



Please refer to StepOne guideline if using ABI PRISM 7500[®] version 2.0.1.

- The use of these guideline is dedicated for Argene kits that mentioned the instrument ABI PRISM 7500 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	15 min.	95°C	1	-
	Denaturation	10 sec.	95°C		-
Amplification	Annealing Elongation	40 sec.	60°C	45	FAM and/or VIC 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 ABI PRISM 7500, Version 1.4 Software.

3. Check if **NONE** is selected in the field **PASSIVE REFERENCE** (see page 3) because amplification premixes do not contain PASSIVE REFERENCE.

ABI7500_DNA_v2bMx_250113

ABI PRISM Programming (i.e.: 7500 Software 1.4)

STARTING THE APPLICATION

- Switch on the computer, its screen and the ABI PRISM real-time PCR platform.
- Enter user name and password if necessary.
- Click on the icon 7500 SYSTEM SOFTWARE (Fig.1).
- A QUICK STARTUP DOCUMENT window appears (Fig.2).
- Select CREATE NEW DOCUMENT...

A NEW DOCUMENT WIZARD window opens (Fig.3).

DEFINE DOCUMENT

- Make sure that:

- In Assay field, **STANDARD CURVE (ABSOLUTE QUANTITATION)** is selected.
- In Container field, 96-WELL CLEAR is selected.
- In Template field, **BLANK DOCUMENT** is selected.
- In Run Mode, **STANDARD 7500** is selected.
- Click on NEXT.

New Document Wizard		
Define Document Select the assay, container, and template for the document, and enter the operator name and comments.	Assay:	Standard Curve (Absolute Quantitation)
Assay Standard Dures (Moskie Quaritation)	Container:	96-Well Clear
Tempike Blank Document T Blowse	Template:	Blank Document
Operator: (RLD	Run Mode:	Standard 7500
Converte: (505 v1.4		
Plate Name Plate1		
Chie Next >> End. Carcel		

Fig.3







Fig.2



CREATE DETECTORS (If you run the program for the first time).

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- Click on **New Detector...** and enter the following data (Fig.4) :
 - In the NAME field enter FAM RGENE (Fig.5).
 - Leave the field **DESCRIPTION** blank.
 - In the field **REPORTER DYE** enter **FAM**.
 - In the field **QUENCHER DYE** enter (NONE).
 - Click on the **COLOR** field and choose green (Fig.6).
 - Click on OK to validate the creation of the detector FAM RGENE.



- In the same way, click again on **NEW DETECTOR...** and enter the following data (Fig.7):

- In the field **NAME** enter **VIC RGENE**.
- Leave the **DESCRIPTION** field blank.
- In the **REPORTER Dye** field enter **VIC**.
- In the **QUENCHER DYE** field enter (NONE).
- Click on the **COLOR** field and choose **YELLOW**.
- Click on **OK** to validate the creation of the detector **VIC RGENE**.
- The two detectors created appear in the list of detectors (Fig.8).
- Select the detectors FAM R-GENE and VIC R-GENE and click on ADD >>.
- The two detectors selected appear now in the field **DETECTORS IN DOCUMENT** (Fig.8).
- Select (NONE) in **PASSIVE REFERENCE** (amplification premix does not contain passive reference).
- Click on **NEXT**.





Fig.4

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SET UP SAMPLE PLATE

- Tick the checkboxes of the two detectors previously selected.
- Click on FINISH (Fig. 9).



ENTRY OF THE PROGRAM DATA

- Click on the **INSTRUMENT** tab (Fig.10).
- Double-click on **STAGE 1** part and click on **DELETE** (Fig11).



Hot Start Taq Activation:

Click on the part **STAGE 1** and enter the value **95.0** in the top part of the window. Then, enter the value **15:00** in the bottom part of the window (Fig.12).

Denaturation step:

Click on the left part of the **STAGE 2**. Enter the value **95.0** in the top part of the window. Enter the value **0:10** in the bottom part of the window.

Annealing/Elongation step:

In the right part of the **STAGE 2**: Enter the value **60.0** in the top part of the window. Enter the value **0:40** in the bottom part of the window.

- **STAGE 2** is repeated 45 times, so type **45** in the blank field **REPS**.



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- Click on the **RAMP RATE** tab (Fig.13).
- Check that the parameter **100%** appears in the second step of the cycling stage (step at 60°C).

up y Instrument VResults			
strument Control	Temperature		
Start Estimated Time Remaining (hhumm):	Sample:	Heat Sink:	
Clon	Cover	Block:	
Stop.	- Cycle		
Disconnect Status:	Stage:	Rep	
	Time (mm:ss):	Step:	
Estend	State:		

- In SETTINGS field enter 25 in SAMPLE VOLUME (µL) (Fig.14).
- In Run Mode select Standard 7500.
- In DATA COLLECTION select STAGE2, STEP2 (60.0@0:40). (Data collection step selected appears in colour.)

Setup Vinstrument (Results)					
Instrument Control	Temperature				
Start Estimated Time Remaining (hh.mm)	Sample: Heat Sink:				
(here	Cover: Block:				
	Cycle				
Disconnect Status:	Stage: Rep:				
	Time (mm:so): Step:				
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Thermal Parties Ander Incommunity Draws Parts			Settings		
Stage 1 Stage 2					
Reps 1 Reps 45			Sample Volume (ul.) :	25	
08.0			o difipio i o difio (piz) :		
16:00 0:10			Dum Manda	Chandred 7500	
10.0		—	Run Mode	Standard 7500	<u> </u>
0.40					
			Data Collection :	Stage 2, Step 2 (60.0 @ 0:40)	-
				,	_
/					
Add Cycle Add Hold Add Step Add Dissoc	ciation Stage Delete Help				
Settings					
Sample Volume (µL) : 25					
Rwn Mode Standard 7500	ㅋ				
Data Callestina : Stars 2 Stars 2 (SDB / 0.0.40)					
Stage 1, Step 1 (95.0 (9 15.00)					
Stage 2, Step 1 (95.0 (e) 0.10)					
\$1309 2, \$399 2 (600 09 0 40)		Ein 44			
		<u>rig.14</u>			

TO SAVE THE PROGRAM:

- Click on **FILE** in the Menu bar and **SAVE AS** (Fig. 15).
- Save your file in TEMPLATES as 60°RGENE and check that SDS TEMPLATES (*.SDT) is selected in TYPE FIELD.

7500 System SDS Software - [Plate1 (Standard Curve)]	Sava Ar		
File View Tools Instrument Analysis W	ndow Help	Save As		
Nov ObieN Open ObieO	e 8	Enregistrer dans :	Conceptates	
Close Save Otri+5	Temperature		AQ RNase P Instal	
Revert To Saved	e: Sample: Heal Sink: Cover: Block:	Mes documents		
Import Sample Setup Otri+I Export View Exported Results	Cycle Skage: Repx Time (mm11) Stepx	Incents I I I I I I I I I I I I I I I I I I I		
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1 2007 05 29 plagar hivt hivt2 vzv 2 6/beckvivs/BDCA 200807 3 2007 05 29 plagar hivt hivt2 vzv 4 6/b000 03 30 plagar citre Nov 6 2006 10 24 plagar Citre Nov		Mes documents		
	Add Dissections Place	Favoris réseau	Nom du fichier : 60° Rgene	
Settings Sample Volume (al.): 25			Lype : SDS Templates (*.sdt)	
Run Mode Standard 7500 Data Collection : Stage 2, Step 2	× 2(60.0 @ 0.40)	Fig 15		



Running the Program

Open

RUNNING THE PRE-STORED PROGRAM

- In the home page of the software when the **QUICK STARTUP** window appears:
 - Select **OPEN EXISTING DOCUMENT** and choose **60° RGENE** in Templates file (Fig.16).



Contraction and	The second second second					All and a second second			
Mes documents récents	▶ 60° Rgene → ▶ AQ RNase P I	Instal							
Bureau Mes documents									
Poste de traval									
Favoris terma	Non-du Echier -	Г				-	Onen		
A DESCRIPTION OF A DESC									
	Fichiers de type :	A	SDS Files (".ods	(f.sdm;f.sdl)		•	Cancel		
/Setup Vinstrume /Plate \	Fichiers de type :		I SDS Files (".ads	;*.sdm;*.sdt)			Cancel		
/Setup Vinstrume /Plate 1	Fichiers de type : nt VResults	A	ISDS Files (".sds Inspector	;".sdm;".sdt)		- L	Cancel		
/ Setup V Instrume /Plate 1 A OS1 5.00e+005	Fichiers de type :	Al Well Well(s): A Sample N	II SDS Files (".ads I Inspector 1 Iame: QS1]	;".sdm;".sdt)			Cancel		
Setup V Instrume Plate 1 A QS1 S.00e+005 U	Fichiers de type :	Well Well(s): A Sample N Use	II SDS Files (".sds I Inspector 1 Jame: 0S1 Detector	(fisdm;fisdk)	Quencher	Task	Cancel	Color]
/ Setup / Instrume / Plate 1 A QS1 3 500e+005 U	Fichiers de type : Il YResults 2 U	Al Well Well(s): A Sample N Use	II SDS Files (".sds Inspector 1 Jame: QS1 Petector FAM Rgene	(fisdm;fisdt) Reporter FAM	Quencher (none)	Task Standard	Cancel Cancel Quantity 5.0e+005	Color	
/ Setup V Instrume / Plate 1 A OS1 B U U	Fichies de type : Fichies de type : 1 YResults 2 1 1 1	All Well(s): A Sample N Use F	ISDS Files (".eds Inspector Jame: QS1 EAM Rgene VIC Rgene	(f.sdm;f.sdk) Reporter FAM VIC	Quencher (none) (none)	Task Standard Unknown	Cancel Cancel Couantity 5.0e+005	Color	1
/Setup Vinstrume /Plate 1 A 25:1 B U B U C	Fichies de type : Fichies de type : 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1	Au Well(s): A Sample N Use F F	ISDS Files (".eds Inspector I ame: QS1 Petector FAM Rgene VIC Rgene	("sdm,"sdt) Reporter FAM VIC	Quencher (nore) (nore)	Task Standard Uninown	Cancel Cancel Coantity 5.0e+005	Color	Passive Re

<u>Fig.16</u>

DEFINE THE SAMPLES

- Click on the **SET UP** tab (Fig.17).
- For each well to define:
 - Double click on the well :
- A new window WELL INSPECTOR appears:
- According to the table below :
 - Select the checkbox(es) of the appropriate(s) detector(s): FAM RGENE and/or VIC RGENE.
 - Enter the name of the sample in **SAMPLE NAME** field.
 - Specify the samples in the TASK column.
 - Specify the concentration of the quantification standard in the QUANTITY column (cf table).
 - Click on **CLOSE**.

		Quantita	tive kits	Qualitat	tive kits		
	Channels	FAM	VIC	FAM	VIC		
Patient sar	nples	UNKNOWN	UNKNOWN	UNKNOWN UNKNOWN			
Quantificat (QS)	ion standard	STANDARD	UNKNOWN	Not applicable			
Sensitivity	control (SC)	UNKNOWN	UNKNOWN	Not app	olicable		
Extraction+ (IC2W0)	inhibition control	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN		
Positive co	ntrol (PC)	Not app	olicable	UNKNOWN	UNKNOWN		
Negative A Control (R	mplification))	NTC (NO TEMPLATE CONTROL)	UNKNOWN	NTC (NO TEMPLATE CONTROL)	UNKNOWN		



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:

		Co	onditions			Standard concentration to be ente			
R-gene [®] Kit	Extractio	n method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
			Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		QIAampDNA	amp DNA Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	OlAcuba	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	e Compact	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
69-002	MagNA Pure	e LC System	Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
EBV R-gene®	NucliSENS®	easyMAG®	Whole blood / Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	BioRobot EZ	1 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	CR Molecular	Plasma	400 μL (extract 250 μL)	65 μL (eluate 50 μL)	2 500 000	250 000	25 000	2 500
			Whole blood						
			Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
		QIAamp DNA	Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	OlAcube	Blood Mini kit	Whole blood Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
	QIACUDE		Serum / Plasma / CSF	200 ul	50 µL	1 250 000	125 000	12 500	1 250
69-003	MagNA Pure	e Compact	Whole blood Amniotic Fluid	200 με	100 µL	2 500 000	250 000	25 000	2 500
CMV R-gene [®] 69-100 CMV HHV6, 7,8 R-gene [®]	MagNA Pure	e LC System	Serum / Plasma / CSF						
			Whole blood		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS®	easyMAG [®]	Serum / Plasma / CSF						
	m2000sp At	obott	Whole blood / plasma / BAL / urine / biopsies / amniotic fluid	800 μL (extract 300 μL)	250 μL (eluate 150 μL)	4 000 000	400 000	40 000	4 000
	Versant kPC	CR Molecular	Plasma	400 µL	65 µL	1 250 000	125 000	12 500	1 250





	Conditions					Standard concentration to be entered			
R-gene [®] Kit	Extractio	on 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®	QIAcube	QIAamp DNA Blood Mini kit QIAamp MinElute Virus Spin Kit QIAamp DNA Blood Mini kit QIAamp MinElute Virus Spin Kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma CSF CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma CSF	200 µL	50 µL	500 000	50 000	5 000	500
	MagNA Pur MagNA Pur	e Compact e LC System	CSF						
	Versant kPe System SP	easyMAG [®] 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 [®]	MagNA Pur	e Compact	Plasma / Respiratory samples *		50 µL	1 250 000	125 000	12 500	1 250
	Magnia Ful	e LC System	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 M	148 QIAGEN	Respiratory samples *		100 µL	2 500 000	250 000	25 000	2 500
	m2000sp A	bbott	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 kP 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.



		Co	nditions			Standard concentration to be entered			
R-gene [®] Ki	t Extractio	Extraction method		Sample volume to be extracted	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	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
00.040		Compact	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
BK Virus R-gene [®]	[®]	Compact	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
	MagNA Pure	e LC System	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 kP0 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

<u>_!</u>

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.

- Click on INSTRUMENT TAB, click CONNECT (if necessary), then insert the microplate in the instrument.

- Launch the run by clicking on **START** (Fig. 18).

Setup VInstrument Results			
Instrument Control Start Stop	Temperature Sample: Cover:	Heat Sink: Block:	
Disconnect Status: Extend	Cycle Stage: Time (mm:ss): State:	Rep: Step:	
	_		

<u>Fig.18</u>



- At the end of the experiment, the **SDS** software automatically opens.
- Click on OK.
- Click on ANALYSIS tab (Fig.19) then on ANALYZE (or click on this button .)

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Setup Instrument Results
Plate
Fig.19

- Click on **RESULTS** tab then **AMPLIFICATION PLOT**.



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LINEAR MODE: QUANTIFICATION OF THE SAMPLES

- In TOOLS in the menu bar, select GRAPH SETTINGS...
- In Post Run Settings field (Fig.20) :
 - In the Y-AXIS tab, select LINEAR radio button.
 - Click on **APPLY** then **OK**.
- Mark all samples in the table that you want to analyze.
- In DATA field select DELTA RN VS CYCLE (Fig.21).
- In DETECTOR field select FAM RGENE.

Roal-Time Sottinge YAxis Auto Scale Minimums C Linear <u>Minimum</u> 0.0001 Maginum 10	Post Run Settings Y-Asis P Auto Scale C Log Micimum 1 Mesingm 1000000
X Avis is autoscaled in RealTime	X-Asis V Auto Scale C Linger Migimum: 1 Masingm: 15
Deplay Options Line <u>W</u> idth: 2 👘 (1 - 10)	

Fig.20



Fig.21

MANUEL BASELINE ADJUSTMENT : IDENTIFICATION OF THE SAMPLES

- Select MANUAL CT (Fig.22) and adjust the <u>noise band</u> (green horizontal line) to a position where it crosses the fluorescence curves of all the samples in their exponential part (Fig.23).
- Click on **ANALYZE**.









- Return in the AMPLIFICATION PLOT tab.
- Click on the DETECTOR field then select the detector VIC R-GENE (Fig.25).

Detector:	VIC Rgene	•
	Fig.25	

- Perform the manual baseline adjustment as described in the previous section.
- Read the calculated CT in the REPORT tab at VIC R-GENE line for each sample inhibition control (IC2sample) and compare its value to the CT value of the reference extraction + inhibition control (IC2W0).
 - NOTE : Do not read the CT of the quantification standards (QS), sensitivity control (SC), positive control (PC) or negative amplification control (R0) with the VIC R-GENE detector (VIC channel).

The interpretation results is 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.

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