

# Applied Biosystems SOLiD™ 4 System

## SOLiD™ SAGE™ Analysis Software v1.10 Guide

SOLiD™ SAGE™  
Tag Preparation

Templated Bead  
Preparation

Instrument  
Operation



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Part no. MAN0001685

Rev. date: 8 July 2010

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# Description of the Software

## Overview

SOLiD™ SAGE™ Analysis Software v1.10 is a Linux-based program that takes the raw data files from SOLiD™ SAGE™ Kit sequencing reads and matches them to known sequences in your reference database of choice. It is designed for use with the SOLiD™ SAGE™ Kit or the SOLiD™ SAGE™ Kit with Barcoding Adaptor Module, which generates libraries of 27-bp tags for all transcripts in a cell.

Using the simple software interface, you create a project name and location, and then identify the directories containing:

- Sequences from a SOLiD™ System read (csfasta formatted)
- A fasta-formatted database of reference sequences (*e.g.*, from RefSeq).

The software then automatically matches the experimental data with the reference data or compares two experimental data sets. The software identifies tags, tabulates tag abundances, and displays associated gene descriptions. It presents the data in a tabular format that is easy to read and export to spreadsheet or other programs for further analysis. It can be used to map single sequencing file or multiple sequencing files at the same time.

## How Tags are Processed

The user selects a sequence file of SOLiD™ SAGE™ Kit reads or a folder containing multiple sequence files, specifies mapping parameters such as tag length and number of mismatches allowed, and selects a directory of reference sequences from a database such as RefSeq. The software then maps the SOLiD™ System reads to the reference database using only a portion of the reference sequences, to greatly reduce processing time. The results of the analysis are saved in the output files described below.

## Output Files

All output files are in tab-delimited text format. The output files for the individual barcoded libraries in a multiplex SOLiD™ System sequencing run are stored in subdirectories of the main project directory. The output files are:

- A mapping output file that lists each tag, its frequency of occurrence, its GenBank Identifier (GI) number, and a description of the identified gene (Map function)
- A mapping results file that provides more detailed information, including SOLiD™ System sequencing read IDs and mismatches (Map function)
- A file comparing the tags in two different samples (Compare function)

To transfer the files to a Windows-based computer for analysis (*e.g.*, by Microsoft Excel software), you can use WinSCP or equivalent.

# Download and Installation

## System Requirements

SOLiD™ SAGE™ Analysis Software v1.10 has the following system requirements:

- Linux operating system
- Perl Tk
- Reference sequences, in NCBI fasta format (e.g., RefSeq files)
- ≥8 gigabytes of memory—varies depending on the size of the reference genome, selected tag length, and selected number of allowed mismatches

## Downloading and Installing SOLiD™ SAGE™ Software

1. To download the software, go to:  
<http://solidsoftwaretools.com/gf/project/solid-sage>
2. Click on the **Files** link in the left navigation bar, and then select the “scripts” tgz file (e.g., **solid.sage.v110.scripts.tgz**) to begin the software download.

The screenshot displays the Forge Advanced Server interface for SOLiD SAGE Analysis Software. The main content area shows a table of packages with the following data:

| Package Name | Latest Release  | Maturity | Files               | FileSize | Downloads |
|--------------|-----------------|----------|---------------------|----------|-----------|
| SOLiDSage    | solid.sage.v110 | 4 - Beta | solid.sage.v110.tgz | 10.18 Kb | 199       |

The 'Files' column for the 'SOLiDSage' package is circled, highlighting the file 'solid.sage.v110.tgz'. The left navigation bar includes links for Summary, Reporting, Search, Forums, Docs and Data, and Files. The top navigation bar includes Home, My Stuff, Search, Projects, and Snippets. The bottom of the page features the Forge Advanced Server logo.

## Completing the Installation

1. Download the **solid.sage.v110.scripts.tgz** file into the directory where you want to install the software.
2. Open **.bash\_profile** in your home directory with a text editor program.
3. Add the following line, substituting the actual directory name from Step 1:  
**PATH=\$PATH:/solid\_sage\_directory**
4. Add the line: **export PATH**
5. Save **.bash\_profile** and exit the text editor.
6. Logout and re-login.
7. Extract the package using the command: **tar xf solid.sage.v110.scripts.tgz**

## Downloading Sample Data

1. To download sample data, go to:  
<http://solidsoftwaretools.com/gf/project/solid-sage>
2. Click on the **Docs and Data** link in the left navigation bar, and then select **Sample Data**.
3. Select the “samples” tgz file (e.g., **solid.sage.v110.samples.tgz**) to begin the data file download.
4. The tgz file contains sample data for each mapping and comparison option, and results are provided in a corresponding directory. An example Description File for mapping multiple sequence files with different parameters is provided as well (see page 12).

# Using the Software

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## Getting Started

### Launching the Program

To launch the software, type **solid.sage.v110.pl**. (The software should be in your working path, so you can launch it from anywhere.)

If you have a problem launching the software, try entering the full path of the directory where you extracted the package followed by the program name.

Example launch commands:

- `solid.sage.v110.pl`
- `solid.sage.v110.pl &`
- `solid_sage_directory/solid.sage.v110.pl &`

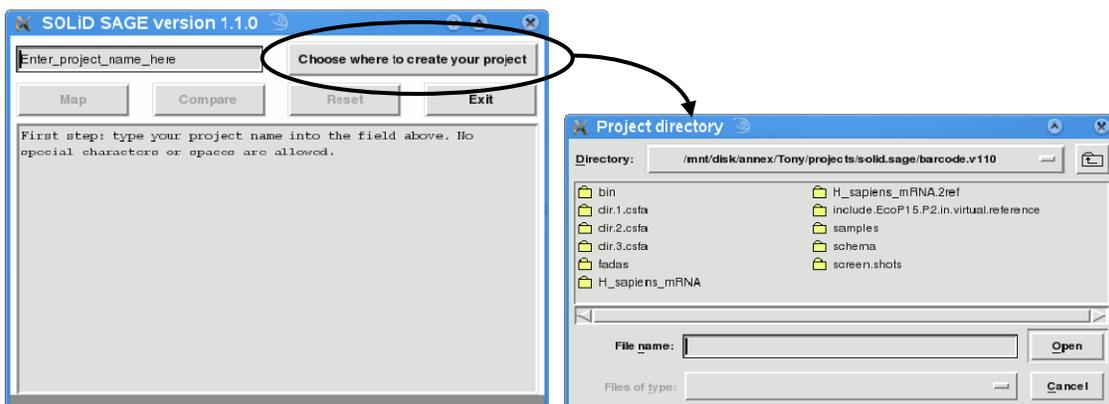
The program screen will open.

### Creating a Project

1. To begin, enter a project name in the top left field, using only alphanumeric characters with no spaces.
2. Next, click on **Choose where to create your project** to specify the file path to the project directory. A directory with the project name you entered will be created containing temporary analysis files and final output files. (Make sure a directory with that project name does not already exist in that location.)

**Note:** You must have “write” permission to the location of the project directory.

3. When you have made your selection, the full path will be displayed in the top left box, and the **Map** and **Compare** buttons will become available.



### Next Steps

With the project name and location created, you can now proceed to:

- **Mapping Sequence Files**, starting on the next page
- **Comparing Two Mapped Samples**, page 12

# Mapping Sequence Files

## Sequence Files from the SOLiD™ System

Sequences files generated by the SOLiD™ System are in the color-space fasta format (.csfasta). SOLiD™ SAGE™ Analysis Software v1.10 can analyze a single .csfasta results file or multiple files (e.g., from different barcoded libraries in a single run or from different runs). This section describes mapping sequence files using a single set of mapping parameters.

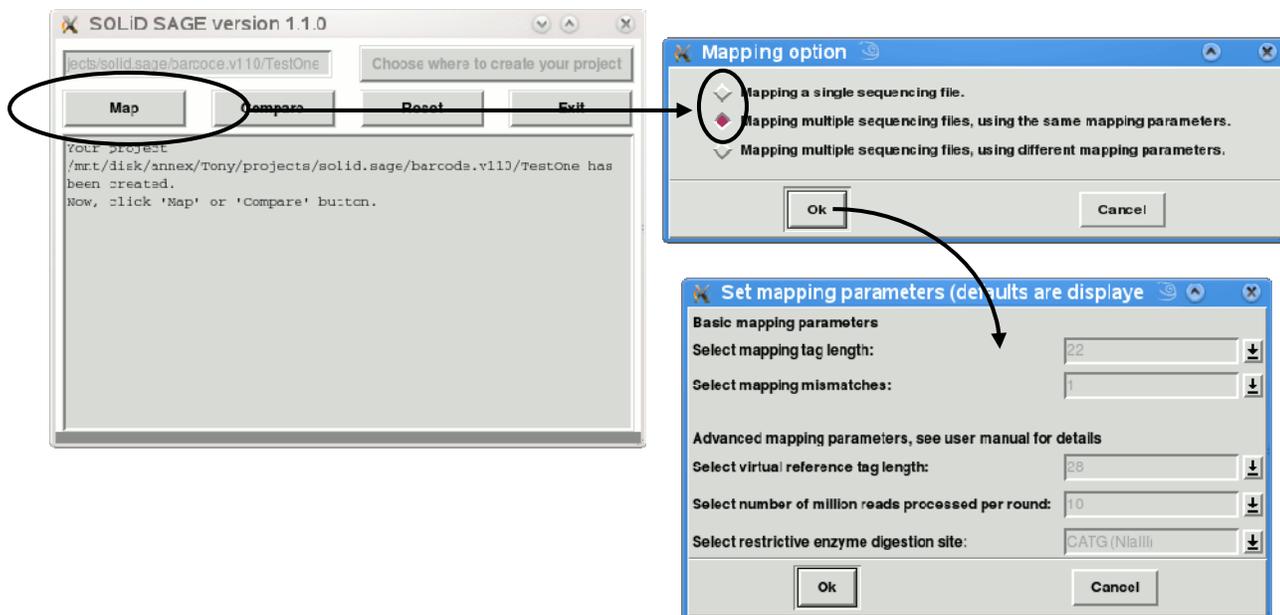


**Note:** All csfasta files to be analyzed must be located in the same directory (unless you are using a Description File as described on page 12).

Besides read definition lines (beginning with a > sign) and the actual read sequence (beginning with T followed by the digit 0/1/2/3), there may be some commenting lines which begin with a # sign at the beginning of the file. The first read definition line should appear within the first 100 lines of the input reads. If it appears later than 100 lines, you will need to trim the preceding comment lines.

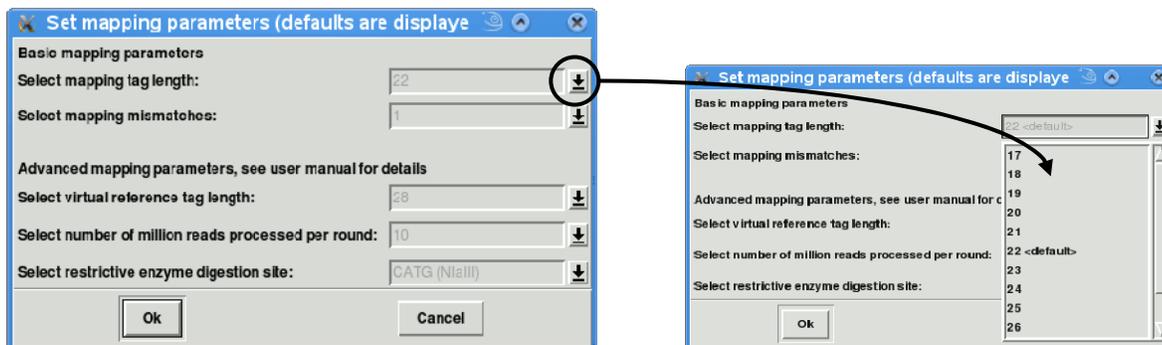
## Mapping a Single File or Multiple Files with the Same Parameters

1. Click on the **Map** button.
2. Select either **Mapping a single sequencing file** or **Mapping multiple sequencing files using the same parameters**.
3. Click on **OK**. The **Set mapping parameters** dialog will open.



## Selecting Mapping Parameters

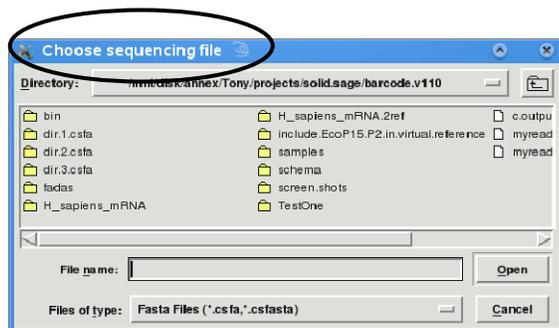
In the **Set mapping parameters** dialog, the default parameters are preselected. Click on the pulldown arrow to change each selection, then click on **OK**.



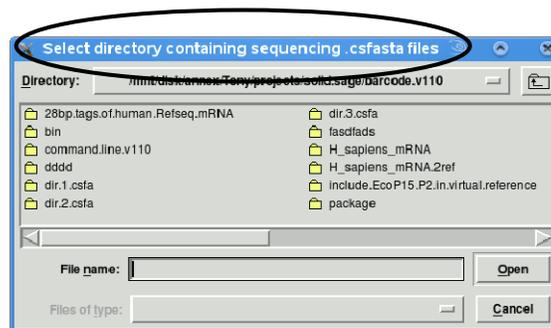
| Parameter                                   | Default                | Explanation   |
|---|------------------------|---|
| Mapping tag length                          | 22                     | The number of bases in each tag counting back from the actual <i>EcoP15</i> I cleavage site towards the <i>Nla</i> III (CATG) restriction site.   |
| Mapping mismatches                          | 1                      | The number of base mismatches allowed during mapping. Empirical data have shown that allowing a 1-base mismatch may provide the optimal balance between mapping accuracy and robustness. Increasing the number of mismatches allowed may decrease accuracy and increase processing time.  |
| Virtual reference tag length                | 28                     | The length of each reference sequence following the <i>Nla</i> III restriction enzyme digestion site that is analyzed for mapping purposes.   |
| Number of million reads processed per round | 10                     | The number of tags (in millions) processed at one time by the software. If this number is less than the total number of tags in a run, the software will process the tags in multiple "rounds" of processing and then combine the data into a single result. This enables slower computers to process large numbers of tags without crashing, but also increases processing time. |
| Restriction enzyme digestion site           | CATG ( <i>Nla</i> III) | The default restriction enzyme used by the SOLiD™ SAGE™ System is <i>Nla</i> III. Alternative workflows may use a different restriction enzyme, such as <i>Sau</i> 3A.  |

### Selecting the Sequencing File(s)

After you select the mapping parameters, you will be prompted to select either a single .csfasta file or the directory containing multiple files, depending on your selection in the **Mapping option** dialog box. Multiple files must be located in the same directory, and all the files in that directory will be analyzed.



or

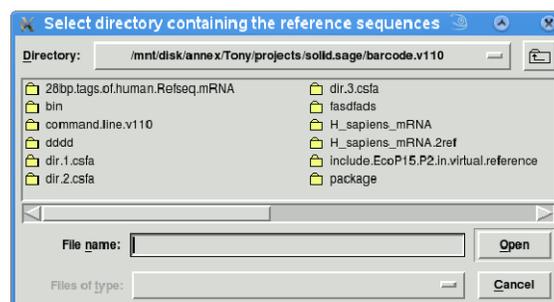


## Selecting the Reference Directory

After you have selected a sequence file or directory and specified the mapping parameters, you will be prompted to select the directory containing the reference sequences.



**Note:** You do not select the reference files themselves—only the directory. The software will use every fasta file in that directory for its analysis. See **Reference File Format** below.



## Reference File Format

In general, the reference files should have the same format as the fasta files from an NCBI RefSeq database. You can simply download the appropriate mRNA or genomic DNA (gDNA) database from NCBI RefSeq and use it as your reference.

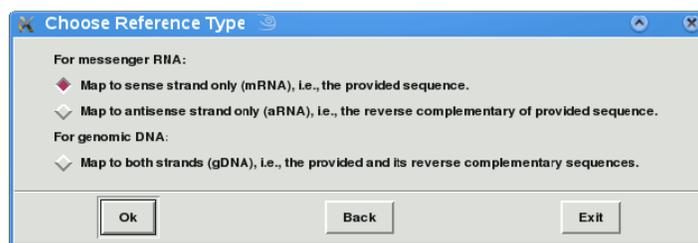
The reference files must be in the fasta format (**.fasta**). The definition lines of the reference sequences should have an NCBI RefSeq sequence format or have the following structure: `>gi | xxx | ref | yyy`

... where xxx is the GI number and yyy is the gene name. There is no length restriction on the GI number or gene name.

The fasta file can have multiple sequences, but each definition line should follow the structure defined above. No comment lines are allowed.

## Selecting the Reference Type

After selecting the reference library, choose whether to map tags to the **sense strand** for mRNA, **antisense strand** for aRNA, or **both strands** for gDNA.



## Note on Virtual Reference Sequences

The program generates a virtual reference sequence that is much shorter than the actual reference sequence so that the analysis will run faster. The virtual reference sequence consists of fragments of a pre-set length—either the default 28 base pairs that follow the *Nla* III anchor site CATG or a user-selected tag length (as selected under **Select mapping parameters**).

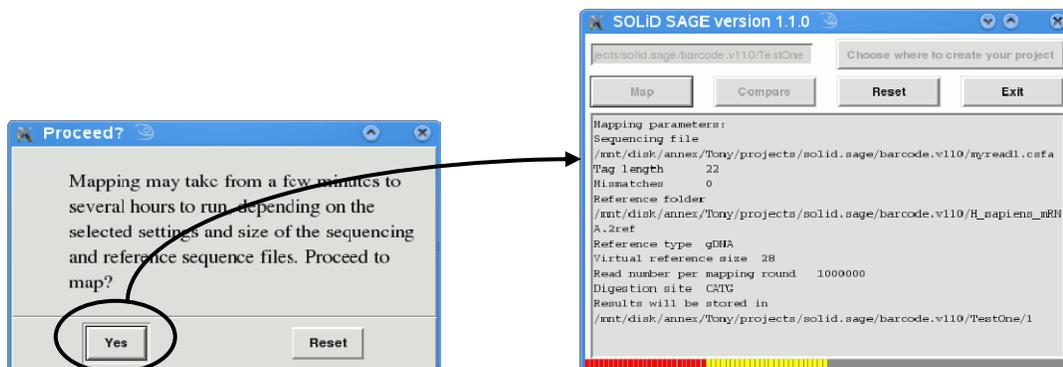
For mRNA or aRNA mapping, only the anchor site CATG and the following bases on the sense or antisense strand will be used as the virtual reference.

If genomic DNA mapping, CATG and the following bases on both strands—the provided sequence and its reverse complementary sequence—will be used as the virtual reference.

## Running the Analysis

After you select the reference type and click on **OK**, you will be prompted to proceed to mapping. Click on **Yes**. After a short processing delay, a progress bar will appear at the bottom of the main software window.

The length of time required for mapping will vary depending on the size of the reference database, the selected tag length, and the number of mismatches allowed. Complex analyses may take several hours.



## Output and Results Files

For each fasta file in the reference directory, the SOLiD™ SAGE™ Analysis Software generates a corresponding subdirectory in the selected project directory.

In each subdirectory, two files are generated by the mapping program:

A mapping Output file (**output.tab**) that lists each tag, its frequency of occurrence, its associated GenBank Identifier (GI) number, and a brief description of the identified gene

A mapping Results file (**results.tab**) that provides additional information, including SOLiD™ System sequencing read IDs and mismatches

### Output file example:

|    | A                            | B     | C                                  | D  |
|----|------------------------------|-------|------------------------------------|--|
| 1  | Tag                          | Count | GI                                 | Description  |
| 2  | CATGAAAAAACCCTCAATAAGAGAATC  | 1     | GI33413399                         | >gi 33413399 ref NM_001984.1  Homo sapiens esterase D/formylglutathione hydrolase (ESD), mRNA  |
| 3  | CATGAAAGGGTCACTTCTGTAATAGTG  | 1     | GI81158221::GI81158223::GI81158225 | >gi 81158221 ref NM_001037133.1  Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 3, mRNA::>gi 81158223 ref NM_005010.3  Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 2, mRNA::>gi 81158225 ref NM_001037132.1  Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 1, mRNA |
| 4  | CATGAACAACCGGCTGGCCGAGACCAG  | 1     | GI11545760                         | >gi 11545760 ref NM_022055.1  Homo sapiens potassium channel, subfamily K, member 12 (KCNK12), mRNA  |
| 5  | CATGAACTTGATACGTCGGTGTCTCC   | 1     | GI53759150                         | >gi 53759150 ref NM_005063.4  Homo sapiens stearyl-CoA desaturase (delta-9-desaturase) (SCD), mRNA   |
| 6  | CATGAAGATGATATGAGGCCGGGGCGG  | 1     | GI52145308                         | >gi 52145308 ref NM_032808.5  Homo sapiens leucine rich repeat and Ig domain containing 1 (LINGO1), mRNA   |
| 7  | CATGAAGGAAGATCCCACAGTCTCAGC  | 1     | GI4758483                          | >gi 4758483 ref NM_004832.1  Homo sapiens glutathione S-transferase omega 1 (GSTO1), mRNA  |
| 8  | CATGACAGCCCTGCTCTTGAGTACC    | 1     | GI39812204                         | >gi 39812204 ref NM_025164.3  Homo sapiens KIAA0999 protein (KIAA0999), mRNA   |
| 9  | CATGACGGAAACAATAGGACTCCCCAGG | 2     | GI38505192                         | >gi 38505192 ref NM_000954.5  Homo sapiens prostaglandin D2 synthase 21kDa (brain) (PTGDS), mRNA   |
| 10 | CATGACGTGTCTATGTCAAAAGTCTT   | 1     | GI115583669                        | >nl 115583669 ref NM_003253.2  Homo sapiens T-cell lymphoma invasion and   |

### Results file example:

|    | A                            | B         | C      | D                 | E        |
|----|------------------------------|-----------|--------|-------------------|----------|
| 1  | Tag_Seq                      | GI_num    | GI_Pos | Read_ID           | Mismatch |
| 2  | CATGTGCAAAATAAATGTGGCTTAGACT | 133908618 | 3298   | >665_1155_42_F3   | 0        |
| 3  | CATGGTAATAAAAATGAATGATAAAA   | 189083841 | 2125   | >665_817_382_F3   | 0        |
| 4  | CATGGTAATAAAAATGAATGATAAAA   | 189083835 | 2280   | >665_817_382_F3   | 0        |
| 5  | CATGGTAATAAAAATGAATGATAAAA   | 189083837 | 2146   | >665_817_382_F3   | 0        |
| 6  | CATGGTAATAAAAATGAATGATAAAA   | 189083839 | 2128   | >665_817_382_F3   | 0        |
| 7  | CATGGTAATAAAAATGAATGATAAAA   | 51593094  | 2283   | >665_817_382_F3   | 0        |
| 8  | CATGGTAATAAAAATGAATGATAAAA   | 132814488 | 2268   | >665_817_382_F3   | 0        |
| 9  | CATGTGATGGCATTGAGCCACACCTC   | 34147410  | 1140   | >665_1807_1132_F3 | 0        |
| 10 | CATGAGGAGCTCGGCTTAAATGTCTT   | 40254847  | 2324   | >665_1994_720_F3  | 0        |
| 11 | CATGAACATGATATGAGCCGGGGCGG   | 53145308  | 1800   | >665_200_447_F3   | 0        |

## **Transferring Files**

To transfer the output files to a Windows-based computer for analysis using a spreadsheet program such as Microsoft Excel software, you can use WinSCP or equivalent.

# Mapping Multiple Sequence Files with Different Parameters

## Description File

You can map multiple sequence files (in .csfasta format) in different directories with different mapping parameters using a **Description File** that contains all the details about each sequence file, the parameters to apply, and the reference directories to use.

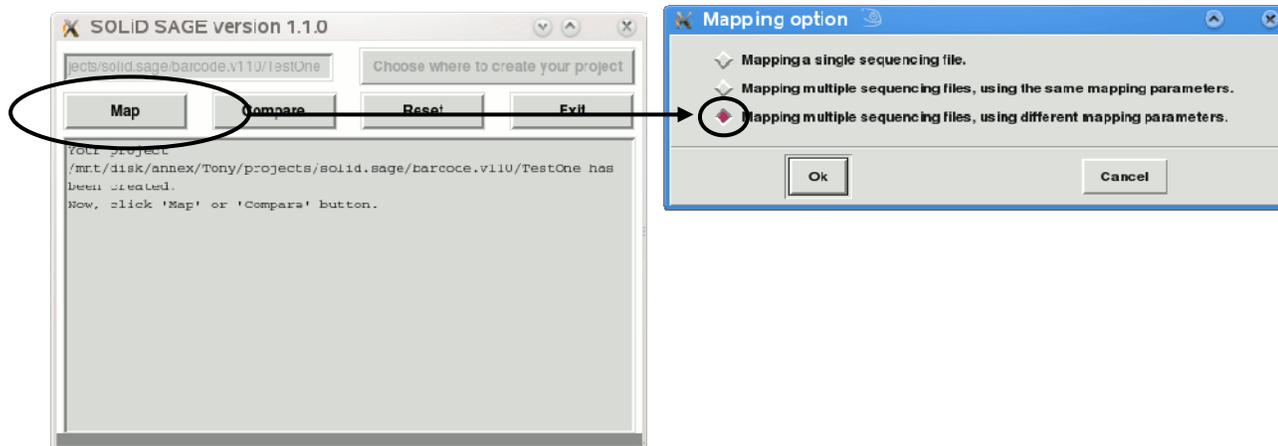
An example Description File is available as part of the sample data download. See **Downloading Sample Data** on page 5.

The example Description File is shown below. The comment lines at the top describe the different elements, and an example format is shown in the circled area, with a single space between each element.

```
#This is a sample description file for barcode/multiple mapping.
#If a line begins with pound sign (#), the program treats it as a comment line.
#The file is supposed to have eight columns, separated by spaces:
#1, csfa file, the input SAGE sequencing read file
#2, mapping tag-length, should be an integer between 21 and 28, inclusive. Default 23.
#3, mapping mismatches allowed, should be 0, 1, 2, or 3. Default 0.
#4, reference directory, the directory where reference sequences are located.
#5, reference type, should be gDNA, mRNA, or aRNA. Default mRNA.
#6, virtual reference tag-length, should be an integer between 27 and 35, inclusive. Default 28.
#7, split number, defines how many reads to be processed for one round mapping. Should be a positive integer or All_reads. Default 'All_reads'.
#8, restrictive enzyme digestion site. Should be CATG for NlaIII or GATC for DpnII. Default CATG.
#An exemplary line with default values:
#myread1.csfa 23 0 myref1.fa mRNA 28 All_reads CATG
#Other exemplary lines:
#myread2.csfa 21 0 myrefdir mRNA 25 1000000 CATG
#myread2.csfa 23 0 myrefdir mRNA 28 All_reads GATC
#myread1.csfa 28 0 myrefdir2 mRNA 35 10000000 CATG
#Please refer to manual and other supporting documents for details on these arguments.
#Following lines will be read into and used by the program.
SeqFolder/BC001.random.1M.csfasta 22 1 /mnt/disk/annex/Tony/database/RefSeq/H_sapiens_mRNA mRNA 28 All_reads CATG
SeqFolder/BC005.random.1M.csfasta 27 0 /mnt/disk/annex/Tony/database/RefSeq/H_sapiens_mRNA mRNA 28 1000000 CATG
SeqFolder/BC005.random.1M.csfasta 27 0 /mnt/disk/annex/Tony/database/RefSeq/H_sapiens_mRNA mRNA 28 1000000 GATC
```

## Creating and Selecting the Description File

1. Create the Description File using the example provided with the software and shown above.
2. Launch the program and create a project, then click on the **Map** button.
3. Select **Mapping multiple sequencing files using different mapping parameters**.
4. Click on **OK**. The **Open mapping description file** dialog will open.
5. Select the Description File you created and click on **Open**.
6. The software will proceed to mapping, as described in **Running the Analysis**.



# Comparing Two Mapped Samples

## Introduction

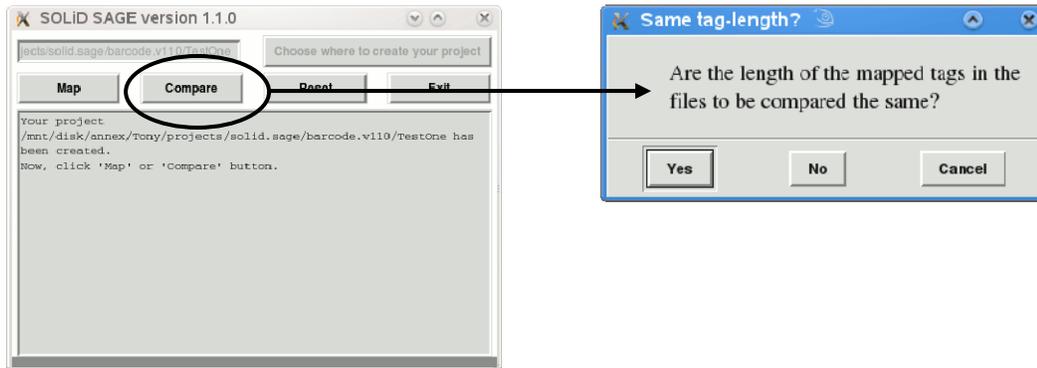
You can compare two previously mapped libraries, as described in this section.

## Selecting the Sequencing Reads to Compare

1. Launch the program and create a project, as described in **Getting Started**.
2. Click on the **Compare** button in the program screen and follow the on-screen steps to select the files to compare. These will be Output files from previous mapping runs (**output.tab** format; see page 10). Do *not* compare results .tab files.
3. The software will find common tags between the libraries and identify the corresponding tag counts and genes. When the comparison is complete, you will receive an alert.



**Note:** If the lengths of the tags in the two libraries are different, all tags will be truncated to the shortest length before comparison. Note that the gene description may not match the truncated tags as well as the non-truncated ones.



## Comparison File

The Comparison file generated by the analysis (**comparison.tab**) lists each tag, its frequency of occurrence in each sample, and its GenBank identification.

|   | A                           | B     | C     | D   |
|---|-----------------------------|-------|-------|---|
| 1 | Tag_Sequence                | File1 | File2 |   |
| 2 | CATGAAGACAGTGGCTGGCGTGCCTG  | 4715  | 5430  | (Based on non-truncated tag) >gi 78214519 ref NM_000998.4  Homo sapiens ribosomal protein L37a (RPL37A), mRNA   |
| 3 | CATGCACAAACGGTAGTTTTGTGTGTT | 2361  | 2604  | (Based on non-truncated tag) >gi 169202035 ref XM_001726056.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA:>gi 169203086 ref XM_001725509.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA:>gi 169203606 ref XM_001726007.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA:>gi 68160923 ref NM_001030.3  Homo sapiens ribosomal protein S27 (metallopanstimulin 1) (RPS27), mRNA |
| 4 | CATGACAACAAGAAAAAGACCTTGTA  | 1736  | 1606  | (Based on non-truncated tag) >gi 56550064 ref NM_001008220.1  Homo sapiens complexin 2 (CPLX2), transcript variant 2, mRNA:>gi 56550103 ref NM_006650.3  Homo sapiens complexin 2 (CPLX2), transcript variant 1, mRNA   |
| 5 | CATGAATATGTGGGCTAAGAAATAGTT | 1659  | 1980  | (Based on non-truncated tag) >gi 17999531 ref NM_004374.2  Homo sapiens cytochrome c oxidase subunit 1c (COX6C), mRNA   |
| 6 | CATGAAGCTGAGGTCTTGAAGCAGCTG | 1558  | 715   | (Based on non-truncated tag) >gi 44889961 ref NM_005563.3  Homo sapiens stathmin 1/oncoprotein 18 (STMN1), transcript variant 3, mRNA:>gi 44890049 ref NM_203399.1  Homo sapiens stathmin 1/oncoprotein 18 (STMN1), transcript variant 2, mRNA:>gi 44890051 ref NM_203401.1  Homo sapiens stathmin 1/oncoprotein 18 (STMN1), transcript variant 1, mRNA   |
| 7 | CATGAACTAATACTACAATAAAGGATG | 1533  | 2163  | (Based on non-truncated tag) >gi 142358075 ref NM_152350.2  Homo sapiens chromosome 17 open reading frame 45 (C17orf45), mRNA   |
| 8 | CATGATCGCTTCTACACTGTATTACA  | 1442  | 1496  | (Based on non-truncated tag) >gi 41406053 ref NM_000484.2  Homo sapiens amyloid beta (A4) precursor protein (APP), transcript variant 1, mRNA:>gi 41406054 ref NM_201413.1  Homo sapiens amyloid beta   |

## Resetting and Exiting the Program

### Reset

Clicking on **Reset** will reset all the options in the program screen, including the project name selection. You will be prompted to complete this action.

### Exiting the Program

Click on **Exit** to exit the program. Depending on the state the program is in, there may be a small or lengthy delay before exiting.

You can also end the program by using standard Unix commands (*e.g.*, CTRL-C) from the terminal where you launch the program.

# Appendix

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## Frequently Asked Questions

### Why is the Program Frozen?

The program will sometimes appear frozen during processing. This often happens when you first load the SOLiD™ System reads or the reference sequences, or when you attempt to switch application windows while the SOLiD™ SAGE™ program is actively running. Allow the process to continue for a few minutes before force-quitting.

### Why Am I Getting An Error Message?

The program includes various error messages, most of which are self-explanatory. If the error says something about “balloon” or “cancellation”, you can ignore it. These errors will not affect your results.

### How Long Does Mapping Take?

The running time of the mapping analysis depends on a few factors:

- Whether you are mapping to mRNA or the genome (mapping to the genome = longer processing time)
- Number of allowed mismatches (more mismatches allowed = longer processing time)
- The number of reads in the input SOLiD™ SAGE™ software file
- The size of the reference database
- Input tag length
- Number of million reads per round of processing (more rounds required = longer processing time)

A typical read file with octet SOLiD™ System data mapped to the human Refseq mRNA dataset with a tag length = 27 and mismatch = 2 takes ~10 minutes on a computer with a 2.33 GHz Duo CPU and 8 GB of memory (note that only one CPU and ~2 GB memory are actually used).

If you are mapping to the human genome with mismatch = 0, it may take 2–3 hours and up to 15 hours with mismatch = 2.

# Examples of Files and Formats

## Input File Formats **SOLiD™ System reads file:**

This is a standard .csfasta file, which looks like:

```
# Tue Dec 23 01:04:30 2008  comments follow
# Cwd: /home/pipeline
# Title: Solid0110_20081218_JMK26_Ribominus_JMK26_C_amp_1_
>443_12_55_F3
T211200121210112020020320000000
>443_12_118_F3
T00000000000000000000000000000000
>443_12_170_F3
T213001130012102033033032131301
>443_12_201_F3
T00300003230000000000000000000000
>443_12_278_F3
T00000000000000000000000000000000
>443_12_294_F3
T00000000000000000000000000000000
>443_12_336_F3
T00000000000000000000000000000000
```

### **Reference files:**

This is a standard NCBI fasta file, part of a database file set in a single directory.

Files look like:

```
>gi|155369268|ref|NM_001100917.1| Homo sapiens tetraspanin 19
(TSPAN19), mRNA
AAACAATCTCGATTCTAAATTG... .. (bases follow) ... .. ACTGGTG
>gi|169212695|ref|XM_001716884.1| PREDICTED: Homo sapiens
hypothetical protein LOC100132679 (LOC100132679), mRNA
ATGTGTGTATATATATATACACATATATATG... .. (bases follow) ... ..
ATGGATGTAT
```

### **Comparison input file:**

Same as standard output file (see next page), with a file name of the format solidsageread.csfasta.taglength.mismatch.output.tab (sample file: solidsageSampleRead.csfasta.27.0.output.tab)

**Output File Names**    **Output file:** File name format:  
 solidsageread.csfasta.taglength.mismatch.output.tab (sample file:  
 solidsageSampleRead.csfasta.27.0.output.tab)

**Results file:** File name format:  
 solidsageread.csfasta.taglength.mismatch.results.tab (sample file:  
 solidsageSampleRead.csfasta.27.0.results.tab)

**Comparison file:** File name: comparison.tab

**Mapping Output  
 File Format**

| Tag                         | Count | GI                                 | Description   |
|-----------------------------|-------|------------------------------------|---|
| CATGAAAAAACTCCAAATAAGAGAATC | 1     | GI33413399                         | >gi   33413399   ref   NM_001984.1   Homo sapiens esterase D/formylglutathione hydrolase (ESD), mRNA  |
| CATGAAAGGGTCACTTCTGTAATAGTG | 1     | GI81158221::GI81158223::GI81158225 | >gi   81158221   ref   NM_001037133.1   Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 3, mRNA::>gi   81158223   ref   NM_005010.3   Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 2, mRNA::>gi   81158225   ref   NM_001037132.1   Homo sapiens neuronal cell adhesion molecule (NRCAM), transcript variant 1, mRNA |
| CATGAACAACCGGCTGGCCGAGACCAG | 1     | GI11545760                         | >gi   11545760   ref   NM_022055.1   Homo sapiens potassium channel, subfamily K, member 12 (KCNK12), mRNA  |
| CATGAACTTGATACGTCCGTGTGTCCC | 1     | GI53759150                         | >gi   53759150   ref   NM_005063.4   Homo sapiens stearyl-CoA desaturase (delta-9-desaturase) (SCD), mRNA   |
| CATGAAGATGATATGAGGCCGGGGCGG | 1     | GI52145308                         | >gi   52145308   ref   NM_032808.5   Homo sapiens leucine rich repeat and Ig domain containing 1 (LINGO1), mRNA   |
| CATGAAGGAAGATCCCACAGTCTCAGC | 1     | GI4758483                          | >gi   4758483   ref   NM_004832.1   Homo sapiens glutathione S-transferase omega 1 (GSTO1), mRNA  |
| CATGACAGCCCTCTGCTCTTGAGTACC | 1     | GI39812204                         | >gi   39812204   ref   NM_025164.3   Homo sapiens KIAA0999 protein (KIAA0999), mRNA   |
| CATGACGGAACAATAGGACTCCCCAGG | 2     | GI38505192                         | >gi   38505192   ref   NM_000954.5   Homo sapiens prostaglandin D2 synthase 21kDa (brain) (PTGDS), mRNA   |
| CATGACGTGTCTATGTCAAAGTTCTT  | 1     | GI115583669                        | >gi   115583669   ref   NM_003253.2   Homo sapiens T-cell lymphoma invasion and metastasis 1 (TIAM1), mRNA  |

**Mapping Results  
File Format**

| Tag_Seq  | GI_num    | GI_Pos | Read_ID | Mismatch |
|--|-----------|--------|---------|----------|
| CATGTGCAAATAAATGTGGCTTAGACT<br>>665_1155_42_F3 0   | 133908618 | 3298   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 189083841 | 2125   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 189083835 | 2280   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 189083837 | 2146   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 189083839 | 2128   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 51593094  | 2283   |         |          |
| CATGGTAATAAAAATATGAATGATAAAA<br>>665_817_382_F3 0  | 132814488 | 2268   |         |          |
| CATGTGATGGGCATTGAGCCACACCTC<br>>665_1807_1132_F3 0 | 34147410  | 1140   |         |          |
| CATGAGGAGCTCGGCTTAAAATGTCTT<br>>665_1994_720_F3 0  | 40254847  | 2324   |         |          |

**Comparison File  
Format**

| Tag_Sequence  | File1 | File2 |
|---|-------|-------|
| CATGAAGACAGTGGCTGGCGGTGCCTG<br>(Based on non-truncated tag) >gi 78214519 ref NM_000998.4  Homo sapiens ribosomal protein L37a (RPL37A), mRNA  | 4715  | 5430  |
| CATGCACAAACGGTAGTTTTGTGTGTT<br>(Based on non-truncated tag) >gi 169202035 ref XM_001726056.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA::>gi 169203086 ref XM_001725509.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA::>gi 169203606 ref XM_001726007.1  PREDICTED: Homo sapiens similar to hCG2027326 (LOC100129905), mRNA::>gi 68160923 ref NM_001030.3  Homo sapiens ribosomal protein S27 (metallopanstimulin 1) (RPS27), mRNA | 2361  | 2604  |
| CATGACAACAAAGAAAAAGACCTTGTA<br>(Based on non-truncated tag) >gi 56550064 ref NM_001008220.1  Homo sapiens complexin 2 (CPLX2), transcript variant 2, mRNA::>gi 56550103 ref NM_006650.3  Homo sapiens complexin 2 (CPLX2), transcript variant 1, mRNA   | 1736  | 1606  |
| CATGAATATGTGGGCTAAGAAATAGTT<br>(Based on non-truncated tag) >gi 17999531 ref NM_004374.2  Homo sapiens cytochrome c oxidase subunit VIc (COX6C), mRNA   | 1659  | 1980  |

# Technical Support

## Web Resources



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Part Number MAN0001685 Rev. date: 8 July 2010

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