

DEVICE MANUAL

ThermoFisher

Applied Biosystems 7500 & 7500 Fast Real-Time PCR System

Designed for GeneProof diagnostic kits

See www.geneproof.com for the current kits list

www.geneproof.com



Molecular diagnostics for your routine

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1. Purpose

This device manual describes in detail the process of using GeneProof PCR kits for microbiological diagnostics with the devices 7500 Real-Time PCR System and 7500 Fast Real-Time PCR System.

1.1. PCR Reaction Preparation

Prepare PCR reaction according to the Instruction for use of the used GeneProof PCR kit.

1.2. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits, or download them from the product site of the used GeneProof PCR kits from the website of the company <u>www.geneproof.com</u>.

Save the downloaded templates on your local disc and open them in the software 7500 Software.exe.

7500 Software v2.3					-	0 ×
File Edit Instrument Analysis		G Event - D Briel Depart				
ing New Experiment • 🗳 Open	Save Close					
Experiment Menu «	Save As	lace HIV-ABI7500	Type: Standard Curve	Reagents: TaqMan® Reagents	START RUH 🅪	()
Setup	Save As Template Experiment Prop	Ctrl+T erties				
Experiment Properties	Enter an experim	ment name, select the instrument type, select the type of experiment to set up	p, then select materials and methods for the PCR reactions and instrument run.			
Plate Setup	How do you want	t to identify this experiment?				
Run Method	* Experiment Name:	150610 validace HIV-ABI7500				
	Barcode (Optional):					
Reaction Setup	User Name (Optiona	al): Administrator				
🫒 Materials List	Comments (Optiona	30): SDS v1.2				Ĵ
Run	• Which instrume	ent are you using to run the experiment?				
Analysis		√ 7500 (96 Wells)	7500 Fast (96 Wells)			
	Set up, run, and an	alyze an experiment using a 4- or 5-color, 96-well system.				
94	• What type of ex	xperiment do you want to set up?				
		(Quantilation Chandrad Quant	Quantitation Datation Chandred Quant			
		V Quantitation - Standard Curve	Quantitation - Relative Standard Curve	Quantiation - Comparativ		
		Melt Curve	Genotyping	Presence/Abser	nce	
	Use standards to d	letermine the absolute quantity of target nucleic acid sequence in samples.				
	• Which reagents	s do you want to use to detect the target sequence?				
		√ TaqMan® Reagents	SYBR® Green Reagents	Other		
	The PCR reactions	contain primers designed to amplify the target sequence and a TaqMan® p	robe designed to detect amplification of the target sequence.			
	*Which ramp spe	eed do you want to use in the instrument run?				
		√ Standard (~ 2 hours to complete a run)	Fast (~ 40 minutes to complete a run)			
	For optimal results	with the standard ramp speed, Applied Biosystems recommends using sta	indard reagents for your PCR reactions.			
*						
A Home W Untitled × 150	610 validace HIV-ABI7500	1 X				

Fig. 1.1: Save template

After saving, the template can be opened from the file Templates. With each next usage of GeneProof PCR kits continue from the chapter **1.3 Starting the PCR amplification**.



1.3. PCR Amplification Start

1.3.1 Starting the software

- 1. Start the 7500 Software.
- 2. In the Set Up column, select Template.
- 3. Open template for the given GeneProof PCR kit.

Set Up	Run	Analyse
· ·		<u> </u>
Design Wizard	QuickStart	Analyse Experiment
	Open X	
	Look in: GeneProof Visit PCR	
	Recett term	
Advanced Setue	Desktop	Create Study
	This PC	
	File name: GeneProof DNA PCR edt gen	
	Nativent Files of type: Experiment Document Template files (* edf) v Cancel	
Template	Fall feet find yest loomet	
	700000 7000 0	Applied Biosystems H

Fig. 1.2 Opening the template



1.3.2 PCR plate editing

- 1. In Experiment Properties, enter experiment name into the Experiment Name row.
- 2. In Plate Setup, use Add New Target to define targets according to the kits used in the experiment.

E.g. for HSV detection (3 channels) set Target Name: **HSV 1**, Reporter: **FAM**, Quencher: **None**; Target Name: **HSV IC**, Reporter: **JOE**, Quencher: **None** and Target Name: **HSV 2**, Reporter: **Cy5**, Quencher: **None**. For MT detection (2 channels) set Target Name: **MT**, Reporter: **FAM**, Quencher: **None** and Target Name: **MT IC**, Reporter: **JOE**, Quencher: **None**. Use **Save Target** and **Add Saved Target** to save and reuse targets.

3. Use Add New Sample to define samples.

4. In the case of qualitative detection, define positive control as a sample, e.g. **MT Positive Control**.

7500 Software v2.3	Table 1995						-	
File Edit Instrument Analysis	Tools Help							
New Experiment • Goven	. 🛃 Save 🔹 🖾 Close 🍕	Export • 🗟 Print Report						
Experiment Menu «	Experiment: YYMMD	D experiment		Type: Standard Cur	ve	Reagents: TaqMan@ Reagents	START RUN 📡	?
Setup	Define Targets and	Samples Assign Targets and Sample	s		_			
Experiment Properties	Instructions: Define the	targets to quantify and the samples to test in the read	ion plate.					
	Define Targets					Define Samples		
Plate Setup	Add New Target Add S	aved Target Save Target Delete Target				Add New Sample Add Saved Sample Save Sample Delete Sample		
Run Method	Target Name	Reporter	Quencher	Colour	11	Sample Name	Color	
🔦 Reaction Setup	HSV 1	FAM	Vone	× -	411	Sample 1		~
	HISV IC	JOE	~ None	v <mark>-</mark> v	411	Sample 2		~
	HSV 2	CY5	~ None	× <mark>-</mark> -	411	Sample 3		~
Mill Pup	MT	FAM	Vone	v		Sample 4		~
	MT IC	JOE	Vone	v		Sample 5		~
Analysis						Sample 6		~
1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -						Sample 7		~
						MT Positive Control		~
C.,								
	Define Biological Repl	licate Groups						
	Instructions: For each	biological replicate group in the reaction plate, click Ad	d Biological Group, then define	the biological group.				
	Add Biological Group	Delete Biological Group						
	Biological Group Name		Color			Comments		
	L				_		(
							Assign Targets and	J Samples
Home SUntitled ×					-			

Fig. 1.3 Define targets and samples

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5. Switch to the Assign Targets and Samples tab.

6. Assign the appropriate targets and samples (controls) for used wells by checking the boxes.

7. For Negative Controls set ${\bf N}$ in the ${\bf Task}$ column of pathogen target.

8. For calibrators (in the case of quantitative detection) set **S** in the **Task** column of pathogen target and enter the corresponding quantity in the **Quantity** column – according to the Package Insert of the used GeneProof PCR kit, e.g.: 10 000, 1 000, 100 a 10.

9. Select **ROX** passive reference (in the case of the Geneproof PCR kit with TEXAS RED detection, select **None**).

Experiment Menu «	Experiment: YYMMDD experiment		Type: Standard Curve	R	eagents: TaqMan	@ Reagents	START RUN ()
Setup	Define Targets and Samples Assign Targets and Sam	ples					
Experiment Properties	Instructions: To set up standards: Click "Define and Set Up Standards." To set up unknowns: Setect wells, assign target(s), setect "U" To set up negative controls: Setect wells, assign target(s), the	Unknown) as the task for each target select "N" (Negative Control) as the f	assignment, then assign a sample. lask for each target assignment.				
Plate Setup	Assign target(s) to the selected wells.	View Plate Layout	iew Well Table				
Run Method	Assign Target Task Quantity			Select Wells With: - Sele	ct item - 🤟 - Select iter	n- ~	
Reaction Setup	HSV 1 10 000	Show in Wells V	ew Legend				111 111 1111
Materials List		1 2	3 4	5 6	7	8 9	10 11 12
		Samole 1 Samole 2	Sample 3 Sample 4	Sample 5 Sample 6	Sample 7	H3V 1	ISV 1 RSV 1 RSV 1
Run			U HAV 2 U HAV 2 U HAV 2 U HAV 2	U HAVE U HAVE	U HSV 2	4 TE3 100	10
Analysis	Mixed 🗓 Unknown 🔂 Standard 💟 Negative Control	в					
	العربي Define and Set Up Standards						
	Assign sample(s) to the selected wells.	C MT IC MT IC	Sample 3 Sample 4	MT IC MT IC	MT IC	MT IC MT IC	
2.01	Assign Sample					MT LLL	
	Sample 1	0					
	Sample 2	0					
	Sample 3						
	· · · · ·	E					
	Assign sample(s) of selected well(s) to biological group.						
	Assign Biological Group	F					
		0					
	And and the state of the second						
	Select the dye to use as the passive reference.	н					

Fig. 1.4 Assign targets and samples



1.3.3 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as the **Experiment Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments folder**.

Save			×
Save <u>i</u> n	: Experime	nts 🗸 🔊 🖓 🔁 🖽 📟	
Recent Items			
Desktop			
Documents			
This PC			
S	File <u>n</u> ame:	YYMMDD experiment.eds	<u>O</u> pen
Network	Files of type:	Experiment Document Single files (*.eds)	Cancel Or

Fig. 1.5 Save experiment





1.4. Qualitative analysis of the result and evaluation of detection for microbiological detection

PCR detection result evaluation must be **always** performed qualitatively first; if you use the PCR kit for quantitative assessment, continue to quantify positive samples in the second step.

When the experiment is finished, Amplification Plot is displayed.



Fig. 1.6 Amplification curves



1.4.4 Analysis settings

- 1. Open Analysis Settings.
- 2. Uncheck Use Default Settings for all targets.
- 3. Uncheck Automatic Threshold and leave original value.
- 4. Uncheck Automatic Baseline and leave Start Cycle 3 and End Cycle 15.
- 5. Click Apply Analysis Settings to confirm.



Fig. 1.7 Analysis settings



1.4.5 Detection analysis of the studied microorganism

1. In Plot Settings, select Plot Type: ∆Rn vs Cycle and Color: Target.

2. In **Options**, select the target microorganism (e.g. CMV) in the **Target** combo box and move the Threshold line just above the reaction basal noise.

3. Click Reanalyse.



Fig. 1.8 Amplification curves of the studied microorganism



4. In Plot Settings, select Graph Type: Linear.

The curves display in a linear scale. The Ct values can be displayed in the View Well Table tab.



Fig. 1.9 Amplification curves of the studied microorganism in linear scale

In the case of multiplex kit, follow the instructions for all the studied microorganisms.

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.



1.4.6 Internal Standard detection analysis

1. In Plot Settings, select Graph Type: Log.

2. In **Options**, select the internal standard (e.g. CMV IC) in the **Target** combo box and move the Threshold line just above the reaction basal noise.

3. Click Reanalyse.



Fig. 1.10 Amplification curves of the internal standard



4. In Plot Settings, select Graph Type: Linear.

The curves display in a linear scale. The Ct values can be displayed in the View Well Table tab.



Fig. 1.11 Amplification curves of the internal standard in linear scale

Perform evaluation according to the Instruction for use of the used GeneProof PCR kit.



1.5. Result quantitative analysis and detection evaluation

1. In **Standard Curve**, evaluate the calibration quality. The R² parameter in a well-performed calibration achieves a minimum value of **0.98** or higher. If the R² parameter is lower than **0.98**, move the **Threshold** and repeat the analysis.



Fig. 1.12 Calibration curve



2. Switch to the **View Well Table** tab. Concentrations of positive samples are displayed in the **Quantity** column of the table.

œeriment Menu≪	Experiment: CMV ABI 7500	Type: Standard Curve		Rea	gents: Taq	Man® Rea	gents			Reanalyse:	Analysis Bettings	
Setup	Amplification Plot		View I	Plate Layout	View W	ell Table					-	
Run	Plot Settings		>			Select Well	s With: - Select iter	n- 4 -5	elect liem -			
MONTRANCO	Plot Type: ARn vs Cycle 💗 Graph Type: Linear 💗 Color: Target		(management	7.000 = 0.000								
Analysis			anow in	Table V Gro	ND R01 A					12	VE TRANSPORTATION AND AND AND AND AND AND AND AND AND AN	(count
	Save current settings as the default		# Name	Task	Dves	CT	Cr Mean Cr S	0 0	Juantity	Quantity M	Quantity 9D HIGH90	0 1
numention Pot		P P B 10 12 12	7	UNKNOWN	JOE-None	30,946	30,849	0,178				
ALMAN SALAR	22 - 25 S C - 2		8	UNKNOWN	FAM-None	30,287	30,261	0,065	30,001	36,713	1,682	
dard Curve	Amplification P	Yot	10	UNKNOWN	JOE None	30.643	30,849	0.178	10.001	36 743	4 682	
Constant of the			11	UNKNOWN	JOE-None	30,958	30,849	0,178				
acomponent Plot	0.60		12	UNKNOWN	FAM-None	30,187	30,261	0.065	38,617	36,713	1,682	
Date Dist.	0.65	1000	13	UNKNOWN	JOE-None	30,624	30,723	0,11	0.046	0.000	0.035	
trata Past		-++++	16	UNKNOWN	JOE-None	30,705	30,723	0.11	s'san	2,309	0,032	
Summary	0.50		16	UNKNOWN	FAM-None	34,470	34,455	0,021	2,286	2,309	0,032	
uninitian y	0.40		17	UNKNOWN	JOE-None	30,842	30,723	0,11				
ple Plots View			18	UNKNOWN	FAM None	34,465	34,455	0.021	2.294	2.309	0,032	
	0.0		20	UNKNOWN	FAM-None	35,349	34,935	0.42	1.29	1.726	0.476	
	0.35		21	UNKNOWN	JOE None	30,020	30,976	0.072				
	2 am		22	UNKNOWN	FAM-None	34,946	34,935	0,42	1,67	1,726	0,476	
	3		23	UNKNOWN	JOE-None	20,950	30,976	0,072	0.000	4.706	0.176	
	0.10		25	UNKNOWN	JOE-None	30.907	30.922	0.034	6,660	1,150	0,475	
	0.30		26	UNKNOWN	FAM-None	23,267	33,125	0,243	5,052	5,598	0,922	
			27	UNKNOWN	JOE-None	30,898	30,922	0.034				
	0.15		28	UNKNOWN	FAM-None	33,263	33,125	0.243	5,067	5,598	0.933	
	0.10		30	UNKNOWN	FAM-None	32.845	33,125	0.243	0.070	5.598	0.933	
			31	UNKNOWN	JOE-None	31.078	30.851	0.219				
	0.05		32	NTC	FAM-None	Undetermi						
	0.00	and the second s	33	UNKNOWN	JOE-None	30,642	30.851	0,219				
	.008		35	UNKNOWN	JOE-None	30.033	30.651	0.219				
	2 4 8 8 10 12 10 10 10 21 22	3 3 3 3 3 3 3 3 3 3 3 6 6 6	30	NTC	FAM-None	Undetermi						
	Cy	rcle	37	UNKNOWN	JOE-None	29,378	30.238	0.839				
			30	STANDARD	FAM-None	21,840	21,036	0,004	10 000			
			40	STANDARD	FAM-None	21.835	21.835	0.004	10 000			
	"monimumer.		41	UNKNOWN	JOE-None	31.054	30,230	0.039				
	Options)		42	STANDARD	FAM-None	21,832	21,836	0,004	10 000			
	Target CMV. V Threshold: Auto 000744	eline	43	UNKNOWN	JOE-None	30,491	30,539	0,042	1.000			
			45	UNKNOWN	JOE-None	20,199	20,100	0.042	1000			
	Show: 🗹 Threshold — 🗌 Baseline Start: Well 🛢 Target 🚲 Baseline End: W	/ell 📕 Target 🚣	48	BTANDARD	FAM-None	25,063	26,166	0.092	1 000			
			47	UNKNOWN	JOE-None	30,566	30,539	0,042				
	Save current settings as the default		48	STANDARD	FAM-None	25,238	25,166	0,092	1 000			
			the second se	a store that have been		and the local division of the local division	30 743	0.087				

Fig. 1.13 Results table

Perform evaluation, including the virus concentration calculation in copies/ml (cp/ml) according to the Instruction for use of the used GeneProof PCR kit.



2. Genetic diagnostics

This chapter describes in detail the process of using GeneProof PCR kits for genetic diagnostics using the instruments 7500 Real-Time PCR Systém a 7500 Fast Real-Time PCR System.

2.1. Device Programming

In case the software does not include predefined templates, it is necessary, before the first use with GeneProof PCR kits, to programme them according to the Instruction for use of the used GeneProof kits, or download them from the product site of the used GeneProof PCR kits from the website of the company www.geneproof.com.

Save the downloaded templates on your local disc and open them in the software 7500 Software.exe.

7500 Software v2.3	Table Hale				-	U X
File Edit Instrument Analysis	Tools Help	0.000				
🔛 New Experiment + 🔄 Open	. 🛃 Save 🕶 Close	And Export				
Experiment Menu «	Save As	dace HIV-ABI7500	Type: Standard Curve Re	agents: TaqMan® Reagents	START RUN 🅪	
100	Save As Template	Ctrl+T				-
Setup	Experiment Prope	rties				
Experiment Properties	Enter an experim	ent name, select the instrument type, select the type of experiment to set up, th	en select materials and methods for the PCR reactions and instrument run.			
Plate Setup	How do you want	to identify this experiment?				
	* Experiment Name:	150610 validace HIV-ABI7500				
	Barcode (Optional):					
Reaction Setup	User Name (Optional	I): Administrator				
Matariala List	Comments (Optional)): SDS v1.2				^
						~
Run	*Which instrumen	nt are you using to run the experiment?				
		it are you asing to run are experiment.				
Analysis		√ 7500 (96 Wells)	7500 Fast (96 Wells)			
	Set up, run, and anal	lyze an experiment using a 4- or 5-color, 96-well system.				
	•What type of exp	periment do you want to set un?				
	initiat type of exp	comment do you want to set up.				
		√ Quantitation - Standard Curve	Quantitation - Relative Standard Curve	Quantitation - Comparative Cτ (ΔΔ	LCT)	
		Melt Curve	Genotyping	Presence/Absence		
	Use standards to de	termine the absolute quantity of target nucleic acid sequence in samples.				
	*Which reagents	do you want to use to detect the target sequence?				
		√ TaqMan® Reagents	SYBR® Green Reagents	Other		
	The PCR reactions of	contain primers designed to amplify the target sequence and a TaqMan® prob	e designed to detect amplification of the target sequence.			
	• Which ramp spec	ed do you want to use in the instrument run?				
		√ Standard (~ 2 hours to complete a run)	Fast (~ 40 minutes to complete a run)			
	For optimal results w	with the standard ramp speed, Applied Biosystems recommends using standa	ard reagents for your PCR reactions.			
*	L					
Home Untitled × 150	610 validace HIV-ABI7500	×				
					Disconnected	

Fig. 2.1 Save template

After saving, the template can be opened from the file **Templates**. With each next usage of GeneProof PCR kits continue from the chapter **1.3 Starting the PCR amplification**.



2.2. Starting the software

- 2.2.1 Opening of the saved template
- 1. Start the 7500 Software.
- 2. In the Set Up column, select Template.
- 3. Open template for the given GeneProof PCR kit.

File Edit Instrument Analysis Tools Help		- U X
😰 New Experiment 🔹 🎯 Open 📓 Save 👻 📸 Close 🛛 🐗 Export 👻 📥 Print Report		
New Experiment • • Open. • We ve • Clear • Least. • Print Report.		Analyse Analyse Experiment
Save current display as the default	7500 & 7500 Fast	Applied Biosystems Home Real-Time PCR Decision Tree

Fig. 2.2 Open template

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Disconnected



1. In Experiment Properties, enter experiment name into the Experiment Name row.

2. In Plate Setup, use Add New Target to define targets according to the kits used in the experiment.

E.g. for FII detection (3 channels) set Target Name: **FII WT**, Reporter: **FAM**, Quencher: **None**; Target Name: **FII MUT IC**, Reporter: **JOE**, Quencher: **None**. Use **Save Target** and **Add Saved Target** to save and reuse targets.

- 3. Use Add New Sample to define samples.
- 4. Define positive controls as a sample, e.g. FII WT, FII MUT and FII HET.

7500 Software v2.3 le Edit Instrument Analysis	Tools Help						-	ø ×
] New Experiment 👻 🎯 Open	. 🛃 Save 🕶 🖆 Close 🛷 Export 🔹	Print Report						
Experiment Menu «	Experiment: jjj		Type: Sta	ndard Curve		Reagents: TaqMan® Reagents	START RUN (\$	2
Jetup	Define Targets and Sample	Assign Targets and Sample:	s					
Experiment Properties	Instructions: Define the targets to	quantify and the samples to test in the reaction	on plate.			Dofine Campler		_
Plate Setup	Denne rargets							
Run Method	Add New Target Add Saved Targe	et Save larget Delete larget	1			Add New Sample Add Saved Sample Save Sample Delete Sample	1	
	Target Name FILWT	Reporter	Quencher Vone	Colour		Sample Name Sample 1	Color	~ ^
Keaction Setup	FILMUT	JOE	~ None	~ <mark>-</mark> ~	-	Sample 2		~
Materials List						Sample 3		~
Run						Sample 5		~
Analysis						FII WT		~
						FII MUT		~
						FILHET		× •
	Define Biological Replicate Gr	oups						
	Add Biological Group Delete Biol	replicate group in the reaction plate, click Add	I Biological Group, then define the b	iological group.				
	Biological Group Name	ogion orotp	Color			Comments		
							Assign Targets a	and Samples
*					_			

Fig. 2.3 Define targets and samples



- 4. Switch to the Assign Targets and Samples tab.
- 5. Assign the appropriate targets, samples and controls for used PCR wells by checking the boxes.
- 6. Select **ROX** passive reference.

 7500 Software v2.3 File Edit Instrument Analysis New Experiment - Content 	Tools Help 			-
Experiment Menu «	Experiment: jjj	Type: Standard Curve	Reagents: TaqMan® Reagents	START RUN (>
Setup	Define Targets and Samples Assign Targets and Sample To set up standards: Click "Define and Set Up Standards."	es		
Plate Setup	To set up negative controls: Select wells, assign target(s), then selected wells.	lect "N" (Negative Control) as the task for each target assignment.		
Run Method	Assign Target Task Quantity	Show in Wells View Legend	Select Wells With: - Select Item - 😒 - Select Item - 💌	
Materials List		1 2 3 4 Sample 1 Sample 2 Sample 3 Sample 4 A II FH MUT II FH MUT II FH MUT	5 6 7 8 Sample 5 FILWT FILMUT FILHET N U FILMUT U FILMUT U FILMUT IFILMUT N	9 10 11 12
Run Analysis	Mixed 11 Unknown S Standard N Negative Control			
	Assign sample(s) to the selected wells.	c		
		D		
	FILHET FILHET Assign sample(s) of selected well(s) to biological group. Elements	e la		
	Assign Biological Group	F		
		0		
	Select the dye to use as the passive reference.	н		
*		Wells: 🔟 8 Unknown <mark>S</mark> 0 Standard 🔝 1 Negative Control		87 Empty

Fig. 2.4 Assign targets and samples



2.2.2 Starting the experiment

Save the experiment before starting the device.

1. Select Save in the main menu and save the created experiment as the **Experiment Document Single files (*.eds)** file type. To make search easier it is recommended to create the **Experiments** folder.

Save					×
Save <u>i</u> n	n: 📙 Experime	nts	\sim	00000	
Recent Items					
Desktop					
Documents					
This PC					
*	File <u>n</u> ame:	YYMMDD experiment.eds			Open
Network	Files of type:	Experiment Document Single files (*.eds)		~	Cancel O

Fig. 2.5 Save experiment





2.3. Analysis of the result and evaluation of detection for genetic detection

For correct parameter setting and evaluation when using multiple MasterMixes in one experiment, for example FII and FV, it is necessary to evaluate the results in groups (separately FII, separately FV...).

- 1. After the protocol is done, swich to the **Analysis** tab and select the **Amplification Plot** tab.
- 2. Plot settings pane set the Linear.



Fig. 2.6 Linear display setting



2.3.3 Setting the Baseline and Treshold parameters

- 1. In **Plot type,** select **ΔRn vs Cycle**.
- 2. In the table next to the graph, mark the samples required for evaluation.
- 3. Click to **Analysis Settings** to select all channels one by one and uncheck **Use Default Settings**. After that uncheck **Automatic Threshold** and **Automatic Baseline**.
- 4. Set **Start Cycle** and **End Cycle** values to cover as much of the curves as possible before the exponential phase and press the **Apply Analysis Settings** button. Incorrect baseline settings will result in lowering curves.



Right



Wrong Fig. 2.7 Right and wrong Baseline Settings

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- 5. In the table below the graph mark only the positive WT, MUT and HET controls of the given examination.
- 6. In the Threshold field, enter any value from the ΔRn axis and move the Threshold line above the non-specific background so that only the FAM amplification curve (eg FII WT) crosses the Threshold line in the case of a positive WT control, only the HEX amplification curve (FII MUT). In the case of a positive HET control, FAM and HEX amplification curves cross the Treshold line.
- 7. Push the button **Reanalyze**.

Implification Plot	
Plot Settings	
Plot Type: ARn vs Cycle V Graph Type: Linear V Color: Well V	
Save current settings as the default	
9 9 A B	× =
Amplification Plot	
0,350	
0.325	
0.300	
0.275	
0.250	
0.225	
0.200	
0.175	
0.180	
0,125	
0,100	
0,075	
0,050	
0,025	
0,000	
2 4 6 8 10 12 14 16 18 20 22 24 28 28 30 22 34 36 Cycle	38
ини – А 📙 В 🚾 С 🖬 D 🗰 Е 🗰 F 📕 G 🗰 Н	
Dptions	
arget HEX V Threshold: Auto 0030227	
now: 🗹 Threshold — 🗌 Baseline Start: Well 🛢 Target 🔺 Baseline End: Well 🛢 Target 🔺	
] Save current settings as the default	

Fig. 2.8 Treshold settings



2.3.4 Evaluation



1. In the table next to the graph, mark the samples required for evaluation.

Fig. 2.9 Evaluated curves after setting Baseline and Threshold

- 2. Switch to View Well Table.
- 3. If the sample in the **Ct** column has a numerical value for the **FAM** detector (**e.g. FII WT**) it is a **standard genotype**. A numerical value for the **HEX** detector (FII MUT)- it is a **mutant genotype** and numerical values **for both detectors** means **heterozygous genotype**.



View Plate Layout View Well Table												
Select Wells With: - Select Item - 🗸 - Select Item - 🗸												
Chauria Table T Craus Du T												
Expand All RC Conapse All												
#	Wall	Omit	Floo	Comple Nome	Torget Nome	Took	Dues	07	Crillian	07.00	Quanti	
#	vven		Flag	Sample Name		LINICHOWN	LOE None	06.040	CT Mean	0.020	Quanti	
10	AD		1	hDNA2	FILMUT	UNKNOWN	JOE-None	20,842	20,825	0,038		$^{\circ}$
10	AO	님	1	HDNA2		UNKNOWN	PAM-None	0000000	06.005	0.020		
12	A0		1	NDINA2	FILMOT	UNKNOWN	JOE-None	20,852	20,825	0,038		
13	A7	님		IDINA3		UNKNOWN	FAM-None	25,208	25,397	0,381		
14	A			NDINA3	FILMOT	UNKNOWN	JOE-None	28,878	29,091	0,302		
15	A8			NDINA3	FILVE	UNKNOWN	FAM-None	25,149	25,397	0,381		
10	A8	님		IDINA3	FILMOT	UNKNOWN	JOE-None	28,880	29,091	0,362		
17	A9			NDINA3	FILVE	UNKNOWN	FAM-None	25,835	25,397	0,381		
18	Ag	님		DUNA3	FILMUT	UNKNOWN	JUE-None	29 509	29,091	0,362		
19	A10			PKWI		UNKNOWN	FAM-None	23,250	I W1	0,04		
20	All	님		PKWL	FILMOT	UNKNOWN	JOE-None	Undetermi	02.040			
21	A11			PKWt	FILWI	UNKNOWN	FAM-None	23,178	23,212	0,04		
22	A11	님		PKWt	FILMUT	UNKNOWN	JOE-None	Undetermi	00.040			
23	A12	님		PKwt	FILWI	UNKNOWN	FAM-None	23,202	23,212	0,04		
24	A12	님		PKwt	FILMUT	UNKNOWN	.IOE-None	Undetermi				
25	B1		- 4	PKmu	FILWT	UNKNOWN	FAM-None	Undetermi	I MI	IT		
26	B1		- 4	PKmu	FILMUT	UNKNOWN	JOE-None	26,555	25,525	0,068		
27	B2		- <u>4</u> -	РКМИ	FILWI	UNKNOWN	FAM-None	Undetermi	- -			
28	B2		<u> </u>	PKmu	FILMUT	UNKNOWN	JOE-None	26,581	26,529	0,068		
29	B3		<u> </u>	PKmu	FILWT	UNKNOWN	FAM-None	Undetermi				
30	B3		- 1 -	PKmu	FILMUT	UNKNOWN	IOE-None	26.452	26,529	0,068		
31	B4			PKhet	FILWT	UNKNOWN	FAM-None	23,447	23 364	0,075		
32	B4		_ L	PKhet	FILMUT	UNKNOWN	JOE-None	26,501	26,508	0,011		
33	B5			PKhet	FILWT	UNKNOWN	FAM-None	23,347	23,364	0,075		
34	B5			PKhet	FILMUT	UNKNOWN	JOE-None	26,521	26,508	0,011		
35	B6			PKhet	FILWT	UNKNOWN	FAM-None	23,299	23,364	0,075		
36	B6			PKhet	FII MUT	UNKNOWN	JOE-None	26,503	26,508	0,011		
37	B7			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,535	29,62	0,116		
38	B7			hDNA3 2ng/ul	FII MUT	UNKNOWN	JOE-None	33,229	33,065	0,152		
39	B8			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,752	29,62	0,116		
40	B8			hDNA3 2ng/ul	FILMUT	UNKNOWN	JOE-None	32,928	33,065	0,152		
41	B9			hDNA3 2ng/ul	FII WT	UNKNOWN	FAM-None	29,573	29,62	0,116		
42	B9			hDNA3 2ng/ul	FII MUT	UNKNOWN	JOE-None	33,040	33,065	0,152		
43	B10		1	NFW	FII WT	UNKNOWN	FAM-None	Undetermi				
44	B10		1	NFW	FII MUT	UNKNOWN	JOE-None	Undetermi				
45	B11											~
	<										>	\square

Fig. 2.10 Evaluation



2.3.5 Examples of typical curves



Fig. 2.11 Typical curves

ThermoFisher ABI 7500/7500Fast

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