



## DEGRADOMETER Version 1.41, SOFTWARE MANUAL

For further details see Auer *et al.*, 2003, Nature Genetics 35: 292-293, Supplementary Methods

Version 1.1 works for multiple versions of the Bioanalyzer software that create different formats of CSV-files.

Version 1.2 contains the following modifications according to feedback from users:

- Detection of the time marker even if the signal is very low
- Baseline adjustments to avoid negative values of the degradation factor and to avoid strong influence of artifacts, e.g. air bubbles

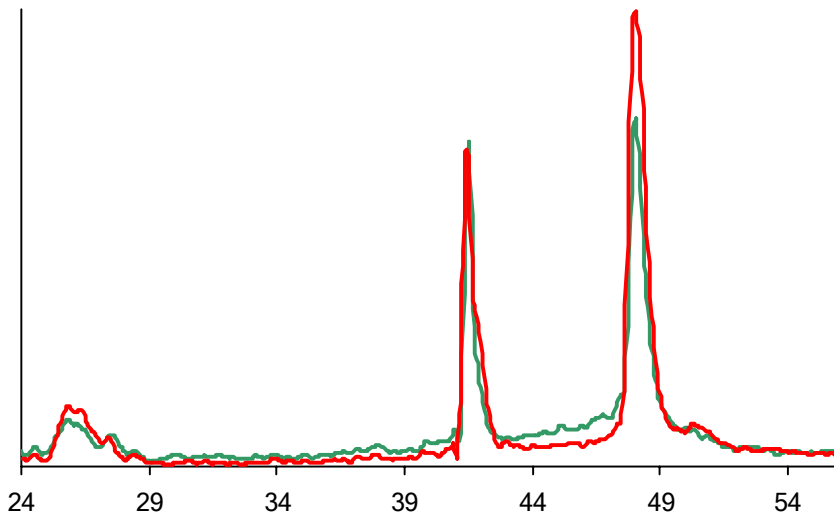
Version 1.3 optimized handling of artifacts

Version 1.4 optimized handling of different formats of CSV-files from new versions of Bioanalyzer software and alternative decimal formats

Version 1.41 optimized handling of variability of migration speed

The Degradometer provides quantitative data about the integrity and concentration of eukaryotic total RNA.

**Attention:** For comparability, RNA isolation of samples should be performed as similar as possible. Even minor differences between isolation procedures will bias the measurement of RNA integrity. The example below shows the alteration of RNA profiles by heat denaturation (2 minutes at 70°C).



**Red:** RNA without heat denaturation, degradation factor = 2

**Green:** RNA after heat denaturation, degradation factor = 4

## 1. Installation

UnZip the Degradometer file; this will create the original “Degradometer.exe” (application) file.

Create a folder named “Degradometer” (e.g. in C:\Program Files\Agilent 2100 Bioanalyzer\Bio Sizing\Data) and copy Degradometer.exe into this folder.

## 2. Analysis of files generated by Bioanalyzer 2100 Bio Sizing software

Using the Bio Sizing software Export the well data as one file per well by selecting “File”, “File-Export”, “Export well data”; save the “well data” (including the ladder) in the Degradometer folder. NOTE: Be sure to uncheck the box entitled: “Create daily subdirectories”.

Open the “Degradometer” folder and double-click on the “Degradometer.exe” icon. A black window will be opened. Write the name of a folder, where the results will be saved into the black window. Click Enter and a few lines of text will be generated in the black window. When prompted, press space bar.

This will automatically create a folder within the folder Degradometer., This new folder will contain the original “well data” and four excel spreadsheets:

- Degradation
- Markers
- Unscaled
- Scaled

## Degradation

This file contains the summary of the analysis. All values are calculated after scaling. Scaling is necessary for the comparison of samples.

Column A (Name): Sample name and file name

Column B (Alert): A degradation alert:

RED – highest alert, strong degradation (Degradation Factor > 24)  
ORANGE – severe degradation (Degradation Factor > 16)  
YELLOW – degradation can be detected (Degradation Factor > 8)  
BLACK – profile could not be reliably interpreted (frequently due to low RNA concentration, unusual migration behavior or shifted baseline values)

Column C (DegFact): The **Degradation Factor**, quantifying the degradation; the higher the number, the more degradation

Column D (CONC): Concentration of RNA, in ng/μl

Column E (T18S): Timing of the 18S rRNA peak; usually approximately 41.4 seconds

Column F (T28S): Timing of the 28S rRNA peak; usually 48 seconds

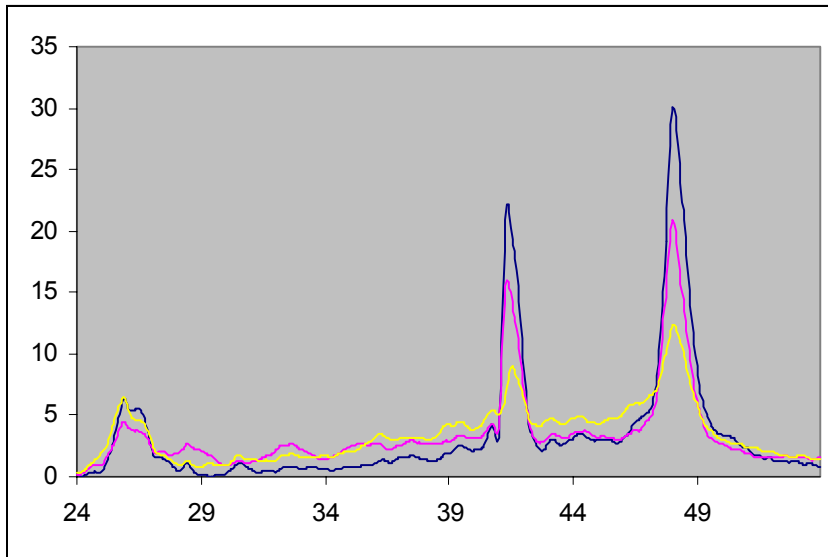
Column G (18S): Signal intensity of the 18S rRNA peak

Column H (28S): Signal intensity of the 28S rRNA peak

Column I (28S/18S): Ratio of 28S/18S signal heights. This number decreases in apoptosis.

## Scaled

For comparison of samples, the original data needed to be scaled. The first step of scaling aligned the graphs for identical timing. (the front marker peak is set to 23 seconds, 28S peak to 48 seconds). The second step of scaling corrected different amounts of RNA loaded in different wells. The "Scaled" file contains time and fluorescence intensity for all samples after scaling. These data are very useful for drawing chromatograms for the comparison of samples such as below.



### Unscaled

This file contains the raw data of all files that have been used for Degradometer analysis.

### Markers

Column A (Name): Sample name and file name

Column B (Mark\_value): Unscaled height of the time marker

Column C (Mark\_time): Location of the time marker on the original time scale

Column D (28S\_value): Unscaled height of the 28S rRNA peak maximum

Column E (28S\_time): Unscaled time of the 28S rRNA peak maximum