DMAN

Analysis of protein thermal denaturation data obtained by multi-well differential scanning fluorimetry

version 5.3

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1. Summary

DMAN is a program to analyse protein thermal denaturation data obtained by multi-well differential scanning fluorimetry. Experimental repetition schemes on multi-well plates can be defined using an ASCII file or through a graphical user interface (GUI). The raw or pre-processed protein denaturation data are read by DMAN, and results of replica wells are averaged with the mean melting temperature and the estimate of standard deviation being calculated. Outliers in a set of replica are automatically excluded based on Grubbs' test using a probability threshold of 95%. The results are annotated to highlight wells that show a significant difference in melting temperature from a reference well, as well as the *p*-value for this difference determined by Welch's *t*-test. The melting curves and the location of determined inflection points can be graphically displayed for each set of replica; the user can also determine the inflection point interactively. When using DMAN, please cite Wang *et al*., 2012 [1].

2. General layout

DMAN has four tabbed panels: **`Input parameters`**, **`Raw data`**, **`Results`** and **`Plate`**. Relevant information for data to be processed and analysed is required by the user on the panel **`Input parameters`**.

In the first stage, DMAN extracts the thermal denaturation data from a file with pre-processed data or determines these parameters from the raw data acquired. The panels **`Raw data`** and **`Plate`** become accessible and show the results of this stage. Outliers are automatically detected using a Grubb's test (95% probability). On the **`Plate`** panel as well as in the graphical display of individual datasets, DMAN will show the suggested wells to include/exclude for analysis. The user can override these suggestions by simply (un-)checking the ***I*** checkboxes. The data are re-analysed, each time a well is included or excluded.

In the **`Results`** panel, the difference of melting temperatures ΔT_m between additive and reference wells are shown as bargraph. Averaged replica datasets are compared to the reference dataset and the two means are subjected to Welch's *t*-test in order to obtain a *p*-value. Individual datasets are labelled for significance as per:

***	$p < 0.001$
**	$0.001 \leq p < 0.01$
*	$0.01 \leq p < 0.05$
R	reference dataset.

3. Command line arguments

DMAN can be started from the terminal using the following command

```
java -jar {dman-archive}.jar
```

Start the GUI and open a DMAN session file:

```
java -jar {dman-archive}.jar {dman-file}.dma
```

Start the GUI with command line options:

```
java -jar {dman-archive}.jar [options]
```

Options:

`-h`	prints the command line help

4. Input parameters

4.1 Input file

The file with protein melt data. DMAN can process ASCII files provided by various real time PCR software.

Roche LC480: In order to obtain raw data straight from the Roche LC480, right-click into the **Data** display window found within the **Experiment Setup** (the window that showed the data being collected in real-time) upon completion of a run, and select **Copy** from the right-click menu. You can then choose to copy the data as a numerical object.

4.2 Input file type

The following ASCII data exports from real-time PCR instruments can be processed:

Agilent/Stratagene Mx3005p raw data
Applied Biosystems 7500 raw data
Applied Biosystems 7900 raw data
Applied Biosystems Step One raw data
Applied Biosystems Step One Plus raw data
Applied Biosystems ViiA7 raw data
BioRad CFX96 raw data
BioRad CFX384 raw data
BioRad MyIQ raw data
QuantStudio 7 Flex Real-Time PCR System
Roche LC480 raw data
Roche Protein Melt output (***.csv** file).

4.3 Plate type

48-well, 96-well or 384-well.

4.4 Plate scheme

If **External plate scheme** is selected from the drop-down list, an ASCII file of a special format that defines the experimental replica scheme, and position of the reference well(s) (see Appendix 1) needs to be defined in text field below.
Three internal pre-defined plate schemes are available from the drop-down list: **48-well, triplicate**; **96-well, triplicate**; or **384-well, quadruplicate** (see Appendix 1 for the graphical layouts).

4.5 Output file

Pre-filled based on the text in **Input file**.
This ASCII output file is automatically generated after each analysis and contains averaged data, as well as raw data.

4.6 Title

Experiment title (free text).

4.7 Screen

Description of the additive screen (free text).

4.8 Slope threshold

Default: 0.1

Only wells for which this value is reported in the raw data file will be included in the DMAN analysis.

5. Generating a plate layout

A plate layout can be generated by writing an ASCII file of the format attached in the Appendix. Alternatively, the item **Design plate scheme** from the **Edit** menu can be used. Here, the user will be presented with a GUI where individual wells that are supposed to be replica need to be labelled with the same number or character. A reference well needs to be defined by clicking **R** at the appropriate position. The replica reference wells will be determined automatically using the user-specified plate layout.

6. Annotating data sets

Comments to individual data sets can be added using the item **Add comments to plate scheme** from the **Edit** menu. **Apply** simply registers the comments for the current analysis. **Save and Apply** also updates the current **Plate scheme** definition file. Alternatively, double-clicking a **Comment** cell in the table shown in the **Results** panel also allows editing the comments (copy/paste operations of multiple cells with **Ctrl-c** and **Ctrl-v** to and from external spreadsheet programs should work properly since version 3.6).

7. Printing

The results of the current analysis can be printed using the **File – Print Results** menu. The plate layout with individual results shown on the Plate panel can be printed by using the **File – Print Plate** menu.

If a PDF printer is installed on your system, you can use this feature to generate a PDF fil. Alternatively, a PDF output of the **Results** table can be generated by exporting the results table as an image (see next section).

On Linux (CUPS), occasional difficulties with printing have been reported that result in an error message “No print services found”. This can be solved by

- editing the CUPS printer configuration file (**/etc/cups/printers.conf**):
  to each printer entry, add the line **Option orientation-requested 3**
- setting CUPS to allow internet printing:
  In a web browser, go to **localhost:631** and choose **Administration**:
  tick **Share printers connected to this system**
  tick **Allow printing from the Internet**

8. Exporting image files

The results of the current analysis can be saved in various image file formats (PDF, PNG, SVG, TIFF), using the **File – Save Results as image** menu.

9. Saving and resuming from previous sessions

The current session can be saved with ***File-Save*** into a binary file. Using the **Open** item from the **File** menu, the binary output file from a previous DMAN session can be loaded. Alternatively, the DMAN file can be loaded when starting the program from the command line:

```
java -jar {dman-archive}.jar {dman-file}.dma
```

10. Operation

After providing the required input, press **Read Data** to process the raw data file. The **Raw Data** and **Plate** panels will become active and display contents. On the **Plate** panel, individual wells can be activated/inactivated for further analysis. Upon inclusion or exclusion of individual wells by (un-)checking the **I** check box, the data are re-analysed.

If a raw data file has been provided as input file, then a **G** check box will appear on each individual well. Activation of the **G** check box will open a graph window where all melt curves of one data set are visualised.

Since version 3.6, the location of current T_m values for each individual curve are visually shown in the graph window as vertical markers. This feature can be turned off in the **Preferences** menu.

In the **Results** panel, a click on the **right mouse button** will open the same graph window. Each individual curve/well can be included or excluded from the analysis by (un-)checking the check boxes shown at the bottom of this panel. In order to return to individual cell selection mode, click the **left mouse button**.

In cases where the apparent inflection point has not been located correctly, the user can highlight the appropriate area by dragging the mouse with the **left mouse button** pressed. Data will be re-analysed after releasing the mouse button. If the search is to be strictly limited in a particular interval, the highlighting can be done with the **right mouse button** pressed.

11. Non-PCSB Java libraries used in this program

DMAN makes use of the following Java libraries not authored by us:

- Apache Batik SVG toolkit (http://xmlgraphics.apache.org/batik/index.html)
- Student's t-distribution cumulative density function from Michael Flanagan's Stat class (http://www.ee.ucl.ac.uk/~mflanaga/java/Stat.html)
- Thierry Lefort's BarChartCellRenderer (http://www.jroller.com/Thierry/).

12. Acknowledgements

We greatly acknowledge support by the many colleagues who provided data files and formats of PCR instruments to help expand the usability of this software.

13. References

1 Wang CK, Weeratunga SK, Pacheco CM & Hofmann A (2012) DMAN: a Java tool for analysis of multi-well differential scanning fluorimetry experiments. *Bioinformatics* **28**, 439–440.

14. Appendix 1: Copyright, License and Disclaimer

15. Appendix: Plate Schemes

15.1 Plate scheme files

A plate scheme file has the following format:

```
12
8
32
31
 1   5   9   *
 2   6  10   *
 3   7  11   *
 4   8  12   *
13  17  21   *
14  18  22   *
15  19  23   *
16  20  24   *
25  29  33   *
26  30  34   *
27  31  35   *
28  32  36   *
37  41  45   *
38  42  46   *
39  43  47   *
40  44  48   *
49  53  57   *
50  54  58   *
51  55  59   *
52  56  60   *
61  65  69   *
62  66  70   *
63  67  71   *
64  68  72   *
73  77  81   *
74  78  82   *
75  79  83   *
76  80  84   *
85  89  93   *
86  90  94   *
87  91  95   Protein buffer control
88  92  96   Protein DMSO control
```

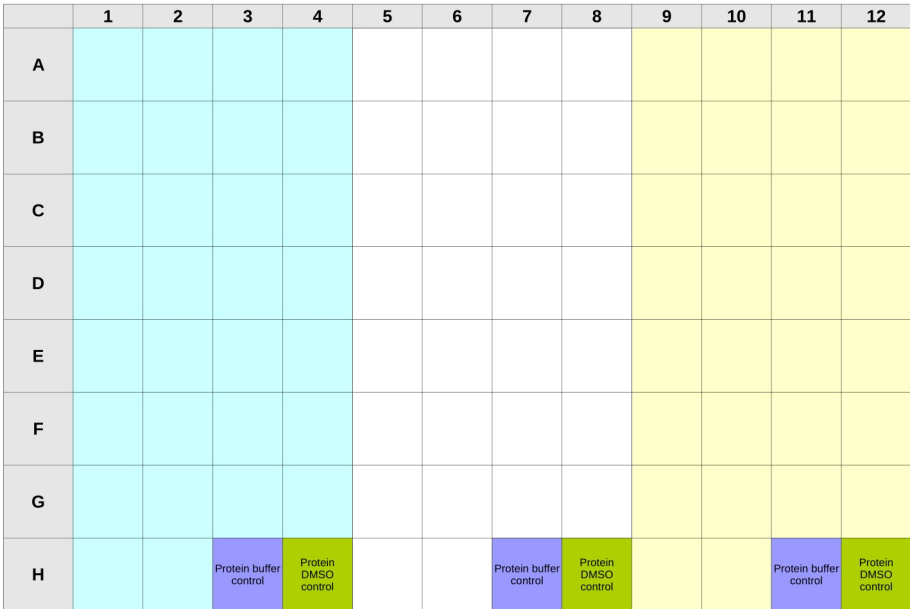

Figure 1: Graphical layout of the plate scheme with three replica as per the plate scheme definition on the left.

Line 1: number of columns on the plate (here: 12)
Line 2: number of rows on the plate (here: 8)
Line 3: total number of independent experiments (here: 32).
Line 4: the set of replica serving as reference for ΔT_m calculation (here: no 31); note that the count starts at 0, i.e. the reference dataset in this example is `88  92  96  Protein DMSO control`.
Lines 5-36: replica definition of the independent experiments. In this example three numbers per line are required, because three replica have been set. Each number describes the well on a 96 well (counted from 1 to 96). In the last column, a comment can be set to describe the well. If '*', the comment will be ignored by DMAN.

15.2 Predefined plate scheme: 48-well, triplicate

	1	2	3	4	5	6	7	8
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
D	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Protein buffer control	Protein DMSO control
E	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Protein buffer control	Protein DMSO control
F	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Protein buffer control	Protein DMSO control

Figure 2: Graphical layout of the predefined internal plate scheme using a 48-well plate with three replicates.

15.3 Predefined plate scheme: 96-well, triplicate

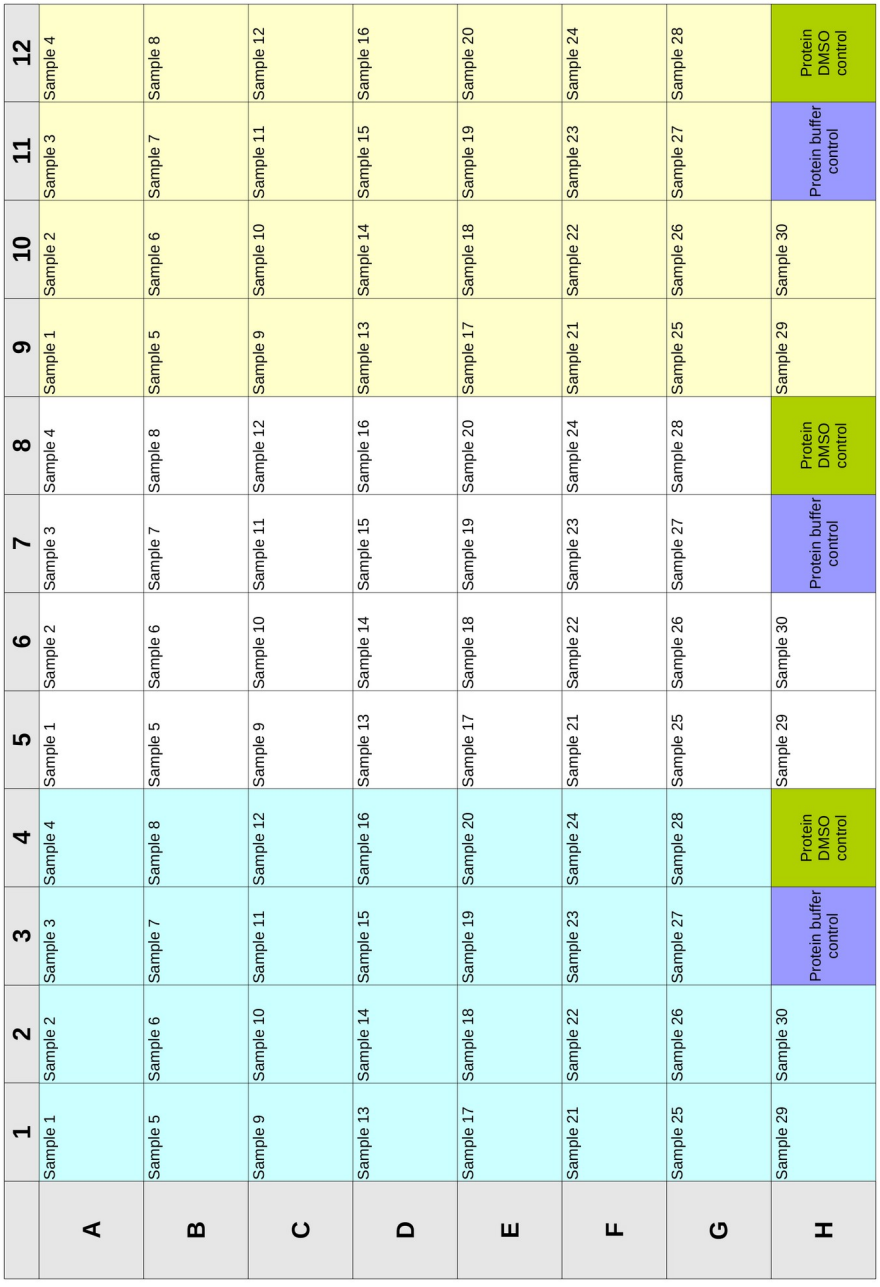

Figure 3: Graphical layout of the predefined internal plate scheme using a 96-well plate with three replicates.

15.4 Predefined plate scheme: 384-well, quadruplicate

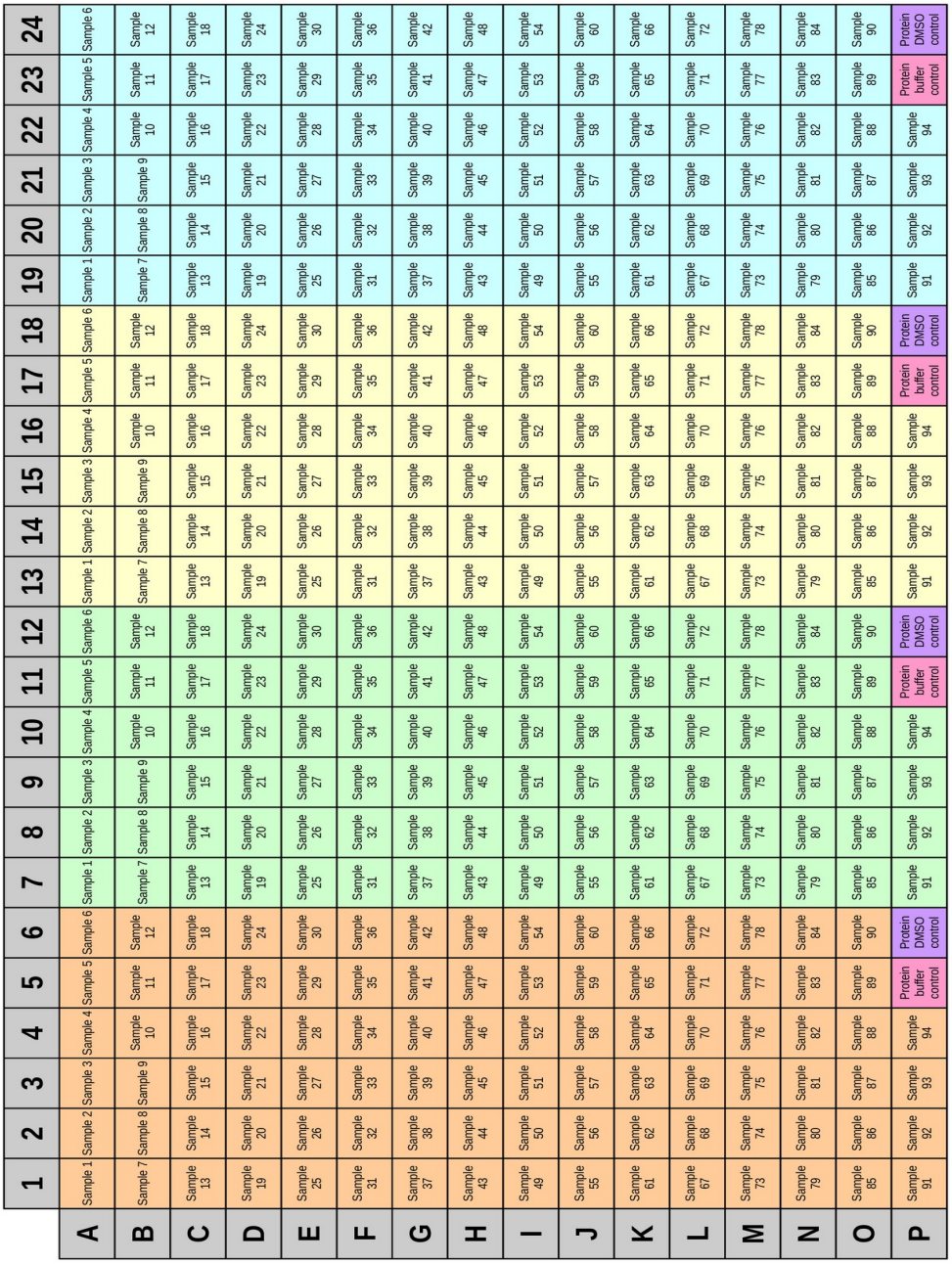

Figure 4: Graphical layout of the predefined internal plate scheme using a 384-well plate with four replicates.