CollecTF Documentation

Release 1.0.0

Sefa Kilic

Mar 28, 2017

Contents

1 Curation submission guide **3**
- 1.1 Data . . . 3
- 1.2 Before you start . . . 3
- 1.3 Curation . . . 5

2 High-throughput submission guide **17**
- 2.1 Why? . . . 17
- 2.2 What? . . . 17
- 2.3 How? . . . 18
- 2.4 The process . . . 18

A play on words using the French *collectif* [collective] and the acronym for transcription factor [TF], CollecTF is a database of prokaryotic transcription factor binding sites (TFBS). Its main aim is to provide high-quality, manually-curated information on the experimental evidence for transcription factor binding sites, and to map these onto reference bacterial genomes for ease of access and processing. The data submitted to CollecTF gets pushed to the major biological sequence databases, where it is embedded as *db_xref* links, maximizing the availability of the TF-binding site data and the impact of the research reported by authors.

CollecTF is accessible at http://www.collectf.org. To read more about CollecTF, please see the correspoding Nucleic Acids Research paper (PMID: 24234444).

Contents:

CHAPTER 1

Curation submission guide

This document is a companion guide for the submission process. The database is accessible at http://www.collectf.org. To read more about CollecTF, please see the correspoding Nucleic Acids Research paper (PMID: 24234444).

Data

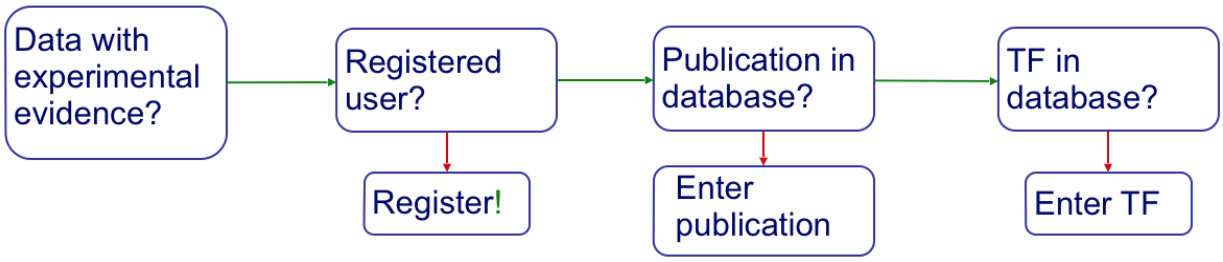
This database only compiles transcription factor binding sites backed by experimental evidence published in peer reviewed articles. CollecTF distinguishes between two main types of experimental support: evidence of binding (e.g. EMSA) and evidence of TF-mediated regulation (e.g. β-gal assay). Identification of TF-binding sites through *in silico* means is recorded as part of the curation process, but not admitted as the *single* source of evidence for a TF-binding site. *Please do not submit data without some form of experimental (i.e. not *in silico*)* evidence, as it will be deleted*.

Before you start

In order to perform a successful submission, several things need to be in place. Namely, you should be a registered user, and your publication and TF should be entered into the system (if not yet there).

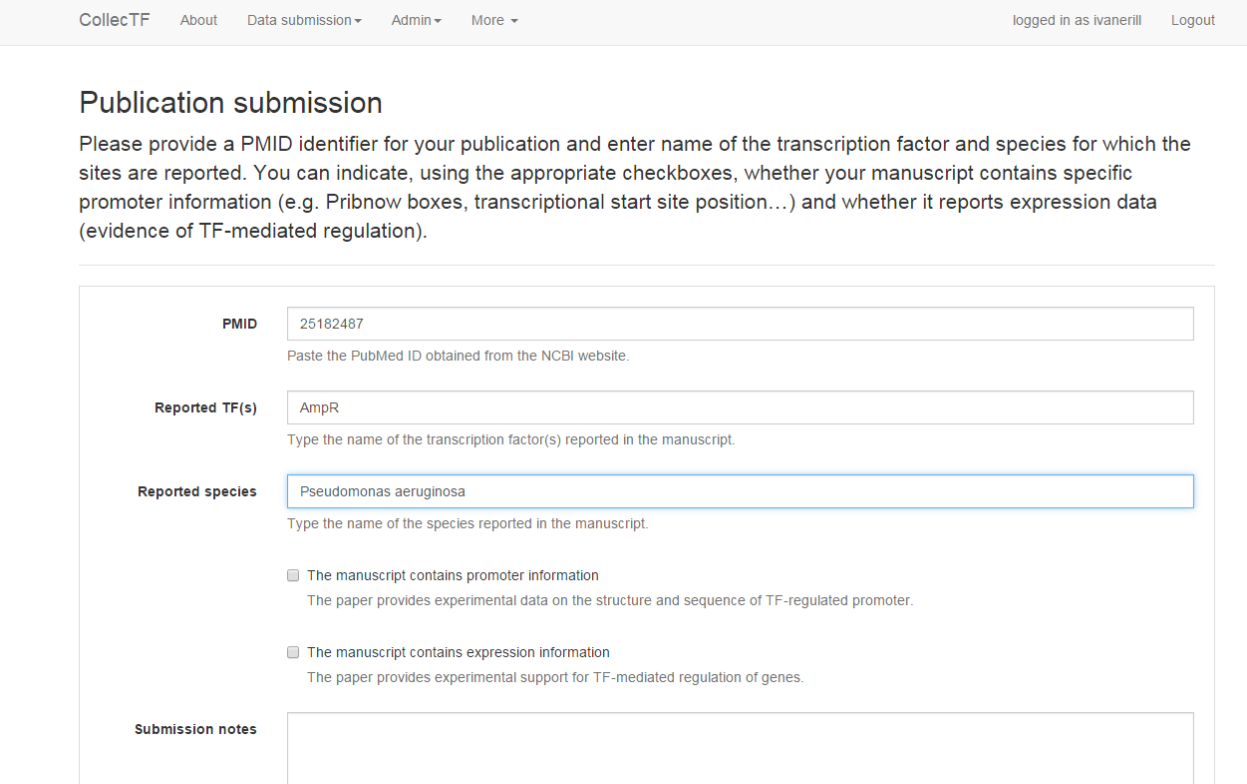
User profiles

Before you can submit data to CollecTF you must first register as a user. To initiate the registration process you must click on the `Register` link at the upper right of the CollecTF main page. A *valid email address* is required for user verification.



Publication submission

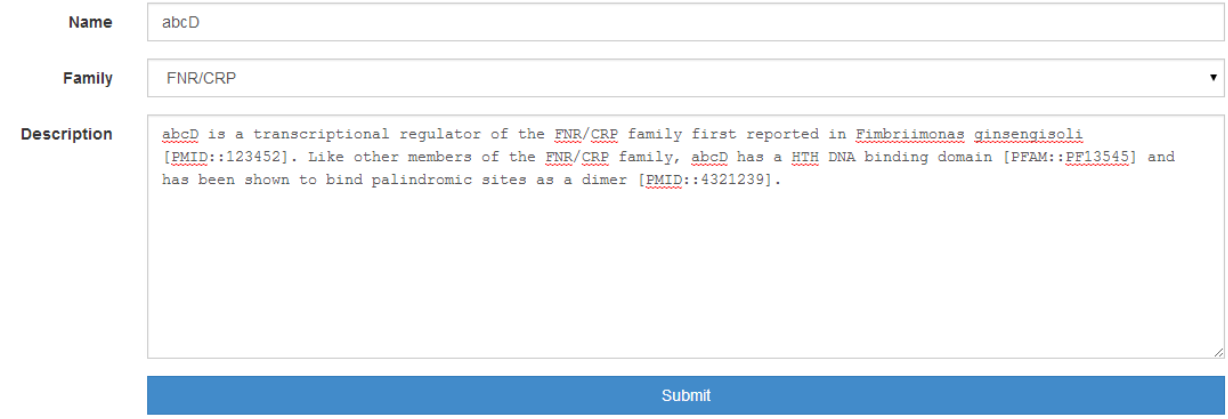
Before submitting a curation, the publication that it reports on must be logged in to the CollecTF database. The easiest way to introduce a publication is using its *PMID identifier*. To enter your publication, simply log in and select `New publication (PubMed)` from the `Data submission` menu. On the dialog that opens, simply enter the PMID (just numbers) for your publication and enter name of the transcription factor and species for which the sites are reported. You can indicate, using the appropriate checkboxes, whether your manuscript contains specific promoter information (e.g. Pribnow boxes, annotated transcriptional start sites...) and whether it reports expression data (evidence of TF-mediated regulation). Once you click Preview, the system will query NCBI PubMed and populate all article fields. If you do not have a PubMed identifier yet, please select `New publication (non-PubMed)` and enter the manuscript data manually.



TF and family information

To submit a curation, you will also need that the TF (and its family) have been added to the database. Please browse the database by TF family and check whether your specific transcription factor is in the database. If it is not, use the `Add TF` and/or the `Add family` options in `Data submission` to include your TF. You can embed

outlinks to PubMed and PFAM in the description of TF and family by using the following double colon notation: `[PMID::pmid_accession]` and `[PFAM::pfam_accession]`.

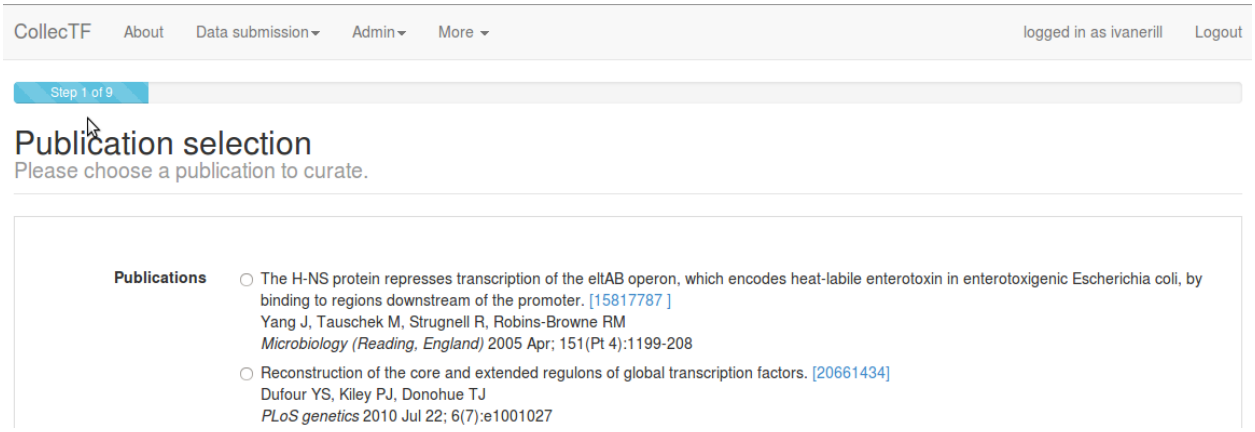


Curation

The initial steps of the submission process require that you select a publication and identify a mapping between the species in which you work and available reference genomes in RefSeq.

Step 0: Publication selection

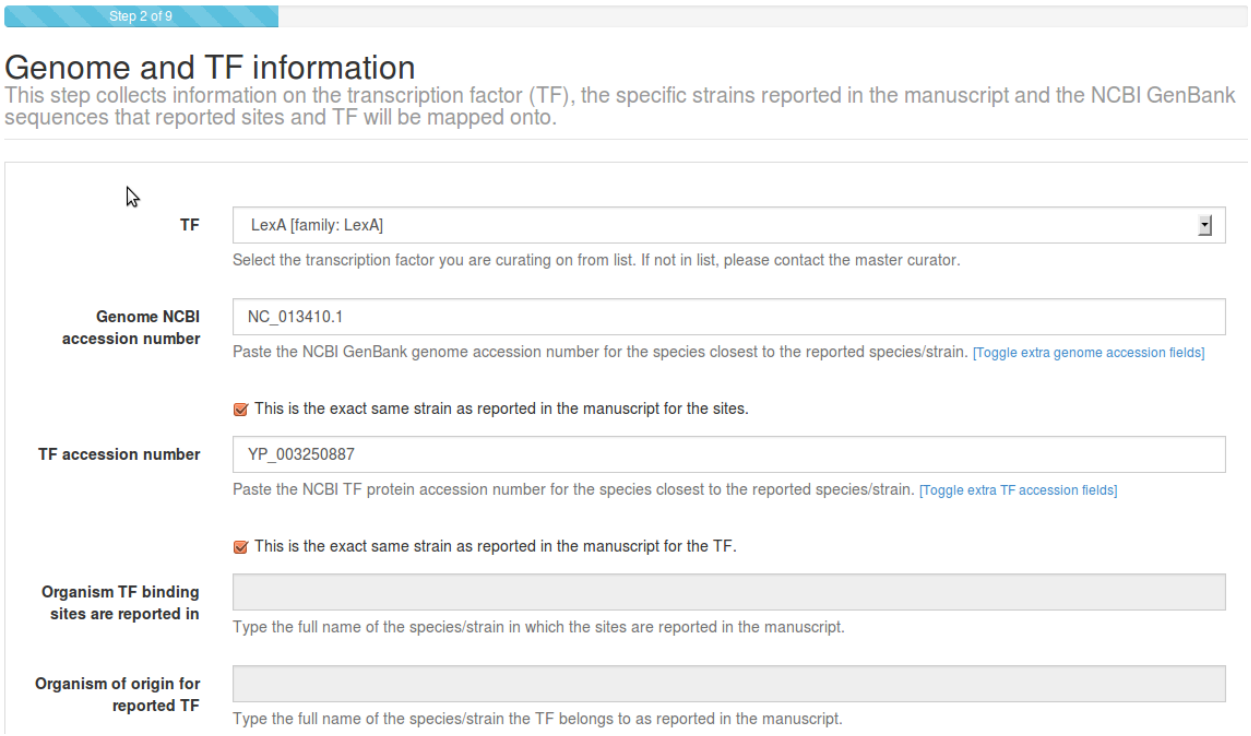
The submission process starts with the submitter selecting a publication for curation. You can upload several publications for curation and perform several curations per publication.



Step 1: Genome and TF information

Once a publication has been selected, the submitter must link the reported species (both for the sites and the transcription factor) to sequences present in the NCBI RefSeq database. This is done by providing RefSeq accession number for the reported chromosomes (e.g. `NC_005363.1`; *including the version number*) and UniProt accession numbers for TF proteins (e.g. `P0A7C2`). Notice that RefSeq accession numbers are designated by an underscore; the version number is the one following the period (e.g. `NC_005363.1`). Only NCBI RefSeq accession numbers are accepted.

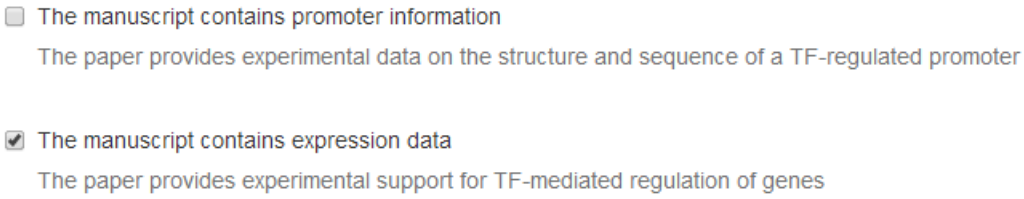
Identifying the RefSeq genome matching your experimental species is often a simple step, but it may become complicated if the sequence for the exact strain used in your work is nopt available as an NCBI RefSeq record. Most often, parental or closely related strains will be available among NCBI RefSeq genomes. As a researcher working hands on with a particular strain, you are best qualified to identify a parental or related strain in NCBI RefSeq Nevertheless, if you are uncertain or there is no clear way to identify a surrogate genome in NCBI RefSeq, please contact the CollecTF team.



If the work you are reporting uses a strain different from the selected RefSeq genome/TF, please type/paste the original strain in the `Organism of origin...` and `Organism TF binding sites...` text fields. Otherwise, click `This is the same strain...` This allows us to keep track of the correspondence between reported and mapped strains. If your TF is a heterodimer or if your species has multiple chromosomes, you can add more than one chromosome/TF accession by clicking on `Toggle extra genome accession fields` / `Toggle extra TF accession fields`.

Additional Fields

The submission process will ask you to verify again if the manuscript reports promoter information or expression data. Please make sure that `The manuscript contains expression data` is checked if you plan to report differential gene expression associated with TF activity.



Step 2: Experimental methods

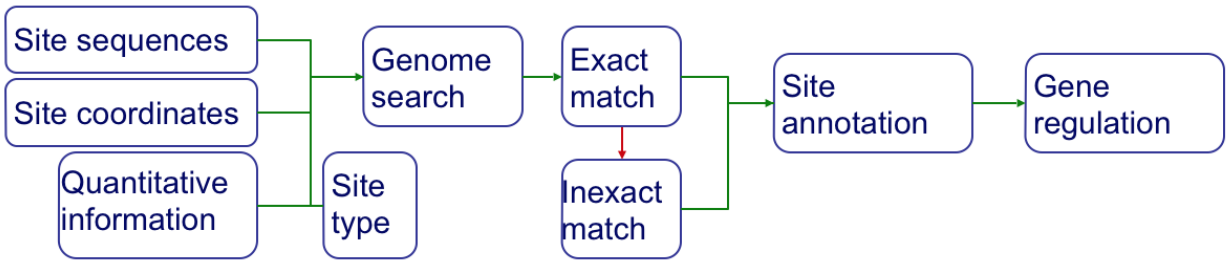
Step 2 requires that you report *all the techniques used in the paper to verify the TFBS* that are being *reported in this submission*. Most work reporting TFbinding sites involves a heterogeneous mix of techniques (e.g. a site is first shown to bind through footprinting and EMSA, then other sites are validated with EMSA alone).

You can select all that apply and you will be able to specify which technique applies to each site at a later step in the curation process. Note that you should only enter techniques used to identify sites, and not any other experimental techniques used in the manuscript for other purposes. In this step we also ask that you provide a *brief written summary* of the process used to verify the submitted TFBS (not the overall experimental process, but just how the selected experimental techniques were combined to define reported TFBS)[1]. Please provide also database accession numbers for externally-linked data if applicable (e.g. GEO, ArrayExpress, PDB) and, if available, details on whether the TF forms complex with other molecules in order to bind.

[1] For instance: "*Sites were first identified using a computer search, then binding was validated with EMSA. TF-mediated expression was confirmed with β-gal assays on w-t vs. tf-mutant*". You can check the provided samples or browse previous curations in the database for additional examples.

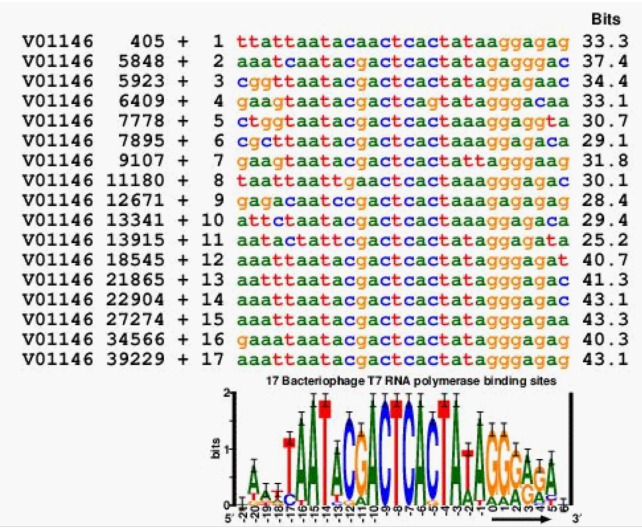
Step 3: Entering reported sites

In this step, you will enter the primary information for CollecTF: binding sites reported in this work *using the techniques specified in Step 2*. Again, you will be able to define what techniques were used specifically for each binding site at a later step.

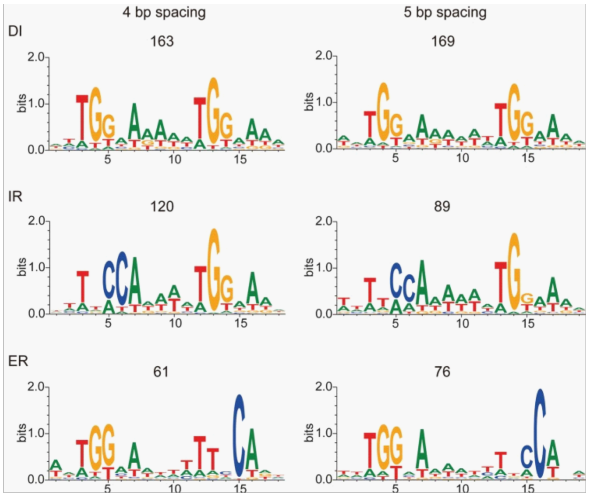


Site types

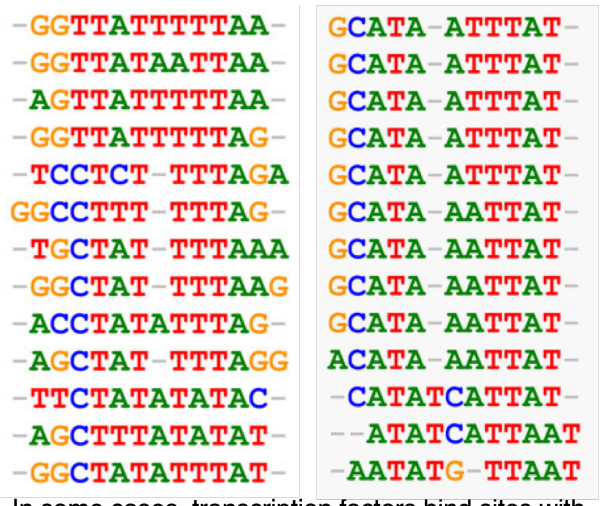
TFbinding sites can be defined at different levels. By definition, a TFbinding site is simply a (relatively short) stretch of DNA to which a transcription factor is shown to bind (e.g. a ChIPSeq peak or a DNAse footprint). Many TFs target known specific sequence patterns in the DNA. Some of these patterns are complex and require gapped alignment (e.g. because of variable spacing) or more complex procedures in order to be defined. Other patterns are simpler and can be represented by a gapless alignment of sites (known as a motif), providing a much more concise definition of TFbinding site. In CollecTF we refer to these site types as motifassociated (for gapless alignments and more complex patterns), variable motifassociated (for complex patterns) and nonmotif associated (for unknown or absent patterns; just evidence of binding). If you are confident that the sites you report conform to a known motif or you establish the binding motif through experimental work (e.g. sitedirected mutagenesis), you should report sites using an existing motif, a new one (`Motif associated (new motif)`) or as `Variable motif associated`. Otherwise, please report them as `Non-motif associated`.



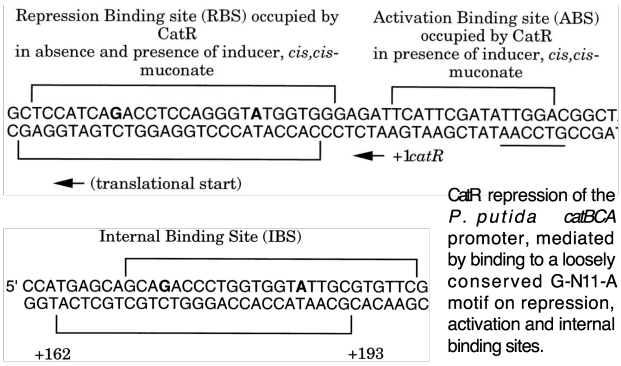
Motif-associated sites can be aligned *without* gaps and are typically represented using sequence logos.
(Image: http://schneider.ncifcrf.gov/glossary.html)



For some transcription factors, sites conforming to *several* un-gapped motifs may have been experimentally described. These sites should be reported as **motif-associated** in *separate curations*.
(Image: Chumsakul *et al.* (2013) *DNA Research*)



In some cases, transcription factors bind sites with variable spacers, without well-defined gapless alignments for different spacer classes. These sites should be reported as **variable motif-associated**.
(Image: Reid *et al*. (2010) *BMC Genomics*)



CatR repression of the *P. putida catBCA* promoter, mediated by binding to a loosely conserved G-N11-A motif on repression, activation and internal binding sites.

Some transcription factors bind loose structures with multiple binding sites adhering somewhat to a sequence motif, but with variation in the positioning of the sub-sites and variable requirement for the presence of each sequence element. Such cases should be reported as **variable motif-associated**.
(Image: Chugani *et al*. (1998) *J. Bacteriol*)

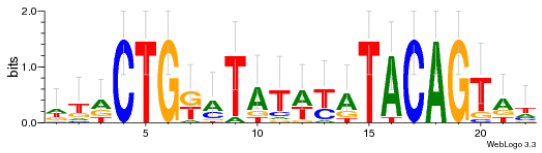
Sequence, coordinates and quantitative data

Sites can be entered as sequences (e.g. `ATCAGACT`) or using genome if they have been mapped to the RefSeq reference strain in the reported work). Sites should be entered one per line (FASTA format is also accepted for sequence entry). In coordinate entry, coordinates are separated by tabs and the first coordinate denotes site start position (e.g. `12280 12260` would denote a 20 bp site in the *reverse* strand starting at position 12280).

If you report quantitative data for sites (e.g. peak intensities, estimated Kd), please append it with a tab/space after the sequence/coordinate entry. A brief description of its nature (method used and range of quantitative data) should be entered in the `Quantitative data format` textbox.

Reported sites [help]

Site type



new motif
variable motif associated
non-motif associated
[motif examples]

Sites

```
TACTGTATATAAAACCAGTT 10.3
TGCTGTGAGTATATACAGCA 12.5
TACTGTATATAAAAACAGTA 15.3
GGCTGTGGTTTTATACAGTC 12.2
TGCTGGATAGATATCCAGCG 11.9
TACTGTATGGATGTACAGTA 12.6
```

Enter the list of sites in FASTA format, raw sequence or coordinate format (one site per line). Sequence entries must be in unambiguous DNA code (A, C, G or T; no degenerate IUPAC codes (e.g. W, Y, N...) or gap symbols (-, _, *). FASTA format does not support quantitative data entry. Quantitative data (q-val) can be added to raw sequence or coordinate entries. All fields (i.e. site & q-val or coordinates & q-val) must be either space or tab separated. [examples]

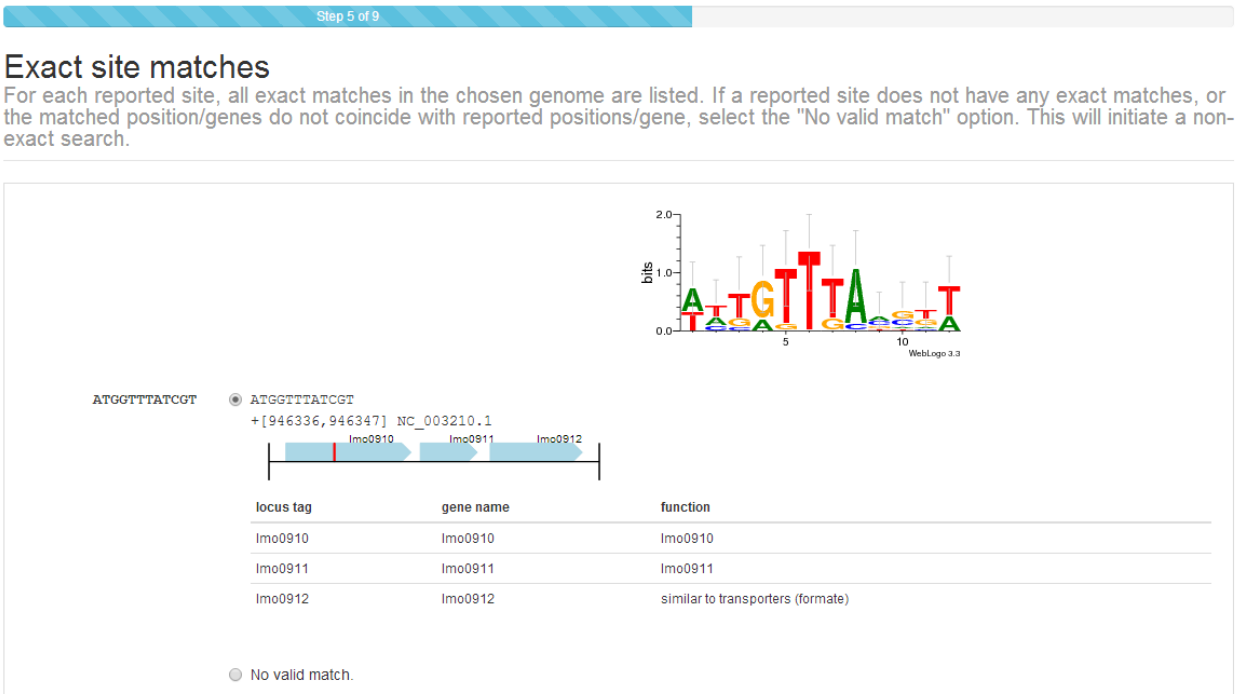
Quantitative data format

Normalized Kd estimated from EMSA (range: 10. 3 - 15.3)

If the manuscript reports quantitative values associated with sites, please enter the quantitative data format here. If not, you can leave this field empty. [example]

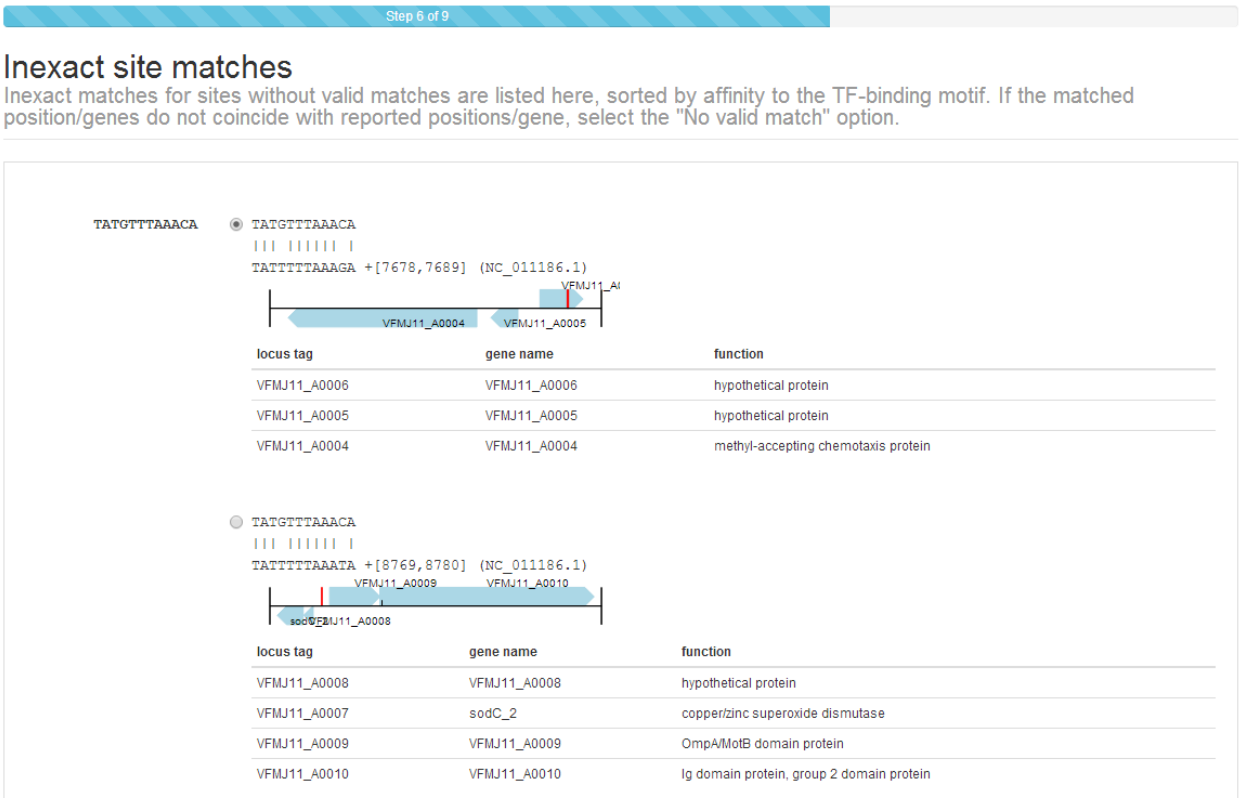
Step 4: Verify sites (exact)

Transcription factor binding sites are often submitted as sequences, of which there may be multiple instances in a genome. After submission, sites submitted as sequences must be manually verified by the submitter to validate that the sites entered correspond to a specific genomic location. The CollecTF submission system will search the genome sequence specified in Step 1 looking for the sequence of each of the sites entered. Exact matches to submitted sites are reported back specifying their location in the genome and nearby genes. Gene annotation details can be accessed by hovering over any gene locus. This information can be used to verify that the sites identified in the NCBI RefSeq genome sequence correspond to the experimentally reported sites.



Step 5: Verify sites (inexact)

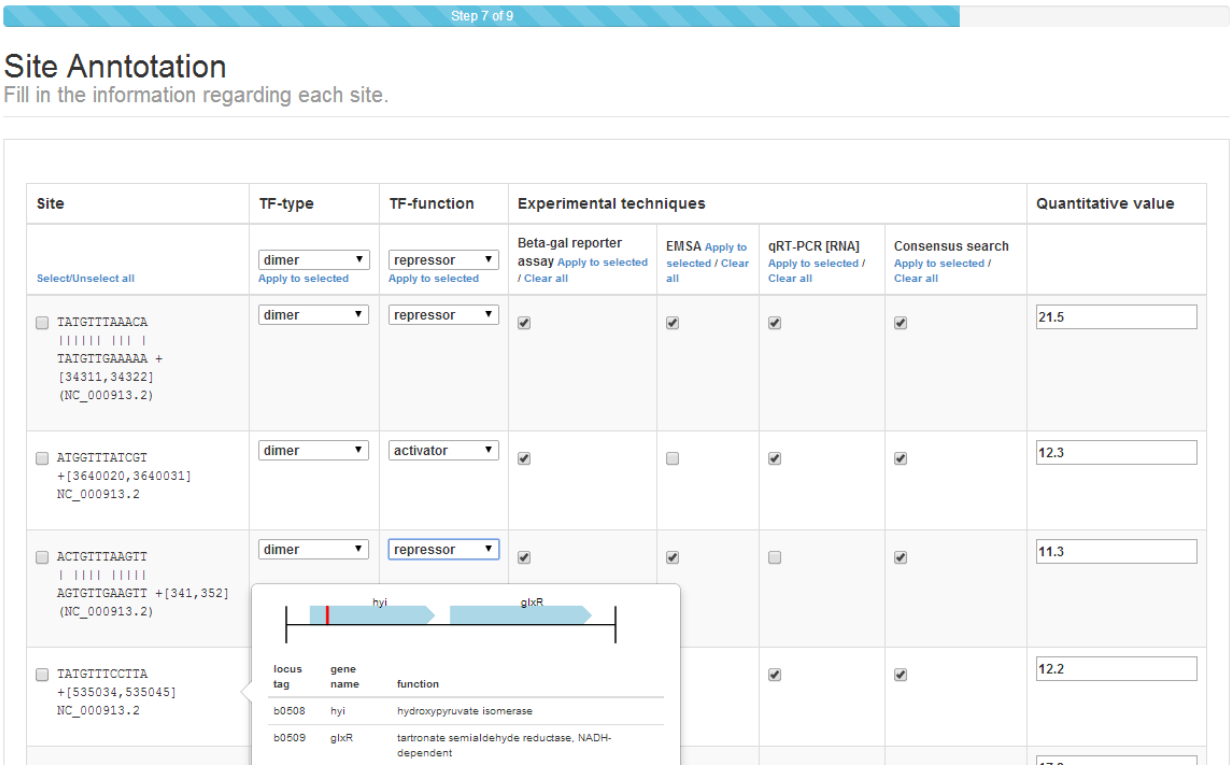
In some cases, especially if using a sequence that is not an exact match to the reported strain, some sites may not be found using an exact search. In this case, the CollecTF submission system will use the available evidence to construct a scoring matrix and search the genome for slightly inexact matches (up to two mismatches away from the reported site). These will be reported in the same way as exact matches and you will be asked to validate them in the same manner.



Step 6: Site annotation

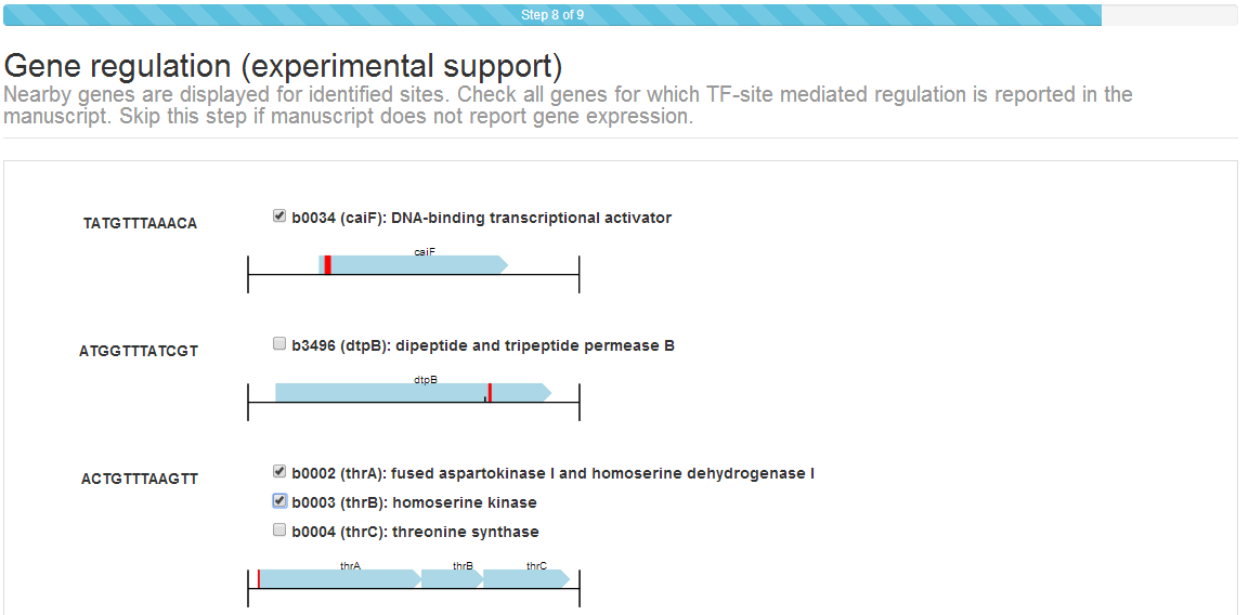
Site annotation step is an essential step for the proper curation of TF-binding site information in CollecTF. During site annotation, specific experimental techniques are matched to individual sites already identified in reference genome. The quaternary structure of the TF when interacting with sites (e.g. dimer), as well as the regulatory mode of TF-binding at each site (e.g. repressor), if known, can also be entered independently for each site. In addition, if quantitative data for sites has been manually entered or mapped from high-throughput data it can also be validated here. The user can select multiple sites using the mouse in combination with the `Shift` key or through the `Select/Unselect all` link to easily assign attributes to several sites at once, using the `Apply to selected` option on each column.

Assigning experimental techniques, TF structure or role independently to each site may require some time, but capturing accurate information on the experimental support and nature of TF-binding sites is the main goal of CollecTF. We therefore kindly request that experimental techniques be completed accurately and that attributes such as quaternary structure be set to default values (`Not specified`) if they cannot be submitted with accuracy. Site annotation can be greatly facilitated by sorting the data before submission, so that sites using similar techniques (or repressed sites, etc.) appear in consecutive order in the `Site Annotation`.



Step 7: Gene regulation

If the manuscript reports experimental evidence for TFmediated regulation of target genes through TFBS, the CollecTF submission system will ask you to specify, for each reported site, which genes have been shown to be regulated by the TF.



Step 8: Curation information

The submission process ends with a final assessment of the curation. You will be asked whether the submission requires review (`Revision required`). Checking this option is indicated in several circumstances. For instance, it is quite possible that no appropriate sequence was identified in NCBI to perform a valid curation. In this case, the curation is marked for revision. The TFBS data is stored, but it will not be linked to a RefSeq sequence until a matching RefSeq record is posted.

You will also be asked whether the curation should be considered for submission to NCBI. Curations will only be considered for submission to NCBI if the sequence for the reported strain is available at NCBI or if a sequence matching the species of the reported strain is *available and at least 90% of the sites you report have been located in the reference RefSeq record as exact matches.*

Multiple curations

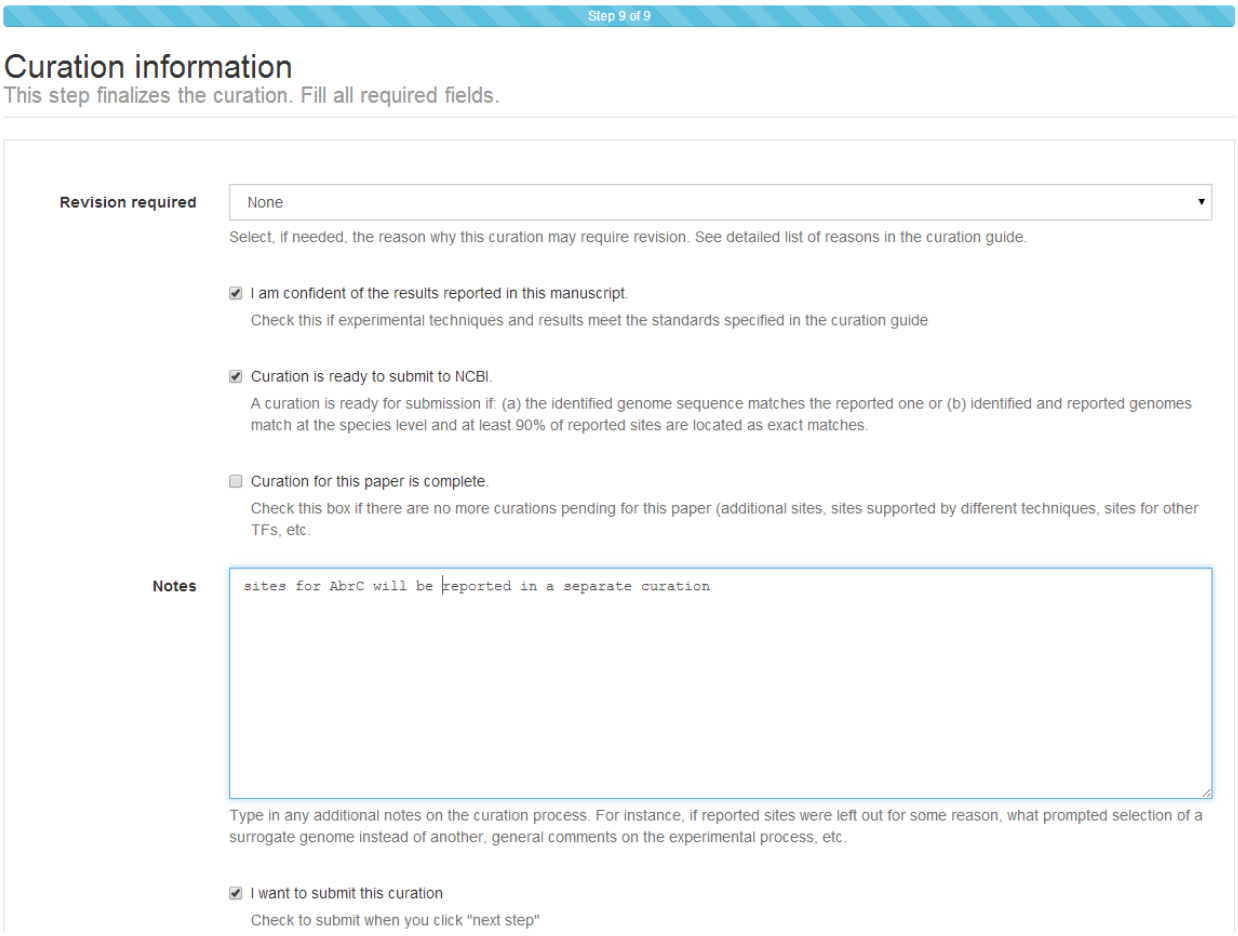
The system also requires that you specify whether the `Curation for this paper is complete`. Do not check this box if, for instance, you want to report additional sites, regulatory modes and/or sources of experimental support in a subsequent curation, or if you are reporting data for more than one TF or species. The CollecTF submission system allows you to submit data from a literature source in as many independent submissions as you require in order to facilitate the `Site Annotation` step in each submission. The submission system will prepopulate fields in subsequent submissions, so that only reported sites and their annotation must be entered anew in each submission (all other fields can, but do not have to, be edited). The same sites can be submitted multiple times (e.g. with different experimental evidence). The CollecTF system will automatically integrate all the data reported for one site.

Revision required

When no genome remotely resembling that of the reported species is available in RefSeq, if sequencing of the genome is still in progress or if the TF of interest is not available in RefSeq, the submission should be tagged as requiring revision. The data for submissions requiring revision is stored in the database, and the CollecTF team periodically assesses whether the conditions for revision are met in order to finalize the submission and link it to RefSeq records.

Final submission

After you check I want to submit this curation and click Next, a summary of your submission will appear for your review. If you spot any errors in the submission, please let us know immediately at collectf@umbc.edu.



Once a submission is completed, the data is uploaded to CollecTF. The submission will be then reviewed by a CollecTF curator and tagged for submission to NCBI. On behalf of the CollecTF team, THANK YOU for your contribution!

CHAPTER 2

High-throughput submission guide

This document is intended as a short annex to the main curation guide, providing specific details regarding the submission of highthroughput data. For further reference on the different aspects of the curation process, please see the CollecTF *Curation submission guide*.

Why?

A significant fraction of the experimental data on transcription factorbinding sites currently being generated relies to more or less extent on highthroughput technologies and, in particular, on ChIPbased methods (e.g., ChIPchip, ChIPSeq). The main goal of CollecTF is to compile and make available through its web interface and through RefSeq genomes as much experimental data as possible on TFbinding sites. The CollecTF highthroughput submission pipeline aims at streamlining the submission of highthroughput data, capturing high throughput specific metadata and incorporating it into highquality annotation for TFbinding sites.

What?

Highthroughput experiments typically generate multiple layers of data. For instance, ChIPSeq experiments generate raw read data, which is mapped to a reference genome. Mapped fragments are typically assigned enrichment values with respect to a control and fed to a peak calling algorithm to identify consistently enriched regions. Authors typically define a minimum threshold for enrichment, and peaks above this threshold are referred to as binding sites. Lastly, researchers may use motif discovery and/or site search algorithms to identify the specific sequence elements targeted by the transcription factor of interest.

CollecTF is not a repository for raw highthroughput data (e.g. ChIPseq reads). We compile only TFbinding sites as defined by the researchers that report them. For ChIP data, this includes peaks above the enrichment threshold defined by the authors as well as specific sequence elements within such bound regions identified by the authors through in silico and/or in vitro methods.

How?

In most highthroughput experiments, both enriched peaks and specific sequence elements are identified through the combination of ChIP protocols with bioinformatics approaches and other experimental sources of evidence. Peaks typically incorporate quantitative enrichment data, which can be transferred to sequence elements identified within the bound region. The CollecTF highthroughput pipeline allows submitting both peak and sequence elements in a single step, and automatically assigns peakassociated data, if available, to sequence elements.

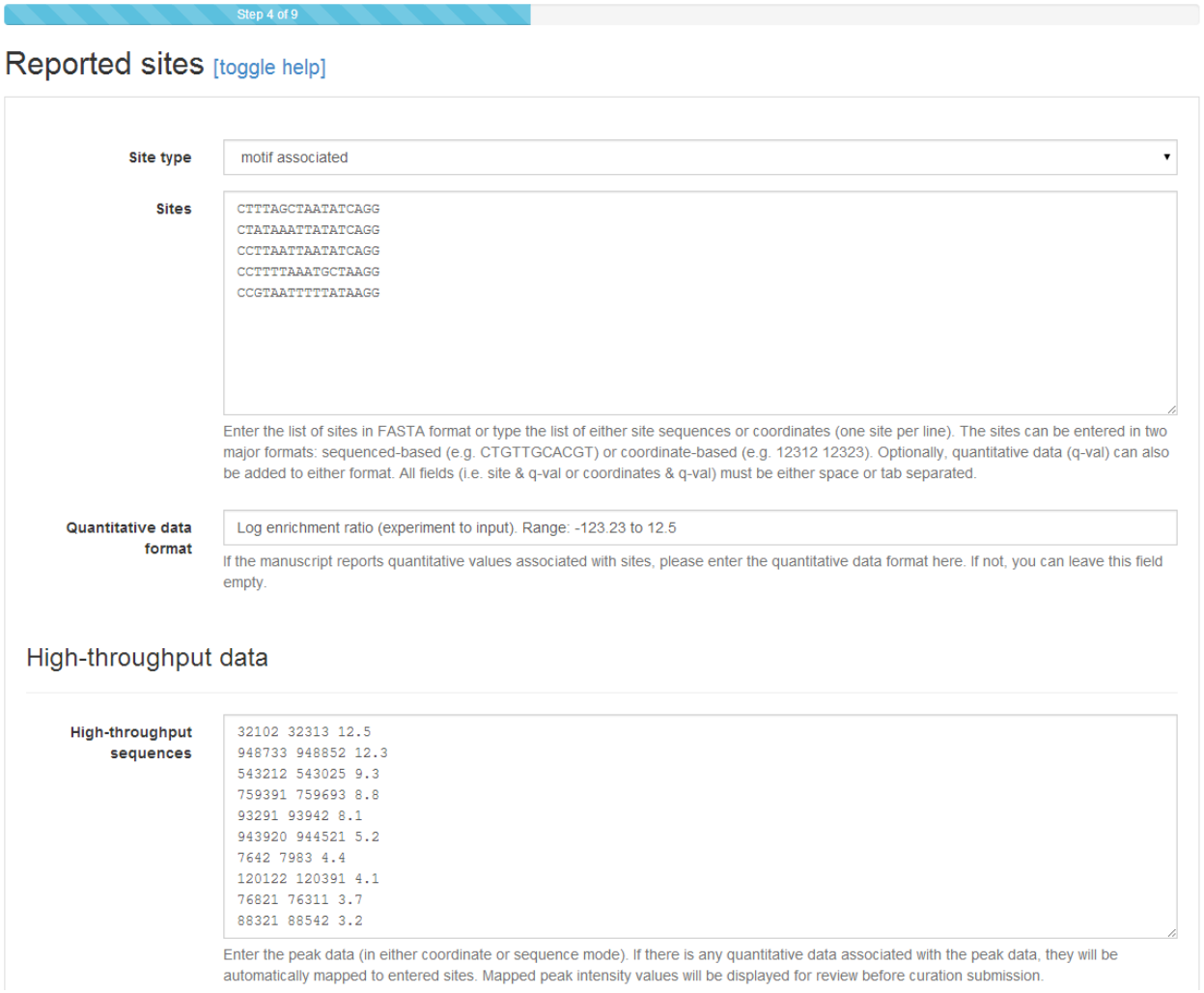
Regulatory mode, additional sources of evidence for specific sites and information on regulated genes can be submitted simultaneously, or may be submitted in a separate curation. CollecTF will seamlessly integrate all available annotation information for TFbinding sites.

The process

Most steps in the CollecTF highthroughput submission process are equivalent to those of normal submissions and the reader is referred to the standard *Curation submission guide* for details.

Entering sites

Beyond making sure to report the accession for the raw highthroughput data in Step 3 (Experimental techniques) through the `High-throughput database accession`, the main difference between standard and high-throughput submissions lies in Step 4 (Reported sites).



The first part of Step 4 is similar to that of standard submissions. Sites (identified sequence elements) can be entered as sequence or coordinates, with or without quantitative data. In highthroughput mode, however, additional space is provided to enter TFbound regions identified through highthroughput methods (e.g. enriched peaks in ChIPseq). These can be again entered as coordinates or sequence, with quantitative data typically appended (tab/space separated) after the last coordinate/base. If entering quantitative data, you will be required to provide brief annotation on its nature and range (e.g. enrichment ratio). Notice that neither field (sites or highthroughput sequences) is strictly required: sites may be submitted without supporting highthroughput data and highthroughput data may be submitted without identified sequence elements.

Detailing highthroughput experiment

Step 4 in highthroughput mode also requires that you enter additional details on the high throughput technique. In particular, two items are required. In `Assay conditions`, you should describe the experimental setup used for the highthroughput step. The aim is to provide a clear description of what was being contrasted (e.g. induced vs. noninduced, wild type vs. mutant) in the highthroughput experiment and its main experimental conditions (e.g. cell growth and isolation, specific strains, definition of control, etc.), so that users browsing the data can easily assess its relevance without needing to read through the entire methodological section.

The `Method notes` section aims at capturing more detail regarding the specifics of the high throughput method. In a ChIPSeq experiment, for instance, it should briefly describe the crosslinking step, the sonication method, immunoprecipitation and crosslink reversion, sequencing, peak calling and motif discovery (if any). Even though a concise synthesis is preferred, direct copying of manuscript methods can be used to define `Method notes`.

The final section of Step 4 for highthroughput asks you to identify the techniques (among those selected in Step 3) that were used to obtain the reported highthroughput data (e.g. enriched peaks). Note that this applies only to the highthroughput data. The techniques used to identify specific sequence elements (sites) can and must be defined in Step 7 (Site annotation).

And that is all. The rest of the highthroughput submission pipeline is equivalent to the standard submission process, and the reader is referred to the general *Curation submission guide* for further details.