SURF

SeqUence Repository and Feature detection

ADMINISTRATOR AND USER GUIDE

Version 1.0

Eddie Iannuccelli

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Revision table:

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Major modifications</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>February 9th 2005</td>
<td>First release</td>
<td>C Dantec, E Iannuccelli</td>
</tr>
<tr>
<td>1.0</td>
<td>October 26 2005</td>
<td>DNA update</td>
<td>E Iannuccelli</td>
</tr>
</tbody>
</table>

Acknowledgment:
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1 Background

cDNA library sequencing has become a popular method to investigate genes through their transcripts, it also provide material for further expression and regulation studies (microarrays). Such libraries are usually sequenced using automated technique that produce huge amount of chromatogram data. These traces are to be processed before any exploitation and the resulting nucleotide sequences can be compared to the public nucleotide databases in order to extract new knowledge. As high flow sequencing can be done through dedicated robots, resulting data storage and processing is not always simple to solve.

2 Introduction

Pertinent nucleotide sequence production commonly involve several dedicated bioinformatic softwares (sequence base calling, vector detection, etc.) : SURF (SeqUence Repository and Feature detection) provide an integrated solution from chromatogram (or other popular format) data storage to cloned insert detection. SURF hosts sequences and manipulates them through two different concepts:

- **library**
  A library is the biological entity that hosts sequences, SURF uses libraries to describe features to detect (vector and adapters) from sequences.

- **Batch**
  A batch is the loading organizational entity which contains sequences that share a format and undergoes a common process (import into system, feature detection and statistic calculation). SURF supports 4 batch format types : Chromatogram, DbEST, EMBL and Fasta.

SURF is developed by SIGENAE team (AGENAE INRA project), SIGENAE extends no warranties of any kind, either expressed or implied.
3 Architecture

3.1 Data structure
SURF provides a multi-instance architecture that allows a complete data isolation between instances. This can be useful to manage various data isolation types (per user, per species, per format, per access level, etc.). An instance is made of:

- A file system data root: it is a directory that manages file type data (chromatograms, FASTA files, DbEST files etc.) using a dedicated hierarchical structure.
- A Postgresql database that contains added value data (features, statistics, etc.) and provides standard SQL searching features.

Instance data root directory path and Postgresql name and location (connection string) are controlled by a dedicated instance section in the main SURF configuration file (surf.conf).

3.2 Application structure
The SURF application is essentially made of Perl programs, they are located in the bin and cgi-bin directories. Here is a basic surf directory as it comes from a fresh installation.

IMPORTANT:
The SURF regexpmodel*.conf files are templates that are copied into instance data root at the instance creation time. So any changes to theses files will only be effective in the future new instances. If you want to change an instance data extraction behavior, you MUST update the dedicated regexpmodel*.conf files stored in the instance data root.
3.3 One instance structure

SURF data is a set of SURF instances. Each instance is made of a Postgresql database and a rooted file system. Here is one instance file system description:

- **banks** directory contains various FASTA files used during contamination detection.

- **Batch** directory contains one directory per loaded batch (named with database batch ID). Each batch contains various subdirectories:
  - `attach`: static HTML files (statistics)
  - `chromatogram`: chromatogram trace files
  - `dbentry`: EMBL or DbEST individual records
  - `load`: files for database load
  - `logs`: batch level log files
  - `processing`: output from various programs
  - `sequence`: individual FASTA sequence files (with their quality files for chromatogram batches)

- **Inbox** directory contains batch source files (zip and gz).
- **Library** directory contains library features fasta files.
- **Workflow_logs** directory contains instance level logs.

3.4 User interface

The user interface of SURF is made of a web graphical user interface plus command line programs. Command line programs are used to load sequences, start feature detection and statistical calculation. Web GUI is mostly designed for consultation and data export. Web interface also exists for adding sequence batch, start feature detection and statistical calculation but since some problems occur with files created by apache user (permissions problems), today these interfaces are considered as experimental.

3.5 User access control

Command line user access is to be managed by operating system (file system permission). If you do not want to manage web user access, SURF can be fully open so everyone is considered as admin user (useful for personal machine install). Admin user can add and update libraries, start sequence load, feature detection and statistical calculation through web interface (experimental!). If you want to manage web site access control, SURF provides a Typo3 CMS integration system based on instance name mapping onto Typo3 user groups. By example, to access a "project1" SURF instance, following conditions must be true:

- user must be logged in typo front end
- user must belong to a typo3 front end user group called "project1" or user name = "admin"
In some circumstances, some instances are to be public (no login in Typo3) while other are still private. SURF provides a simple way to grant anonymous access to instance while others are still access controlled. Simply prefix instance name with a dedicated string (default is `public`, stored in `surf.conf`) and that's it. Let's say that prefix string equals `public`, `publicProject1` instance will be available to any anonymous user.
4 Installation

4.1 Prerequisite's

4.1.1 Perl modules

The following Perl modules must be installed:

<table>
<thead>
<tr>
<th>Module</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaseLib</td>
<td></td>
</tr>
<tr>
<td>Carp</td>
<td></td>
</tr>
<tr>
<td>CGI</td>
<td></td>
</tr>
<tr>
<td>DBI</td>
<td></td>
</tr>
<tr>
<td>Class::Struct</td>
<td></td>
</tr>
<tr>
<td>Config::Simple</td>
<td></td>
</tr>
<tr>
<td>Exporter</td>
<td></td>
</tr>
<tr>
<td>File::Spec</td>
<td></td>
</tr>
<tr>
<td>Getopt::Long</td>
<td></td>
</tr>
<tr>
<td>IO::File</td>
<td></td>
</tr>
<tr>
<td>Pod::Text</td>
<td></td>
</tr>
<tr>
<td>Pod::Usage</td>
<td></td>
</tr>
<tr>
<td>POSIX &quot;:sys_wait_h&quot;</td>
<td></td>
</tr>
<tr>
<td>Time::localtime</td>
<td></td>
</tr>
<tr>
<td>XML::Simple</td>
<td></td>
</tr>
</tbody>
</table>

Perl tested version is Perl 5.8

4.1.2 Postgresql database

SURF uses one Postgresql database per instance, so you need a Postgresql account with all privileges on databases you will use (see instance creation section later in this document). Postgresql tested version is 5.3.4

4.1.3 Third party programs

SURF uses some third party programs to produce data, here is the list.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>version</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phred</td>
<td>chromatogram basecalling, 0.020425.c</td>
<td></td>
</tr>
<tr>
<td>(phred/phrap/consed)</td>
<td>Checks that the phredpar.dat file supports your chemistry and sequencing machine in order to avoid phred default parameters usage for basecalling (or empty sequences).</td>
<td></td>
</tr>
<tr>
<td>Crossmatch</td>
<td>Vector and features detection in sequences (can be crossmatch manyreads for a lot of sequences)</td>
<td>0.990329</td>
</tr>
<tr>
<td>RepeatMasker</td>
<td>Low complexity zones detection 00.3.0</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>Statistic calculation</td>
<td>2.0.0</td>
</tr>
</tbody>
</table>

Check these programs manually before using SURF because it will be more difficult to investigate third party programs problems from SURF integration.

4.1.4 Integrated programs

Most SURF integrated programs are Perl programs and are embedded in the bin directory. The only exception is a C program (called 'polySmurtsh') used for single nucleotide stretches detection. The polySmurtsh distributed binary is 32 bits/Linux (you can recompile it using sources available in the surf/src directory).
4.2 Files and directories

You can install SURF in your home public_html directory or in another dedicated folder on webserver. The $home/public_html directory installation can be attractive in a personal usage way. Here are the two installation type procedures.

4.2.1 Installing SURF in your home directory

NB: In the following procedure, all paths are relative to your home directory.

1. Copy the distribution (entire surf directory) in your public_html directory (the extracted directory should be called ‘surf, do not rename it!).

2. Check that public_htm/surf directory permissions are set to 755. If not, run:
   
   ```
   chmod 755 ./public_html/surf
   ```

3. Check that public_html/surf/cgi-bin directory AND its content (CGI files) permissions are set to 755. If not, run:
   
   ```
   chmod -R 755 ./public_html/surf/cgi-bin
   ```

4. Check that public_html/surf/temp directory permissions are set to 777 in order to grant apache user with write access. If not, run:
   
   ```
   chmod 777 ./public_html/surf/temp.
   ```

5. Create a symbolic link of public_html/surf/cgi-bin in public_html/cgi-bin directory called surf
   
   ```
   cd ./public_html/cgi-bin
   ln -s ../surf/cgi-bin surf
   ```

6. Update the surf.conf file paths and URLs sections as following. Do not forget to replace $HOME with your home directory path and $user_name with your Linux account name.
   
   ```
   ;--paths
   systemroot=$HOME/public_html/surf
   default_dataroot=$HOME/public_html/surf/data
   temp=$HOME/public_html/surf/temp
   ;--URLs
   server_url=http://myserver
   htdocs_path_url=~/$user_name/surf/htdocs
   cgi_path_url=~/$user_name/cgi-bin/surf
   style_url=http://myserver/~/$user_name/surf/htdocs/style.css
   ```
### 4.2.2 Installing SURF in a dedicated directory

**NB:** In the following procedure, we will install SURF in the /project directory, all paths are absolute.

1. Copy the distribution (entire `surf` directory) in the target directory `/project`, the extracted directory should be called 'surf' so do not rename it!

2. Check that `/project/surf` directory permissions are set to 755. If not, run:

   ```bash
   chmod 755 /project/surf
   ``

3. Check that `/project/surf/cgi-bin` directory AND its content (CGI files) permissions are set to 755. If not, run:

   ```bash
   chmod -R 755 /project/surf/cgi-bin.
   ``

4. Check that `/project/surf/temp` directory permissions are set to 777 in order to grant apache user with write access. If not, run:

   ```bash
   chmod 777 /project/surf/temp
   ``

5. Update the apache configuration file to set an alias of the `/project/surf/` directory and to make `/project/surf/cgi-bin` a server side execution directory. Here are the `httpd.conf` updates to make.

   ```
   Alias /surf "/project/surf"
   <Directory "/project/surf">
       Options Indexes FollowSymLinks
       AllowOverride None
       Order allow,deny
       Allow from all
   </Directory>
   <Directory "/project/surf/cgi-bin">
       Options FollowSymLinks ExecCGI
       SetHandler cgi-script
       AllowOverride None
       Allow from all
       Order allow,deny
   </Directory>
   ```

6. Update the `surf.conf` file as following:

   ```
   ;--paths
   systemroot=/project/surf
   default_dataroot=/project/surf/data
   temp=/project/surf/temp
   
   ;--URLs
   server_url=http://myserver
   htdocs_path_url=/surf/htdocs
   cgi_path_url=/surf/cgi-bin
   style_url=http://myserver/surf/htdocs/style.css
   ```
4.3 Configuration files update

In previous section, we updated the Paths and URLs surf.conf file sections. In order to fit your server configuration (third party programs location, database connection, etc.), you must check and update the following surf.conf parameters groups (a group is prefixed by a ;) from the [default] surf.conf section. Here are the groups you need to check and update:

- Shell
- Postgresql
- Phred
- Crossmatch
- RepeatMasker
- Statistics by R
- Typo3 integration
- web interface

For detailed informations about these parameters, see Configuration files section.

In addition, you can change input data parsing behavior using regexpmodel*.conf files. The section 4.4 provides configuration files detailed description.
## 4.4 Configuration files

SURF configuration files are text files using the Windows INI style structure. A section is enclosed in square brackets and keys are structure as KEY=VALUE.

### 4.4.1 Surf.conf

This file contains one default section that provides main informations for global system and one section per instance containing instance specific parameters (paths, style, etc.)

#### 4.4.1.1 Default section

<table>
<thead>
<tr>
<th>group</th>
<th>name</th>
<th>description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell</td>
<td>zip_command</td>
<td>Path to zip program</td>
<td>/usr/bin/zip</td>
</tr>
<tr>
<td></td>
<td>unzip_command</td>
<td>Path to unzip program</td>
<td>/usr/bin/unzip</td>
</tr>
<tr>
<td></td>
<td>gzip_command</td>
<td>Path to gzip program</td>
<td>/usr/bin/gzip</td>
</tr>
<tr>
<td></td>
<td>tar_command</td>
<td>Path to tar program</td>
<td>/bin/tar</td>
</tr>
<tr>
<td></td>
<td>gunzip_command</td>
<td>Path to gunzip program</td>
<td>/usr/bin/gunzip</td>
</tr>
<tr>
<td>paths</td>
<td>systemroot</td>
<td>Path to surf directory</td>
<td>/project/surf</td>
</tr>
<tr>
<td></td>
<td>default_dataroot</td>
<td>Path to default data root directory</td>
<td>/project/surf_data</td>
</tr>
<tr>
<td></td>
<td>temp</td>
<td>Path to temp directory</td>
<td>/project/surf_temp</td>
</tr>
<tr>
<td>URLs</td>
<td>server_url</td>
<td>Server URL</td>
<td><a href="Http://myserver">Http://myserver</a></td>
</tr>
<tr>
<td></td>
<td>htdocs_path_url</td>
<td>htdocs directory URL part</td>
<td>/surf/htdocs</td>
</tr>
<tr>
<td></td>
<td>cgi_path_url</td>
<td>cgi-bin directory URL part</td>
<td>/surf/cgi-bin</td>
</tr>
<tr>
<td></td>
<td>style_url</td>
<td>Full URL to CSS style sheet</td>
<td><a href="http://myserver/surf/htdocs/style.css">http://myserver/surf/htdocs/style.css</a></td>
</tr>
<tr>
<td>Postgresql</td>
<td>psql_command</td>
<td>Path to psql program</td>
<td>/usr/bin/psql</td>
</tr>
<tr>
<td></td>
<td>createdb_command</td>
<td>Path to Postgresql createdb program</td>
<td>/usr/bin/createdb</td>
</tr>
<tr>
<td></td>
<td>dbhost</td>
<td>Postgresql host name</td>
<td>localhost</td>
</tr>
<tr>
<td></td>
<td>dbport</td>
<td>Postgresql TCP/IP port</td>
<td>5432 (pg default value)</td>
</tr>
<tr>
<td></td>
<td>dbname</td>
<td>Postgresql user name</td>
<td>john</td>
</tr>
<tr>
<td></td>
<td>dbpassword</td>
<td>Postgresql password</td>
<td>joe_passwd</td>
</tr>
<tr>
<td>Phred</td>
<td>phred_command</td>
<td>Path to phred program</td>
<td>/usr/bin/phred</td>
</tr>
<tr>
<td></td>
<td>phred_parameter_file</td>
<td>Path to phredpar.dat file (phred parameter file)</td>
<td>/usr/phredpar.dat</td>
</tr>
<tr>
<td></td>
<td>trace_extension</td>
<td>List of accepted chromatogram file extensions</td>
<td>SCF, ABI, AB1, ABD</td>
</tr>
<tr>
<td>Crossmatch</td>
<td>crossmatch_command</td>
<td>Path to crossmatch program</td>
<td>/usr/bin/cross_match</td>
</tr>
<tr>
<td></td>
<td>crossmatch_minscore</td>
<td>Crossmatch minscore parameter</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>crossmatch_minmatch</td>
<td>Crossmatch minmatch parameter</td>
<td>10</td>
</tr>
<tr>
<td>group</td>
<td>name</td>
<td>description</td>
<td>Example</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>RepeatMasker</td>
<td>repeatmasker_command</td>
<td>Path to RepeatMasker program</td>
<td>/usr/bin/RepeatMasker</td>
</tr>
<tr>
<td>Statistics by R</td>
<td>R_bin_path</td>
<td>path to R program</td>
<td>usr/bin/R</td>
</tr>
<tr>
<td></td>
<td>R_quality_length</td>
<td>Basecalling quality length to analyse</td>
<td>1000</td>
</tr>
<tr>
<td>Typo3 integration</td>
<td>typo_activated</td>
<td>Typo3 user access control activation</td>
<td>true</td>
</tr>
<tr>
<td></td>
<td>typo_dsn</td>
<td>DBI dsn string to typo3 mysql database</td>
<td>DBI:mysql:database =typo_database_name;host=typo_host_name</td>
</tr>
<tr>
<td></td>
<td>typo_db_username</td>
<td>Typo3 database user name</td>
<td>bobby</td>
</tr>
<tr>
<td></td>
<td>typo_db_password</td>
<td>Typo3 database user password</td>
<td>lapointe</td>
</tr>
<tr>
<td></td>
<td>typo_login_url</td>
<td>URL to typo3 front end login page</td>
<td><a href="http://myserver/typo_site">http://myserver/typo_site</a></td>
</tr>
<tr>
<td></td>
<td>typo_public_instance_prefix</td>
<td>Anonymous access for instances having name starting by this value.</td>
<td>public</td>
</tr>
<tr>
<td></td>
<td>typo_grouped_instance_prefix</td>
<td>Instances name starting by one of these prefix will check user presence in prefix Typo3 group (rather than in instance Typo3 group).</td>
<td>project1,project2</td>
</tr>
<tr>
<td></td>
<td>typo_instance_translation</td>
<td>Regular expression used to modify each instance name before Typo3 group mapping. This mechanism is run before public instance and grouped instance functionalities.</td>
<td>'s/sigenae/Agenae/'</td>
</tr>
<tr>
<td>web interface</td>
<td>displayed_name</td>
<td>Default sequence alias type to use for sequence name display, can be 'default,accession_number','accession number secondary','published accession number','est name','genbank gi' or 'surf name'.</td>
<td>default</td>
</tr>
<tr>
<td></td>
<td>selector_mode</td>
<td>Default pagination type for web interface:full show all pages, light show only previous and next page. light is useful for instance with a lot of sequences (&gt; 100 000)</td>
<td>full</td>
</tr>
<tr>
<td></td>
<td>export_limit</td>
<td>maximum number of item (chromatograms, fasta files, etc.) per download (preserve your server health)</td>
<td>100</td>
</tr>
</tbody>
</table>
**WARNING:** before update the following parameters, be sure to understand their impact!

<table>
<thead>
<tr>
<th>Group</th>
<th>Name</th>
<th>Description</th>
<th>Default Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makequality and polySmursh</td>
<td>makequality_param</td>
<td>Drive the way SURF emulates quality files for sequences without basecalling quality files (Cf quality emulation section)</td>
<td>'30:10,530:25 15</td>
</tr>
<tr>
<td></td>
<td>makequality_param_mRNA</td>
<td>Same as before but only for mRNA sequence type</td>
<td>'10:30 30'</td>
</tr>
<tr>
<td></td>
<td>makequality_param_genomicDNA</td>
<td>Same as before but only for genomic DNA sequence type</td>
<td>'30:25,530:25 15'</td>
</tr>
<tr>
<td>Vector detection</td>
<td>vector_detection_zone_start</td>
<td>Cf insert detection section</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>vector_detection_zone_end</td>
<td>Cf insert detection section</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>vector_detection_gap</td>
<td>Cf insert detection section</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>vector_feature_name</td>
<td>Expected feature name for cloning vector (cf library web interface)</td>
<td>vector</td>
</tr>
<tr>
<td></td>
<td>insert_feature_name</td>
<td>Feature name of insert</td>
<td>insert</td>
</tr>
<tr>
<td></td>
<td>adapter_feature_name</td>
<td>Expected feature name for cloning adapters (cf library web interface)</td>
<td>adapter</td>
</tr>
<tr>
<td></td>
<td>use_lucy</td>
<td>Use lucy to detect insert</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lucy_as_insert</td>
<td>Use lucy result as SURF insert</td>
<td></td>
</tr>
<tr>
<td>Status calculation</td>
<td>mingoodbase</td>
<td>Cf status calculation section</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>contaminants</td>
<td>Cf status calculation section</td>
<td>colli, yeast, mitoch ondry, ribosome, univec</td>
</tr>
<tr>
<td>Others</td>
<td>prim5value</td>
<td>Orientation field value for 5 prim sequences</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>prim3value</td>
<td>Orientation field value for 3 prim sequences</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>minqual</td>
<td>Basecalling phred value under which a nucleotide is considered as a bad base.</td>
<td>20</td>
</tr>
</tbody>
</table>
### 4.4.1.2 Instance section

Each instance is driven by a section named as instance, instance section must provide the following parameters:

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>dataroot</td>
<td>Path to directory containing instance data</td>
<td>/project/surf/data/myinstance</td>
</tr>
<tr>
<td>dbname</td>
<td>Postgresql instance database name</td>
<td>SURF_myinstance</td>
</tr>
<tr>
<td>species</td>
<td>Provide a valid NCBI Taxonomy Database species name to use for RepeatMasker program and must be contained in the RepeatMasker repeat database.</td>
<td>'sus scrofa'</td>
</tr>
</tbody>
</table>

**NB : It is possible to override any default section parameter in an instance section.**

Example:

You want the pig instance to use a dedicated style sheet, to use accession_number as default displayed name and to use 20 as crossmatch_minscore parameter instead of the 15 global value. To do that, just add the following lines in the pig section of surf.conf file:

```
style_url=http://sigena/surf/htdocs/pig.css
displayed_name='accession number'
crossmatch_minscore=20
```

### 4.4.2 regexpmodel_chromato.conf

This file contains Perl regular expressions used to extract data (library, plate, etc ...) from chromatogram filenames. These regular expressions are grouped into models (in square brackets). Regular expression model name is one of the parameters of batch load command line. By default, a default section must exists to provide data extraction rules from chromatogram file names. As an example, here are the default regular expressions provided:

```
library='s/^\(.4\)\.*$/\1/e'         ->: extract four characters from start
plate='s/^\(.9\)\.*$/\1/e'          ->: extract nine characters from start
strand='s/^.*_\(\)\..*$/$1/e'      ->: extract one character between_ and .
clone='s/^\(.8\)\.(\[5\]).*$/$1.$2/e'-> merge characters [1..8] and [9..13]
row='s/^\[10\](\w).*$/\1/e'    -> extract the eleventh character
col='s/^\[12\]\\d(20).*$/\1/e'  -> extract [13,14] characters
```

All these values can be empty if necessary.

### 4.4.3 regexpmodel_dbest.conf

This file contains Perl regular expressions used to extract data (sequence names,library, etc ...) from DbEST file content. These regular expressions are grouped into models. Regular expression model name is one of the parameters of batch load command line. By default, a default section must exists to provide data extraction rules from DbEST files. Here are the default regular expressions provided. In normal circumstances, you should not update that file because DbEST file format is quite stable ;)

```
[id='s/\^\dBEST\^\dId:\s*+\(\S+)\$/\1/e'
est_name='s/\^\EST\^\name:\s*+\(\S+)\$/\1/e'
genBank_acc='s/\^\GenBank\^\Acc:\s*+\(\S+)\$/\1/e'
genbank_gi='s/\^\GenBank\^\gi:\s*+\(\S+)\$/\1/e'
close='s/\^\Clone\^\Id:\s*+\(\S+)\$/\1/e'
mol_type='s/\^\DNA\^\type:\s*+\(\S+)\$/\1/e'
creation_date='s/\^\Entry\^\Created:\s*+\(\S+)\$/\1/e'
```

SURF User's Guide
4.4.4 regexpmodel_embl.conf

This file contains Perl regular expressions used to extract data (sequence names, clone, etc ...) from EMBL file content. These regular expressions are grouped into models. Regular expression model name is one of the parameters of batch load command line. By default, a default section must exists to provide data extraction rules from EMBL files. Here are the default regular expressions provided. In normal circumstances, you should not update that file because EMBL file format is quite stable ;)

```perl
library='s/^dbEST\sid:.*$/\1/e'
library_name='s/^Lib\sName:\.*$/\1/e'
tissue_type='s/^(?:Tissue\stype|Organ):.*$/\1/e'
develop_stage='s/\sDevelop\ ss\stage:\.*$/\1/e'
seq_exclusion='/AGENAE/i'
plate='s/Plate:.*Row:.*Column:.*$/\1.\2.\3/e'
```

**WARNING**: `tissue_type` and `develop_stage` are NOT missing one double quote, they are multi lines features so closing double quote can be found in further line.

4.4.5 regexpmodel_fasta.conf

This file contains Perl regular expressions used to extract data (sequence names, clone, etc ...) from FASTA files header description (1). SURF assumed FASTA files to have the following header line structure:

```
>ADDKK_45  bla bla bla bla bla bla bla bla bla bla ...
```

**Seq. Name**  **description (1)**

One or many whitespace characters

Theses regular expressions are grouped into models. Regular expression model name is one of the parameters of batch load command line. By default, a default section must exists to provide data extraction rules.

```perl
[default]
library='s/.*(4)\*$/\1/e'
strand='s/.*(9)\*$/\1/e'
clone='s/.*(8)\*$/\1.\2/e'
row='s/.*(10)\*$/\1/e'
col='s/.*(12)\*$/\1/e'
```
5 User guide

5.1 Managing libraries

As the library drive the way the sequences are 'built', it is the best place to describe the expected features. Basic features are vector and adapters, but it is possible to add any kind of feature since a Fasta file is available. SURF uses Crossmatch software to detect features, this software needs two parameters (per feature):

- \textit{minmatch} : the minimum number of contiguous nucleotide to match before trying to extend match
- \textit{minscore} : the minimum score to get in order to keep the hit

Feature can be freely named but some values are reserved for putative insert detection, we will call them 'construction features' later in this document. The following names (case sensitive) are used to detect putative insert (other features are also used, see the Insert detection chapter for more details):

- 'vector' is used to designate the cloning vector feature.
- any feature starting by 'adapter' (ex : adapter5, adapter3)
- any feature ending by a '!' (ex : primer1!, primerA!)

Here is the library list screen, header provide criteria selection. In the list, the library name link provide a detailed library view page. The \textit{update} link and \textit{Add} button are reserved to system administrators.
## One library detailed view

<table>
<thead>
<tr>
<th><strong>Available informations are :</strong></th>
<th><strong>Comments</strong></th>
<th><strong>Features to detect</strong></th>
<th><strong>Custom properties</strong></th>
<th><strong>Library features combinations</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong>: The library name</td>
<td></td>
<td><strong>Name</strong>: vector; <strong>Minmatch</strong>: 10; <strong>Minscore</strong>: 15; <strong>File</strong>: vector 2640-2650; E5; 2667-2742; E3;</td>
<td><strong>No data</strong></td>
<td><strong>Library features combinations</strong></td>
</tr>
<tr>
<td><strong>Code</strong>: string used to connect a sequence to its library during data loading</td>
<td></td>
<td><strong>Minmatch</strong>: 9; <strong>File</strong>: adapter; <strong>Subfeatures</strong>: lucy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Description</strong>: any descriptive data</td>
<td></td>
<td><strong>Minscore</strong>: 15; <strong>File</strong>: lucy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Comments</strong>: any comment</td>
<td></td>
<td><strong>Subfeatures</strong>: formatted as START-END:NAME ex: 10-15:EcoR1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Features to detect</strong></td>
<td></td>
<td><strong>Custom properties</strong>: tissue_type and organ data only for imported DbEST libraries.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Features to detect</strong></td>
<td></td>
<td><strong>Library feature combinations</strong>: link to library statistical page (also available from batch statistics, see statistical calculation chapter for details).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2 Managing batches

A batch contains sequences that shipped together during the primary data import from chromatogram, Dbest, Embl or fasta raw data. Batch sequences are also processed together for further processing (features detection and statistic calculation). The batch list screen provide following data:

- ID (SURF internal identifier)
- name
- number of sequences
- a link to see batch sequences: bring to sequence list screen
- a link to see batch statistics
- a pop up menu plus a ‘Go’ button to see processing logs (data loading, feature detection and statistic processing)

Here is a screen capture of the batch management tool.

<table>
<thead>
<tr>
<th>ID</th>
<th>Name</th>
<th>Nb Seq.</th>
<th>Sequences</th>
<th>Statistics</th>
<th>Processing log</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>test-fasta</td>
<td>20</td>
<td>test-fasta sequences</td>
<td>test-fasta statistics</td>
<td>Sequence load</td>
</tr>
<tr>
<td>2</td>
<td>test-chromato</td>
<td>30</td>
<td>test-chromato sequences</td>
<td>test-chromato statistics</td>
<td>Sequence load</td>
</tr>
<tr>
<td>3</td>
<td>dbest1</td>
<td>1836</td>
<td>dbest1 sequences</td>
<td>dbest1 statistics</td>
<td>Sequence load</td>
</tr>
<tr>
<td>4</td>
<td>fasta2</td>
<td>0</td>
<td>fasta2 sequences</td>
<td>Not available</td>
<td>Sequence load</td>
</tr>
<tr>
<td>5</td>
<td>aze</td>
<td>0</td>
<td>aze sequences</td>
<td>Not available</td>
<td></td>
</tr>
</tbody>
</table>

Here is a screen capture of the batch management tool.
The batch statistics provide the following information:

- Sequence number
- Null sequence count
- Count per status (see status chapter)
- Count per contaminant (see status chapter)
- Batch libraries features combinations
- Full length sequence list
- Sequence length histogram
- Sequence Nb good base histogram (see status chapter)
- Basecalling quality histogram
Batch libraries features combinations

Full length sequence screen
5.3 Managing sequences

Sequence is the SURF central entity, it is accessible through the following list screen which provide various selection criteria and export capabilities.

Sequence can be displayed using various names:

- **SURF name** (chromatogram file name, DbEST ID field, EMBL ID field or FASTA ID)
- **Accession number**
- **Genbank Gi**
- **Est name**

NB:
Selecting an alias in this screen will restrict results to sequence having such alias. By example, if you select 'Display sequence name using accession number', chromatogram sequences without accession number alias will simply be hidden.
One sequence details
5.4 Loading sequence

Sequences are not loaded into SURF one by one but all together through a batch. A batch is a set of sequences that share a common format and, most of the time, were created through the same production process (same automatic sequencer machine or/and run, same PCR reaction set, etc.). SURF does not use batch format only as a formatting specification, format also change the way SURF manages sequence versioning. Each batch format is loaded through dedicated command line programs, here are the various batch format SURF can load.

```
| NB: command line programs are located in the SURF /bin directory. |
```

Programs will ask for common parameters:

- **instance**: SURF instance name
- **batch name**: batch name, note that SURF checks batch existence using batch name so if you want to override existing batch with another one, use the same name.
- **library**: existing library for batch sequence to link to (feature detection). Default value is 'autodetect' which tries to parse library name from batch source (file name or content).
- **regular expression model name**: corresponding regexpmodel_*conf section to use for parsing (see configuration files). Default is `default` section.
- **Workflow step number to start**: step rank to run, any previous step is ignored (useful to "debug" some huge batches). Default value is 'autodetect' which means that any previous failed step will be used as value.
- **Address to send email notification when finished**: e-mail address to which success or failure notifications are sent.
5.4.1 Chromatogram batch

5.4.1.1 Launching the program

Use the `run_chromato.sh` to start loading batch, here are the specific parameters:

- **zip file path**: path to the zip file containing the chromatograms. This file is a zip archive, not a gzip archive. It is very important that archive **DO NOT CONTAINS DIRECTORIES**, all chromatogram files **MUST** be at the zip archive root level (this constraint force file name unicity). Any zip archive sub-directory will be simply ignored, SURF will load SCF, ABI, AB1, ABD extension zip files only (see `surf.conf`).
- **default sequence type**: default molecule type for standalone sequence (not linked to a library). Usually, when a sequence is linked to a library, the library molecule type is used as template for sequence molecule type. Default is cDNA.
- **plate name**: string to put as plate name, default is 'autodetect' which means that SURF will try to parse plate name from batch source (file name or content).
- **strand**: string to put as strand value, default is 'autodetect' which means that SURF will try to parse strand value from batch source (file name or content).
- **clone name auto detection**: tells SURF to parse batch source for clone name, default is 'yes'.
- **row and column values auto detection**: tells SURF to parse batch source for row and col values, default is 'yes'.

5.4.1.2 Sequence name version rules

SURF tries to keep memory of sequence modifications so a version value is inserted in the sequence file name, before the file extension (ex: the file name of fourth version of `scac0001.a.04_5.scf` sequence will be `scac0001.a.04_5.4.scf`). Any chromatogram sequence input is assumed to be a new version sequence (n+1) except when loading already existing batch (check based on the batch name). Typically, if you load a batch using an existing batch name, if sequence name are conflicting, SURF will overwrite existing batch sequences with new chromatogram data but will keep previous version values.

5.4.1.3 Libraries management:

SURF tries to link chromatogram sequences to their libraries at loading time. For each sequence, 'library' regular expression is run onto chromatogram file name, resulting string is used for a library 'code' field lookup. If a library match, the link is made, it will bring valuable informations at feature detection time.
5.4.2 DbEST batch

5.4.2.1 Launching the program
Use the `run_dbest.sh` to start loading a batch. The only specific parameter is `gz file path`, path to a tar.gz file containing DbEST files.

5.4.2.2 Sequence name and versions
SURF uses `dbEST Id` tag as sequence name. Other DbEST identifiers (`EST name, GenBank Acc and GenBank gi`) are stored as sequence aliases. If a DbEST candidate sequence name already exists in SURF and if the two FASTA sequences are strictly equals, the candidate sequence is excluded from load.
If a DbEST candidate sequence name corresponds to an existing chromatogram sequence alias, the candidate DbEST sequence is loaded ONLY when its FASTA nucleotidic sequence does not equal any existing chromatogram sequence 'publication' feature FASTA. Publication feature FASTA sequence can be customized by a 'FASTA' tag stored in `publication` feature 'comment' field (Ex: `fasta (20..150,AATTT,200..210)`). This can be useful to avoid sequence duplication (private chromatogram version + public version return through DbEST batch). When 'publication' feature provides a FASTA comment, FASTA comment overrides 'publication' feature start and end positions. If a new sequence version is loaded, '.version' string is appended at the end of the sequence file name (Ex : `XXX.2`).

5.4.2.3 Libraries management
Libraries are parsed from DbEST files and new libraries are added. If a library already exists in the system, the library `custom properties` are updated using DbEST library records `dev_stage` and `tissue_type` tags.

5.4.2.4 Basecalling quality management emulation
Special '.type' file are also generated in order to emulate quality at sequence extraction time. For each sequence, the program creates a file named `[sequence_name].fasta.type` and writes the `molecule_type` value on the first line. That file will be used later to generate quality on the fly during sequence quality extraction (see `get_sequence.pl` program).

5.4.2.5 DbEST -> SURF fields mapping

<table>
<thead>
<tr>
<th>DbEST file fields</th>
<th>SURF</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>DbEST Id</code></td>
<td>SURF sequence name</td>
</tr>
<tr>
<td><code>est_name</code></td>
<td>'est_name' type alias</td>
</tr>
<tr>
<td><code>genbank_acc</code></td>
<td>'genbank_acc' type alias</td>
</tr>
<tr>
<td><code>genbank_gi</code></td>
<td>'genbank_gi' type alias</td>
</tr>
<tr>
<td><code>Clone Id</code></td>
<td>Sequence clone</td>
</tr>
<tr>
<td>DNA type</td>
<td>Molecule type</td>
</tr>
<tr>
<td><code>SEQUENCE (1)</code></td>
<td>Sequence nucleotidic sequence</td>
</tr>
<tr>
<td>Plate Row Column</td>
<td>Sequence plate, row, and column</td>
</tr>
<tr>
<td>Entry Created</td>
<td>creation date</td>
</tr>
<tr>
<td><code>DbEST lib id</code></td>
<td>Library name</td>
</tr>
<tr>
<td>Lib Name</td>
<td>Library description</td>
</tr>
<tr>
<td>Description (1)</td>
<td>Library comments</td>
</tr>
<tr>
<td>Tissue type</td>
<td>Organ</td>
</tr>
<tr>
<td>Develop stage</td>
<td>Library &quot;dev_stage&quot; custom properties</td>
</tr>
</tbody>
</table>

(1) hard coded, not stored in `regexp_dbest.conf`
5.4.3 EMBL batch

5.4.3.1 Launching the program
Use the `run_embl.sh` script to start loading a batch. The only specific parameter is *gz file path*, which is the path to a tar.gz file containing EMBL files.

5.4.3.2 Sequence name and versions
SURF uses EMBL *ID* tag as sequence name. The First EMBL accession number is stored as an 'accession number' alias type and others accession numbers are stored as 'secondary accession number' alias type.

If an EMBL candidate sequence name already exists in SURF and if the two FASTA sequences are strictly equals, the candidate sequence is excluded from load. If an EMBL candidate sequence name corresponds to an existing chromatogram sequence alias, the candidate sequence is loaded ONLY when its FASTA nucleotidic sequence does not equal any existing chromatogram sequence 'publication' feature FASTA. *Publication* feature FASTA sequence can be customized by a 'FASTA' tag stored in *publication* feature 'comment' field (Ex : fasta (20..150,AATTT,200..210)). This can be useful to avoid sequence duplication (private chromatogram version + public version return through EMBL batch). When *publication* feature provides a FASTA comment, FASTA comment overrides *publication* feature start and end positions. If a new sequence version is loaded, '.version' string is appended at the end of sequence file name (Ex : XXX.2).

5.4.3.3 Libraries management:
As EMBL file format is generalist and do not give reliable structured library informations, SURF only parse *dev_stage* and *tissue_type* tags and place them into sequence custom properties (instead of library custom properties for DbEST).

5.4.3.4 Basecalling quality management emulation
Special '.type' file are also generated in order to emulate quality at sequence extraction time. For each sequence, the program creates a file named `[sequence_name].fasta.type` and writes the *molecule_type* value in first line. That file will be used later to generate quality on the fly during sequence quality extraction (see `get_sequence.pl` program).

5.4.3.5 EMBL -> SURF fields mapping

<table>
<thead>
<tr>
<th>EMBL file fields</th>
<th>SURF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>SURF sequence name</td>
</tr>
<tr>
<td>AC</td>
<td>First is &quot;accession number&quot; type alias, others (if applicable) are &quot;accession number secondary&quot; type aliases.</td>
</tr>
<tr>
<td>DE</td>
<td>Stored in FASTA file header</td>
</tr>
<tr>
<td>DT Rel. Created</td>
<td>Creation date</td>
</tr>
<tr>
<td>SQ (multi-lines)</td>
<td>Sequence</td>
</tr>
<tr>
<td>FT /clone</td>
<td>Clone</td>
</tr>
<tr>
<td>FT /tissue_type</td>
<td>Sequence &quot;tissue_type&quot; custom properties</td>
</tr>
<tr>
<td>FT /dev_stage</td>
<td>Sequence &quot;dev_stage&quot; custom properties</td>
</tr>
<tr>
<td>FT /mol_type</td>
<td>Molecule type</td>
</tr>
</tbody>
</table>

5.4.3.6 Molecule type EMBL specific rule
We assume that mRNA sequences lower than 1000 base pairs without 'complete' keyword in description field are not credible, so we change 'mol type' field from "mRNA" to cDNA for such sequences.
5.4.4 FASTA batch

5.4.4.1 Launching the program

Use the `run_fasta.sh` script to start loading a batch. Here are the specific parameters:

- **gz file path**: path to a gz archive containing the FASTA files.
- **default sequence type**: default molecule type for standalone sequence (not linked to a library). When a sequence is linked to a library, the library molecule type is used as a template for sequence molecule type. Default is cDNA.
- **plate name**: string to put as plate name. Default is 'autodetect' which means that SURF will try to parse plate name from batch source (file name or content).
- **strand**: string to put as strand value. Default is 'autodetect' which means that SURF will try to parse strand value from batch source (file name or content).
- **clone name auto detection**: tells SURF to parse batch source for clone name. Default is 'yes'.
- **row and column values auto detection**: tells SURF to parse batch source for row and column values. Default is 'yes'.

5.4.4.2 Sequence name and versions

If a FASTA candidate sequence name already exists in SURF and if the two FASTA sequences are strictly equals, the candidate sequence is excluded from load. The only exception is when a batch is reloaded (i.e. when using the same batch name). In that case, the new batch sequences always overwrite the previous ones. If a FASTA candidate sequence name corresponds to an existing chromatogram sequence alias, the candidate sequence is loaded ONLY when its FASTA nucleotidic sequence does not equal any existing chromatogram sequence 'publication' feature FASTA. Publication feature FASTA sequence can be customized by a 'FASTA' tag stored in publication feature 'comment' field (Ex: `fasta (20..150,AATTT,200..210)`). This can be useful to avoid sequence duplication (private chromatogram version + public version return through FASTA batch). When publication feature provides a FASTA comment, FASTA comment overrides publication feature start and end positions.

5.4.4.3 Libraries management

SURF tries to link FASTA sequences to their libraries at loading time. For each sequence, 'library' regular expression is run onto FASTA file name. The resulting string is used for a library 'code' field lookup. If a library matches, the link is made. It will bring valuable informations at feature detection time.

5.4.4.4 Basecalling quality management emulation

Special '.type' file are also generated in order to emulate quality at sequence extraction time. For each sequence, the program creates a file named `[sequence_name].fasta.type` and writes the `molecule_type` value in first line. That file will be used later to generate quality on the fly during sequence quality extraction (see `get_sequence.pl` program).
5.5 Features detection

Once sequences are load into SURF, only basic informations are available (name, sequences, quality, etc), no added value data (as vector, adapter, repeats) exist in the system. To get these data, you must start the feature detection process. This process will use various programs to characterize each sequence and will try to detect a putative insert. At the end, a status, reflecting sequence validity, is calculated.

NB: command line programs are located in the SURF /bin directory.

5.5.1 Launching the program

Use the run_feature.sh script to start feature detection. Here are the parameters:

- **instance**: SURF instance name.
- **batch ID**: batch ID.
- **Workflow step number to start**: step rank to run. Any previous steps are ignored (useful to “debug” some huge batches). Default value is ‘autodetect’ which means that any previous failed step will be used as value.
- **Workflow step number to stop at**: step rank to run as last step.
- **Address to send email notification when finished**: email address to which success or failure notifications are sent.

5.5.2 Feature detection steps

5.5.2.1 Library specific vector detection

The aim of this step is library specific construction detection using Crossmatch program. When a sequence is linked to a library which provides a ‘vector’ feature, the ‘vector’ feature FASTA file is used to detect cloning vector hits. If sequence is not linked to a library or if the library does not contain a ‘vector’ feature, UNIVEC database is used to detect cloning vector hits. Linked library can also provide other features (adapters, etc.), these features are also tested against each batch sequence in this step. Each feature can be described more finely using ‘sub-features’ field. A sub-feature is a string containing one or more [start-stop:label;] descriptors (ex: 10-16:EcoR1;20-25:tag). These sub-features are considered as features when they are fully included into parent feature hit.

5.5.2.2 Low complexity sequence detection

This step uses RepeatMasker program to detect low complexity sequences: LINE, SINE, (CAAAA)n#Simple_repeat, (AGCTGTGGGGC)n#Satellite, etc. Note that SURF uses the open 3.0 version that can use WU-Blast as sequence comparison engine instead of Crossmatch.

5.5.2.3 Bad quality detection

This step uses phred quality files to produce bad quality features. Any phred value strictly lower than minqual surf.conf file key is considered as a bad base. Bad bases are clustered into bad quality features using a 10 base pairs maximum gap. Non chromatogram sequences bad quality features are emulated using makequality_param [molttype] surf.conf keys. makequality_param is used when makequality_param_moleculeType key does not exist (ex makequality_param_mrna key will override makequality_param key for mRNA quality emulation).

These keys are formatted as: makequality_param,= param1 param2 where:

- **param 1**: something like ‘p1:v1,p2:v2,...,pn:vn\nwhere pi<p(i+1), in fact a comma delimited list of couples pi:vi where pi is a position on the sequence and vi is the phred value that will be given to all bases located before the specified position. The list can be empty.
- **param 2**: the default phred value for other bases.
5.5.2.4 Single nucleotide repetition detection
Since Crossmatch does not always report this kind of repeat (by example when no other hit is detected) and since RepeatMasker does not always report small single nucleotide repetition, we decide to write a SURF specific program (Polysmurtsh) to detect single nucleotide repetition (15 bp minimum PolyN anchored by a 8 bp minimum with a maximum gap of 4 bp). For this reason, you can find some PolyN twice in sequences features, one found by RepeatMasker, one found by Polysmurtsh.

5.5.2.5 Initial database loading
First feature database load, these features will serve as data source for the next insert detection step.

5.5.2.6 Insert position detection
SURF detects insert position by using its own algorithm or using TIGR lucy program.

**Using TIGR lucy**

In order to use lucy as insert detection tool, you must update surf.conf file as following:

```
use_lucy=true
lucy_as_insert=true  # (if false, SURF will detect a useless lucy feature)
```

You also need to create a dedicated lucy feature which contains splicing sites (30 bp flanking regions minimum) for sens AND reverse so 4 FASTA entries (5S,5R,3S,3R) in one fasta file for EACH library.

**NB: SURF will stop processing if a library does not provide the lucy feature.**

Lucy also need a vector so if you do not add a library 'vector' feature UNIVEC will be used.

**Using SURF algorithm**

SURF tries to extend the vector from the sequence extremities using 'construction features' (described in the related library, usually vector and adapters plus polyA and polyT, see library).

Theses features are joined if they are :
- overlapping or
- separated by 20 bp maximum or
- separated by a minimum 60% bad quality zone.

SURF processes these features in a two process way : 5' and 3'
- 5' features are processed from the beginning to 20% of the sequence, they are joined as previously described.
- 3' features are processed from 60% of the sequence to the end or from any feature to the end since the feature has not been used by 5' side process and if bad quality between feature and sequence end is greater than 80%.

After putative insert has been detected, SURF calculates the number of good bases. The number of good bases is the count of sequence nucleotides that :
- belong to insert
- have a phred quality >= minqual surf.conf key (usually 20)
- are not included in a repeat (only for cDNA and mRNA molecule types)

This value reflects the sequence pertinence regarding it quality and composition.

5.5.2.7 UNIVEC contamination detection
SURF uses UNIVEC database to detect any cloning vector contamination in putative insert. This method is an indirect way for chimera detection since a vector hit (using UNIVEC) located into putative insert often denote chimera presence. Crossmatch program is used with the following parameters:
**minmatch** =10 and **minscore**=25. UNIVEC database is reached via a symbolic link, called *univec.fasta*, located in the instance *banks* directory, the targeted file is located into SURF dataroot *banks* directory. UNIVEC can be detected but not considered as a contaminant simply by removing its name from the *contaminants* *surf.conf* key.

### 5.5.2.8 E. Coli contamination detection

SURF uses E.Coli genome sequence database to detect any contamination in putative insert. *Crossmatch* program is used with the following parameters: **minmatch** =100 and **minscore**=150. E. Coli database is reached via a symbolic link, called *coli.fasta*, located in the instance *banks* directory, the targeted file is located into SURF dataroot *banks* directory. E.Coli can be detected but not considered as a contaminant simply by removing its name from the *contaminants* *surf.conf* key.

### 5.5.2.9 Yeast contamination detection

SURF uses yeast genome sequence database to detect any contamination in putative insert. *Crossmatch* program is used with the following parameters: **minmatch** =100 and **minscore**=150. Yeast database is reached via a symbolic link, called *yeast.fasta*, located in the instance *banks* directory, the targeted file is located into SURF dataroot *banks* directory. Yeast can be detected but not considered as a contaminant simply by removing its name from the *contaminants* *surf.conf* key.

### 5.5.2.10 Ribosome contamination detection

SURF uses dedicated species ribosome sequence database (should be set at instance creation) to detect any contamination in putative insert. *Crossmatch* program is used with the following parameters: **minmatch** =100 and **minscore**=150. Ribosome database is a file called *ribo.fasta* located in the instance *banks* directory. Ribosome can be detected but not considered as a contaminant simply by removing its name from the *contaminants* *surf.conf* key.

### 5.5.2.11 Mitochondry contamination detection

SURF uses dedicated species mitochondry sequence database (should be set at instance creation) to detect any contamination in putative insert. *Crossmatch* program is used with the following parameters: **minmatch** =100 and **minscore**=150. Mitochondry database is a file called *mito.fasta* located in the instance *banks* directory. Mitochondry can be detected but not considered as a contaminant simply by removing its name from the *contaminants* *surf.conf* key.

### 5.5.2.12 Poly-Adenylation signal detection

This step detects any poly-adenylation signal (AATAAA / ATTAAA) in the 30 base pairs before any polyA/polyT feature.

### 5.5.2.13 Final database loading

This step loads from UNIVEC step to *Poly-adenylation signal detection* step detected features into database.

### 5.5.2.14 Status update

In order to quickly sort good and bad sequences, SURF provides a sequence status. This status is strictly greater than 0 (1 or greater) when sequence is acceptable for further studies (clustering, assemblies, etc.). Status equal 0 when at least one of the following condition is true:

- **sequence contains contaminant** such as E. Coli, Yeast, Mitochondry, Ribosome or UNIVEC. UNIVEC is assumed as a contamination when it is found into putative insert (see UNIVEC contamination detection section), which means that sequence has a good chance to be a chimera.
- **Sequence having a good base number lower than 100 base pairs** are assumed to be too small to provide pertinent information.

Status is lower than 0 when sequence was correct but now have a newest active version.
5.6 Batch statistics

After batch is loaded, SURF can calculate various statistics reflecting batch global quality.

5.6.1 Launching the program

Use the run_stat.sh script to start feature detection. Here are the parameters:

- **instance**: SURF instance name
- **batch ID**: batch ID
- **Workflow step number to start**: step rank to run. Any previous steps are ignored (useful to “debug” some huge batches). Default value is 'autodetect' which means that any previous failed step will be used as value.
- **Workflow step number to stop at**: step rank to run as last step.
- **Address to send email notification when finished**: email address to which success or failure notifications are sent.

5.6.2 Statistics calculation steps

5.6.2.1 File system creation

The aim of this step is to create a statistic.html and a statistic_files directory under the batch attach folder. Some of the statistical data will be stored there.

5.6.2.2 General statistics calculation

This step calculates basic indicators such as sequence count, null sequence count, count per status and count per contaminant.

5.6.2.3 Full length sequence detection

This step counts and detects full length sequences.

5.6.2.4 Library feature combinations

This step compiles all feature combinations (including number of features) per batch library and count them, related sequence list is provided for each feature pattern.

5.6.2.5 Graphics production

This step uses R software to produce sequence length histogram, sequence number of good bases histogram, insert size histogram and basecalling quality / base pair position plot.
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Version 1.2, November 2002

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