

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.

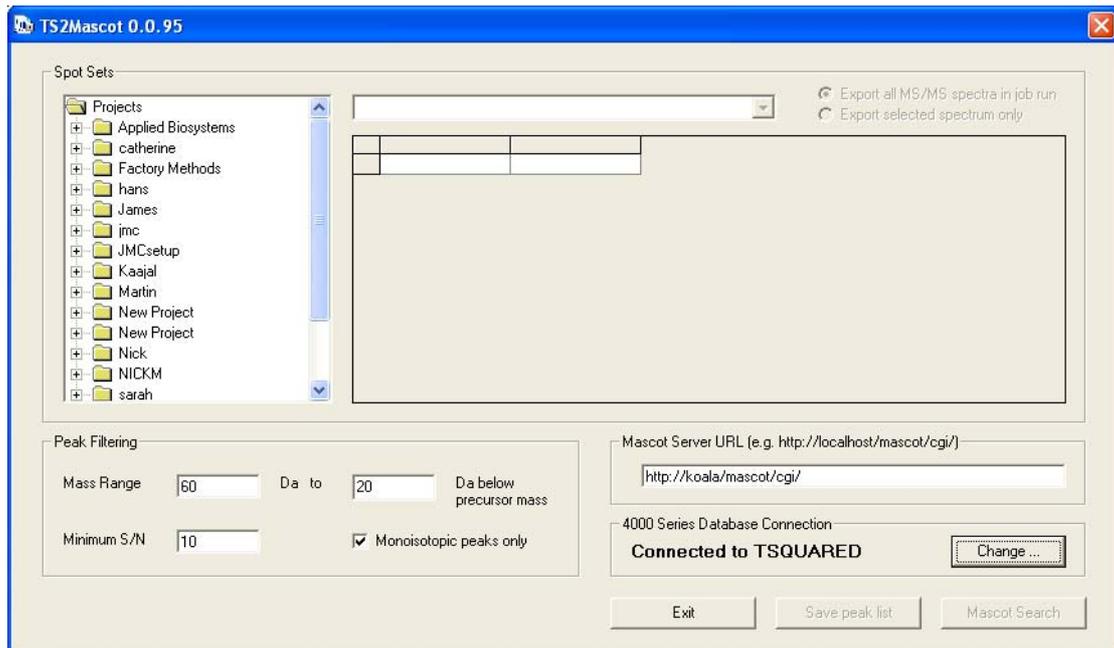
Installation

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

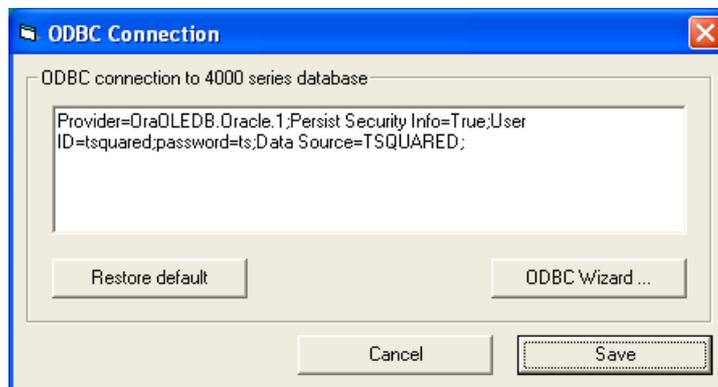
When launched (Start menu; Programs; Mascot; TS2Mascot), the main window has this appearance:



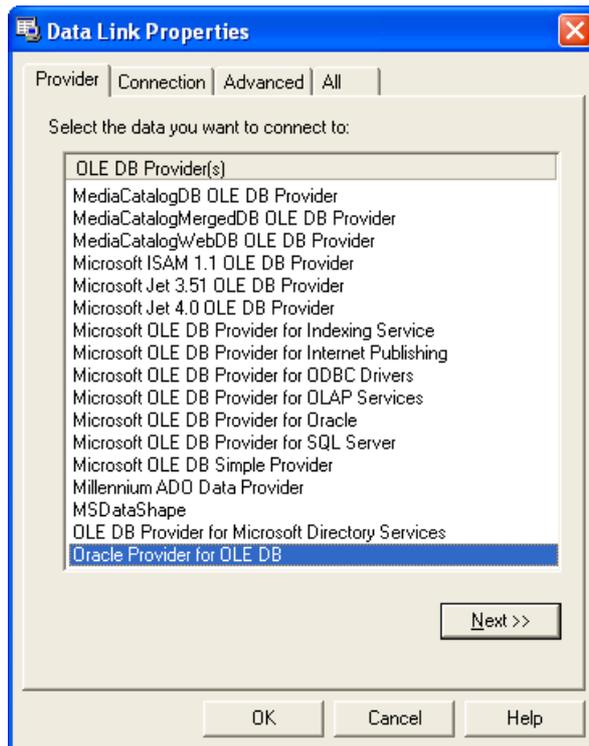
The Oracle service name is displayed towards the lower right. The folder tree in the *Spot Sets* frame is populated with project folders from the 4000 series database.

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:

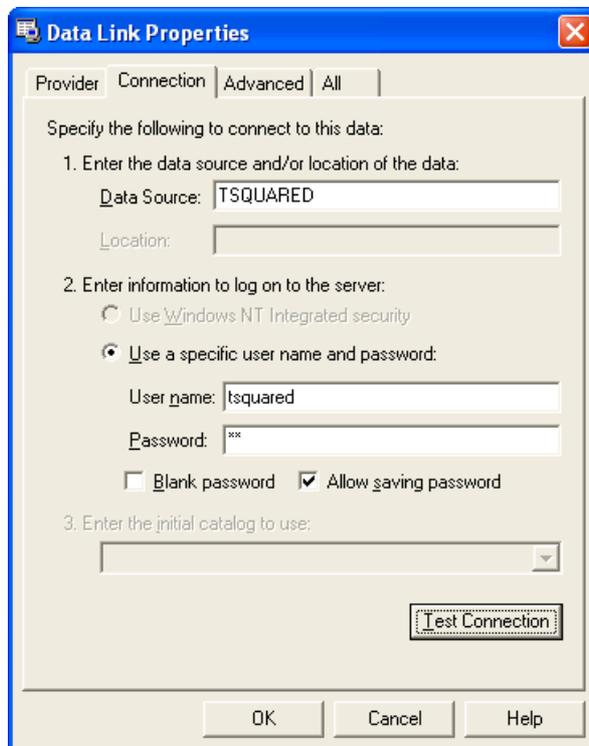


You can edit the connection string in place, or choose *ODBC Wizard* to create a new connection string:



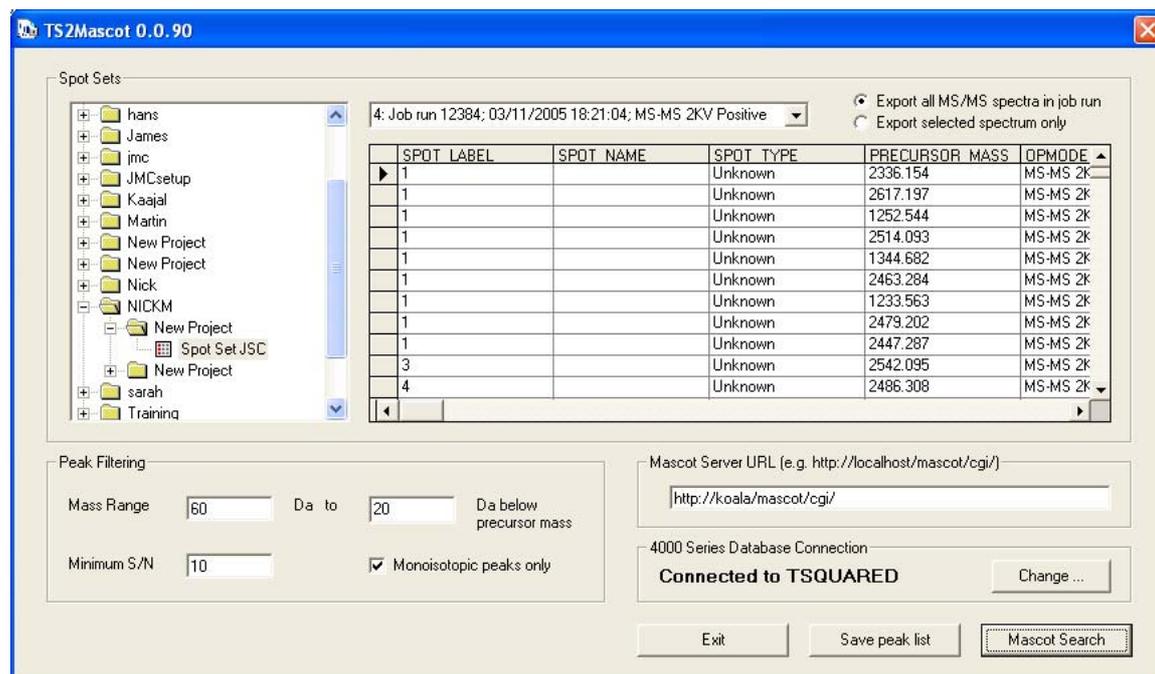
If you create a new connection string, it is advisable to choose the Oracle Provider for OLE DB. The Microsoft OLE DB Provider for Oracle may work, but has not been thoroughly tested.

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



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.



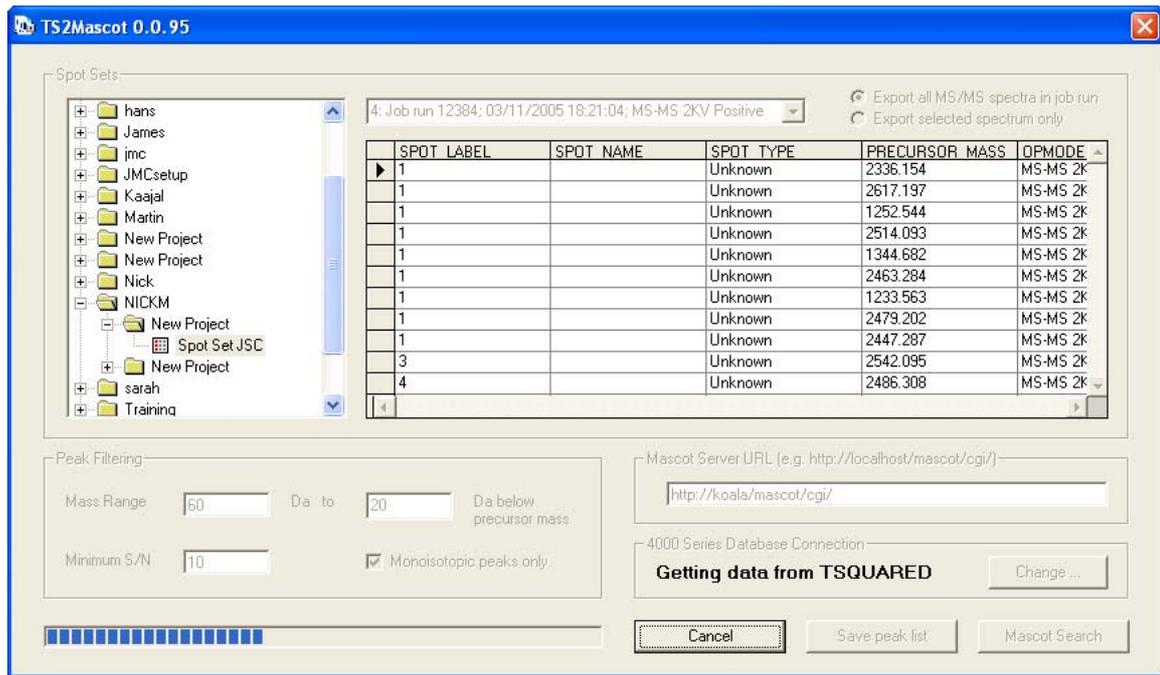
The radio buttons are used to choose whether to export all of the spectra in the job run, which only works for MS/MS data, or a selected spectrum, which works for both MS and MS/MS. The selected spectrum is indicated by the marker in the first column of the grid. Click in this column to select a different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

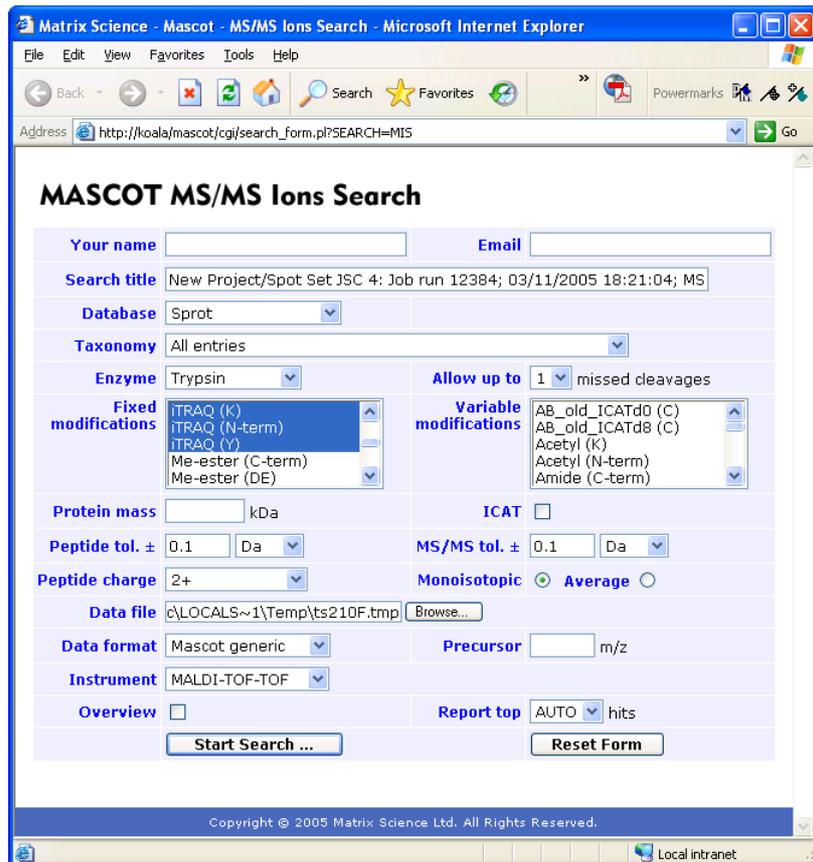
Save peak list invokes a standard file selection dialog before processing the data.

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.



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:



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  
            MsgBox "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

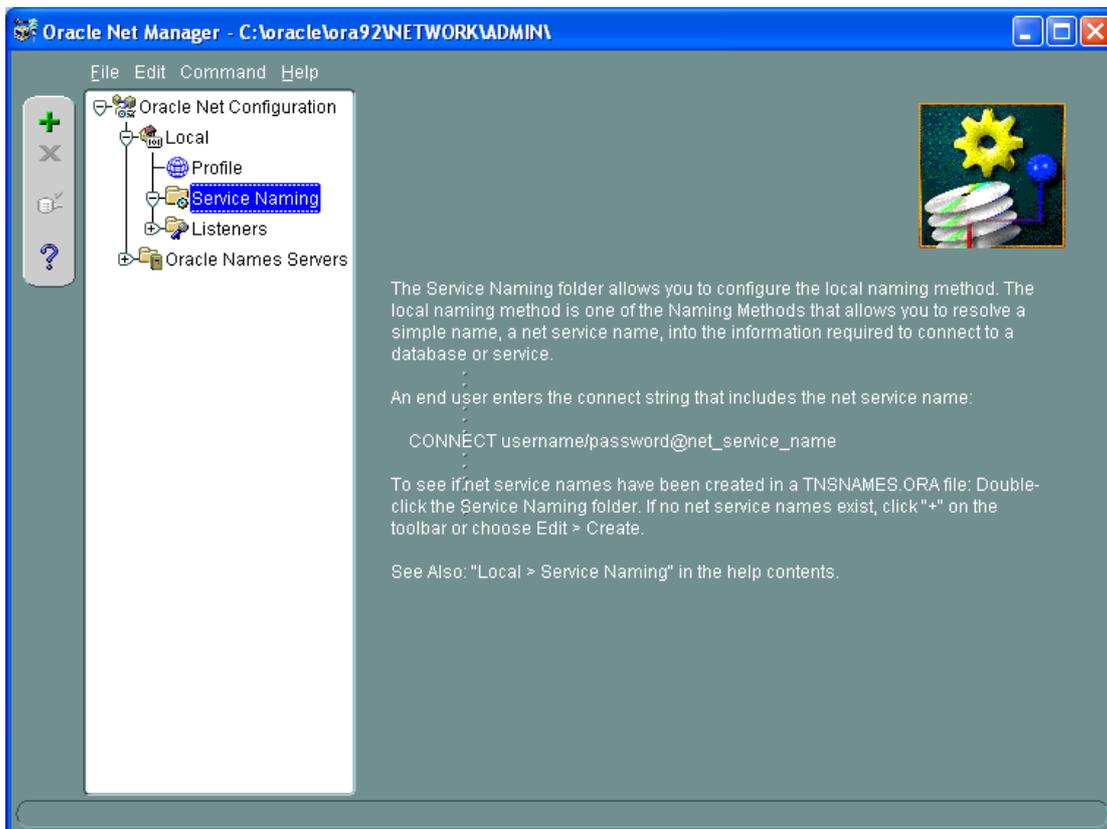
```

End Sub

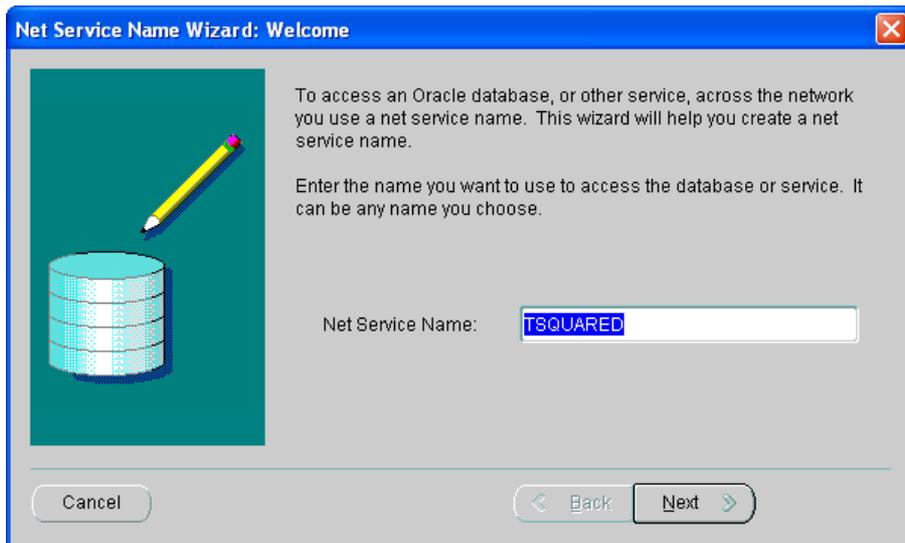
The only other method is Cancel, which is used to interrupt DoExport if it can be called in a separate thread. All properties are read and write except DllVersion, which is read only.

Appendix: Creating a local Oracle Service Name

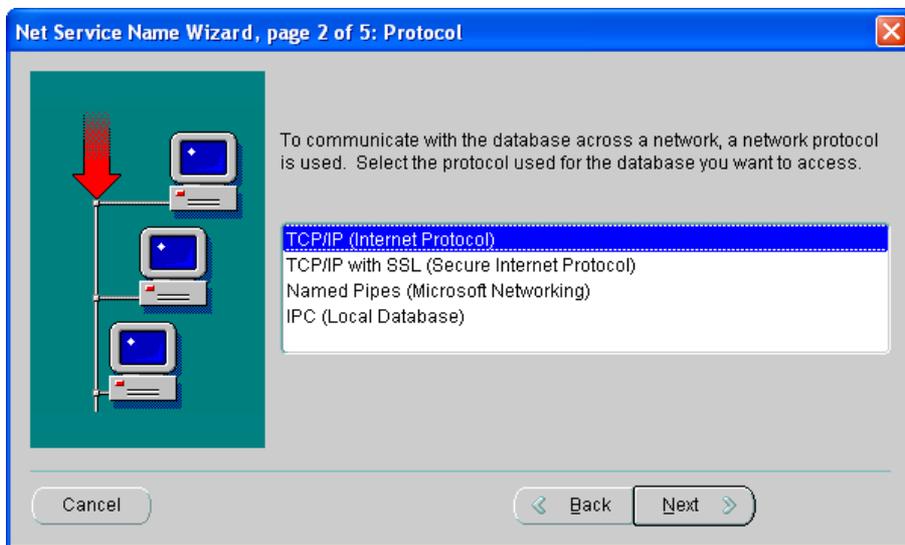
Launch the Oracle Net Manager, Select *Service Naming*, and choose the plus tool button to add a new net service name



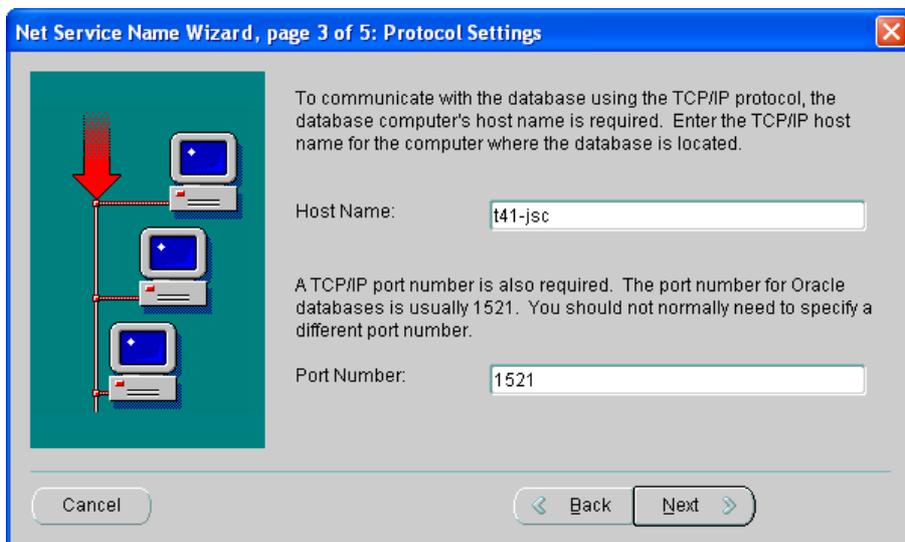
You can use any name you like. The advantage of choosing TSQUARED is that you won't need to change the default name used by TS2Mascot



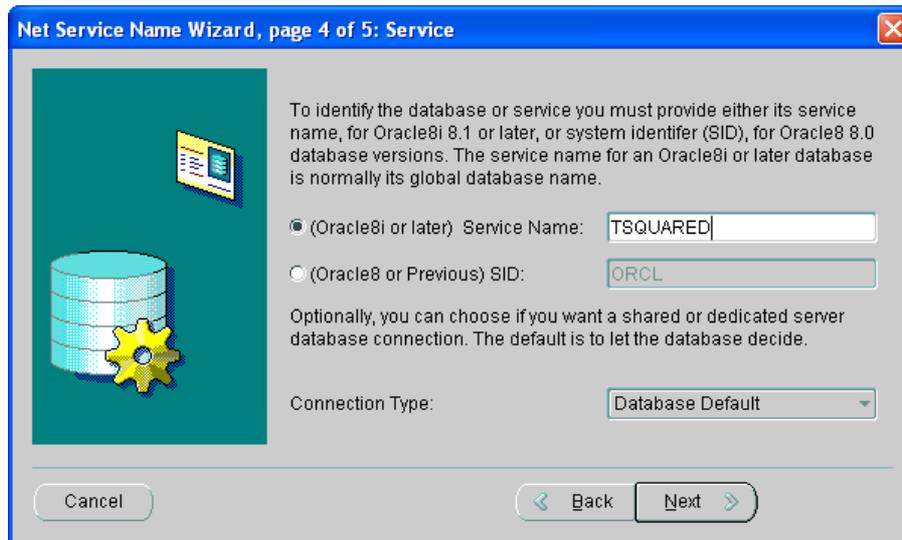
If you are unsure what the network protocol is, try TCP/IP first



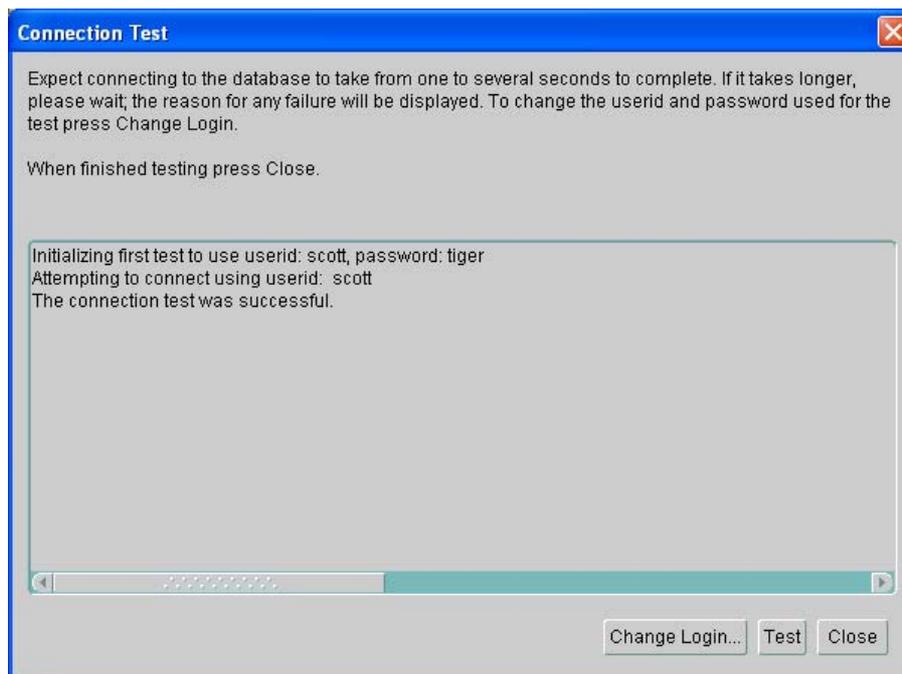
Enter the host name of the computer which hosts the 4000 Series database. The default port number will usually be correct



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



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



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