TS2Mascot

Introduction

TS2Mascot is a simple utility to export peak lists from an Applied Biosystems 4000 Series database. The peak list is saved as a Mascot Generic Format (MGF) file. This can be a file in a user-selected location or it can be a temporary file for immediate submission to Mascot.

This utility fulfils two requirements that are not met elsewhere:

- 1. TS2Mascot can be used as a data import filter, enabling automated search submission from Mascot Daemon. This is particularly important if you are using Mascot Integra for automation and data management.
- 2. TS2Mascot exports a peak list that has accurate peak areas for the iTRAQ reporter peaks. The peak areas in a peak list created by *Peaks to Mascot* are not the same as those used by GPS Explorer for quantitation. This is particularly important if you wish to perform iTRAQ quantitation outside of GPS Explorer.

The utility has two components. An executable for stand-alone, interactive use and a dynamic link library, (DLL), that can be called by Mascot Daemon or any software that supports COM

System Requirements

TS2Mascot will run under Microsoft Windows NT 4.0 SP6, Windows 2000, Windows XP Professional, and Windows 2003 Server (32 bit only).

TS2Mascot communicates with the Applied Biosystems 4000 Series database using ODBC. The PC will need to have Oracle client software installed, including the Oracle ODBC driver.

To submit a Mascot search from TS2Mascot, Microsoft Internet Explorer 4.01 SP2 or later must be installed and operational.

Installation

Install TS2Mascot by executing TS2Mascot.msi and choosing a suitable location for the installation.

Unless 4000 Series Explorer or GPS Explorer is already installed, you may need to create a local Oracle service name, (see Appendix).

Interactive Use

When launched (Start menu; Programs; Mascot; TS2Mascot), the main window has this appearance:

| L Applied Riccustores | | × c | Export all MS7MS spectra in job run Export selected spectrum only |
|--------------------------------------|-------------------------------|--|--|
| Applied Blosystems Catherine | | | |
| Factory Methods Hans | | | |
| 🗄 🦲 James | | | |
| | | | |
| 🗄 🦲 Kaajal | | | |
| | | | |
| New Project | | | |
| | | | |
| La rectan | | | |
| 🗄 🧰 sarah 🔛 | | | |
| n - initiation - sarah | | ⊢ Mascot Server URL (e.g. http://lo | calhost/mascot/cgi/) |
| eak Filtering | | Mascot Server URL (e.g. http://lo | calhost/mascot/cgi/) |
| eak Filtering Mass Range 60 Da to | 20 Da below precursor mass | Mascot Server URL (e.g. http://lo | calhost/mascot/cgi/) |
| eak Filtering Mass Range 60 Da to | 20 Da below precursor mass | Mascot Server URL (e.g. http://lo http://koala/mascot/cgi/ 4000 Series Database Connection | calhost/mascot/cgi/] |

The Oracle service name is displayed towards the lower right. The folder tree in the *Spot Sets* frame is populated with project folders from the 4000 series database.

If you have more than one 4000 series database, choose *Change* to connect to a different database.

The default connection is to a data source called TSQUARED with User ID TSQUARED and password ts. If this is not correct, the ODBC connection dialog will be displayed when TS2Mascot is first launched:

| ODBC Connection | | |
|---|--|-------------|
| ODBC connection to 4000 series | database | |
| Provider=OraOLEDB.Oracle.1; ID=tsquared;password=ts;Data | Persist Security Info=True Source=TSQUARED; | ;User |
| Restore default | | ODBC Wizard |
| | Cancel | Save |

You can edit the connection string in place, or choose *ODBC Wizard* to create a new connection string:

| 🗟 Data Link Properties 🛛 🗙 | | | | | | | |
|---|--|--|--|--|--|--|--|
| Provider Connection Advanced All | | | | | | | |
| Select the data you want to connect to: | | | | | | | |
| OLE DB Provider(s) | | | | | | | |
| MediaCatalogDB OLE DB Provider | | | | | | | |
| MediaCatalogMergedDB OLE DB Provider | | | | | | | |
| Microsoft ISAM 1.1 OLE DB Provider | | | | | | | |
| Microsoft Jet 3.51 OLE DB Provider | | | | | | | |
| Microsoft Jet 4.0 OLE DB Provider | | | | | | | |
| Microsoft OLE DB Provider for Internet Publishing | | | | | | | |
| Microsoft OLE DB Provider for ODBC Drivers | | | | | | | |
| Microsoft OLE DB Provider for OLAP Services | | | | | | | |
| Microsoft OLE DB Provider for SQL Server | | | | | | | |
| Microsoft OLE DB Simple Provider | | | | | | | |
| Millennium ADO Data Provider | | | | | | | |
| OLE DB Provider for Microsoft Directory Services | | | | | | | |
| Oracle Provider for OLE DB | | | | | | | |
| , | | | | | | | |
| Next >> | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| OK Cancel Help | | | | | | | |

If you create a new connection string, it is advisable to choose the Oracle Provider for OLE DB. The Microsoft OLE DB Provider for Oracle may work, but has not been thoroughly tested.

If you use the ODBC Wizard, make sure you check *Allow saving password* and test the connection before saving it.

| 평 Data Link Properties 🛛 💈 💈 | | | | | | |
|---|--|--|--|--|--|--|
| Provider Connection Advanced All | | | | | | |
| Specify the following to connect to this data: | | | | | | |
| 1. Enter the data source and/or location of the data: | | | | | | |
| Data Source: TSQUARED | | | | | | |
| Location: | | | | | | |
| 2. Enter information to log on to the server: | | | | | | |
| C Use Windows NT Integrated security | | | | | | |
| Use a specific user name and password: | | | | | | |
| User <u>n</u> ame: Isquared | | | | | | |
| Password: ** | | | | | | |
| □ Blank password Allow saving password | | | | | | |
| 3. Enter the initial catalog to use: | | | | | | |
| | | | | | | |
| (<u>Test Connection</u>) | | | | | | |
| OK Cancel Help | | | | | | |

Once connected to the database, simply navigate the folder tree and select a spot set icon. This will populate the adjacent drop down list with the job runs for the spot set. The grid area displays details of individual job run items for the selected job run.

Whenever the grid is populated, the Save peak list and Mascot Search buttons are enabled.

| 🕀 🧰 hans | | ~ | 4: Job ru | n 12384; 03/11/ | 2005 18:2 | 1:04; MS-MS 2 | KV Positive 💌 | Export all MS/MS spe Export selected spects | ctra in job run rum only |
|-----------------------------|--------------|-------|-----------|-----------------------|-----------|-----------------|--|--|-----------------------------|
| H James | | | SPO | T LABEL | ISPOT | NAME | SPOT TYPE | PRECURSOR MASS | |
| | hun | | 1 | | | | Unknown | 2336.154 | MS-MS 2K |
| 🕀 🧰 Kaaial | p | | 1 | | _ | | Unknown | 2617.197 | MS-MS 2K |
| 🖾 🦲 Martin | | | 1 | | | | Unknown | 1252.544 | MS-MS 2K |
| 🕀 🧰 New F | roject | | 1 | | | | Unknown | 2514.093 | MS-MS 2K |
| E O New F | roject | | 1 | | | | Unknown | 1344.682 | MS-MS 2K |
| I Nick | rojoot | | 1 | | _ | | Unknown | 2463.284 | MS-MS 2K |
| | 1 | | 1 | | | | Unknown | 1233.563 | MS-MS 2K |
| | av Project | | 1 | | | | Unknown | 2479.202 | MS-MS 2K |
| | Spot Set JSC | | 1 | | | | Unknown | 2447.287 | MS-MS 2K |
| 🖽 🧰 🔤 | Project | _ | 3 | | - | | Unknown | 2542.095 | MS-MS 2K |
| E _ h | SWI TOJOOC | | 4 | | - | | Unknown | 2486.308 | MS-MS 2K + |
| 🗄 🦲 Trainin | q | ~ | • | 1 | - | | 1. | 1 | • |
| eak Filtering Mass Range | 60 | Da to | 20 | Da below precursor | mass | Masco | t Server URL (e.g. http p://koala/mascot/cgi/ |)://localhost/mascot/cgi/) — , | |
| dinimum S/N | 10 | | 🔽 Mono | isotopic peaks or | nly | - 4000 S Con | eries Database Conne nected to TSQU | JARED | Change |

The radio buttons are used to choose whether to export all of the spectra in the job run, which only works for MS/MS data, or a selected spectrum, which works for both MS and MS/MS. The selected spectrum is indicated by the marker in the first column of the grid. Click in this column to select a different spectrum.

Peak filtering settings are equivalent to those used in Applied Biosystems GPS Explorer. The mass range limits only apply to MS/MS fragment ion peaks. The settings shown above are suitable for Mascot searching of iTRAQ data. The contents of the Peak Filtering and Mascot Server URL fields are sticky. If you change them, then process a spot set, the new values will be remembered.

Save peak list invokes a standard file selection dialog before processing the data.

Before choosing *Mascot Search*, ensure that you have entered a valid URL for a Mascot server. You can enter the URL of the Matrix Science public web site, but remember that this has a limit of 300 spectra in a single search.

While the peak list is being created, a progress bar is displayed, and all the controls are disabled apart from a *Cancel* button.

| 🛨 🦲 hans | ▲ 4: J | ob run 12384; 03/1 | 1/2005 18:21:04; MS-M | 6 2KV Positive 💽 | Export all M5/M5 spe Export selected spect | otra in job ru rum only |
|----------------------|----------|--------------------|-----------------------|---|---|----------------------------|
| 🛨 🛄 James 王 📄 jmc | | SPOT LABEL | SPOT NAME | SPOT TYPE | PRECURSOR MASS | OPMODE |
| 🗄 🦳 JMCsetup | | 1 | | Unknown | 2336.154 | MS-MS 2K |
| 🕂 🧰 Kaajal | | 1 | | Unknown | 2617.197 | MS-MS 2K |
| 🕂 🧰 Martin | | 1 | | Unknown | 1252.544 | MS-MS 2K |
| 🕂 🦳 New Project | | 1 | | Unknown | 2514.093 | MS-MS 2K |
| 🕂 🦳 New Project | | 1 | | Unknown | 1344.682 | MS-MS 2K |
| F 🔲 Nick | | 1 | | Unknown | 2463.284 | MS-MS 2K |
| | | 1 | | Unknown | 1233.563 | MS-MS 2K |
| New Project | | 1 | | Unknown | 2479.202 | MS-MS 2K |
| Spot Set JSC | | 1 | | Unknown | 2447.287 | MS-MS 2K |
| E New Project | | 3 | | Unknown | 2542.095 | MS-MS 2K |
| E Sarah | | 4 | | Unknown | 2486.308 | MS-MS 2K |
| ak Filtering | Da to 20 | Da bel precurs | ow sor mass | cot Server URL (e.g. http: http://koala/mascot/cgi/ 0 Series Database Conne | :://localhost/mascot/cgi/)— , section— | |

Once peak list export is complete, if you had chosen *Mascot Search*, the default web browser will be launched and the Mascot MS/MS search form displayed:

| 🕘 Matrix Science - | Mascot - MS/MS lons Search - Mic | rosoft Internet I | Explorer 📃 🗖 🔀 | | | | | | |
|-----------------------------------|--|---------------------------|---|--|--|--|--|--|--|
| <u>File E</u> dit <u>V</u> iew Fa | Eile Edit View Favorites Iools Help | | | | | | | | |
| G Back • O | C Back · O · E C · Search * Favorites 6 Powermarks 1 A % | | | | | | | | |
| Address 🍓 http://koal | a/mascot/cgi/search_form.pl?SEARCH=MI | 5 | 💌 🄁 Go | | | | | | |
| маѕсот | MS/MS Ions Searc | h | | | | | | | |
| Your name | | Email | | | | | | | |
| Search title | New Project/Spot Set JSC 4: Jol | o run 12384; 03 | /11/2005 18:21:04; MS | | | | | | |
| Database | Sprot 💌 | | | | | | | | |
| Taxonomy | All entries | | ▼ | | | | | | |
| Enzyme | Trypsin 💌 | Allow up to | 1 💌 missed cleavages | | | | | | |
| Fixed modifications | ITRAQ (K) ITRAQ (N-term) ITRAQ (Y) Me-ester (C-term) Me-ester (DE) ✓ | Variable modifications | AB_old_ICATd0 (C) AB_old_ICATd8 (C) Acetyl (K) Acetyl (N-term) Amide (C-term) | | | | | | |
| Protein mass | kDa | ICAT | | | | | | | |
| Peptide tol. ± | 0.1 Da 💌 | MS/MS tol. \pm | 0.1 Da 💌 | | | | | | |
| Peptide charge | 2+ 💌 | Monoisotopic | ⊙ Average ○ | | | | | | |
| Data file | c\LOCALS~1\Temp\ts210F.tmp | Browse | | | | | | | |
| Data format | Mascot generic 🛛 💌 | Precursor | m/z | | | | | | |
| Instrument | MALDI-TOF-TOF | | | | | | | | |
| Overview | | Report top | AUTO 🔽 hits | | | | | | |
| | Start Search | | Reset Form | | | | | | |
| | | | | | | | | | |
| | Consulable @ 2005 Mattin Cala | معامر ما م | Reserved | | | | | | |
| | Copyright © 2005 Matrix Sciel | ice Lta. All Rights | Keserveo. | | | | | | |

The search title and data file path are filled in automatically. The other settings are the search form defaults, (which can be customised by following a link on the search form selection page).

Make changes to the search parameters as required and choose *Start Search* to submit the search to Mascot.

Calling TS2Mascot.dll

This code fragment in Visual Basic illustrates the properties and methods:

```
Private Sub Form Load()
  Dim TS2 As TS2Mascot
  'Create a TS2Mascot object
  Set TS2 = New TS2Mascot
  'Might want to check the dll version before proceeding
  Debug.Print TS2.DllVersion
  'Invoke the TS2Mascot database browser window to select
  'individual spectra or a complete job run
  On Error Resume Next
  If TS2.ShowDataBrowser = True Then
    'connect to the 4000 series database
    If TS2.Connect = True Then
      'export the peak lists(s) to an MGF file
      If TS2.DoExport("c:\temp\xxx.mgf") = False Then
        MsgBox "DoExport method failed with error: "
        & Err.Description
      End If
    Else
      MsqBox "Connect method failed with error: " & Err.Description
    End If
  Else
   MsgBox "User chose Cancel return from ShowDataBrowser"
  End If
  On Error GoTo 0
  Exit Sub
  'Choosing Select from the data browser sets the following
  'properties. If the data browser is not used, some or all
  'of these properties must be set before calling DoExport.
  'A description for use in scan titles, (optional)
  'ShowDataBrowser sets this to the spot set path
  TS2.Descriptor = "NICKM/New Project/Spot Set JSC"
  'The job run ID (required)
  TS2.JobRunID = 12345
  'The job run item ID to export a single spectrum
  '(this is ignored if AllSpectra = True)
  TS2.JobRunItemID = 0
  'Whether to export a complete job run (True)
  'or an individual spectrum (False)
  TS2.AllSpectra = True
  'The type of spectrum, either "MS" or "MS-MS"
  '(if AllSpectra = True, this must be "MS-MS")
  TS2.MSType = "MS-MS"
  'Choosing Select from the data browser also returns
  'these values as a colon delimited string in the
  'DescriptorList property
  '"Descriptor:JobRunID:JobRunItemID:AllSpectra:MSType"
```

```
'If multiple rows are selected, the strings for
'all rows are concatenated, delimited by Chr(0)
Dim sArray() As String
sArray() = Split(TS2.DescriptorList, Chr(0))
'The following properties are initialised from values
'saved to the registry by TS2Mascot.exe. They can be
'read or set in code as required
'ODBC connection string
TS2.ODBCString = "Provider=OraOLEDB.Oracle.1;Persist Security
    Info=True;User ID=tsquared;password=ts;Data Source=TSQUARED;"
'peak filter properties
TS2.Monoisotopic = 1 '1=checked, 0=unchecked
TS2.maxMass = 20
                     'below the precursor mass
TS2.minMass = 60
TS2.S2N = 10
                      'min S/N
```

End Sub

The only other method is Cancel, which is used to interrupt DoExport if it can be called in a separate thread. All properties are read and write except DllVersion, which is read only.

Appendix: Creating a local Oracle Service Name

Launch the Oracle Net Manager, Select *Service Naming*, and choose the plus tool button to add a new net service name



You can use any name you like. The advantage of choosing TSQUARED is that you won't need to change the default name used by TS2Mascot



If you are unsure what the network protocol is, try TCP/IP first

| Net Service Name Wizard, | page 2 of 5: Protocol | × |
|--------------------------|---|---|
| | To communicate with the database across a network, a network protocol is used. Select the protocol used for the database you want to access. TCP/IP (Internet Protocol) TCP/IP with SSL (Secure Internet Protocol) Named Pipes (Microsoft Networking) IPC (Local Database) | |
| Cancel | 🔇 Back 🛛 Next 📎 | |

Enter the host name of the computer which hosts the 4000 Series database. The default port number will usually be correct

| Net Service Name Wizard, | page 3 of 5: Protocol Settings 🛛 🗙 |
|--------------------------|---|
| | To communicate with the database using the TCP/IP protocol, the database computer's host name is required. Enter the TCP/IP host name for the computer where the database is located. |
| | Host Name: t41-jsc |
| | A TCP/IP port number is also required. The port number for Oracle databases is usually 1521. You should not normally need to specify a different port number. |
| | Port Number: 1521 |
| | |
| Cancel | 🔇 Back Next >> |

You must enter the actual service name of the 4000 Series database on the Oracle server. In this example, it is also called TSQUARED

| Net Service Name Wizard, | page 4 of 5: Service | | × |
|--------------------------|---|--|---|
| | To identify the database or service you name, for Oracle8i 8.1 or later, or syste database versions. The service name is normally its global database name. (Oracle8i or later) Service Name: (Oracle8 or Previous) SID: Optionally, you can choose if you want database connection. The default is to Connection Type: | I must provide either its service em identifer (SID), for Oracle8 8.0 for an Oracle8 i or later database TSQUARED ORCL a shared or dedicated server let the database decide. Database Default | |
| Cancel | Bac | k <u>N</u> ext ≫ | |

Always test the connection. If the default user ID and password (scott, tiger) fails, try the standard user ID and password set on installation: tsquared and ts

| Connectio | n Test | | | |
|---|--|---|--|--|
| Expect cor please wa test press | nnecting to the database it; the reason for any failu Change Login. | to take from one to sev ure will be displayed. T | reral seconds to complete. If o change the userid and pas | it takes longer, sword used for the |
| When finis | shed testing press Close | 9. | | |
| Initializing Attempting The conne |) first test to use userid: s g to connect using useric ection test was successf | scott, password: tiger d: scott ful. | | |
| वा | | 1 Alexandre | | D |
| | | | Change Login | . Test Close |

If the test is successful, save the changes. You should now be able to connect to the remote 4000 database using TS2Mascot.