

## Application notes Agilent AriaMx™

### Instructions for Use with Eurofins GeneScan Kits

Cycler application notes AriaMx™

V1 (17.01.2017)

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Instructions for Set up and Evaluation

## Instructions for Setup and Evaluation on Agilent AriaMx™

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### 1. INTENDED USE

These instructions are meant for users of Stratagene (Agilent) AriaMx™.

The following instructions are based on Agilent AriaMx software version 1.0.


## 2. RUN SETUP AND PROGRAMMING OF A TEMPLATE FILE

If you want to use template files for the AriaMx™, a template needs to be programmed and saved. Alternatively, send an e-mail to [kits@eurofins.com](mailto:kits@eurofins.com) with information of your cycler model.

### 2.1. Setup of the AriaMx™ template file (\*.amxt) in the AriaMx™ software

1. Open Agilent AriaMx™ software.
2. Click „Experiment 1” and select “Quantitative PCR” (Fluorescence Probe). Choose an Experiment Name and Click “Create”.
3. A previously created template (\*.amxt) can be opened by “File” → Select “Open”.
4. The blank Plate Setup screen opens. Setup the wells as described in the respective kit manual.
5. Select the respective “Well Type” (“Buffer” or “NTC” for Negative Controls and “Unknown” for your samples) in the “Properties” panel on the right. Mark the wells and click on the dyes which are used in the kit under “Add Dyes” in the same panel.

6. If you want to use the automated Excel Evaluation sheets Qualitative/ Quantitative PCR (MACRO Versions), please specify detector names/ identifiers as mentioned in the respective kit manual by clicking on the arrow besides “Targets”. There, the detector names/ identifier should be entered under “Target Name”.
7. Please specify if and which reference dye is used in the respective kit under “Reference Dye”.
8. Adapt the thermal and time settings as described in the manual by selecting “Thermal Profile” on the left in the panel “Setup” (below the “Plate Setup”). If not stated differently in the kit manual, the run parameters are to be programmed as follows:

Thermal Cycler Times and Temperatures		
1 HOLD	CYCLE (45 repeats)	
<i>enzyme act.</i>	<i>denaturation</i>	<i>ann. &amp; extension</i>
<b>10 min at 95 °C</b>	<b>15 sec at</b>	<b>90 sec at 60 °C</b>
no data collection	no data collection	<b>data collection</b> 

9. The file can now be saved as template. Go to “File” → “Save as template” and select the corresponding folder for the (\*.amxt) template.

10. Start the run by Clicking on “Run”  
Run status and raw data plots can be accessed in the left panel under “Run”

Please refer to 2.2. to start from PC and 2.3. to start the experiment from the AriaMx™ machine.

### 2.2. Start a run from PC in the AriaMx™ software

1. Click “Run”.  
The Instrument Explorer dialog box opens.
2. In the dialog box, locate the instrument and select “Send Config”.
  - If you have not already logged in to the selected instrument, you will be prompted to log in before continuing.
  - If you have not already saved the experiment, you will be prompted to save it before continuing.
3. Load your samples onto the thermal block.
4. At the bottom of the instrument touchscreen, press the icon shown below.



5. In the pop-up menu that opens, press “Open Primed Experiment”. The experiment opens on the touchscreen.
6. On the Thermal Profile screen of the touchscreen, press “Run Experiment”.
7. The Raw Data Plots screen opens, allowing you to monitor the progress of the run.

### 2.3. Setup of a run directly from the AriaMx™ machine

1. Use the touchscreen to set up and run an experiment.
2. Load Plate and select “Run Experiment” on the Thermal Profile screen.
3. A message box opens asking to save the experiment. Click OK to open the saved experiment
4. Select a folder for the experiment and save it.

### 3. ANALYSIS OF A SAMPLE RUN

When running the AriaMx™ without a connected PC, export the run data first:

- Select your run file from “Saved Experiments” and copy the run file to your personal computer.

1. Open Agilent AriaMx™ software and select the run data file (\*.amxd) by going to “File” → “Open”. The “Plate Setup” of your experiment will be displayed.

**OPTIONAL:**

To import sample names, a text file (\*.txt) needs to be prepared with Well Name, Sample ID (e.g. A1, Sample X; B1, Sample Y); Please note: use a blank between comma and Sample ID). Name the wells by right-click into the wells and select “Import Well Name...” and select the folder in which you saved the prepared text file (\*.txt)

2. For analysis of the run, mark the wells you want to analyse and then go to “Graphical Displays” under the left panel “Analysis”.
3. If ROX was used as reference dye in the respective kit, the normalized  $\Delta R_n$  values are displayed. If no reference dye was selected,  $\Delta R$  values are shown.

4. Smoothing should be switched “On”.
5. Click on the grey arrow to show and adapt settings for “Baseline Correction”, “Threshold Fluorescence” and “Background Based Threshold” (Cycle Range, Sigma Multiplier).
6. The threshold can manually be set according to the kit manual and for each detector individually using the linear view.
7. If appropriate, the auto calculated threshold can be used with the following settings below the “Background Based Threshold”:
  - Cycle range: 5 thru 9
  - Sigma Multiplier: 10

### 4. EXPORT OF ANALYSED DATA

1. For data export, go to “Export Data” under the left panel “Results”.
2. From the “Export Configuration” menu on the right, an Export file can be defined by selecting the drop-down menu besides “Definition” and select “Add New”.

3. Select Text file (\*.txt) as File Type and from the Items list “Tabular Results”.
4. If the settings are set, click on the memory card symbol to save these definitions.
5. Click on the blue pen besides the “Tabular Results” to select the following column options before export:
  - Well
  - Well Type
  - Sample Name
  - Well Name
  - Dye
  - Target
  - $\Delta R(n)$  Last\*
  - Threshold  $\Delta R(n)$ \*
  - Cq  $\Delta R(n)$ \*

\* If no reference dye was selected,  $\Delta R$  values need to be exported, if a reference dye was used, select  $\Delta R_n$  values for export.

6. Click “Export Data” and select the respective folder in which the data shall be saved
7. Export data is now saved and can now be used for evaluation of the run.

### 5. EVALUATION

Refer to your cycler’s manual for details. An evaluation (Excel™) sheet can be requested at [kits@eurofins.com](mailto:kits@eurofins.com). If you use the Eurofins GeneScan evaluation spreadsheet, please follow strictly the spreadsheet instructions of the respective Excel™ sheet.

### 6. TECHNICAL SUPPORT

If you have any questions or experience any difficulties regarding the use of Eurofins GeneScan products in general, please contact Eurofins GeneScan or your local distributor.

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