



## Trace File Submission Protocol

This protocol assists in the submission of trace files to BOLD. It describes the necessary format of the files and the ancillary data that is required for the correct submission.

### I. Register Primers:

Be sure that your primer codes are registered with BOLD before assembling the submission package. To register your primers, send the following information to [sratnasi@uoguelph.ca](mailto:sratnasi@uoguelph.ca)

- Desired primer code
- Primer sequence
- Reference (if from a manuscript) or designer's name

If the primer you used has already been registered under a different name, you will be provided with the registered code to be used in your submission. Primer codes are case sensitive and must be used exactly as registered or provided.

See the steps for assembling the package below.

### 2. Assemble Package:

The image submission package should consist of trace files (.ab1), corresponding phred files (.phd.1) and a spreadsheet with the file names and ancillary data. The submission spreadsheet should be named **data.xls** and contains the columns described below.

|                     |  |
|---------------------|--|
| Trace File          | complete (incl. extension) and identical file name(case sensitive) |
| Score File          | This field is currently optional but will be required              |
| PCR Primers Fwd/Rev | Primer codes are case sensitive                                    |
| Seq Primer          | This field is currently optional, but will be required             |
| Read Direction      | Forward or Reverse   |
| Process Id          | Process Id of specimen, must match Process Id in BOLD              |

Table 1 : Field definitions for accompanying spreadsheet

### Steps:

A. Fill in the data.xls data sheet with all the data about your files.

To create the list of the files in a folder, you need to open a terminal window (run cmd in windows), move to the folder where the trace and score files have been placed and then run one set of the following commands:

```
Windows      dir /b *.ab1>ab1.txt and dir /b *.phd.1 >phd.txt
MacOS        ls *.ab1>ab1.txt and ls *.phd.1 > phd.txt
Linux/Unix   ls *.ab1>ab1.txt and ls *.phd.1 > phd.txt
```

These commands will generate lists of all the files in the current folder and save it ab1.txt and phd.txt. You can then open the text files and move the data into the appropriate columns.

| Trace File           | Score File             | PCR Fwd | PCR Rev | Seq Primer | Read Direction | Process Id  |
|----------------------|------------------------|---------|---------|------------|----------------|-------------|
| KKBNA001-04_H01.ab1  | KKBNA001-04_H01.phd.1  | BirdF1  | BirdR1  | BirdF1     | Forward        | KKBNA001-04 |
| KKBNA001-04r_H07.ab1 | KKBNA001-04r_H07.phd.1 | BirdF1  | BirdR1  | BirdR1     | Reverse        | KKBNA001-04 |
| KKBNA002-04_G01.ab1  | KKBNA002-04_G01.phd.1  | BirdF1  | BirdR1  | BirdF1     | Forward        | KKBNA002-04 |
| KKBNA002-04r_G07.ab1 | KKBNA002-04r_G07.phd.1 | BirdF1  | BirdR1  | BirdR1     | Reverse        | KKBNA002-04 |
| KKBNA003-04_F01.ab1  | KKBNA003-04_F01.phd.1  | BirdF1  | BirdR1  | BirdF1     | Forward        | KKBNA003-04 |
| KKBNA003-04r_F07.ab1 | KKBNA003-04r_F07.phd.1 | BirdF1  | BirdR1  | BirdR1     | Reverse        | KKBNA003-04 |
| KKBNA004-04_E01.ab1  | KKBNA004-04_E01.phd.1  | BirdF1  | BirdR1  | BirdF1     | Forward        | KKBNA004-04 |

Example 1: data.xls with sample data filled in

B. These components (Trace files, Score files and Spreadsheet) need to be placed in a single folder. Compress them all into a single file before submitting. The following free tools are available to provide this functionality:

WinZip - [http://www.download.com/WinZip/3000-2250\\_4-1045671.html?tag=lst-0-1](http://www.download.com/WinZip/3000-2250_4-1045671.html?tag=lst-0-1)  
 WinRar - [http://www.download.com/WinRAR/3000-2250\\_4-1045671.html?tag=lst-0-1](http://www.download.com/WinRAR/3000-2250_4-1045671.html?tag=lst-0-1)  
 MacZipIt - <http://www.maxzipit.com>

C. BOLD will accept a maximum file size of 200MB. Upload the images to BOLD by clicking on the link "Trace Files" in the Uploads panel of the desired project. Select the zipped folder of files and the hit "submit".

## Trace File Submission Tips

This information describes the most common issues with trace file uploads.

- Primers must be registered before upload. If the primers are not registered, there will be an error. Please refer to Step 1 in the Trace File Submission Protocol.
- Zipped file must be under 200mb in size. If the upload fails to initialize, it is probably because the zipped file is too large. Try breaking it into two uploads.
- No formulas can be in the spreadsheet. If the upload program can not find the files, it is possibly because it can not read the names. Make sure that you have text values only in the spreadsheet.
- Full filenames must be used in excel sheet. The extension (.ab1, .phd.1) must be included in the file name (case sensitive).
- Spreadsheet must be named data.xls. If the upload program can not find the excel sheet, confirm that it is named correctly (case sensitive).
- Max of 30 characters in the free text fields of the excel sheet. Verify that the data length in these fields and make adjustments if necessary.
- Data must start on the second line of the spreadsheet. There is only one line for the column headers.