a new copy of the database file, select *Save database as* in the *File* menu.
17. The manipulation actions form together a tree structure, where the root of the tree represents the final manipulated worm, and the leaves represent the starting worm morphologies. In this way, complex manipulations can be unambiguously formalized with the interface by combining multiple actions (e.g., multiple graftings from different source worms).

18. Notice that a crop action amputates a section of the morphology and discards the rest of the morphology (e.g., amputating the head region to produce a head fragment). In contrast, a removal action amputates a section from the morphology, discards it, and keeps the rest of the morphology (e.g., removing the tail region and keeping the head and trunk regions).

19. The sum of all the frequencies in a regeneration period must be one.

20. Researchers are encouraged to contribute their curated experiments to the centralized databases of Planform and Limbform. For this, please follow the instructions in the submission page of their respective websites.

21. While both worm and limb morphologies are defined with mathematical graphs, notice that the nodes of the graph define regions in a worm but joints in a limb.

22. A limb segment can have four configurations: normal, reversed, inverted, and reversed and inverted. When formalizing a limb morphology, these attributes are defined directly on each segment. When formalizing a limb manipulation, the reverse action affects the entire morphology (to reverse a smaller section, apply a crop action previous to the reverse action), and rotations can be freely applied to any graft using the join and relocation actions.

23. The file extension for PlanGexQ databases is *gdb*. During the installation of PlanGexQ, a file type and extension association are automatically created in the operating system so that double clicking PlanGexQ database files automatically open them in the PlanGexQ tool. Similar to Planform and Limbform, these database files are locally stored in the SQLite version 3 (public domain) format.

24. The form to input new genes facilitates the search of their accession number from the Uniprot, SmedGD, and GenBank databases. After writing the gene name in the *Name* field, a link is shown that directly searches Uniprot and GenBank within the appropriate planarian species for the gene name entered. The link to SmedGD directs to the general search in that database. After entering the appropriate accession number from these databases, the link changes to a direct access to the appropriate gene information page. These direct links are also shown in the main expression pattern form.

25. The *Scan Caption* function searches in the caption from all the images for relevant keywords that are related to anatomical ontology terms and then suggest them for inclusion in the annotation. In addition, this function can suggest gene names for the experiment as found in the caption using a pattern matching approach. The *Scan Expression* function suggests

anatomical ontology terms according to the worm regions that include gene expression as currently input in the standard morphology. This function also updates the relative gene expression levels for each worm region of the current expression pattern.

26. The check marks in the ontology tree terms, selected terms, and suggested terms are always synchronized so it is clear which terms are assigned to the current expression pattern. To facilitate the annotation process, hovering the mouse over a term shows the description of the ontology term.

Acknowledgments

I thank the members of the Lobo Lab for their dedicated work and discussions. This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number R35GM137953. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Li Q, Yang H, Zhong TP (2015) Regeneration across metazoan phylogeny: lessons from model organisms. J Genet Genomics 42:57–70. https://doi.org/10.1016/j.jgg.2014.12.002
2. Lobo D, Beane WS, Levin M (2012) Modeling planarian regeneration: a primer for reverse-engineering the worm. PLoS Comput Biol 8:e1002481. https://doi.org/10.1371/journal.pcbi.1002481
3. Rubin BP, Brockes J, Galliot B et al (2015) A dynamic architecture of life. F1000Research 4. https://doi.org/10.12688/f1000research.7315.1
4. Sánchez Alvarado A (2006) Planarian regeneration: its end is its beginning. Cell 124:241–245
5. Ivankovic M, Haneckova R, Thommen A et al (2019) Model systems for regeneration: planarians. Development 146:dev167684. https://doi.org/10.1242/dev.167684
6. Birkholz TR, Van Huizen AV, Beane WS (2019) Staying in shape: planarians as a model for understanding regenerative morphology. Semin Cell Dev Biol 87:105–115. https://doi.org/10.1016/j.semcdb.2018.04.014
7. Reddien PW (2018) The cellular and molecular basis for planarian regeneration. Cell 175:327–345. https://doi.org/10.1016/j.cell.2018.09.021
8. Brockes JR, Kumar A (2008) Comparative aspects of animal regeneration. Annu Rev Cell Dev Biol 24:525–549. https://doi.org/10.1146/annurev.cellbio.24.110707.175336
9. Joven A, Elewa A, Simon A (2019) Model systems for regeneration: salamanders. Development 146(14):dev1677000. https://doi.org/10.1242/dev.167700
10. Miller BM, Johnson K, Whited JL (2019) Common themes in tetrapod appendage regeneration: a cellular perspective. Evodevo 10:1–13. https://doi.org/10.1186/s13227-019-0124-7
11. Kato K, Orii H, Watanabe K et al (1999) The role of dorsoventral interaction in the onset of planarian regeneration. Development 126:1031–1040. https://doi.org/10.1242/dev.126.5.1031
12. Maden M, Turner RN (1978) Supernumerary limbs in the axolotl. Nature 273:232–235. https://doi.org/10.1038/273232a0
13. French V (1976) Leg regeneration in the cockroach, Blatella germanica. II. Regeneration from a noncongruent tibial graft/host junction. J Embryol Exp Morphol 35:267–301
14. Mace DL, Varnado N, Zhang W et al (2009) Extraction and comparison of gene expression

patterns from 2D RNA in situ hybridization images. Bioinformatics 26:761–769. https://doi.org/10.1093/bioinformatics/btp658

15. Deans AR, Lewis SE, Huala E et al (2015) Finding our way through phenotypes. PLoS Biol 13. https://doi.org/10.1371/journal.pbio.1002033
16. Christiansen JH, Yang Y, Venkataraman S et al (2006) EMAGE: a spatial database of gene expression patterns during mouse embryo development. Nucleic Acids Res 34: D637–D641. https://doi.org/10.1093/nar/gkj006
17. Lobo D, Malone TJ, Levin M (2013) Towards a bioinformatics of patterning: a computational approach to understanding regulative morphogenesis. Biol Open 2:156–169. https://doi.org/10.1242/bio.20123400
18. Lobo D, Feldman EB, Shah M et al (2014) A bioinformatics expert system linking functional data to anatomical outcomes in limb regeneration. Regeneration 1:37–56. https://doi.org/10.1002/reg2.13
19. Lobo D, Malone TJ, Levin M (2013) Planform: an application and database of graph-encoded planarian regenerative experiments. Bioinformatics 29:1098–1100. https://doi.org/10.1093/bioinformatics/btt088
20. Lobo D, Feldman EB, Shah M et al (2014) Limbform: a functional ontology-based database of limb regeneration experiments. Bioinformatics 30:3598–3600. https://doi.org/10.1093/bioinformatics/btu582
21. Broitman-Maduro G, Maduro MF (2011) In situ hybridization of embryos with antisense RNA probes. In: Rothman JH, Singson A (eds) Caenorhabditis elegans: molecular genetics and development. Academic Press, Waltham, MA, pp 253–270. https://doi.org/10.1016/B978-0-12-544172-8.00009-8
22. King RS, Newmark PA (2018) Whole-mount in situ hybridization of planarians. In: Rink JC (ed) Planarian regeneration: methods and protocols. Humana Press, New York, pp 379–392. https://doi.org/10.1007/978-1-4939-7802-1_12
23. Adell T, Barberán S, Sureda-Gómez M et al (2018) Immunohistochemistry on paraffin-embedded planarian tissue sections. In: Rink JC (ed) Planarian regeneration: methods and protocols. Humana Press, New York, pp 367–378. https://doi.org/10.1007/978-1-4939-7802-1_11
24. Forsthoefel DJ, Ross KG, Newmark PA et al (2018) Fixation, processing, and immunofluorescent labeling of whole mount planarians. In: Rink JC (ed) Planarian regeneration: methods and protocols. Humana Press, New York, pp 353–366. https://doi.org/10.1007/978-1-4939-7802-1_10
25. Ramos JA (2005) Technical aspects of immunohistochemistry. Vet Pathol 426:405–426. https://doi.org/10.1354/vp.42-4-405
26. Roy J, Cheung E, Bhatti J et al (2020) Curation and annotation of planarian gene expression patterns with segmented reference morphologies. Bioinformatics 36:2881–2887. https://doi.org/10.1093/bioinformatics/btaa023
27. Nowotarski SH, Davies EL, Robb SMC et al (2020) The planarian anatomy ontology: a resource to connect data within and across experimental platforms. Running title Keywords. bioRxiv. https://doi.org/10.1101/2020.08.14.251579
28. Lobo D, Levin M (2017) Computing a worm: reverse-engineering planarian regeneration. In: Adamatzky A (ed) Advances in unconventional computing, vol 2: prototypes, models and algorithms. Springer, Cham, pp 637–654. https://doi.org/10.1007/978-3-319-33921-4_24
29. Herath S, Lobo D (2020) Cross-inhibition of Turing patterns explains the self-organized regulatory mechanism of planarian fission. J Theor Biol 485:110042. https://doi.org/10.1016/j.jtbi.2019.110042
30. Ko JM, Lobo D (2019) Continuous dynamic modeling of regulated cell adhesion: sorting, intercalation, and involution. Biophys J 117:2166–2179. https://doi.org/10.1016/j.bpj.2019.10.032
31. García-Quismondo M, Levin M, Lobo D (2017) Modeling regenerative processes with Membrane Computing. Inf Sci (Ny) 381:229–249. https://doi.org/10.1016/j.ins.2016.11.017
32. Lobikin M, Lobo D, Blackiston DJ et al (2015) Serotonergic regulation of melanocyte conversion: a bioelectrically regulated network for stochastic all-or-none hyperpigmentation. Sci Signal 8:ra99. https://doi.org/10.1126/scisignal.aac6609
33. Lobo D, Levin M (2015) Inferring regulatory networks from experimental morphological phenotypes: a computational method reverse-engineers planarian regeneration. PLoS Comput Biol 11:e1004295. https://doi.org/10.1371/journal.pcbi.1004295
34. Mousavi R, Konuru SH, Lobo D (2021) Inference of dynamic spatial GRN models with multi-GPU evolutionary computation. Brief Bioinform 22(bbab104):1–11. https://doi.org/10.1093/bib/bbab104

35. Lobo D, Lobikin M, Levin M (2017) Discovering novel phenotypes with automatically inferred dynamic models: a partial melanocyte conversion in Xenopus. Sci Rep 7:41339. https://doi.org/10.1038/srep41339

36. Lobo D, Morokuma J, Levin M (2016) Computational discovery and in vivo validation of hnf4 as a regulatory gene in planarian regeneration. Bioinformatics 32:2681–2685. https://doi.org/10.1093/bioinformatics/btw299

37. Lobo D, Hammelman J, Levin M (2016) MoCha: molecular characterization of unknown pathways. J Comput Biol 23:291–297. https://doi.org/10.1089/cmb.2015.0211

38. UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515. https://doi.org/10.1093/nar/gky1049

39. Robb SMC, Gotting K, Ross E et al (2015) SmedGD 2.0: the Schmidtea mediterranea genome database. Genesis 53:535–546. https://doi.org/10.1002/dvg.22872

40. Sayers EW, Cavanaugh M, Clark K et al (2019) GenBank. Nucleic Acids Res 47:D94–D99. https://doi.org/10.1093/nar/gky989

41. Giglio M, Tauber R, Nadendla S et al (2019) Eco, the evidence & conclusion ontology: community standard for evidence information. Nucleic Acids Res 47:D1186–D1194. https://doi.org/10.1093/nar/gky1036