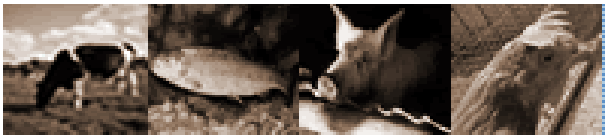
SURF

SeqUence Repository and Feature detection

USER GUIDE

Version 1.0.1

Eddie Iannuccelli



Revision table :

Version	*Date*	*Major modifications*	*Author(s)*
0.1	February 9th 2005	First release	C Dantec, E Iannuccelli
1.0	October 26 2005	DNA update	E Iannuccelli
1.0.1	16/02/2006	Instance creation description	E Iannuccelli

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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 though 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 User guide

3.1 Managing libraries

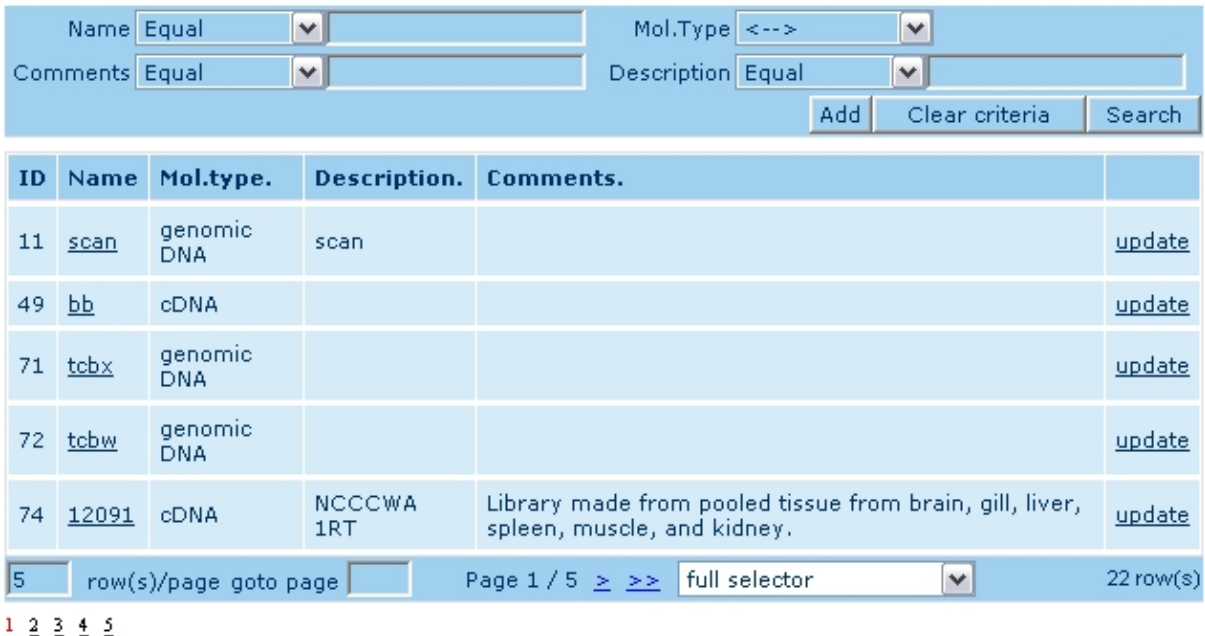
As the library drive the way the sequences are 'built', it is the best place to describe the expected features. Basic features are vector and adapters, but it is possible to add any kind of feature since a Fasta file is available. SURF uses *Crossmatch* software to detect features , this software needs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

- *minmatch* : the minimum number of contiguous nucleotide to match before trying to extend match
- *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) :

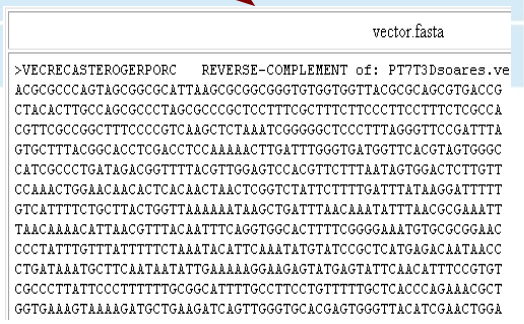
- 'vector' is used to designate the cloning vector feature.
- any feature starting by 'adapter' (ex : adapter5, adapter3)
- any feature ending by a '!' (ex : primer1!, primerA!)

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



One library detailed view

Name	scan
Code	scan
Molecule type	genomic DNA
description	scan
comments	
Features to detect	***Name*** ***Minmatch*** ***Minscore*** ***File*** ***SubFeatures*** vector 10 15 vector 2640-2660:E5;2667-2742:E3; adapter 3 9 adapter lucy 10 15 lucy
Custom properties	No data
Back	Library features combinations


vector.fasta

>VECRECASTEROGERPORC REVERSE-COMPLEMENT of: PT7T3Dsoares.ve
ACGCGCCCAGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCA
CGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTA
GTGCTTTACGGCACCTCGACCTCCAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGC
CATCGCCCTGATAGACGGTTTTACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTT
CCAAACTGGAACAACACTCACAACTAACTCGGTCTATTCTTTTGATTTATAAGGATTTTT
GTCATTTTCTGCTTACTGGTTAAAAAATAAGCTGATTTAACAAATATTTAACGCGAAATT
TAACAAAACATTAACGTTTACAATTTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAAC
CCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACC
CTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGT
CGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCT
GGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGA

Available informations are :

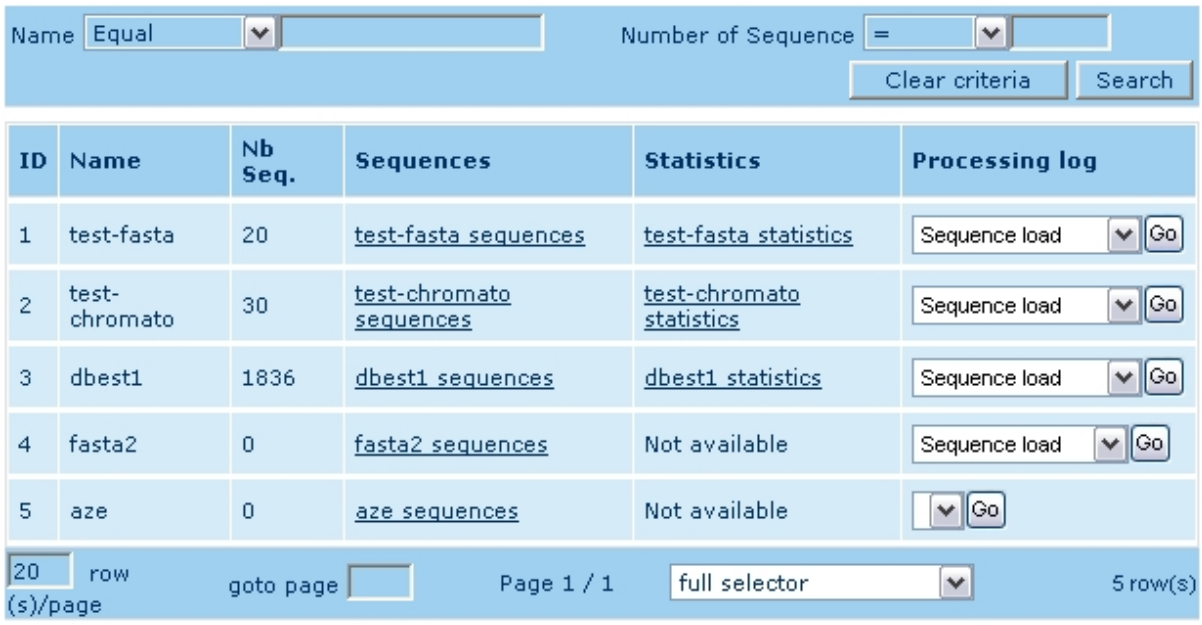
- *Name* : The library name
- *Code* : string used to connect a sequence to its library during data loading
- *Description* : any descriptive data
- *Comments* : any comment
- *Features* to detect list :
  - *Name*
  - *Minmatch* :crossmatch parameter
  - *Minscore* : crossmatch parameter
  - *File* : fasta file containing feature sequence
  - *Subfeatures* : sub features coordinates
    formatted as START-END:NAME ex : 10-15:EcoR1
- *Custom properties* : *tissue_type* and *organ* data only for imported DbEST libraries.
- *Library feature combinations* : link to library statistical page (also available from batch statistics, see *statistical calculation* chapter for details).

3.2 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

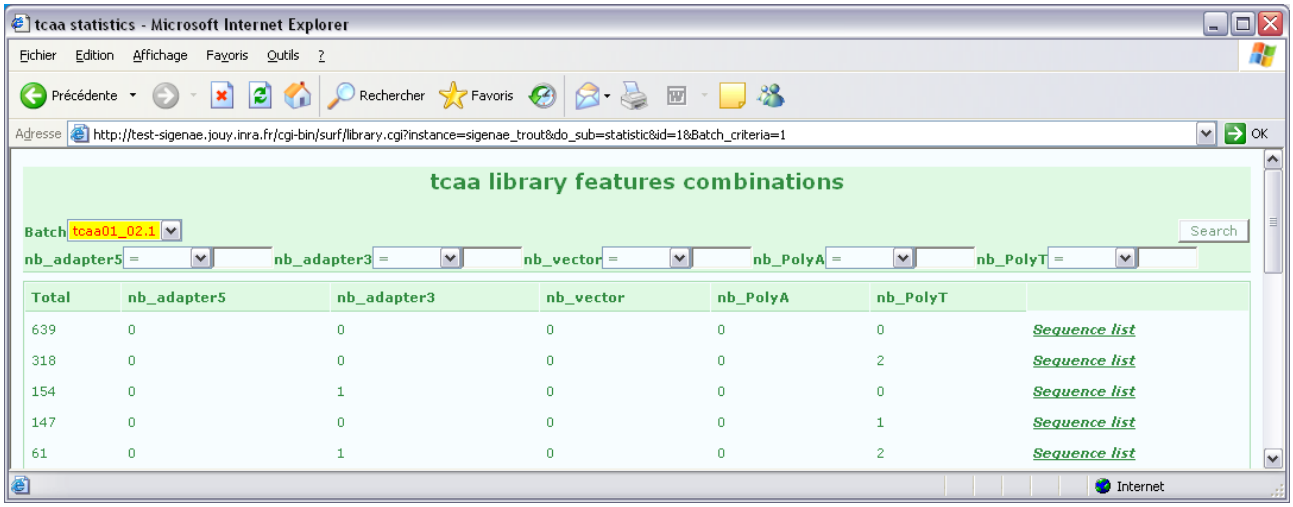
Here is a screen capture of the batch management tool.



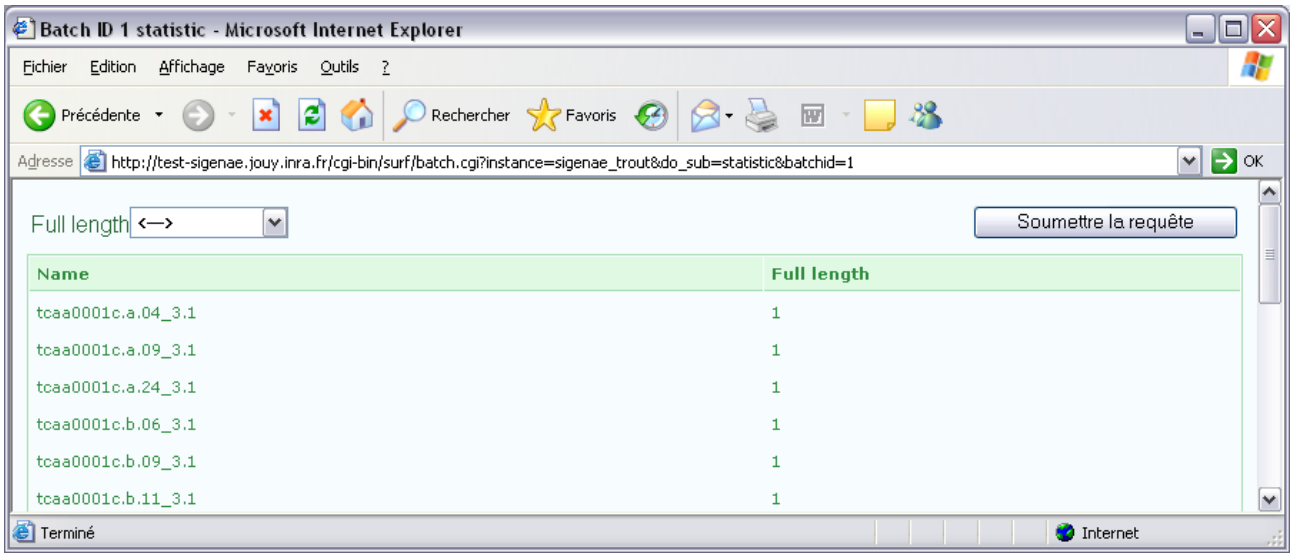
The batch statistics provide following informations :

- *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

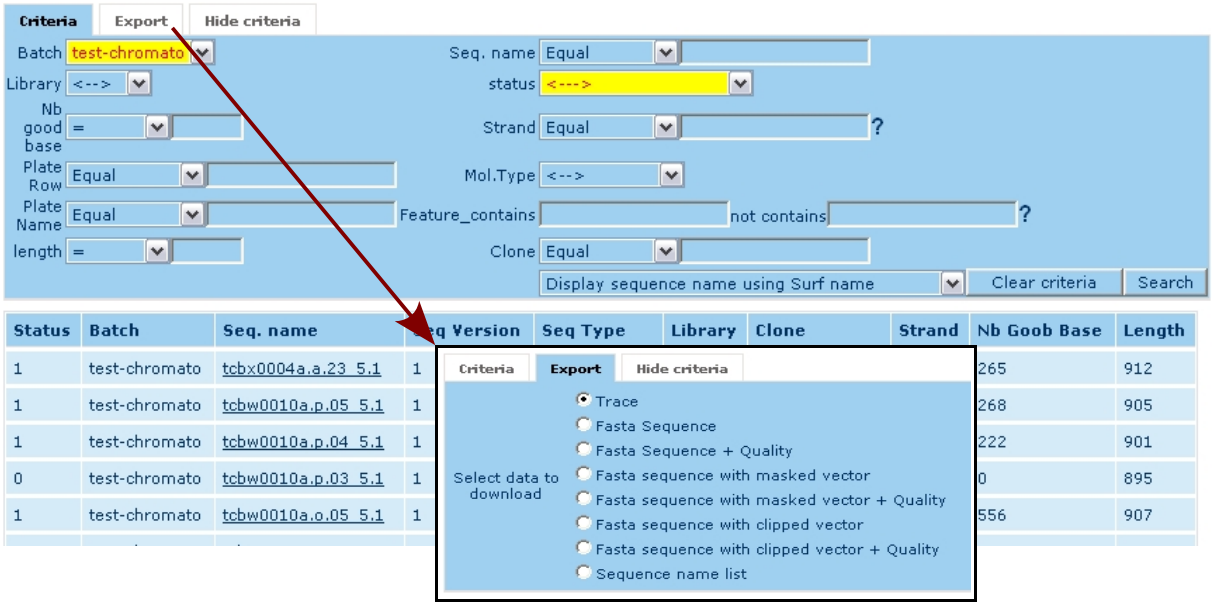


Full length sequence screen



3.3Managing 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

3.4 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.

3.4.1 Chromatogram batch

3.4.1.1 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'.

3.4.1.2 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 wil 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.

3.4.1.3 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.

3.4.2DbEST batch

3.4.2.1Launching 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.

3.4.2.2Sequence 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).

3.4.2.3Libraries 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.

3.4.2.4Basecalling 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).

3.4.2.5DbEST -> SURF fields mapping

DbEST file fields	*SURF*
DbEST Id	SURF sequence name
est_name	'est_name' type alias
genbank_acc	'genbank_acc' type alias
genbank_gi	'genbank_gi' type alias
Clone Id	Sequence clone
DNA type	Molecule type
SEQUENCE (1)	Sequence nucleotidic sequence
Plate Row Column	Sequence plate, row, and column
Entry Created	creation date
DbEST lib id	Library name
Lib Name	Library description
Description (1)	Library comments
Tissue type | Organ	**Library** “tissue_type” custom properties
Develop stage	**Library** “dev_stage” custom properties

(1) hard coded, not stored in *regexp_dbest.conf*

3.4.3EMBL batch

3.4.3.1Launching 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.

3.4.3.2Sequence 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).

3.4.3.3Libraries 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).

3.4.3.4 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).

3.4.3.5EMBL -> SURF fields mapping

EMBL file fields	*SURF*
ID	SURF sequence name
AC	First is “accession number” type alias, others (if applicable) are “accession number secondary” type aliases.
DE	Stored in FASTA file header
DT Rel. Created.	Creation date
SQ (multi-lines)	Sequence
FT /clone	Clone
FT /tissue_type	**Sequence** “tissue_type” custom properties
FT /dev_stage	**Sequence** “dev_stage” custom properties
FT /mol_type	Molecule type

3.4.3.6Molecule 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.

3.4.4FASTA batch

3.4.4.1Launching 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'.

3.4.4.2Sequence 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 p*ublication* 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.

3.4.4.3Libraries 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.

3.4.4.4 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).

3.5 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.

3.5.1 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*** : e mail address to which success or failure notifications are sent.

3.5.2 Feature detection steps

3.5.2.1 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.

3.5.2.2 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*.

3.5.2.3 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_[moltype] 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.

3.5.2.4 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*.

3.5.2.5 Initial database loading

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

3.5.2.6 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 (usualy 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.

3.5.2.7 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.

3.5.2.8 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.

3.5.2.9 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.

3.5.2.10 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.

3.5.2.11 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.

3.5.2.12 Poly-Adenylation signal detection

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

3.5.2.13 Final database loading

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

3.5.2.14 Status update

In order to quickly sortgood 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

3.6 Batch statistics

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

3.6.1 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*** : e mail address to which success or failure notifications are sent.

3.6.2 Statistics calculation steps

3.6.2.1 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.

3.6.2.2 General statistics calculation

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

3.6.2.3 Full length sequence detection

This step counts and detects full length sequences.

3.6.2.4 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.

3.6.2.5 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.

4 Licence

Version 1.2, November 2002

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