TreeLimbs User Guide

Information highlighted in blue is specific to the Cooke Lab TreeLimbs database.

Contents

1. Access

You will need a username and password to access the system. Email

royko@ualberta.ca

to get access. Once you have your username and password, point your web browser to

http://cookelab.biology.ualberta.ca/lims

to get to the main page. The recommended browser is Firefox, through Internet Explorer, Chrome, or Safari should also work.

2. Sample Codes

The TreeLimbs database is sample-centric, so interacting with it involves finding, entering or retrieving data about samples or sets of samples. A four part code is used to identify samples within the database.

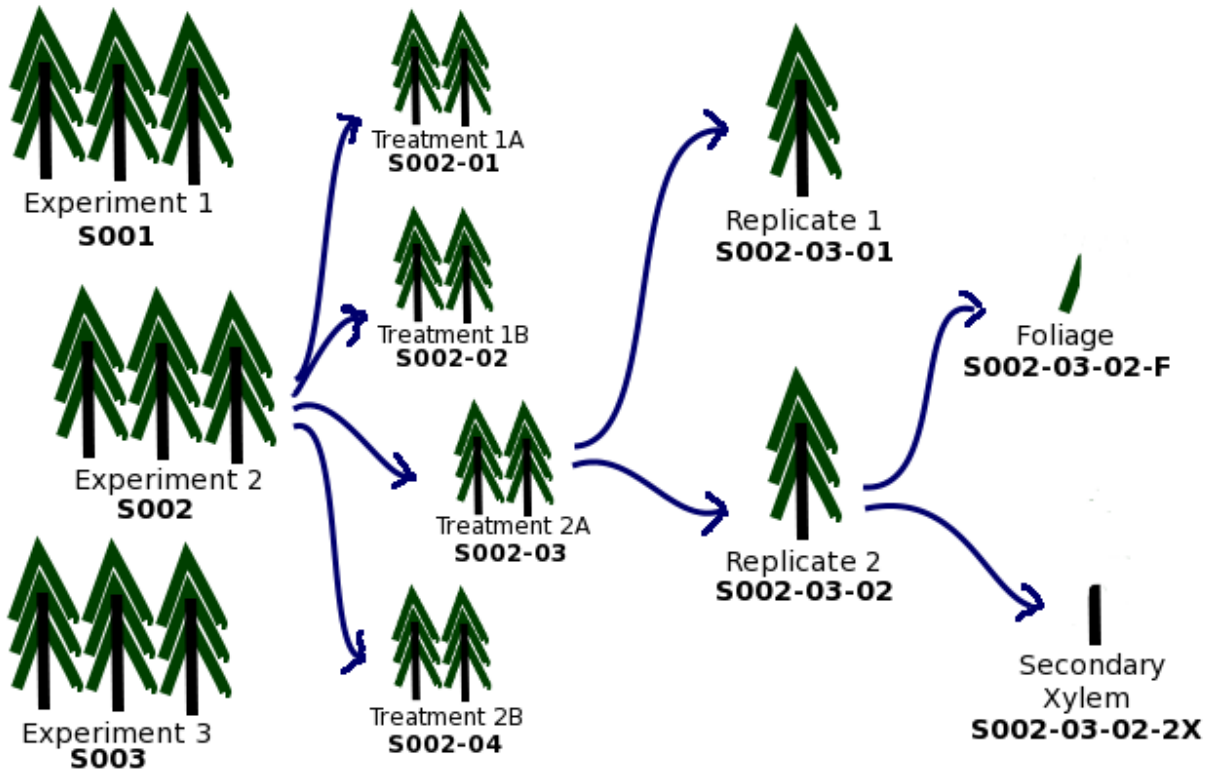


Figure 2.1 Hierarchical nature of sample codes. Experiments are given sequential codes (1 letter for the project + 3 digits). Each experiment is then split into sequentially numbered groups of samples based on the combination of treatments each group receives (2 digits). Within each treatment group, replicates are numbered sequentially (2 digits). Finally, a tissue code (alpha-numeric) is assigned to the end of the code based on the tissue sampled.

Figure 2.1 illustrates the hierarchical nature of the four part code. From left to right, each element of the code denotes an increasingly specific set of samples. Every individual sample code is unique, and yet contains enough detail to determine which experiment the sample belongs to, what treatments it has undergone, and which tree and tissue it came from.

A typical sample code:

S002-03-02-F

The first part, **S002**, denotes the experiment. One letter indicates the project (in this case, S for Spruce), and three digits are a sequential experiment number, automatically generated when the experiment is created.

The second part, **03**, denotes the treatment code. All samples undergoing the same treatments are given the same treatment code. The number of treatment codes will be equal to the product of the number of possible treatments for each experimental factor. For example, if the experimental factors are time of harvest (day 0, day 1, day 2), water availability (well watered, water deficit), and nitrogen availability (high, low), then there will be 3 x 2 x 2 = 12 treatment codes, running from **01** to **12**. The treatment codes are also generated automatically when an experiment is created.

The third part, **02**, denotes the replicate. This can be multiple samples taken from the

same tree (technical replicates), or samples from multiple trees that were subject to the same set of treatments (biological replicates). Note that your statistical analysis will be different depending on whether you use technical or biological replicates. The LIMS system does not intrinsically distinguish between biological and technical replicates.

The last part, **F**, denotes the tissue harvested. Existing tissue codes we use are:

Code	Description
BP	shoot tip
SBP	axillary buds
BU	branch terminal bud lateral stem from the current year's growth
F	foliage from current year's growth
YF	young foliage
2F	foliage from previous year's growth
MF	mature foliage
OF	older foliage
MY	Mycelium (fungal root)
2P	secondary phloem
R	roots
T	unlignified stem from current year's growth
TB	lateral stem from the current year's growth
Tp	primary stem from current year's growth
Ts	secondary stem from current year's growth
1T	lignifying stem, this year's growth
2T	lignified stem, last year's growth
2X	secondary xylem

3. General Layout

The page can be divided into three regions: *menu*, *main content* and *personal information*.

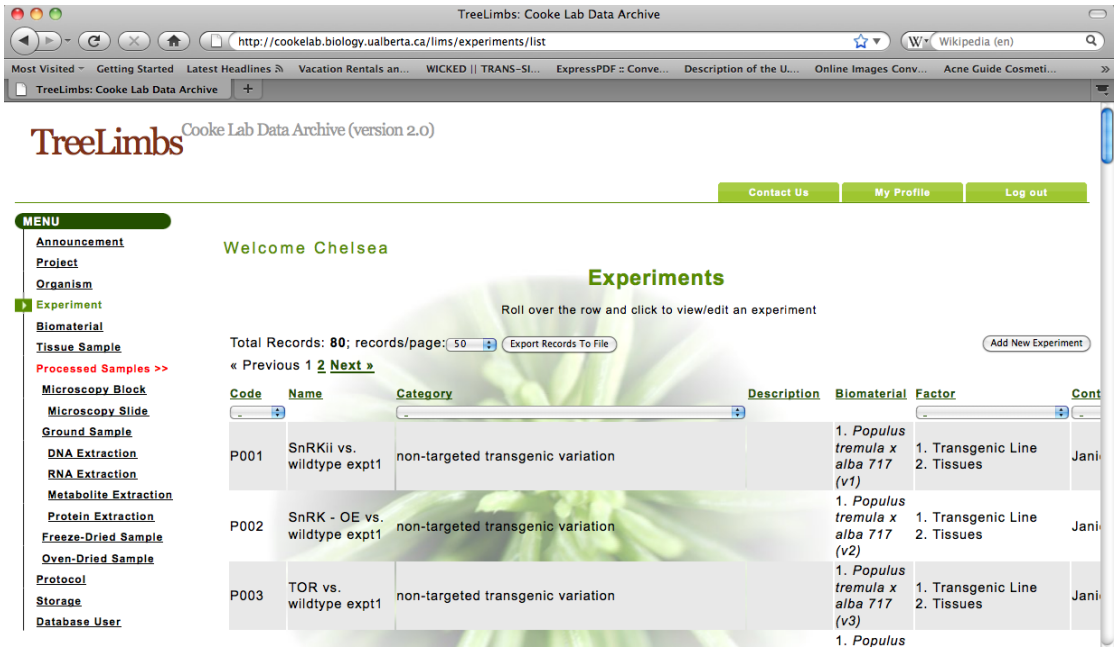


Figure 3.1 A typical view of TreeLimbs.

3.1. Menu

The menu provides a list of the categories of data stored in the database, and allows users to navigate to different pages. A green arrow icon on the left indicates the current menu selection, highlighted in green. Under the *processed samples* heading, primary processes (those performed on unprocessed collected tissue samples) are indented once, and secondary processes (those performed on processed samples) are indented twice. For example, *microscopy block*, *ground sample*, *freeze-dried sample*, and *oven-dried sample* are recorded as the primary processes applied to tissue samples; *microscopy slide* is the secondary process after the *microscopy block* is created.

3.2. Main Content

Data are displayed in this section, and each row represents one record.

3.2.1. Record Navigation

The top left corner shows the total number of records in the database which meet the selected filter criteria, and the drop down box allows users to select the number of records to be displayed in one page. If there is more than one page of records, users can navigate to different pages through the "<< Previous" and "Next >>" hyperlinks, or to a specific page number.

3.2.2. Sort and Filter

Data can be sorted by clicking on the hyperlink of a green column name. To sort the data in a descending order, click on the column name for the second time. Data can also be filtered by selecting information on the dropdown boxes below the column names. Multiple filtering can be achieved by selecting information from two or more dropdown boxes. Due to the complexity of the database, the sort and filter functions may only be available on certain columns.

3.2.3. View and Edit Record

Each row in a table represents a record. Click on a record to open a new pop-up window displaying the details of this record. In the detail view, users may hide or display sections of the page through the "hide" or "show" hyperlinks. The *Edit* button at the bottom of the detail view allows users to edit the information associated with this record. More information may be displayed than can be edited. For example, viewing a sample record will display information about the experiment as a whole, but only allow editing of information specific to the sample selected. To edit information about the experiment, the user must select the experiment's record from the **Experiments** page.

3.2.4. Add Record

To add a new entry to the database, click on the *Add* button on the top right corner of the records table, and follow the instructions in the new pop-up window. (See sections below for details on adding new experiments and samples.)

3.2.5. Export Records to File

To create a text file containing the information displayed in a table, click the *Export Records to File* button near the top of the page. A small popup window will appear while the file is being generated, and your web browser will probably give you the option of saving or opening the file. You can import the resulting file into most spreadsheet applications as a tab-delimited text file. The first line of the output file will consist of the column names describing the values in each column on subsequent lines of the output file. The output file may contain additional information which can only be viewed on the detail page (see section 3.2.3) in the web interface. Note that your filters will apply to the exported data, but all records in the filtered list will be exported. Even though the list may require several pages in your browser, all the data will be stored as one file.

3.3. Personal Information

Three green tabs at the top of the page allow users to contact the administrator of the database through email, edit their personal information, and log out of TreeLimbs.

3.3.1. My Profile

The *My Profile* tab displays personal information about the current user. Users may edit their first name, last name, email address, and password in a pop-up window by clicking on the *Edit* or *Change Password* buttons.

3.3.2. Log out

The *Log Out* tab clears out information stored by your web browser about the current session.

4. Planning Experiments (Adding New Experiments)

Before adding a group of samples to TreeLimbs, you will need to add an experiment to which the samples will be linked. You will need to know the project that this experiment falls under, and what experimental factors are going to be manipulated. The data you will enter will be common to all samples in the experiment. On each screen, fields that are

required will be marked with a red asterisk. Other fields are optional.

Figure 4.1 Required fields are marked with red asterisks.

To begin, select *Experiments* from the menu of the left to see the list of experiments in the system. Then click the *Add New Experiment* button near the top right.

4.1 Step 1: Description of Experiment

***Project:** If your project is not in the list, you will need to contact an Administrator. Otherwise, select the project, and the next available sequential experiment number within that project will automatically be assigned.

***Contact person**: Select the primary contact for the experiment from the list of users.

***Code**: An experiment code will be created automatically by TreeLimbs. (Administrators can change this number if necessary, but the system will not allow duplicate experiment codes.

Category: This field will give you hint of existing categories after you start typing. If your experiment falls into one of the listed categories, select it from the list, to make it easier to search for all experiments within a category. If your experiment falls into a category as specified by MIAME (Minimum Information About a Microarray Experiment; http://www.mged.org/Workgroups/MIAME/miame.html; http://www.plantmethods.com/content/2/1/1), use this. Example MIAME experiment types include: "normal-versus-diseased comparison", "time course", and "dose response". Additional examples of categories we've used previously in the Cooke lab include: "non-targeted transgenic variation", "Organism part comparison design", and "developmental or differentiation design".

Name: Add a short name for the experiment which describes the main factor(s) being tested. This should be concise, and searchable.

Description: Describe the conditions of the experiment and any additional useful information about your experiment. This should be detailed enough to allow someone else to replicate the experiment.

Comment: Any other comments about your experiment that don't belong elsewhere can be placed here.

Start date: The start date for the experiment as a whole. (Harvest dates will be captured when creating sample entries.)

Location: Where is the experiment taking place, i.e. specific field site, room number of growth chamber, greenhouse, etc.

Click the "*Save & Proceed*" button.

4.2 Step 2: Experiment Factors

If you are running a time course experiment, select Time Point or Date of Collection as your first factor. This will keep your sample codes sequential as you harvest. Subsequent

factors should be listed in order of application. For example, if you will be applying various levels of fertilizer throughout the experiment, and inoculating plants just before harvest, list fertilizer before inoculation in the factor list.

Factor: Choose a factor from the drop-down list to auto-fill the **Name** and **Description**, or if none apply, select New, and enter the following:

*__Name__: Factor name, e.g. Day Length, Nitrogen Level, Transgenic Line, etc.

Description: Detailed information about the factor (e.g., what is 'water deficit' or 'short day'?)

Variables: This list of treatments will be generated as the samples are entered.

Tissue is always the last factor (even if only one tissue is being harvested), since it generates the last part of the sample code. (See section 2 for a discussion of tissue codes.)

Note: It is important to plan experimental factors carefully, and to ensure that all factors are entered into the database *before* sample records are created. Because the codes assigned to samples are automatically generated based on the experimental factors, it is not possible to modify the list of factors after sample collection has started, and sample codes have been generated in the database. On the other hand, comments can be added to the experiment at any point. For example, if a power outage causes the lights in the growth chamber to be off for a prolonged period, this is worth noting even after the experiment is under way. (To add comments to an existing experiment, find the experiment in the **Experiments** list, click its line to see the detailed view, and click the *Edit* button at the bottom of the page.)

Click the "*Save & Proceed*" button.

4.3 Step 3: Biomaterial

You will need to specify the biomaterial you are using, even if it is not a variable factor in the experiment. The biomaterial is the combination of species, family/clone, source, and handling of the plants to be used in the experiment. If you are using the same biomaterial as a previous experiment, select it from the drop-down list. As you mouse over items in a list, a hint box will appear, listing the experiments in which the biomaterial was used. (If the hint box is partially obscured, try resizing your window.) For additional detail, you can switch to the main browser window and refer to the list of biomaterials without closing the experiment entry page. If you select an existing biomaterial, the **Organism Description**, **Details of Culture and Collection**, and **Comments** will be auto-filled with the information from the existing biomaterial record. Verify this information carefully. If you need to make changes, tick the "Modify..." checkbox, and the fields will be unlocked.

If none of the biomaterials listed are apropriate, select New, and enter the following fields:

***Species**: Select a species from the drop-down list. If you are using a species not listed, contact an Administrator to create it for you.

*__Clone/Family__: If you are using a transgenic line, enter the clone or family.

***Organism description**: e.g. size, height, diameter, age, seed source, genetic information, etc.

***Details of culture or collection**: e.g. date when material arrived, potting conditions, etc.

Comments: any additional information about the biomaterial

Click the "*Save & Proceed*" button.

A verification page will display all information for the experiment. Here, you can edit the general information or experimental factors. You can also click *Delete Experiment* at the bottom of the page if you prefer to start over. When you are happy, click the *OK* button at the bottom of the page to save the experiment in the database.

Once the experiment record has been created, you will need to contact an Administrator to delete it.

5. Sample Collection (Adding New Tissue Samples)

Once the experiment is created, you are able to create new tissue samples. Select **Tissue Samples** from the menu on the left hand side, and click the *"Add New Tissue Samples"* button near the top right.

5.1 Step 1: General Information

***Experiment**: Choose the experiment from the drop-down list (A hint box will pop up giving details of the experiment if you don't remember the code. If the hint box is partly obscured, try resizing your window. To get rid of the tool-tip, click the close window link at the bottom of the box.)

Note: The **Harvest Date**, **Harvest Time**, **Harvest People**, and **Comment** fields are optional. However it is recommended that you fill in these fields for more useful information about your samples.

Storage Location: This field will provide you with hints after a few seconds once you start typing. If you see your storage location already in the list, simply select it from the list. Not only will this save you some typing, but it will also make it easier to search the database for all items stored in a particular location (when spacing and capitalisation are uniform).

If any of these fields are not the same for all samples you are about to create, select the values which apply to most of the samples. You will be able to change the fields one by one for individual samples in Step 3.

Click the "*Save & Proceed*" button.

5.2 Step 2: Define Factors: Treatments and Tissues

Factors: Each of your factors (i.e. biomaterials, treatments, time of collection, etc) will be defined in this section.
You will specify all the treatments for each factor. Click the *"More ..."* button to add treatments. For example, for factor Time Point, you will click the *More Time Points* button to add each individual treatment, such as Day 1, Day 3, Day 7, etc.

For each treatment, you need to enter a name and (optionally) a description.
Name: It is a good idea to keep your treatment names clustered alphabetically. This will make using the filter functions in other parts of TreeLimbs simpler. For example, use "Day

1", "Day 3", "Day 7", and "Week 1", "Week 2", "Week 3" as opposed to "1st Day", "2nd Week", etc. The alphabetical filter list will then list the "Day x" treatments in a group, and likewise the "Week x" treatments, rather than having "1st Day" interleaved with "1st Week", etc.
Description: Here, give a detailed description of what a treatment name like "short day", or "low nitrogen" means.

For factor **Tissue**, the **Code** field will provide hints of existing codes used when you start typing. If you are creating a new code, enter its description.

The information you enter on this page will be used to generate the sample codes described in Section 2.

Click the "*Save & Proceed*" button.

5.3 Step 3: Replicates and Modification

The treatment codes generated on this page will be based on the information from the previous page, and will form the second part of the resulting sample codes (experiment-treatment-replicate-tissue). The total number of codes should be equal to the number of treatments for each factor (except Tissue) multiplied together. Tissue is not included in the treatment code because it forms the last part of the sample code.

For example, if you have factors Time Point (with treatments Day 1, Day 3, Day7), Nitrogen Level (High and Low), Inoculation (Wounded, Not Wounded), and Tissue (Foliage, Root), then the number of treatment codes would be 3 Time Points x 2 Nitrogen Levels x 2 Inoculations = 12. The meaning of each generated code is explicitly listed in the second column on this page.

Select the number of replicates for each combined treatment. When you do, several fields will appear, automatically populated from the information given in Step 1. If you need to modify or add to the information for a particular treatment, you can do so here.

Limbs will create a sample code based on your entered information.

Click the "*Save & Proceed*" button.

5.4 Step 4: Replicates Information

On this page, you can enter a **Tree Number**, **Replicate Description**, and **Comments** specific to each replicate.

Click the "*Save & Proceed*" button.

5.5 Step 5: Confirmation

This screen lists all the information you entered. You can make any modifications (which are specific for individual samples) to the information you entered previously. If you accidentally created more replicates than necessary, or if some samples were lost or

destroyed, you can delete individual samples by clicking the check box beside the sample code. Otherwise, if everything is to your liking, click the *Save* button at the bottom of the page.

5.6 Pooling tissue samples

From the **Tissue Sample** list, you also have the option of pooling tissue samples. Some reasons for doing this may be that you do not have enough tissue in one sample to perform the extraction you need, that you want to analyse more samples than you have space for in your equipment, or that you want to get a "wholistic" picture of a plant or portion of a plant for which component tissues were sampled individually. It is possible to pool samples both within and among experiments. To pool samples, click the *Pool Tissue Samples* button near the top right of the **Tissue Sample** list.

Select a sample: Select the first sample from the drop-down list. The experiment and treatment information for the selected sample will be copied to the pooled sample.
Highlight multiple samples: You can select multiple samples to pool with the first sample from this list by using Click+Drag, Shift+Click, Ctrl+Click, or Command+Click. Once you have selected all the samples involved, click on the *"Generate code for pooled samples"* button. A code will appear in the field below.

Modify the code for pooled sample: This is not necessary, unless you wish to change the default code. The default code replaces the replicate number with "Z#", where # is a sequential digit. If samples from multiple experiments are involved in the pool, a Z will also appear in the experiment portion of the sample code.

Click the "*Save & Proceed*" button to continue.

On the second page, you can modify the information copied from the first source sample you selected. This information will be stored with the new pooled sample.

Click the "*Save & Proceed*" button to save the information and create the sample.

You will then be shown the new detail page for the pooled sample record. Verify that all the information is correct. If it is not, click the *Edit Sample* button at the bottom of the page and make corrections. If all is well, click the *OK* button instead.

Pooled samples will be displayed in the **Tissue Sample** list just as regular samples are. You can bring up the details, edit, or delete them by clicking on the record just as with regular samples. Additional information will be displayed indicating which samples were pooled to generate the new samples. This allows you to trace back the history of all the components of the pooled sample.

5.7 Deleting tissue samples

To delete a tissue sample, find the sample in the **Tissue Sample** list, and click its row to bring up the detail view. At the bottom of the page, you should see several buttons. If a *Delete Sample* button is available, you can click it to delete the tissue sample. You will be asked for your password to confirm that you want to delete the sample. If you need to remove a lot of samples from the system, it is easier to contact the LIMS administrator and

bribe them with chocolate.

If a *Delete Sample* button is not visible at the bottom of the sample detail view, it is likely because the sample has already been processed. You will first need to delete the entry for the processed sample (e.g. a ground sample or microscopy block) before deleting the tissue sample. This is a safety precaution to prevent accidental deletion of information which has already been used as the basis of further work. Again, if you legitimately need to do this, it may be easier to contact the LIMS administrator.

6. Sample Processing (Adding New Processed Samples)

6.1 Microscopy

6.1.1 Microscopy Blocks

Processing samples is divided into several stages. The procedure is similar to creating new samples during sample collection. If you are creating **Microscopy Slides**, first you will need to create a **Microscopy Block**. From the left hand menu, select **Microscopy Block**, and click the *Add Microscopy Block* button. Most of the fields are self-explanatory. The first page refers to all the blocks created from this sample. You will be able to enter details specific to different blocks on the second page.

Select a sample: Note that you can only process samples that have not been marked as "Finished". If the sample you would like to use is gray in the drop-down list, it has probably been previously marked as consumed. (If this is an error, select **Tissue Samples** from the left hand menu, find the sample, and click to see the detail view. Click on the *Edit Sample* button at the bottom of the detail view. This will allow you to change the "Finished" tag.)

Processed Date & **Processed Person:** If not all the blocks were created on the same day or by the same person, select the values which apply to most of the blocks. You can change the values for individual blocks one by one on the second page. Another strategy is to create one set of blocks for one date/person, then go through the process again to create another set of blocks for the second date/person.

Processed Protocol: If you are using a protocol which is not in the list, please upload a copy. Select **Protocol** from the left hand menu, and click the *Add New Protocol* button. This will allow you to select a file saved on your computer with a description of the protocol, and upload it to the TreeLimbs server. (You do not have to leave the "Add Microscopy Blocks" page in order to upload a protocol. If your new protocol does not show up in the drop-down list when you come back to this page, try reloading by right-clicking on the page and selecting "Reload" from the pop-up menu.)

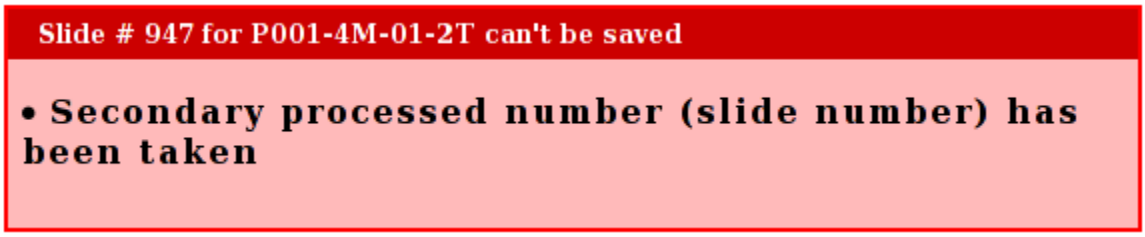
Comments: These comments will be copied into the comment field for *all* the blocks. (You can make comments about individual blocks on the second page.)

Click the *Save and Proceed* button near the bottom of the page to go to page 2. On page 2 you can change the **Comments**, **Process Date**, and **Processed Person** for individual blocks. You can also indicate whether the sample is consumed. If you mark the sample as consumed, it will be grayed out in lists of samples for processing, and will not show up as being in storage. When you are happy, click the *Save* button.

6.1.2 Microscopy Slides

Once you have made **Microscopy Blocks**, you can make **Microscopy Slides** by selecting **Microscopy Slide** from the left and menu and clicking the *Add Microscopy Slide* button near the top right. Again, the first page requests information pertaining to *all* the slides, and you can modify the **Comments**, **Quality**, **Stainer**, **Date Stained**, **Stained With**, **Sectioner**, **Date Sectioned**, **Section Type**, and **Embedding Material** fields individually for each slide on the second page. On the second page, you can also indicate whether the specified microscopy block has been consumed.

Slide Numbers are assigned automatically to be sequential by default. You may change the **Slide Number** on page 2, but if there is a conflict (multiple slides from the same block , the system may give you an error message.



Simply change your slide number, and all will be well. The number must be an integer.

6.2 Ground Samples and Extractions

6.2.1 Ground Samples

Gound Samples are currently the basis of most of our extractions. You need to create **Ground Samples** before you can create **DNA/RNA/Metabolite/Protein Extraction** entries. Select **Ground Samples** from the left hand menu, and click the *Add Ground Samples* button. As before, the first page requests information pertaining to *all* the samples, and you can edit fields for individual samples on subsequent pages.

Select Sample(s): You can only create ground sample entries based on tissue samples which have already been entered into the system. If the samples you wish to grind are not in the list, you first need to create tissue sample entries from the **Tissue Samples** page. You can select multiple samples to process at once from this list by using Click+Drag, Shift+Click, Ctrl+Click, or Command+Click. Note that you can only process samples that have not been marked as "Finished". If the sample you would like to use is gray in the drop-down list, it has probably been previously marked as consumed. (If this is an error, select **Tissue Samples** from the left hand menu, find the sample, and click to see the detail view. Click on the *Edit Sample* button at the bottom of the detail view. This will allow you to change the "Finished" tag.)

Processed Date & **Processed Person:** If not all the samples were ground on the same day or by the same person, select the values which apply to most of the blocks. You can change the values for individual samples one by one on the second page. Another strategy is to create one set of samples for one date/person, then go through the process again to create another set of blocks for the second date/person.

Processed Protocol: If you are using a protocol which is not in the list, please upload a

copy. Select **Protocol** from the left hand menu, and click the *Add New Protocol* button. This will allow you to select a file saved on your computer with a description of the protocol, and upload it to the TreeLimbs server. (You do not have to leave the "Add Ground Samples" page in order to upload a protocol. If your new protocol does not show up in the drop-down list when you come back to this page, try reloading by right-clicking on the page and selecting "Reload" from the pop-up menu.)

Storage: This field will provide you with hints after a few seconds once you start typing. If you see your storage location already in the list, simply select it from the list. Not only will this save you some typing, but it will also make it easier to search the database for all items stored in a particular location (when spacing and capitalisation are uniform).

Comments: This field will be copied to *all* ground sample entries you create. You can modify the comments for individual entries on subsequent pages.

Clicking *Save and Proceed* will take you to page 2. On page 2 you can change the **Comments**, **Storage**, **Process Date**, and **Processed Person** for individual ground samples. If you have ground up all of the original tissue for a particular sample code, mark the tick box indicating that the tissue sample is consumed. When you are happy, click the *Save* button.

6.2.2 DNA/RNA/Metabolite/Protein Extractions

You can only create **Extraction** entries based on **Ground Sample** entries. Select your **Extraction** type from the left hand menu, and click the *Add Extractions* button near the top right of the list. As before, the first page requests information pertaining to *all* the samples, and you can edit fields for individual samples on subsequent pages.

Select Sample(s): At present, you can only create extraction sample entries based on ground samples which have already been entered into the system, since extractions are considered a secondary processing step. You can select multiple samples to process at once from this list by using Click+Drag, Shift+Click, Ctrl+Click, or Command+Click. Note that you can only process samples that have not been marked as "Finished". If the sample you would like to use is gray in the drop-down list, it has probably been previously marked as consumed. (If this is an error, select **Ground Samples** from the left hand menu, find the sample, and click to see the detail view. Click on the *Edit Sample* button at the bottom of the detail view. This will allow you to change the "Finished" tag.)

Extracted Date & **Extracted Person:** If not all the samples were created on the same day or by the same person, select the values which apply to most of the samples. You can change the values for individual samples one by one on the second page. Another strategy is to create one set of samples for one date/person, then go through the process again to create another set of samples for the second date/person.

Extracted Protocol: If you are using a protocol which is not in the list, please upload a copy. Select **Protocol** from the left hand menu, and click the *Add New Protocol* button. This will allow you to select a file saved on your computer with a description of the protocol, and upload it to the TreeLimbs server. (You do not have to leave the "Add Extractions" page in order to upload a protocol. If your new protocol does not show up in the drop-down list when you come back to this page, try reloading by right-clicking on the page and selecting "Reload" from the pop-up menu.)

Storage: This field will provide you with hints after a few seconds once you start typing. If you see your storage location already in the list, simply select it from the list. Not only will this save you some typing, but it will also make it easier to search the database for all items stored in a particular location (when spacing and capitalisation are uniform).

Comments: These comments will be copied into the comment field for *all* the samples. (You can make comments about individual samples on the second page.)

Click *Save and Proceed* near the bottom of the page to go to page 2. On page 2 you can change the **Comments**, **Storage**, **Extracted Date**, and **Extracted Person** for individual samples. You can also indicate whether the sample is consumed. If you mark the sample as consumed, it will not show up as being in storage. There are additional fields where you can optionally specify the **Initial Amount** and **Amount Left**, **Spectrophotometry A260/ A280 Ratio**, and **Spectrophotometry Concentration**. When you are finished, click the *Save* button.

6.3 Freeze-Dried Samples

To create entries for lyophised samples, select your **Freeze-Dried Samples** from the left hand menu, and click the *Add Freeze-Dried Samples* button near the top right of the list. As before, the first page requests information pertaining to *all* the samples, and you can edit fields for individual samples on subsequent pages.

Select Sample(s): At present, you can only create freeze-dried sample entries based on tissue samples which have already been entered into the system, since freeze-drying is considered a primary process, like grinding and microscopy block creation. You can select multiple samples to process at once from this list by using Click+Drag, Shift+Click, Ctrl+Click, or Command+Click. Note that you can only process samples that have not been marked as "Finished". If the sample you would like to use is gray in the drop-down list, it has probably been previously marked as consumed. (If this is an error, select **Tissue Samples** from the left hand menu, find the sample, and click to see the detail view. Click on the *Edit Sample* button at the bottom of the detail view. This will allow you to change the "Finished" tag.)

Processed Date & **Processed Person:** If not all the samples were created on the same day or by the same person, select the values which apply to most of the blocks. You can change the values for individual samples one by one on the second page. Another strategy is to create one set of samples for one date/person, then go through the process again to create another set of blocks for the second date/person.

Processed Protocol: If you are using a protocol which is not in the list, please upload a copy. Select **Protocol** from the left hand menu, and click the *Add New Protocol* button. This will allow you to select a file saved on your computer with a description of the protocol, and upload it to the TreeLimbs server. (You do not have to leave the "Add Freeze-Dried Samples" page in order to upload a protocol. If your new protocol does not show up in the drop-down list when you come back to this page, try reloading by right-clicking on the page and selecting "Reload" from the pop-up menu.)
Comments: This field will be copied to *all* freeze-dried sample entries you create. You can modify the comments for individual entries on subsequent pages.

Clicking *Save and Proceed* will take you to page 2. On page 2 you can change the **Comments**, **Process Date**, and **Processed Person** for individual freeze-dried samples. If

you have used all of the original tissue for a particular sample code, mark the tick box indicating that the tissue sample is consumed. When you are happy, click the *Save* button.

6.4 Oven-Dried Samples

We do not currently use **Oven-Dried Samples** in our lab, but the procedure for creating TreeLimbs entries for them is similar to that for **Freeze-Dried Samples**. See section 6.3.

7. Physical Sample Storage: Finding and Moving Samples

If you select **Storage** from near the bottom of the left hand menu, you will find a list of all storage locations, and all sample codes which are marked in the database as being in storage (i.e., have been entered, but have not been marked as consumed). You can find all the samples which can be found in a particular freezer box by filtering the **Storage** column. Conversely, if you would like to find where a particular sample is stored, you can filter the **Sample Code** column. (This information is also accessible through the appropriate **Tissue Sample/Ground Sample/Extraction** list.)

Near the top right of the storage list is a *Move Samples* button. Clicking this pops up a page which allows you to move multiple samples from one location to another. For a single sample, this can be done by finding the sample in the appropriate list, clicking it to bring up the detail view, clicking the *Edit* button, and changing the storage location. However, if an entire box of samples needs to be moved from one freezer to another, the "Sample Relocation" page offers an easier way. You can either select samples from the list of all samples in storage (which comes up by default), or you can filter the list by selecting a storage location from the **Search samples from a rack** drop-down list. You can select multiple samples to move by using Click+Drag, Shift+Click, Ctrl+Click, or Command+Click. If a sample you expect is not in the list, it may have been marked as consumed in the database. (If this is an error, find the sample in the appropriate list - Tissue/Ground/ Extraction, etc. - and click it to see the detail view. Click on the *Edit Sample* button at the bottom of the detail view. This will allow you to change the "Finished" tag.)

Once you have selected the samples you wish to move, simply enter their new location, and click the *MOVE* button.

8. Administrative Tasks

There are a number of tasks which only "Administrator" users can do. The intent is to maintain a "controlled vocabulary", i.e., to have some items, such as **Organism** and **Project** names, have only one spelling within the database. This makes certain columns easier to filter, and allows us to have drop-down lists of choices in some data entry fields, so that you can select, rather than having to type in the information.

Only Administrators can add new **Projects**, **Organisms**, and **Database Users** (who are not organisms). Except for **Database Users**, the process is trivial. When adding **Database Users**, you will be asked to indicate which projects the user participates in. Check all that apply. If a user is not marked as participating in a project, they cannot view or add data related to that project. There are also three **Role**s to choose from - Guest, Staff, or Administrator. Guests can only view data, but not add new entries. This might be

useful for demonstrating the LIMS, or for outside read-only access. Staff is the default role, allowing users to view, enter, edit, and delete data for the projects in which they participate. Finally, Administrators can do all that Staff can do, plus the tasks described here.