

TechNote TN-02: Analysis of data generated using the osteomiR[™] workflow

Part I: Instructions for qPCR analysis of osteomiR[™]plates: Roche[®] LightCycler[®] 480 II

Part II: Using the osteomiR[™] software application for raw data import, quality control analysis and data export

Version 1 Released: December 2016



Table of Contents

| 1 | Introduction | 2 |
|------------|---------------|---|
| I . | 111(10uuction | 3 |

| 2. R | oche® LightCycler® 480 II | 5 |
|------|---------------------------------------------------------------------------|---|
| 2.1. | Implementation of the osteomiR™ macro on your LightCycler 480 II platform | 5 |
| 2.2. | Run the osteomiR qPCR experiment and save raw qPCR run .txt file | 8 |

| 3. ost | eon | niR™ App | 11 |
|--------|-----|--------------------------------------|----|
| 3.1. | Cre | eation of the clinical data file | 11 |
| 3.1. | 1. | Create clinical data file in Excel | 11 |
| 3.1. | 2. | Save clinical data file as .txt file | 12 |
| 3.2. | Da | ata import into the osteomiR™ App | 13 |
| 3.3. | Qu | ality Control and Data normalization | 13 |

| | FAQ on the use of the osteomi R^{TI} | ¹ app | 17 |
|--|----------------------------------------|------------------|----|
|--|----------------------------------------|------------------|----|



1. Introduction

This TechNote guides you through all steps of required for analyzing osteomiR[™] qPCR plates on a LightCycler 480 II platform:

- 1. Setup of a qPCR run on a Roche LightCycler 480 II
- 2. Export of raw qPCR data
- 3. Importing of raw qPCR data into the osteomiR[™] App provided by TamiRNA

To facilitate the use of the osteomiR[™] kit and make your workflow more robust and safe TAmiRNA provides an osteomiR[™] specific **Light Cycler 480 II macro**, which is a template that contains all experimental settings for the qPCR run (specified in Annex I) and alleviates the need for programming the LightCycler 480 II yourself.

Macros offer a convenient way of speeding up the creation of an experiment. We have generated the osteomiR[™] macro with respective qPCR cycling conditions and microRNA subset information. After initial implementation of the macro on the LightCycler 480 II platform you will only have to click on the macro for starting the experiment, with the proper experimental settings and microRNA subset information already being predefined.

The experimental settings used in the osteomiR™ macro are summarized in Annex I.

The **osteomiRTM App** is a software for standardized analysis of data generated using the osteomiRTM kit and osteomiRTM plates. This software automatically calculates Cq values, performs melting curve (T_m) analysis, allows to check the quality of your samples and subsequently select samples for normalization and data export.

For using the software you will have to upload two types of data: i) the raw qPCR data exported from the Roche LightCycler 480 II as text-file and ii) a file specifying clinical parameters for the respective samples analyzed in .txt format.

An overview of the entire osteomiR[™] workflow is given on the next page.

Overview of the osteomiR[™] workflow

osteomıR™

Biomarkers of Bone Quality

Clinical specification .txt file



2. Roche[®] LightCycler[®] 480 II

2.1. Implementation of the osteomiR[™] macro on your LightCycler 480 II platform

Upon purchase of the osteomiR[™] kit you will receive the osteomiR[™] macro via email. The following steps guide you through the implementation and use of the macro.

- 1. Download the osteomiR[™] macro and copy it to the LC 480 II workstation.
- 2. Start LightCycler480 II software.
- 3. Enter "username" and "password" to login and proceed to the overview window (Fig. 1)
- 4. Click on "Navigator" button 🛞 and select "Import".
- 5. Go to the folder where you have stored the osteomiR.ixo file you received via email and select it (Fig. 2).
- 6. An overview window displaying the experimental settings provided with the osteomiR[™] macro appears (Figure 5). As only one reporter dye (SYBR green I) is used, options for color compensation on the top left are set to "none". Click on "Save" button □ , the window shown in Figure 3 will appear. Choose the directory /Root/System Admin/Macros (Fig. 4), confirm with on and go back to the Overview window by clicking the "Overview" button □ .



Figure 1: Overview window.

osteomiR™

| | Bio | omarkers of Bone | e Quality |
|---------------------------------------------|------------------------------------------|---------------------------|-----------------------|
| Navigator | Query | | No summary available. |
| E → BOOC CUser01 Experiments Acros | | | _ |
| Preferences Special Data | t File | × | J |
| ⊡ Templates ⊡ Roche | Look in: 🚺 OsteomiR Macro 💌 | ⇐ 🗈 📸 🎫 | |
| i ⊕ System Admin | Name | Date modified Type | |
| Recent | Places OsteomiR Macro.ixo | 09/14/2016 16:02 IXO File | |
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Figure 2: Select osteomiR[™] Macro for import.

| C Aut | tomatically select color compensation. Iude "fixed" color compensation objects. | 2] |
|-------|-------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| lotes | Save Macro | 67 |
| | □- ⊕ Root □- □ LCUse(01 ⊕ Experiments - □ Macros ⊕ Preferences | 器 |
| | Br—Special Data Br—Templates | (€) □ |
| | | |
| | | \otimes |
| | | <u>C</u> |
| | | 5 |
| | Name OsteomiR Macro | |
| | | |

Figure 3: Select a directory (see also Figure 4) for saving the osteomiR™ Macro and confirm with 🥑

| Root Administration System Admin System Admin Macros Preferences Preferences Templates |
|--------------------------------------------------------------------------------------------------------------------|
| |

Figure 4: Proposed directory /Root/System Admin/Macros for saving the osteomiR[™] macro in the LightCycler480 II software.

| 9 | LightCycler® 480 So | oftware relea | se 1.5.1.62 | | | | | | | - 8 × |
|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------|-------------------------|---------------------|----------|----------------------|---------------------|--------------------------|-----------|
| Instrument: No active instrument | | | | | | Database | e: My Compu | iter (Traceable) | | Roche |
| Window: OsteomiR Macro | | | | | - | User: | System Ac | Imin | | |
| C Automatically select color compensation. C Include "fixed" color compensation objects. | | | | | | | | | | Ð |
| (None. | | | | | | | | | | |
| Notes | Summary | | | | | | | | | 0 <u></u> |
| | Macro name: OsteomiR Macro | D | | | | | | | | \sim |
| | Created on: 14.09.2016 15:59 Created by: LCUser01 Original Experiment: OsteomiR Last modified or: 22.09.2016 1 Last modified by: System Admin | 9:09 _Template 16:00:01 n | | | | | | | | B |
| | Software version: LUS480 1.5. | 1.62 | | | | | | | | (4) |
| | Programs: 1: Aktivation 1 Target(10) Ac 95 No | cycle(s) cquisition Mode one | None Hold (hh:mm:ss) 00:10:00 | Ramp Rate (*C/s) 4.4 | Acquisitions (per * | C) (| Sec Target (°C) D | Step Size (*C) 0 | Step Delay (cycles) 0 | |
| | .2: Cycles 45 Target("C) Ac 95 No CO CO | 5 cycle(s) cquisition Mode one | Quantification Hold (hh:mm:ss) 00:00:10 00:01:00 | Ramp Rate (°C/s) 4.4 | Acquisitions (per ° | C) | Sec Target ("C) D | Step Size (*C) 0 | Step Delay (cycles) 0 | |
| | .3: Melting Curve 1 Target("C) Ac 95 No | cycle(s) cquisition Mode | Melting Curves Hold (hh:mm:ss) 00-00-10 | Ramp Rate (*C/s) | Acquisitions (per * | C) : | Sec Target ("C) | Step Size (*C) | Step Delay (cycles) | |
| | 55 No 99 Co | one | 00:01:00 | 22 | 5 | |) n | Ŭ N | Ŏ | \otimes |
| | .4: Cooling 1 Target("C) Ac 40 No | cycle(s) cquisition Made one | None Hold (hh:mm:ss) 00:00:01 | Ramp Rate ("C/s) 2.2 | Acquisitions (per * | C) | Sec Target ("C) D | Step Size (*C) 0 | Step Delay (cycles) 0 | |
| | Block tune: 96 wells (silver) | | | | | | | | | |
| Revision History | · · · · · · · · · · · · · · · · · · · | | | | | | | | | L |
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Figure 5: Overview of experimental settings deposited in the osteomiR[™] macro (highlighted in red box).

Biomarkers of Bone Quality

2.2. How to tun the osteomiR[™] qPCR experiment and save qPCR data as text-file

Important notes before starting

- Turn on your LightCycler480 II system before proceeding with the following steps.
 - Wait for the initialization of the instrument to finish the left LED on the front of the instrument should become a steady green while the right LED should be a steady orange.
 - Macros can only be selected when the LightCycler480 II system is turned on and initialized.
- As the experiment run automatically starts as soon as the osteomiR[™] macro is selected, the qPCR 96-well plate must be prepared **before** proceeding with the following steps.
- 1. Start LightCycler480 II software and enter "Username" and "password" to log in and proceed to the Overview window (Figure 1).
- 2. Put the prepared osteomiR[™] 96-well plate into the LightCycler480 II instrument.
- 3. Select "New Experiment from Macro". The window shown in Figure 6 will appear.
- 4. Select the osteomiR[™] macro.
- 5. The window shown in Figure 7 appears- Choose a directory for the experiment to be saved and proceed with 🕢 , the qPCR run starts.
- Replace pre-annotated sample names (Patient 1 to Patient 6) by your own sample annotation. Please remember the sample names must be matched with the clinical specification file.
- 7. After the run is finished, the window shown in Figure 8 opens up. You can now save the generated data as .txt file by clicking on €. The window shown in Figure 9 will appear for choosing a directory for saving the qPCR data and importantly choosing the required data format as .txt.
 - ◆ There is no need in analyzing the Cq values (by using e.g. Second Derivative Maximum method) in the LightCycler 480 II software, as the osteomiR[™] app does this by using the raw data of the amplification curves by applying the Second Derivative Maximum method.
 - The same applies for Melting Curve analysis- Melting curves are computed and can be checked when the data is plugged into the osteomiR[™] software.



Figure 6: Start the osteomiR™ Macro by selecting it and confirm with 🚺



Figure 7: Choose a directory for your osteomiR[™] experiment to be saved and proceed with

| | | | | | | RIOL | lark | ers of Bone | Quality |
|--------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|--------------------------------|----------|------|----------|-------------------------|---------|
| 9 | | | Lig | htCycler® 480 Software release | 1.5.1.62 | | | | - 0 × |
| Instrument | : No active instrument | | | | | 0 | atabase: | My Computer (Traceable) | Boche |
| Window: | OsteomiR_Test | | | | | • U | lser: | System Admin | |
| Experiment Subset Editor Analysis Report Sum. | Logenomine_lease Loge | d completed at 21.01.2016 18:40: None Quantification Melting Curves None Melt factor 1.2 | 33. Quart factor Maxintegration 30 I second(s) Created | ne | Reason | | 381. | | |
| | | | | | | | | | |
| | Save as Macro | | | | | | | | |
| | | | | | | | | | |

Figure 8: Window indicating finish of the qPCR run- data ready for export as .txt file. Red arrow indicates the necessary button for proceeding with data export



Figure 9: Choosing a directory for saving the generated qPCR data and the required data format as .txt (indicated by red arrow).

3. osteomiR[™] App

3.1. Creation of the clinical data file

3.1.1. Create clinical data file in Excel

For using the osteomiR[™] app, a clinical data file matched to the samples analyzed on the osteomiR[™] 96-well plate(s) needs to be provided in addition to the qPCR run .txt files.

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|------|--------------------------------|-------------------------|------------------------|---------------------------|
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| F1 | 0 - | : 🗙 | s fx | |
| | А | В | С | D |
| 1 | Sample ID | Age | Group | |
| 2 | Patient 1 | 77 | Case | |
| 3 | Paitent 2 | 53 | Control | |
| 4 | Patient 3 | 68 | Case | |
| 5 | Patient 4 | 44 | Control | |
| 6 | Paitent 5 | 59 | Case | |
| 7 | Patient 6 | 81 | Case | |
| 8 | Patient 7 | 66 | Case | |
| 9 | Patient 8 | 69 | Case | |
| 10 | Patient 9 | 71 | Case | |
| 11 | Patient 10 | 58 | Case | |
| 12 | Paitent 11 | 55 | Control | |
| 13 | Patient 12 | 68 | Case | |
| 14 | Patient 13 | 69 | Case | |
| 15 | Paitent 14 | 72 | Control | |
| 16 | Patient 15 | 63 | Case | |
| 17 | Patient 16 | 61 | Control | |
| 18 | Paitent 17 | 59 | Case | |
| 19 | Patient 18 | 42 | Control | |
| 20 | | | | |

The Excel screenshot on the left (Figure 10) gives an example of how the clinical data file needs to be prepared- in this case shown for the analysis of three qPCR run .txt files and accordingly 18 samples.

In the first column (column A) provide the sample IDs.

Important note: The sample IDs provided in the clinical data file need to be exactly matched with the sample IDs assigned in the LightCycler 480 II software (the sequence of samples in the clinical specification file and in the qPCR run file does NOT matter).

In the second column (column B) enter the age of the respective patients.

In the third column (column C) define whether the sample analyzed is regarded as Case or Control sample.

Figure 10: Example Excel sheet for creating the Clinical Specification File.

3.1.2. Save clinical data file as .txt file

For importing the clinical specification file into the osteomiRTM app, the file needs to be saved as .txt file. For this purpose choose the required .txt format when clicking on *Saving as* in Excel as shown in Figure 11 below (indicated by red box).

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| TI30992300A (C:) | Programme (x86) Recovery | 22.10.2016 23:30 16.09.2015 12:32 | Dateiordner Dateiordner | | ~ | | | |
| Dateiname: Clini Dateityp: Text (| cal Specification File (Tabstopp-getrennt) | | | | ~ | | | |
| Autoren: Montz Weigi Markierungen: Markierung minzurugen Intel: Intel minzurugen Ordner ausblenden Tools Speichern Abbrechen | | | | | | | | |

Figure 11: Save Clinical Specification File as .txt file (selection of .txt format is shown in red box, English: "tabdelimited").

3.2. Data import into the osteomiR[™] App

- 1. Open Google Chrome and go to *tamirna.platomics.com* and log in via your user ID + password to access the Overview window (Figure 13).
 - a. Important Note: For full functionality please use Google Chrome to access the osteomiR[™] App.
- 2. On the left side find the Workspace strip, click on *Project* and select *osteomiR* (Figure 14).
- 3. Choose an experiment name and upload the clinical data file (Figure 15).
- 4. Upload the qPCR run .txt file(s). You can also upload and analyze data from multiple qPCR runs, be careful to annotate all samples from the qPCR plates in the clinical specification file (Figure 16).
 - Be aware that it is very important that sample names from the qPCR run.txt files (derived from the LightCycler 480 II software) and sample names specified in the clinical data file exactly match.
- 5. Click Start and wait for the quality control table to be generated (Figure 17).

3.3. Quality Control and Data normalization

- 1. Browse the quality of your data by checking the Cq values of osteomiR microRNAs and spiked control oligonucleotides. Clicking on Cq values accesses amplification and melting curves.
 - a. Mouse-over Cq Scores Hemolysis Scores will give you information on how to interpret Cq values regarding quality parameters.
- 2. Select samples on the right strip *Include* (Figure 12) destined for further analysis and normalization.



Figure 12: Red box indicates strip for selection of samples destined for further analysis.



Biomarkers of Bone Quality to normalize the selected samples, the normalized data

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table is generated (Figure 18).

4. The normalized data table can now be downloaded as .xls file by clicking on Download



Figure 13: Overview window of osteomiR[™] software.

| Ś | PLATOMICS | | |
|---|------------|----|------|
| W | ORKSPACE | + | HOME |
| > | PROJECT-11 | ł | |
| > | PROJECT-10 | ** | |
| > | PROJECT-8 | ŧ | |
| > | PROJECT-7 | : | |

Figure 14: Zoom of workspace strip in Overview window. Click on Project and select osteomiR[™] for proceeding to the data upload interface.

Biomarkers of Bone Quality

| OSTEOMIK [™] Biomarkers of Bone Quality | App: ap-000001323 Description: App for osteomiR kit: as-321221 Biomarkers for Bone Quality | Stability for life. | | |
|-----------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------|--|--|
| Input | | Start | | |
| Experiment Name: | | | | |
| Clinical Data File: * | Datei auswählen Keine ausgewählt | | | |
| qPCR Run Files: * | Dateien auswählen Keine ausgewählt | | | |
| | | | | |
| | | | | |

Figure 15: Choose an experiment name and upload the clinical data .txt file + qPCR .txt files.

| OSTEOMIR TM Biomarkers of Bone Quality | | | | App: ap-00000 Description: A Quality |)1323 pp for osteomiR kit: as-321221 Bioma | rkers for Bone | Stability for life. |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|---------------------------------------------|-----------------------------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|---------------------|
| Input | | | | | | | Start |
| | | Experimen | it Name: | Demo | | | |
| | | Clinical Da | .ta File: * in Files: * | Datei auswä | hlen 160811_Preanalyticnnotation ALLtxt wählen 3 Dateien | | |
| | | | | | | | |
| | PREVIEW | | | | | Valid files: 3/3 | |
| | PREVIEW | age | group | some.data | runld | Valid files: 3/3 | |
| | Id Serum 1 | age 6 | <i>group</i> Case | <i>some.data</i> asd | runid 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | id Serum 1 Serum 2 | age 6 4 | group Case Case | <i>some.data</i> asd fgh | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | id Serum 1 Serum 2 Serum 3 | age 6 4 7 | group Case Case Case | <i>some.data</i> asd fgh dfg | runld 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | id Serum 1 Serum 2 Serum 3 Plasma 1 PRP RT | <i>age</i> 6 4 7 3 | group Case Case Case Case | some.data asd fgh dfg xfgn vb | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | Id Serum 1 Serum 2 Serum 3 Plasma 1 PRP RT Plasma 2 PRP RT | age 6 4 7 3 9 | group Case Case Case Case Case | some.data asd fgh dfg xfgn vb hd | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | id Serum 1 Serum 2 Serum 3 Plasma 1 PRP RT Plasma 2 PRP RT Plasma 3 PRP RT | age 6 4 7 3 9 6 | group Case Case Case Case Case Case | some.data asd fgh dfg xfgn vb hd frz | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | Id Serum 1 Serum 2 Serum 3 Plasma 1 PRP RT Plasma 3 PRP RT Plasma 1 PPP RT | age 6 4 7 3 9 6 5 | group Case Case Case Case Case Case Case | some.data asd fgh dfg xfgn vb hd frz dg | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 1.txt | Valid files: 3/3 | |
| | Id Serum 1 Serum 2 Serum 3 Plasma 1 PRP RT Plasma 2 PRP RT Plasma 1 PPP RT Plasma 1 PPP RT | age 6 4 7 3 9 6 5 4 | group Case Case Case Case Case Case Case Case | some.data asd fgh dfg xfgn vb hd frz dg hdc | runid 160811_Preanalytics OsteomiR 1.txt 160811_Preanalytics OsteomiR 2.txt 160811_Preanalytics OsteomiR 2.txt | Valid files: 3/3 | |

Figure 16: Uploaded clinical data .txt file + assigned qPCR .txt files ready for analysis.

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| PUT RESULT_ OSTEOMIR™ Biomarkers of Bone Quality | | | | | A | App: ap-000001323 Description: App for osteomiR kit: as-321221 Biomarkers for Bone | | | | | | | | | | | | | |
|--------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|----------------------|-------------------------|----------|------------|---------------------------------------------------------------------------------------|------------|-------------|------------|-------------|------------|-------------------|--------------------|----------------------------|---------------|-----------|--------------------|----------------|---|
| Bro | Quality Quality Quality Control Output Browse your data quality and select samples for data normalization | | | | | | | | | | | Start N | stability for life | | | | | | |
| C _q Sc Sample | oues | Hemoly dc-981-Jiu | sis Scores dS-IE-Viu | oir.203a | niR-335-5p | et-7b-5p | niR-127-3p | niR-550a-5p | niR-155-5p | niR-199b-5p | niR-29b-3p | niR-451a Imbnt | ation strat | ea hi <i>R-39-3p CP</i> | 33 <i>1PC</i> | not imput | e missing Score | values (defaul | T |
| ID Filter. | hsa-n | hsa-n | hsa-n | hsa-n | hsa-n | hsa-le | hsa-n | hsa-n | hsa-n | hsa-n | hsa-n | hsa-n | hsa-n | cel-m | Unisp | UniSp | Hemo | Include | _ |
| Serum 1 | 38.67 | NA | NA | 36.91 | 35.53 | 28.61 | 35.73 | 37.07 | 36.68 | 38.13 | 31.37 | 20.50 | 27.09 | 28.12 | 20.48 | 26.95 | 6.59 | | Ê |
| Serum 2 | 36.58 | 35.04 | 35.02 | 36.73 | 33.39 | 27.14 | 32.73 | 38.27 | 33.06 | 32.58 | 28.66 | 19.11 | 24.59 | 25.52 | 20.03 | 25.30 | 5.48 | | |
| Serum 3 | 36.42 | 35.09 | NA | 38.61 | 34.32 | 27.04 | 34.06 | 41.62 | 35.09 | 33.20 | 29.45 | 18.62 | 25.43 | 25.83 | 20.06 | 25.51 | 6.81 | • | |
| Plasma 1 PRP RT | 36.91 | 34.99 | 34.83 | NA | 34.05 | 27.08 | 35.71 | 38.99 | 34.87 | 37.00 | 29.28 | 18.65 | 26.08 | 25.45 | 20.13 | 25.58 | 7.43 | | |
| Plasma 2 PRP RT | NA | 36.29 | 34.48 | 35.47 | 34.91 | 26.62 | 33.72 | 35.03 | 33.32 | 36.04 | 28.41 | 17.96 | 26.33 | 25.54 | 20.03 | 25.60 | 8.37 | | |
| Plasma 3 PRP RT | 38.59 | NA | 36.24 | 35.77 | 34.50 | 27.14 | 34.59 | 40.85 | 34.60 | 37.29 | 29.19 | 18.56 | 25.53 | 25.89 | 20.44 | 25.97 | 6.97 | | - |

Figure 17: Quality Control data table.

| INPUT | RESULT_ OSTEOMIRTM Biomarkers of Bone Quality Quality Control Output | | App: a Descr Quali | App: ap-000001323 Description: App for osteomiR kit: as-321221 Biomarkers for Bone Quality | | | | | | | | | | stability for life. | | |
|-------|-------------------------------------------------------------------------------|----------------|--------------------------|--------------------------------------------------------------------------------------------------|--------------|----------------|---------------|----------------|-----------------|----------------|-----------------|----------------|------------------|---------------------|-----------|-----------------|
| | Sample ID | hsa-miR-214-3p | hsa-miR-188-3p | hsa-miR-31-5p | hsa-miR-203a | hsa-miR-335-5p | hsa-let-7b-5p | hsa-miR-127-3p | hsa-miR-550a-5p | hsa-miR-155-5p | hsa-miR-199b-5p | hsa-miR-29b-3p | cel-miR-39-3p CP | UniSp3 IPC | UniSp4 CP | Hemolysis Score |
| | Serum 1 | 34.28 | NA | NA | 32.52 | 31.14 | 24.22 | 31.34 | 32.68 | 32.29 | 33.74 | 26.98 | 28.12 | 20.48 | 26.95 | 6.5! |
| | Serum 2 | 36.89 | 35.35 | 35.33 | 37.04 | 33.70 | 27.45 | 33.04 | 38.58 | 33.37 | 32.89 | 28.97 | 25.52 | 20.03 | 25.30 | 5.41 |
| | Serum 3 | 36.18 | 34.85 | NA | 38.37 | 34.08 | 26.80 | 33.82 | 41.38 | 34.85 | 32.96 | 29.21 | 25.83 | 20.06 | 25.51 | 6.8: |
| | Plasma 1 PRP RT | 36.91 | 34.99 | 34.83 | NA | 34.05 | 27.08 | 35.71 | 38.99 | 34.87 | 37.00 | 29.28 | 25.45 | 20.13 | 25.58 | 7.4: |
| | Plasma 2 PRP RT | NA | 36.28 | 34.47 | 35.46 | 34.90 | 26.61 | 33.71 | 35.02 | 33.31 | 36.03 | 28.40 | 25.54 | 20.03 | 25.60 | 8.3 |
| | Plasma 3 PRP RT | 37.45 | NA | 35.10 | 34.63 | 33.36 | 26.00 | 33.45 | 39.71 | 33.46 | 36.15 | 28.05 | 25.89 | 20.44 | 25.97 | 6.91 |
| | Plasma 1 PPP RT | 38.19 | 36.17 | 35.57 | 37.41 | NA | 26.90 | 35.83 | 37.41 | 34.45 | 38.32 | 29.51 | 25.58 | 20.01 | 25.48 | 7.78 |
| | Plasma 2 PPP RT | 35.34 | NA | 38.12 | 35.56 | 34.13 | 26.66 | 34.58 | NA | 33.35 | 34.74 | 28.11 | 26.39 | 20.30 | 25.91 | 8.3 |
| | Plasma 3 PPP RT | 37.98 | 35.20 | 40.47 | NA | 35.70 | 26.45 | 34.77 | NA | 37.28 | 35.27 | 28.93 | 26.02 | 20.12 | 26.08 | 8.1. |
| | Plasma 1 PRP 4*C | 35.57 | 35.22 | 34.43 | 36.05 | 33.94 | 26.02 | 34.89 | 35.98 | 32.79 | 36.24 | 28.75 | 25.65 | 19.83 | 25.51 | 7.3(|
| | Plasma 2 PRP 4°C | 39.96 | 35.01 | 36.21 | 37.44 | 33.11 | 25.79 | 31.71 | 37.92 | 33.04 | 35.55 | 28.16 | 25.47 | 20.04 | 25.71 | 6.8! |
| | Plasma 3 PRP 4°C | 37.64 | 35.20 | 35.38 | 35.82 | 32.91 | 25.93 | 32.29 | 37.21 | 33.15 | 34.57 | 28.84 | 25.39 | 20.13 | 25.59 | 6.1. 👻 |

Figure 18: Table with normalized data, ready for download as .xls Excel file.

OSTEOMIRTM Biomarkers of Bone Quality

FAQ on the use of the osteomiR[™] app

What happens in case of pipetting errors e.g. accidental mix up of wells in the lab?

At the moment you will have to exclude the whole sample from further analysis with the osteomiR[™] app. We will eventually implement a "Flag as error" possibility for single wells, though it has to be emphasized that this might lead to negative effects on normalization and if at all should only be done for Research Use Only experiments.

The bottom line is that accurate pipetting and analysis of all osteomiR[™] microRNAs constitutes a prerequisite for making quality data driven assumptions.

Why are some of the Cq values in the downloaded raw/normalized data tables displayed as dates when viewed in Excel?

The Cq values use dots " . " as comma separator. This problem relates to your Excel settings and appears when you have chosen comma " , " as comma separator. Please change your settings to using a dot " . " as comma separator- then this problem should not occur anymore.

Once I have uploaded qPCR run.txt files or the clinical specification file on the upload interface (Figure 15), can I remove/change the files destined for analysis in case of miss-clicking before proceeding and pressing Start?

Yes. Just select "Select Data" again, choose the respective files for analysis and the prior uploaded files will be replaced by those now chosen.

Do I have to use a specific browser for optimal function of the osteomiR[™] app?

Yes. Please only use Google Chrome. Using Internet Explorer, Mozilla Firefox or other browsers might lead to functional problems with the software.

Is it possible to analyze data from multiple osteomiR[™] 96-well plates at once?

Yes. As long as the clinical specification file matches to the qPCR data you can upload and analyze multiple 96-well plates by simply choosing more qPCR .txt files on the upload interface (Figure 15).

Biomarkers of Bone Quality

Annex I: Specification of the qPCR protocol deposited in the osteomiR™ macro

Setup parameters

- Detection format: SYBR Green I / HRM Dye
- Block Type: 96
- Plate ID (optional)
- Reaction Volume: 10µl

Four program lines are specified in the osteomiR[™] experimental protocol

1. Heat activation

Analysis mode: None Cycles: 1

| Target | Acquisition | Hold | Ramp | Acquisition | Sec | Step | Step |
|--------|-------------|------------|--------|-------------|--------|------|----------|
| (°C) | mode | (hh:mm:ss) | rate | (per °C) | target | size | delay |
| | | | (°C/s) | | (°Č) | (°C) | (cycles) |
| 95 | None | 00:10:00 | 4.4 | | 0 | 0 | 0 |

2. Cycles

Analysis mode: Quantification Cycles: 45

| Target (°C) | Acquisition mode | Hold (hh:mm:ss) | Ramp rate (°C/s) | Acquisition (per °C) | Sec target (°C) | Step size (°C) | Step delay (cycles) |
|----------------|---------------------|--------------------|------------------------|-------------------------|-----------------------|----------------------|---------------------------|
| 95 | None | 00:00:10 | 4.4 | | 0 | 0 | 0 |
| 60 | Single | 00:01:00 | 1.6 | | 0 | 0 | 0 |

3. Melt curve

Analysis mode: Melting curves Cycles: 1

| Target (°C) | Acquisition mode | Hold (hh:mm:ss) | Ramp rate (°C/s) | Acquisition (per °C) | Sec target (°C) | Step size (°C) | Step delay (cycles) |
|----------------|---------------------|--------------------|------------------------|-------------------------|-----------------------|----------------------|---------------------------|
| 95 | None | 00:00:10 | 4.4 | |) Ó |) O | 0 |
| 55 | None | 00:01:00 | 2.2 | | 0 | 0 | 0 |
| 99 | Continuous | 00:00:01 | 0.11 | 5 | 0 | 0 | 0 |

4. Cooling

Analysis mode: None Cycles: 1

| Target | Acquisition | Hold | Ramp | Acquisition | Sec | Step | Step |
|--------|-------------|------------|--------|-------------|--------|------|----------|
| (°C) | mode | (hh:mm:ss) | rate | (per °C) | target | size | delay |
| | | | (°C/s) | | (°C) | (°C) | (cycles) |
| 40 | None | 00:00:01 | 2.2 | | 0 | 0 | 0 |