

# SmartCycler 2.0 DNA R-gene<sup>®</sup> 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 Polymera	se Activation	900 sec.	95°C	1	-
	Denaturation	10 sec.	95°C		-
Amplification	Annealing Elongation	40 sec.	60°C	45	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.
- 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.

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.

# Jenaturation step

Enter "95.0" in the 1<sup>st</sup> row of the TEMP field.

Enter "10" in the 1<sup>st</sup> row of the **SECS** field.

Select **OFF** in the 1<sup>st</sup> row of the **OPTICS** field.

# Annealing/ Elongation step

Enter "60.0" in the  $2^{nd}$  row of the **TEMP** field. Enter "40" in the  $2^{nd}$  row of the **SECS** field. Select **ON** in the  $2^{nd}$  row of the **OPTICS** field.

Select ON In the 2 row of the OPTICS

- Click SAVE PROTOCOL.



Fig.3



Fig.1





# 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 chang	gec	to to
UNUSED.		

Create Run Che	ck Status		Stop P	Suus .	View	Results	L.L.	Locots De	fine Graphs	(f)2 Maintenan	-	
Ran Nama: EBV RGENE YYYY-MM-D Notes:		Site ID	Pri	otocol	Sample	e ID Sarr	iple Type	Notes	FAM Std Conc	Cy3 Std TuF Come C	8 Std Cy onc C	6 Std Ionc
		AddRei	nove Site	es			Ļ	Ļ	Ļ			1
Dye Set FCTC25	• 1	FAM	Assay	Sub ON	Min Cycle 5	Max Cycle 40 40	Primary Curve	Manual Manual	Fluor Units 30.0	#SD's NA	Auto Min Cycle 5	C)
Protocols: Protocol Lot Number	3	DiR Cy6	Assay Assay	ON ON	5	40 40	Primary Curve Primary Curve	Manual Manual	30.0 30.0	NA NA	5	10
Graphs:		U										

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

Select Protocols and Sites	×	Select Protocols and Sites	×
Protocols:	Selections:	Protocols:	Selections:
FAM RGENE	Site Protocol	FAM RGENE	Site Protocol   A1 EBV R-gene   A2 EBV R-gene   A3 EBV R-gene   A4 EBV R-gene   A5 EBV R-gene
Sites:     Image: Constraint of the site o		Sites:       A6       A7       A8       A9       A10       A11       A12       A13	
	Cancel		Cancel OK
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 b 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.

		Quant	itative kits	Qualitative kits				
	Channels	FAM	Сүз	FAM	Сүз			
Patient sa	mples	UNKN	UNKN	UNKN	UNKN			
Quantification standard (QS)		STD	UNKN	Not app	olicable			
Sensitivity	r control (SC)	UNKN	UNKN	Not applicable				
Extraction (IC2W0)	+inhibition control	UNKN	UNKN	UNKN	UNKN			
Positive control (PC)		Not a	applicable	UNKN UNKN				
Negative / Control (R	Amplification (0)	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.

		Standard concentration to be entered							
R-gene <sup>®</sup> Kit	Kit Extraction method		Sample Specimen volume to be Elution volume extracted		Elution volume	QS1	QS2	QS3	QS4
			Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		QIAampDNA	Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
		Blood Mini kit	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
	QIACUDE		Plasma / CSF	200 µL	50 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure	Compact	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
69-002	MagNA Pure	LC System	Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
EBV R-gene®	NucliSENS®	easyMAG <sup>®</sup>	Whole blood / Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	BioRobot EZ	Workstation	Whole blood	350 µL	200 µL	6 000 000	600 000	60 000	6 000
	m2000sp At	obott	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 kPC System SP	R Molecular	Plasma	400 μL (extract 250 μL)	65 μL (eluate 50 μL)	2 500 000	250 000	25 000	2 500

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		Co	onditions			Standard concentration to be entered				
R-gene <sup>®</sup> Kit	e <sup>®</sup> Kit Extraction method Specimen		Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4		
		QlAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma							
60-004		QIAamp MinElute Virus Spin Kit	CSF							
03-004       HSV1 HSV2 VZV       R-gene®       71-015       HSV1 r-gene®       71-016       HSV2 r-gene®       71-017       VZV r-gene®	QIAcube	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							
	MagNA Pure MagNA Pure	e Compact e LC System	CSF	CSF						
NucliSEN Versant k System S		easy MAG <sup>∞</sup> CR Molecular	CSF CSF	400 μL (extract 250 μL)	65 μL (eluate 50 μL)					
		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	
		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	
		QIAamp DNA Stool Mini kit	Stool*	200 µL	100 µL	2 500 000	250 000	25 000	2 500	
69-010 ADENOVIRUS R-gene <sup>®</sup>	MagNA Pure	e Compact	Plasma / Respiratory samples *		50 µL	1 250 000	125 000	12 500	1 250	
	MagNA Pure	e LC System	Whole blood / Stool*		100 µL	2 500 000	250 000	25 000	2 500	
	NucliSENS®	easyMAG <sup>®</sup>	Respiratory samples*/ Whole blood / Stool*		50 µL	1 250 000	125 000	12 500	1 250	
	BioRobot M	48 QIAGEN	Respiratory samples *		100 µL	2 500 000	250 000	25 000	2 500	
	m2000sp Al	obott	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 kP0 System SP	CR Molecular	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 <sup>®</sup> Kit	it Extraction method		Specimen Volume to extractor		Elution volume	QS1	QS2	QS3	QS4					
			Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500					
		QIAamp DNA	Plasma		50 µL	1 250 000	125 000	12 500	1 250					
	QIAcube	Blood Mini kit	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					
CD 012	MagNA Pur	Compact	Whole blood / Urine	200 µL	100 µL	2 500 000	250 000	25 000	2 500					
69-013 BK Virus R-gene <sup>®</sup>	Magrover an	oompuor	Plasma		50 µL	1 250 000	125 000	12 500	1 250					
			Whole blood		100 µL	2 500 000	250 000	25 000	2 500					
	Magina Pur	e LC System	Plasma / Urine		50 µL	1 250 000	125 000	12 500	1 250					
	NucliSENS®	easyMAG <sup>®</sup>	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 kP System SP	CR Molecular	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).

-	▼ 4000000000000000000000000000000000000	222	000000						0000000			0002	*******		00000	inns
R	Miewe esults Table	1	Site	Protocol	Sample	Sample Type	Notes	s Statu	s FAI	/I Std/Res	FAM Ct	Γ	Cy3	Cy3 Ct		
			01	COVICIONIC	Duplos	OTAL CLA		VIC	221		51.55	- 17	-	92.14		
	naiysis settings		A8	EBV R-gene	Duplex	UNKN		0K	191	0.515	35.18	Ν	D	32.73		E
P	rotocols		A9	EBV R-gene	Duplex	UNKN		0K	ND		37.16	N	D	32.48		
T I	emperature		A10	EBV R-gene	Simple	LINKN		0K	335	39.431	30.98	N	1	0.00		
E,	AM Standard Curve		A11	EDV R gono	Simple	LINIZNI		OK	627	0.000	22.67	Ň	5	0.00		
E,	AM Primary Curve		011	EDV Regene	Oimple			OK	204	0.000	33.07	-	-	0.00		
E/	AM Second Derivati		ALZ	EBV R-gene	simple	UNKN		UK	134	3.033	33.21	P.	<u> </u>	0.00		
ш	EV Primary Curve		A13	EBV R-gene	Simple	UNKN		OK	ND		0.00	P	2	0.00		
	EX Cocord Derivet		A14	EBV R-gene	Simple	STD		OK	250	0000.00	24.57	Ν	D	0.00		
<u>п</u>	EX Second Derivat		A15	EBV R-gene	Simple	STD		OK	250	000.00	28.13	Ν	D	0.00		
S	tandard Curve HEX		A16	EBV R-gene	Simple	STD		0K	250	00.00	31.55	N	D	0.00		
			B1	EBV R-gene	Simple	STD		OK	250	0.00	34.66	N	5	0.00		
			B2	EBV R-gene	Simple	UNKN		OK	ND		39.92	٨	D	0.00		
			B3	EBV R-gene	Simple	UNKN		OK	ND		0.00	Ν	D	29.17		
			B4	EBV R-gene	 Simple	UNKN		OK	ND		0.00	Ν	D	28.54		
			DA	EDI/ D gono	Qimplo	LINIZNI		OK	ND		0.00	A	2	20.12	1	
_			•													
t	Select Graphs	;		View Anothe	r Run	Delete F	Run(s)	Update An	nlysis	In	port Std Cur	ve	C	ompare Rui	n	

#### **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  $\ensuremath{\mathsf{FAM}}\xspace{\mathsf{CT}}$  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**<sup>®</sup> 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





- 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<sup>®</sup>, Argene) has to be read in the FAM channel.
- Click CY3 PRIMARY CURVE in order to check the final fluorescence values (fig.8).



<u>NOTE</u>: 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).



# E

# Interpretation of Results

- Detailed interpretation is described in each corresponding datasheet.

	<u>)</u> :						
In the case of ve The SmartCycle interpret these p	ery I r sc osi	high p oftwar tive sa	ositive e can ample	e samı give s s.	oles (CT trange c	< 13) (s curves of	ee fig opposite), r even not correctly
To calculate a co - Replace BKGN - Replace BKGN	orre 1D 1D	ect CT MIN C MAX	, click CYCLI CYCL	on An E defa E defa	NALYSI ult value ault value	s SETTI e 5 with e 10 wit	NGS 1 th 5
	Ch # 1	Dye Name FAM	Usage Assay	Bkgnd Sub ON	Bkgnd Min Cycle 1	Bkgnd Max Cycle 5	

-AM	Assay	ON	1	5
Суз	Unus	ON	5	40
TxR	Unus	ON	5	40
Cy5	Unus	ON	5	40
	Cy3 FxR Cy5	Dy3 Unus TxR Unus Dy5 Unus	Cy3 Unus ON Cy3 Unus ON Cy5 Unus ON Cy5 Unus ON	Cy3     Unus     ON     5       Cy4     Unus     ON     5       Cy5     Unus     ON     5

- 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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