SmartCycler 2.0
DNA R-gene® Kits
Programming

- **The use of these guideline is dedicated for Argene kits that mentioned the instrument SmartCycler 2.0 in their package insert.**

- **Some products have their own amplification program following the same principle. In this case, please refer to their Outlined procedure.**

Products to be amplified relate to the extracted DNA obtained with the extraction methods recommended in the corresponding datasheet.

Plan the experiment as described in the datasheet.

- **AMPLIFICATION PROGRAM: "60°C R-gene" Program**

Steps		Time	Temperature	Cycles	Wavelength for signal reading
Taq Polymerase Activation		900 sec.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing Elongation	40 sec.	60°C		FAM and/or Cy3 end of the annealing

1. Throughout the patient follow-up, it is imperative to use the same protocol and to use the same extraction and amplification instrument.

2. This guideline is based on the SmartCycler, Version 2.0d Software.

3. In the case of very high positive samples (CT< 13), the SmartCycler software can give strange curves or even not correctly interpret these positive samples. To calculate a correct Ct, follow the procedure described on the last page.

SmartCycler 2.0 Programming

In the room reserved for amplification

STARTING THE SMARTCYCLER SOFTWARE

- Switch on the computer, its screen, the SmartCycler 2.0 real-time PCR platform(s).
- Click on the SMARTCYCLER icon (fig.1).
- Enter the user name and the software password, if required.

Fig.1

- Click on the DEFINE PROTOCOL button then NEW PROTOCOL to open the programming module and enter the programming data.

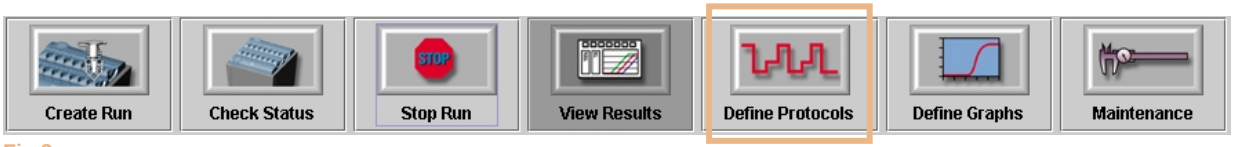


Fig.2

ENTRY OF THE PROGRAM DATA

- Type 60°C RGENE in the PLEASE ENTER A NEW NAME FOR THE PROTOCOL field in the NEW PROTOCOL window.
- Click OK.

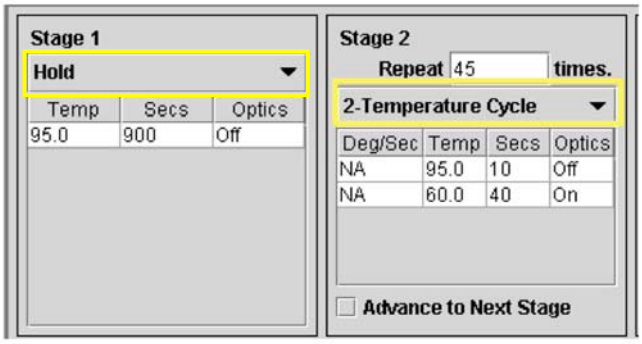


Fig.3

In the STAGE 1 section (fig.3):

Hot Start Taq Activation:

Select the HOLD tab.

Enter "95.0" in the TEMP field.

Enter "900" in the SECS field.

Select OFF in the OPTICS field.

In the STAGE 2 section (fig.3):

Select the 2-TEMPERATURE CYCLE tab.

Enter "45" in the REPEAT TIMES field.

Denaturation step

Enter "95.0" in the 1st row of the TEMP field.

Enter "10" in the 1st row of the SECS field.

Select OFF in the 1st row of the OPTICS field.

Annealing/ Elongation step

Enter "60.0" in the 2nd row of the TEMP field.

Enter "40" in the 2nd row of the SECS field.

Select ON in the 2nd row of the OPTICS field.

- Click SAVE PROTOCOL.

LAUNCH THE EXPERIMENT:

- Click **CREATE RUN**.

- In the **RUN NAME** field, type the name of the experiment (ex: **EBV RGENE YYY-MM-DD**) (fig.4).

- Make sure the "**DYE SET**" field is set on **FCTC 25**.

- Make sure the **GRAPHS** field includes, at least, the following parameters: **FAM, CY3**, **STANDARD** and **TEMPERATURE**.

- Make sure that:
  - **PRIMARY CURVE** is set in the **CURVE ANALYSIS** column.
  - **MANUAL** is set in the **THRESH SETTING** column.
  - **30.0** is set in the **MANUAL THRESH FLUOR UNITS** column.

- Check **CH1 FAM** and **CH2 CY3** are set on **ASSAY** in the **ADD/REMOVE SITES** section.

*Optional: **CH3 TXR** and **CH4 CY5**, in the **USAGE** column, **ASSAY** can be changed to **UNUSED**.*

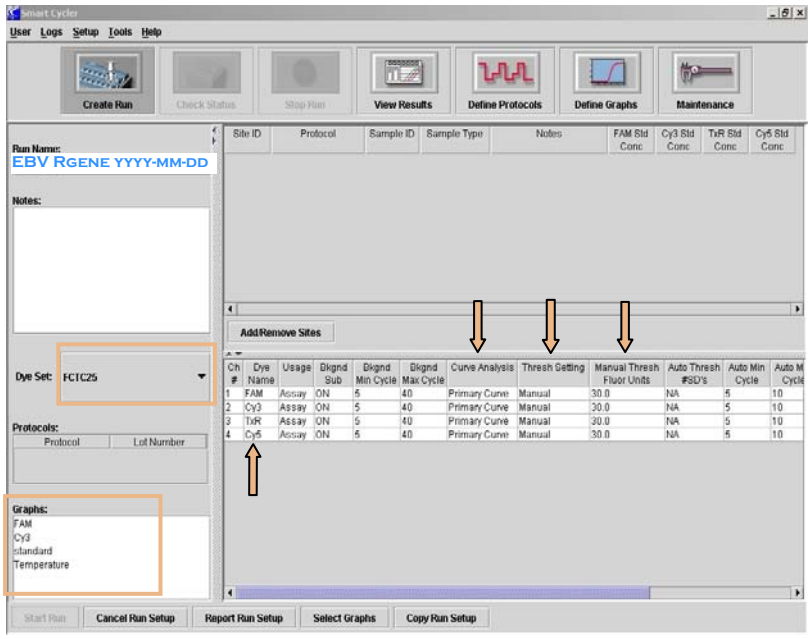


Fig.4

- Click on **ADD/REMOVE SITES**. A window appears: **SELECT PROTOCOLS AND SITES** (fig.5).

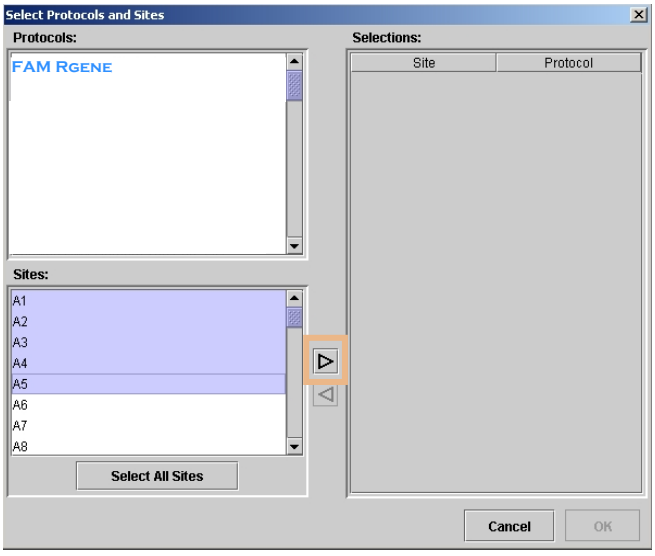


Fig.5

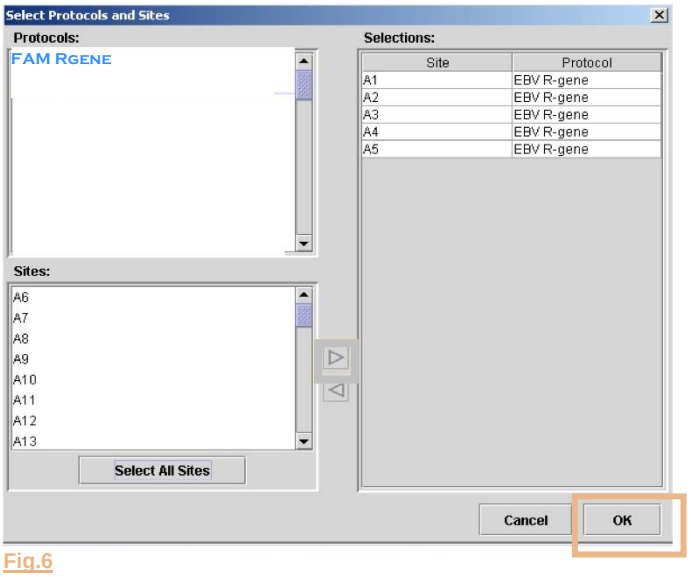


Fig.6

- Select the protocol: "**60°C RGENE**".

- Select the sites (**A1, A2, A3,** etc...)" corresponding to the samples and validate by clicking ▷ then **OK**.

- Insert the tubes in the selected I-Core sites.

- Launch the experiment by clicking **START RUN**.

Running the program

- In the **VIEW** tab of the **VIEWS RESULTS** section, select **RESULTS TABLE** and define samples names, standards and controls to be amplified as described below.

DEFINE THE SAMPLES

- Fill the **SAMPLE ID** column in a way which matches each I-Core sites to the corresponding sample.
- Select the correct type of sample in the **SAMPLE TYPE** column as described in the table below.

	Quantitative kits		Qualitative kits	
Channels	FAM	Cy3	FAM	Cy3
Patient samples	UNKN	UNKN	UNKN	UNKN
Quantification standard (**QS**)	STD	UNKN	Not applicable	
Sensitivity control (**SC**)	UNKN	UNKN	Not applicable	
Extraction+inhibition control (**IC2W0**)	UNKN	UNKN	UNKN	UNKN
Positive control (**PC**)	Not applicable		UNKN	UNKN
Negative Amplification Control (**R0**)	UNKN	UNKN	UNKN	UNKN

- Fill the **FAM STD / RES** column with the concentration of the standards.

- If the external standard curve is imported, QS3 is the only standard which needs to be tested. In this case it is defined as UNKN.
- If several parameters are detected in the same experiment, each quantification standard range has to be identified one by one. Not applicable for qualitative detection kits.

Only for quantification:

- Enter the concentration of the quantification standard(s) in the **FAM STD RES** column.

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-002 EBV R-gene®	QIAamp DNA Blood Mini kit / QIAcube	Whole blood	200 µL	100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
		Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact / MagNA Pure LC System	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®	Whole blood / Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	BioRobot EZ1 Workstation	Whole blood	350 µL	200 µL	6 000 000	600 000	60 000	6 000
	m2000sp Abbott	Whole blood / plasma / CSF / BAL / biopsies	800 µL (extract 300 µL)	250 µL (eluate 150 µL)	8 000 000	800 000	80 000	8 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	2 500 000	250 000	25 000	2 500

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-004 HSV1 HSV2 VZV R-gene® **71-015** HSV1 r-gene® **71-016** HSV2 r-gene® **71-017** VZV r-gene®	QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma	200 µL	50 µL	500 000	50 000	5 000	500
	QIAamp MinElute Virus Spin Kit	CSF						
	QIAcube + QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma						
	QIAcube + QIAamp MinElute Virus Spin Kit	CSF						
	MagNA Pure Compact MagNA Pure LC System	CSF						
	NucliSENS® easy MAG®	CSF						
	Versant kPCR Molecular System SP	CSF	400 µL (extract 250 µL)	65 µL (eluate 50 µL)				
69-010 ADENOVIRUS R-gene®	QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	QIAcube + QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies		100 µL	2 500 000	250 000	25 000	2 500
		Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube + QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact MagNA Pure LC System	Plasma / Respiratory samples*		50 µL	1 250 000	125 000	12 500	1 250
		Whole blood / Stool*		100 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®	Respiratory samples* / Whole blood / Stool*		50 µL	1 250 000	125 000	12 500	1 250
	BioRobot M48 QIAGEN	Respiratory samples*		100 µL	2 500 000	250 000	25 000	2 500
	m2000sp Abbott	Whole blood / biopsies / Respiratory samples*	800 µL (extract 300 µL)	250 µL (eluate 150 µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP	Plasma / CSF	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	1 250 000	125 000	12 500	1 250

***** *For a quantitative detection in cp/PCR, see 69-010 datasheet, section 11.3.*

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-013 BK Virus R-gene®	QIAamp DNA Blood Mini kit	Whole blood / Urine	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure Compact	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure LC System	Whole blood		100 µL	2 500 000	250 000	25 000	2 500
		Plasma / Urine		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS® easyMAG®	Whole blood / Plasma		50 µL	1 250 000	125 000	12 500	1 250
		Urine		100 µL	2 500 000	250 000	25 000	2 500
	Versant kPCR Molecular System SP	Plasma / Urine	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	1 250 000	125 000	12 500	1 250

If several parameters are detected in the same experiment, each quantification standard range has to be identified one by one. Not applicable for qualitative detection kits.

Data Analysis

ANALYSIS OF SAMPLES AND STANDARDS

- At the end of the run click on **RESULT TABLE** in the **VIEWS** section (fig.7).

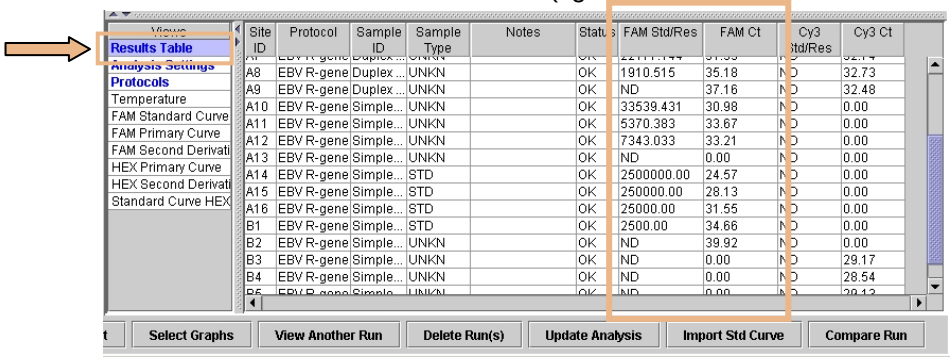


Fig.7

ONLY FOR QUANTIFICATION:

The standard curve is created with all 4 quantification standards (QS1, QS2, QS3, QS4)

- A calculated concentration is displayed for each tube in the column titled **FAM STD/RES** (fig.7).

- Read the calculated concentration of positive samples.

- A sample defined as "**ND**" in the column **FAM STD/RES** is:
  - negative if no CT value is calculated in the column **FAM CT**
  or
  - positive if a CT value is calculated in the column **FAM CT**, even if the software cannot give quantification result.

For standard curve importation

- Select **IMPORT STD CURVE** tab;

- Choose the run in which a full validated standard curve can be used (same **R-gene®** kit, same batch number and less than 3 months).

- The standards appear in yellow.

- Then, follow the explanation described above.

ONLY FOR QUALITATIVE DETECTION KITS:

- A positive sample is defined as **POS** in the column **FAM STD/RES** and a Ct value is displayed in the column **FAM CT**.

- A negative sample is defined as **NEG** in the column **FAM STD/RES** and "**0.00**" value is displayed in the column **FAM CT**.

	POS	26.27
	POS	29.58
	NEG	0.00
	NEG	0.00

ANALYSIS OF CONTROLS (IC2sample, IC2W0)

- Read the calculated CT at **Cy3** in the **Cy3 Ct** column in the **Result table** window for each sample inhibition control (**IC2sample**) and compare its value to the CT value of the reference extraction+inhibition control (**IC2W0**).

⚠ ***71-101*** *(DICO Extra r-gene®, Argene) has to be read in the FAM channel.*

- Click **Cy3 Primary curve** in order to check the final fluorescence values (fig.8).

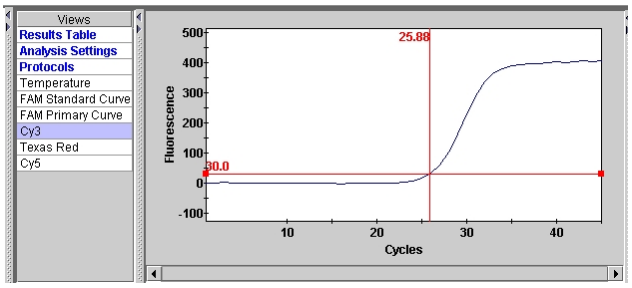


Fig.8

NOTE : **Do not read the CT of the quantification standards (QS), sensitivity control (SC), positive control (PC) and negative amplification control (R0) in the Cy3 channel.**

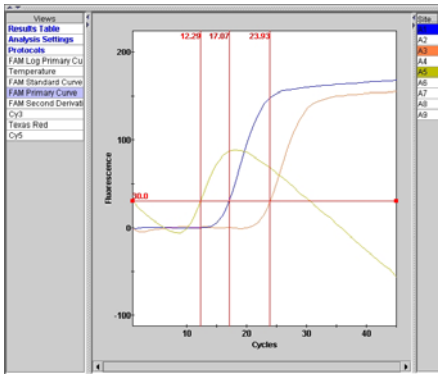
The interpretation results are only based on the comparison between the CT obtained for each sample extraction+inhibition control (IC2sample) and the CT obtained with reference extraction+inhibition control (IC2W0).

Interpretation of Results

- Detailed interpretation is described in each corresponding datasheet.

WARNING:

In the case of very high positive samples (CT< 13) (see fig opposite), The SmartCycler software can give strange curves or even not correctly interpret these positive samples.

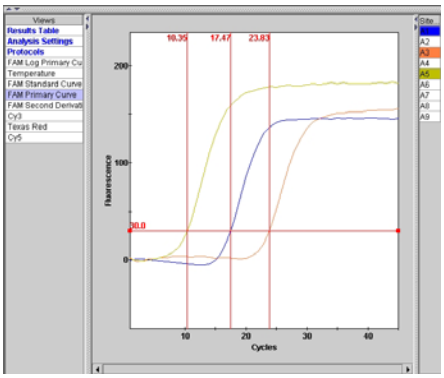


To calculate a correct CT, click on **Analysis Settings**
- Replace **Bkgnd Min Cycle** default value **5** with **1**
- Replace **Bkgnd Max Cycle** default value **10** with **5**

Ch #	Dye Name	Usage	Bkgnd Sub	Bkgnd Min Cycle	Bkgnd Max Cycle
1	FAM	Assay	ON	1	5
2	Cy3	Unus...	ON	5	40
3	TxR	Unus...	ON	5	40
4	Cy5	Unus...	ON	5	40

- Click on **Update Analysis**

The strange curve becomes normal (see fig opposite) and the CT can be validated.



This analysis must be done only for strange curves.

For the other curves, reset the default values (5 and 10).

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