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Chapter 1. Introduction

DAX can be used to read and analyse trace files such as the files that are created by the Amersham MegaBACE™ and ABI Genescan® systems.

The MegaBACE™ and ABI systems, like other DNA analysis systems, can analyse four or five traces in a single run, using four (five) different (coloured) labels. One way to use this feature is to include a standard sample with known DNA fragment sizes as one of the traces, and apply this standard to the other traces. The standard is used to ascertain the fragment sizes of the components in the unknown samples.

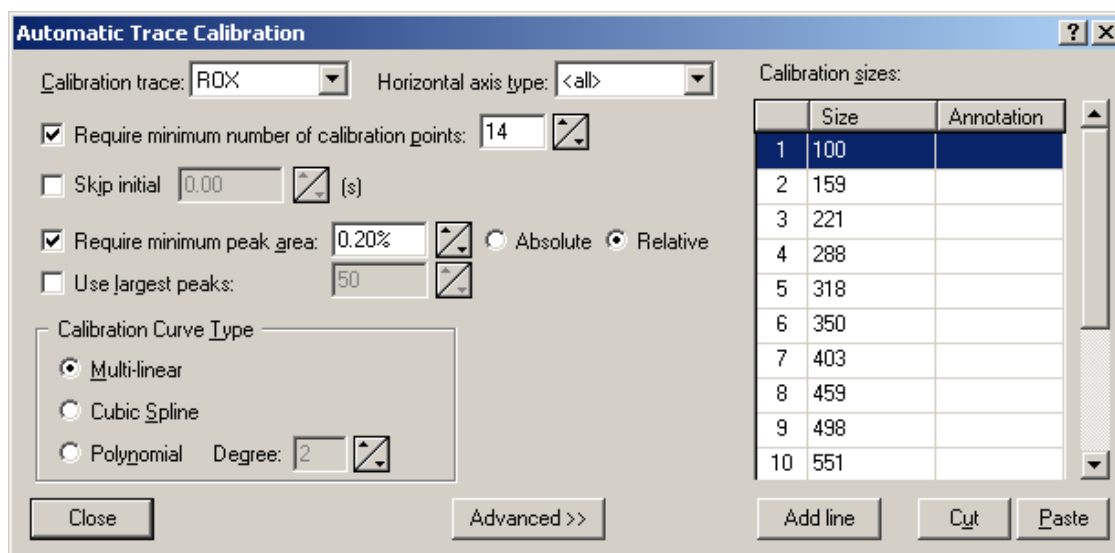
Chapter 2. Defining the Standard

This chapter describes the steps needed to set up the analysis of the sizing standard contained in trace files. Typically, you will go through this process once for a particular sizing standard.

DAX uses a heuristic method called Automatic Trace Calibration to recognise the sizing standard's fragments¹.

Chapter 2.1. Defining the ATC parameters

Use the **Analysis | Edit ATC ..** menu option to invoke the ATC² dialog box. The dialog box looks like this:



The dialog box titled "Automatic Trace Calibration" contains the following settings:

- Calibration trace: ROX
- Horizontal axis type: <all>
- Require minimum number of calibration points: 14
- Skip initial: 0.00 (s)
- Require minimum peak area: 0.20% (Absolute/Relative radio buttons)
- Use largest peaks: 50
- Calibration Curve Type: Multi-linear (selected), Cubic Spline, Polynomial (Degree: 2)

On the right, a table lists calibration sizes:

	Size	Annotation
1	100	
2	159	
3	221	
4	288	
5	318	
6	350	
7	403	
8	459	
9	498	
10	551	

Buttons at the bottom include Close, Advanced >>, Add line, Cut, and Paste.

The items in this dialog box have the following meaning:

- the **Calibration trace** defines the trace that contains the sizing standard, in this instance ROX.
- Require minimum number of calibration points** will often be set to the number of standard sizes, but can be set to a lower value if the sample may not have been analysed completely (i.e. if the analysis was cut short).

¹ Earlier versions of DAX used a process that recognised the sizing standard's peaks, essentially, by their positions; refer to daxtrace.pdf for an explanation of this procedure.

² ATC is short for Automatic Trace Calibration.

- **Skip initial ...** can be used to disregard the early part of a measurement, which speeds up the analysis, but is not required.
- **Require minimum peak area ...** can be used to disregard small peaks, which again speeds up the analysis, but is not required. Values of 0.2% or 0.4% generally work well.
- The **Use largest peaks** option is an alternative way of disregarding small peaks. If **n** standard peaks are present, a value of **2n** generally works well.
- The **Calibration curve type** defines what sort of curve will be drawn through the sizing calibration points.
- The **Calibration sizes** are simply a list of the sizes of the fragments in the sizing standard. For the example file L0083.rsd, these sizes are: 100, 159, 221, 288, 318, 350, 403, 459, 498, 551, 600, 633, 700, 726

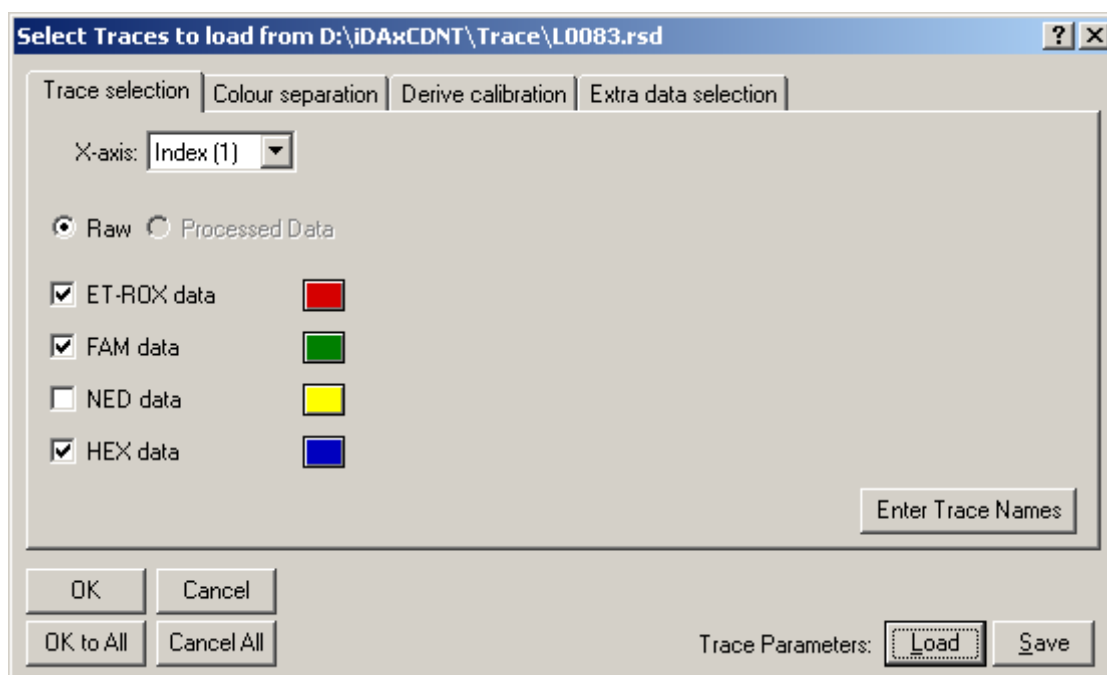
Once all fields have been filled, click the **Close** button to close the ATC dialog. You should now use **Analysis | Save ATC parameters** to store this setup on disk.

The file ATC.atc also contains these settings.

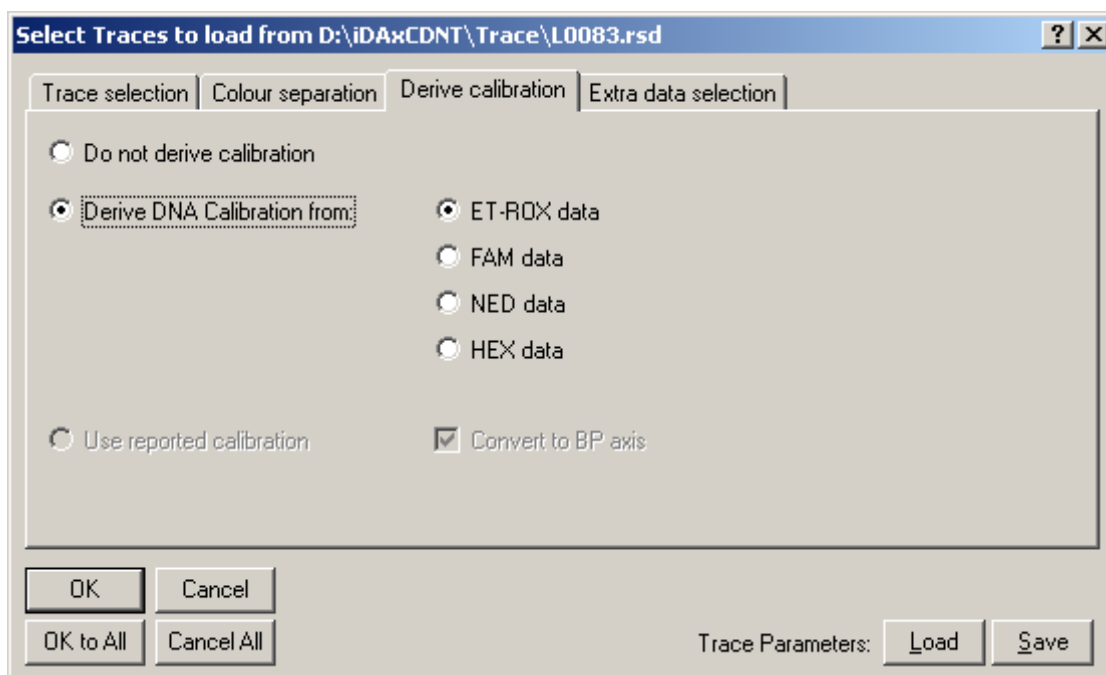
Chapter 3. Analysis of the Unknown

To analyse a file, follow the following procedure.

1. Invoke the **File | Open** command. Select **Files of Type MegaBACE™**. Select the file or files you want to analyse.
2. Check the **AutoAnalyse** button.
3. Click the **Config** button next to the AutoAnalyse button. The AutoAnalysis setup dialog box appears.
4. Make sure that at least **Construct Baseline** and **Detect Peaks** are checked. Additional items can be checked also. However, **Derive DNA Calibration** should not be checked. For now, do not check **Convert Axis**.
5. Click the **OK** button.
6. Click the **Open** button. This will start loading the trace file.
7. The Trace Analysis Parameters dialog box is displayed. Set it up as shown below. Alternatively, the settings can be loaded from the file UNKNOWN.TAP.



On the **Trace selection** tab, the ROX, FAM and HEX traces have been checked to be loaded.



On the **Derive calibration** tab, **Derive DNA Calibration** has been checked, and **ROX** has been selected as the trace to derive the size standard from³.

8. Click the **OK** button.

Now, the following will happen in succession.

- the ET-ROX trace is loaded. It is analysed using the current settings. Since the Automatic Trace Calibration parameters define ROX as the calibration trace, the sizing standard will be recognised, and a calibration relating time coordinates to fragment sizes is derived from the ROX trace.
- the FAM and HEX traces are loaded and analysed using the current settings.

You can now click any of the peak list buttons at the left side of the data tags for the ET-ROX, FAM, or HEX traces. You will note that the correct fragment sizes are listed in the Base Pairs column.

It is also possible to display the fragment sizes of the peaks that were found in the graphics window. To do this, invoke the **File | Customise** menu option. Select the **Plotting Peaks** tab. Under Peak Labeling, select **Component Name** as the first peak label.

³ The trace you select here must match the trace selected in the ATC dialog box (cf. Chapter 2).

Select **Base Pairs** as the second peak label (it is near the bottom of the list of possible peak labels). Click the **OK** button.

The graphic window will now display base pair counts for all the peaks. There will be very many peaks displayed. You can click and drag the mouse on the graph to zoom in on the peaks, and see more detail.

Chapter 3.1. Converting to a Base Pair axis

In the example above, fragment sizes (base pair counts) are labeled for the peaks, but the horizontal axis displays time coordinates. For gene analysis, this often does not make much sense. Often, the horizontal axis does not correspond to specific time coordinates.

For this reason, DAX can convert the horizontal axis to fragment size units. Use the **Calibration | Axis Conversion** menu option to do this. Make sure to check the **New Window** check box, and then make sure that all data lines are selected.

A more convenient way to convert the axis is to check the **Convert Axis** item in the AutoAnalysis setup dialog box. Use the following procedure.

1. Invoke the **File | Open** command. Select **Files of Type** MegaBACE™. Select the file or files you want to analyse.
2. Check the **AutoAnalyse** button.
3. Click the **Config** button next to the AutoAnalyse button. The AutoAnalysis setup dialog box appears.
4. Make sure that at least **Construct Baseline** and **Detect Peaks** are checked. Check **Convert Axis**.
5. Click the **OK** button.
6. Click the **Open** button. This will start loading the trace file.
7. The Trace Analysis Parameters dialog box is displayed. Set it up as before. The settings can be loaded from the file UNKNOWN.TAP.
8. Click the **OK** button.

The analysis will be performed as before, but the horizontal axis will be converted to fragment units at the end of the analysis.

Chapter 4. Conclusion

DAX can be used to perform highly complex analyses of trace files with relatively little effort.

There are a large number of additional features that have not been discussed in this manual. Examples are:

- peak width standardisation. If a sample is known to contain mostly peaks that have a width of 1 fragment, DAX can be told to preferentially assign peak widths of 1 fragment. If a peak is *clearly* wider, its width will not be affected.
- command line analyses. Typically, trace analysers will be used to perform dozens to hundreds of analyses per day. To make it possible to analyse such large quantities of data, DAX can be run from batch files. All the data on an entire hard disk can be analysed with a single command, while highly flexible report files are created.

These features exceed the scope of this manual. Refer to the DAX User's Manual for details.