For Professional Use Only

Sacace Molecular Genetics

Handbook

Real Time PCR kits for detection of Single Nucleotide Polymorphisms (SNPs)

REF **see below kits table**

60

96

NAME

Sacace Molecular Genetics

INTRODUCTION

A single nucleotide polymorphism (SNP pronounced "snip") is a DNA polymorphisms at the level of a single nucleotide, a single base mutation in DNA. SNPs are ‘conserved’ across the genome and represent the most simple form and most common source of genetic polymorphism in the human genome: 90% of all human DNA polymorphisms are associated with SNPs and variation frequency is about 1 every 1000bp in the human genome (Sachidanandam et al.,2001).

The SNPs in the genome can affect gene functions, protein structure or expression and for these reasons they are used as markers in genetic disease studies (Kim & Mishra, 2007).

It’s sometimes possible to correlate a SNP with a particular trait or disease: susceptibility to disease may also be described as an ‘unfortunate trait’ that can be assessed checking if the mutated (unfortunate) polymorphism is carried in both alleles.

SNPs testing can be applied to:

- Diagnostics / risk profiling
- Drug response prediction
- Gene function identification

Several SNPs have been associated to genetic susceptibility to different diseases and disorders like for example:

- Hypertension
- Fibrinolysis
- Myocardial infarction
- Ischemic stroke
- Cancer
- Metabolic disorders

In order to perform SNP genotyping, two specific probes labeled with different dyes are used, the first for the “wild type” allele and the second for the “mutant” allele. If the assay results in the emission of only the first fluorescent color, then the individual is homozygous “wild type” at that locus. If the assay results in the emission of only the second fluorescent color, then the individual is homozygous “mutant” at that locus. And finally, if both fluorescent colors are produced, then the individual is heterozygous for that locus.

INTENDED USE

Sacace Molecular Genetics Kits are intended for detection and allelic discrimination of genetic polymorphisms associated with inherited susceptibility to increased risk of disease, or to different response to drugs (see KITS TABLE for details).

PRINCIPLE OF ASSAY

Sacace Molecular Genetics Kits are qualitative tests that allow the detection by Real Time PCR based on the amplification of the genome specific region using specific primers associated to specific alleles. In Real Time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product. The real-time monitoring of the fluorescence intensities during the reaction allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run.

MATERIALS PROVIDED

Option No.1: Ready to use 0,2 ml tube format (TXXXXX-50-T)

- **60 ready to use 0,2 ml PCR tubes** (each PCR tube contains 15 µl of PCR mix)
- **Taq polymerase,** 0,3 ml (1 vial)
- **Negative control C-**, 0,1 mL (1 vial)
- **C+ Homozygous Wild Type (allele 1-1)**, 50 µL (1 vial)
- **C+ Heterozygous (allele 1-2)**, 50 µL (1 vial)
- **C+ Homozygous Mutant (allele 2-2)**, 50 µL (1 vial)

Contains reagents for 60 tests.

Option No.2: Ready to use 12x8 strip format (TXXXXX-96-S)

- **12 x 8 strip ready to use** (each PCR tube contains 15 µl of PCR mix)
- **Taq polymerase,** 0,5 ml (1 vial)
- **Negative control C-**, 0,1 mL (1 vial)
- **C+ Homozygous Wild Type (allele 1-1)**, 50 µL (1 vial)
- **C+ Heterozygous (allele 1-2)**, 50 µL (1 vial)
- **C+ Homozygous Mutant (allele 2-2)**, 50 µL (1 vial)

Contains reagents for 96 tests.

KITS TABLE

Code	Gene	Polymorphism details	Fluorescence Channel / Allele
T01273	**MTHFR**	Glu429Ala 1298 A>C Rs1801131	HEX: Glu (A) – allele 1
			FAM: Ala (C) – allele 2
T01105	**F7**	Arg 353 Gln CGG 353 CAG rs6046	HEX: Arg (G) – allele 1
			FAM: Gln (A) – allele 2
T01106	**ITGB3**	Leu 33 Pro CTG 33 CCG rs5918	HEX: Leu (T) – allele 1
			FAM: Pro (C) – allele 2
T01107	**FGB**	G-455A rs1800790	HEX: G – allele 1
			FAM: A – allele 2
T01120	**PAI**	-675 5G/4G rs1799768	HEX: 5G – allele 1
			FAM: 4G – allele 2
T01124	**MTRR**	Ile 22 Met ATA 22 ATG A 66 G rs1801394	HEX: Ile (A) – allele 1
			FAM: Met (G) – allele 2
T01143	**MTR**	Asp 919 Gly GAC 919 GGC rs1805087	HEX: Asp (A) – allele 1
			FAM: Gly (G) – allele 2
T01155	**ITGA2**	Phe 224 Phe TTC 224 TTT C807T rs1126643	HEX: C – allele 1
			FAM: T – allele 2
T01179	**GPIBA**	Thr 145 Met C482T ACG 145 ATG rs6065	HEX: Thr (C) – allele 1
			FAM: Met (T) – allele 2
T01354	**GPIBA**	T -5 C rs2243093	HEX: T – allele 1
			FAM: C – allele 2
T01355	**F13A1**	Val35Leu GTG 35 TTG rs5985	HEX: Val (G) – allele 1
			FAM: Leu (T) – allele 2
T01356	**F12**	C -4 T rs1801020	HEX: C – allele 1
			FAM: T – allele 2
T01329	**FTO ***	A 23525 T rs9939609	HEX: A – allele 1
			FAM: T - allele 2
T01331	**CYP3A5 ***	G 6986 A rs776746	HEX: G – allele 1
			FAM: A – allele 2
T01182	**NOS3 ***	C786T	HEX: C – allele 1
			FAM: T – allele 2

Code	Gene	Polymorphism details	Fluorescence Channel / allele
T01357	**SELPLG**	Met 62 Ile ATG 62 ATA rs2228315	HEX: Met (G) – allele 1 FAM: Ile (A) – allele 2
T01104	**CYP2C9**	Arg 144 Cys CGT 144 TGT rs1799853	HEX: Arg (C) – allele 1 FAM: Cys (T) – allele 2
T01111	**CYP2C9**	Ile 359 Leu ATT 359 CTT rs1057910	HEX: Ile (A) – allele 1 FAM: Leu (C) – allele 2
T01144	**VKORC1**	C1173T rs9934438	HEX: C – allele 1 FAM: T – allele 2
T01145	**VKORC1**	G3730A rs7294	HEX: G – allele 1 FAM: A – allele 2
T01335	**PPARG2**	Pro12Ala CCA 12 GCA rs1801282	HEX: C – allele 1 FAM: G – allele 2
T01358	**ADRB2**	Gln27Glu rs1042714	HEX: C – allele 1 FAM: G – allele 2
T01359	**ADRB2**	Arg16Gly rs1042713	HEX: T – allele 1 FAM: C – allele 2
T01360	**ADRB3**	Trp64Arg rs4994	HEX: A – allele 1 FAM: G – allele 2
T01361	**FABP2**	Ala54Thr rs1799883	HEX: G – allele 1 FAM: A – allele 2
T01349	**IL28B**	rs8099917 T>G	HEX: T – allele 1 FAM: G – allele 2
T01371	**IL28B**	rs12979860 C>T	HEX: C – allele 1 FAM: T – allele 2
T01303	**SLCO1B1**	Val 174 Ala GTG 521 GCG rs 4149056	HEX: Val(T) – allele 1 FAM: Ala (C) – allele 2

Code	Gene	Polymorphism details	Fluorescence Channel / allele
T01118	**AGT (1)**	Thr174Met rs4762	HEX: Thr(C) – allele 1 FAM: Met(T) – allele 2
T01119	**AGT (2)**	Met235Thr Rs699	HEX: Met(T) – allele 1 FAM: Thr(C) – allele 2
T01131	**AGTR1**	A1166C Rs51186	HEX: A – allele 1 FAM: C – allele 2
T01148	**ApoE**	Leu28Pro rs769452	HEX: Leu(T) – allele 1 FAM: Pro(C) – allele 2
T01149	**LPL**	Ser474Ter Rs328	HEX: Ser(C) – allele 1 FAM: Ter(G) – allele 2
T01323	**CYP2C19(*2)**	G681A rs4244285	HEX: G – allele 1 FAM: A – allele 2
T01324	**CYP2C19(*3)**	Trp212Ter TGG 212 TGA rs4986893	HEX: Trp(G) – allele 1 FAM: Ter(A) – allele 2
T01171	**IL17A**	IL17A G-197A	HEX: G – allele 1 FAM: A – allele 2
T01352	**COMT**	Val158Met rs4680	HEX: Val (G) – allele 1 FAM: Met (A) – allele 2
T01272	**ACE**	Alu Ins/Del rs4646994	HEX: Ins – allele 1 FAM: Del – allele 2
T01177	**TNF**	G-308A rs1800629	HEX: G – allele 1 FAM: A – allele 2
S01191	**HFE**	His63Asp rs1799945	HEX: C – allele 1 FAM: G – allele 2
S01192	**HFE**	Ser65Cys rs1800730	HEX: A – allele 1 FAM: T – allele 2
S01193	**HFE**	Cys282Tyr rs1800562	HEX: G – allele 1 FAM: A – allele 2

** in those kits Allele 1 on HEX identifies the "mutant" genotype and Allele 2 on FAM identifies the "wild type" genotype: always refer to the allele base name in the results (for example CC or CG or GG); refer to the reference rs number (for example* rs12979860) *as indicated in the "kits table" for details about the polymorphism.*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation

- DNA extraction kit
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- Dry heat block
- Vortex mixer
- Pipettes
- Sterile pipette tips with filters
- 1,5 ml polypropylene sterile tubes
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: Real Time amplification

- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Freezer, refrigerator
- Tube racks

STORAGE INSTRUCTIONS

Sacace Molecular Genetics kits must be stored at 2-8°C. The kits can be shipped at 2-8°C and stored as indicated immediately on receipt.

STABILITY

Sacace Molecular Genetics kits are stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

RUO For Research Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

PRODUCT USE LIMITATIONS

Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Sacace Molecular Genetics Kits can analyze genomic DNA extracted from:

- *whole blood* collected in EDTA tubes;
- *Buccal swab*: insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

Specimens can be stored at +2-8°C for no longer than 24 hours, or freeze at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kits are recommended:

⇒ **Genomic column DNA Express** – spin column extraction kit (Sacace, REF K-1-1/E)

⇒ **SaMag Blood DNA extraction kit** (Sacace, REF SM001);

⇒ **QIAamp DNA Blood mini kit** (Qiagen, REF 51104);

⇒ **DNA-Sorb-A** (Sacace, REF K-1-1/A) for buccal swab;

Please carry out DNA extraction according to the manufacturer's instruction.

PROTOCOL

Sacace Molecular Genetics kits do not include reagents required for sample preparation and DNA extraction. Blood samples and biological materials must be processed by using the recommended kits or those with similar performances of quality and quantity of extracted DNA. Use of blood samples collected in tubes containing heparin is not recommended.

The analysis of the genomic DNA specimens using **Sacace Molecular Genetics** kits includes the following stages:

1. Preparing the Real Time PCR;
2. Real Time PCR analysis;
3. Data analysis with the software of Real Time PCR instrument;
4. Results analysis and conclusions.

EXPERIMENTAL PROTOCOL

Total reaction volume: 25 µl

1. Prepare the necessary number of ready-to-use PCR tubes (samples + 3 pos controls + 1 neg control).
2. Spin for 3-5 sec the **Taq polymerase**, mix by pipetting and **add 5 µl** to each PCR tube.
3. Add into the corresponding PCR tubes **5.0 µl** of extracted DNA from sample:
   - **DNA** sample

   Add into the corresponding PCR tubes **5.0 µl** of controls:
   - **C+ Homozygous (allele 1-1)**
   - **C+ Heterozygous (allele 1-2)**
   - **C+ Homozygous (allele 2-2)**
   - **Negative Control C-**
4. Spin the tubes for 3–5 seconds to collect the drops.
5. Insert the tubes in the Real-time PCR instrument.

Amplification

Create a temperature profile on your instrument as follows:

<table>
<tr><th rowspan="2">Step</th><th colspan="3">Plate or modular type instruments[1]</th><th colspan="3">Rotor type instruments[2]</th></tr>
<tr><th>Temperature, °C</th><th>Time</th><th>Cycles</th><th>Temperature, °C</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Hold</td><td>80</td><td>2 min</td><td>1</td><td>80</td><td>2 min</td><td>1</td></tr>
<tr><td>Hold</td><td>94</td><td>3 min</td><td>1</td><td>95</td><td>3 min</td><td>1</td></tr>
<tr><td rowspan="2">Cycling</td><td>94</td><td>15 s</td><td rowspan="2">5</td><td>95</td><td>10 s</td><td rowspan="2">40</td></tr>
<tr><td>64</td><td>40 s</td><td>60</td><td>40 s
fluorescence detection</td></tr>
<tr><td rowspan="2">Cycling 2</td><td>94</td><td>15 s</td><td rowspan="2">35</td><td colspan="3" rowspan="2"></td></tr>
<tr><td>64</td><td>40 s
fluorescence detection</td></tr>
</table>

[1] For example, SaCycler-96™ (Sacace); CFX-96 / iQ5™ (BioRad); Mx3005P™ (Agilent); ABI® 7500 Real Time PCR (Applied); LightCycler® 96 (Roche).*

[2] For example Rotor-Gene™ 6000/Q (Corbett Research, Qiagen); when using Rotor Gene instrument with strips the tube caps may be marked and it's recommended to cut the strip into two equal parts (4 tubes each)

** To perform the test with ABI 7500 (Applied) a disposable adapter provided with the kit has to be used. Additional adapters can be purchased separately.*

Fluorescence is detected in FAM/Green, JOE/Yellow/HEX fluorescence channels.

DATA ANALYSIS

The fluorescent signal intensity is detected in 2 channels as shown in the table below:

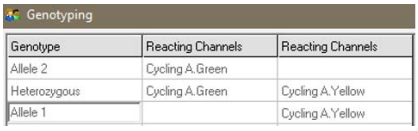
FAM	HEX
Allele 2 *("mutant"*)*	**Allele 1** *("wild type"*)*

Note: Please refer to the "Kits Table" at the beginning of this manual to check the details of the nucleotides substitution for each polymorphism.

** in some kits Allele 1 on HEX identifies the "mutant" genotype and Allele 2 on FAM identifies the "wild type" genotype: always refer to the "kits table" and the rs polymorphism code in the kits table at the beginning of the manual for details about the polymorphism.*

Interpretation of results for Rotorgene 6000/Q (Corbett Research, Qiagen):

Principle of interpretation:



- **Signal only in allele 1 (Yellow) : homozygous "***wild type"*
- **Signal only in allele 2 (Green) : homozygous "***mutant"*
- **Signal in both allele 1 and allele 2 : heterozygous**

Click **Analysis**, click **Other**, select **Allelic Discrimination**, select **Slope Correct**, click **Eliminate cycles before / Ignore first** and insert value. Insert the **Threshold** and **Outlier removal** values as in the following table:

Code	Gene	Polymorphism	Channel / allele	Threshold	Slope Correct	Outlier Removal	Ignore first
T01105	*F7*	Arg 353 Gln CGG 353 CAG rs6046	Yellow: Arg (G) Green: Gln (A)	0,03	On	10%	10
T01358	*ADRB2*	Gln27Glu rs1042714	Yellow: C Green: G	0,03	On	15%	5
T01359	*ADRB2*	Arg16Gly rs1042713	Yellow: T Green: C	0,03	On	15%	5
T01360	*ADRB3*	Trp64Arg rs4994	Yellow: A Green: G	0,03	On	15%	5
T01349	*IL28B*	rs8099917 T>G	Yellow: T Green: G	0,03	On	15%	5
T01371	*IL28B*	rs12979860 C>T	Yellow: C Green: T	0,03	On	0%	0
T01106	*ITGB3*	Leu 33 Pro CTG 33 CCG rs5918	Yellow: Leu (T) Green: Pro (C)	0,1	On	5%	5
T01107	*FGB*	G-455A rs1800790	Yellow: G Green: A	0,03	On	10%	10
T01120	*PAI*	-675 5G/4G rs1799768	Yellow: 5G Green: 4G	0,15	On	10%	5

Code	Gene	Polymorphism	Channel/allele	Threshold	Slope Correct	Outlier Removal	Ignore first
T01143	*MTR*	Asp 919 Gly GAC 919 GGC rs1805087	Yellow: Asp(A) Green: Gly (G)	0,03	On	15%	5
T01124	*MTRR*	Ile 22 Met ATA 22 ATG A 66 G rs1801394	Yellow: Ile (A) Green: Met(G)	0,03	On	15%	5
T01155	*ITGA2*	Phe 224 Phe TTC 224 TTT C807T rs1126643	Yellow: C Green: T	0,03	On	10%	10
T01179	*GPIBA*	Thr 145 Met ACG 145 ATG rs6065	Yellow: Thr (C) Green: Met (T)	0,03	On	15%	5
T01354	*GPIBA*	T -5 C rs2243093	Yellow: Thr (C) Green: Met (T)	0,03	On	15%	5
T01273	*MTHFR*	Glu429Ala 1298 A>C	Yellow: Glu (A) Green: Ala (C)	0,03	On	15%	5
T01355	*F13A1*	Val35Leu GTG 35 TTG rs5985	Yellow: Val (G) Green: Leu (T)	0,03	On	10%	10
T01356	*F12*	C -4 T rs1801020	Yellow: C Green: T	0,03	On	15%	5
T01329	*FTO*	A 23525 T rs9939609	Yellow: A Green: T	0,03	On	10%	5
T01331	*CYP3A5*	G 6986 A rs776746	Yellow: G Green: A	0,015	On	0%	5
T01104	*CYP2C9*	Arg 144 Cys CGT 144 TGT rs1799853	Yellow: Arg(C) Green: Cys(T)	0,03	On	20%	5
T01111	*CYP2C9*	Ile 359 Leu ATT 359 CTT rs1057910	Yellow: Ile(A) Green: Leu(C)	0,03	On	20%	15
T01144	*VKORC1*	C1173T rs9934438	Yellow: C Green: T	0,03	On	0%	0
T01145	*VKORC1*	G3730A rs7294	Yellow: G Green: A	0,03	On	10%	0
T01335	*PPARG2*	Pro12Ala CCA 12 GCA rs1801282	Yellow: C Green: G	0,03	On	15%	5
T01361	*FABP2*	Ala54Thr rs1799883	Yellow: G Green: A	0,03	On	15%	5
T01303	*SLCO1B1*	Val 174 Ala GTG 521 GCG rs 4149056	Yellow: Val(T) Green: Ala(C)	0,03	On	10%	5
T01182	*NOS3*	C786T	Yellow: C Green: T	0,05	On	10%	5
T01118	*AGT (1)*	Thr174Met rs4762	Yellow: Thr(C) Green: Met(T)	0,03	On	10%	5
T01119	*AGT (2)*	Met235Thr Rs699	Yellow: Met(T) Green: Thr(C)	0,03	On	10%	5
T01131	*AGTR1*	A1166C Rs51186	Yellow: A Green: C	0,03	On	10%	5
T01148	*ApoE*	Leu28Pro	Yellow: Leu(T) Green: Pro(C)	0,03	On	10%	5
T01149	*LPL*	Ser447Ter	Yellow: Ser(C) Green: Ter(G)	0,03	On	10%	5
T01323	CYP2C19(*2)	G681A rs4244285	Yellow: G Green: A	0,06	On	15%	10
T01324	CYP2C19(*3)	Trp212Ter TGG 212 TGA rs4986893	Yellow: Trp (G) Green: Ter (A)	0,06	On	15%	10
T01171	IL17A	IL17A G-197A	Yellow: G Green: A	0,06	On	15%	0

T01352	*COMT*	Val158Met rs4680	Yellow: Val(G) Green: Met (A)	0,03	On	15%	0
T01272	*ACE*	Alu Ins/Del rs4646994	Yellow: Ins Green: Del	0,03	On	10%	5
T01177	TNF	G-308A rs1800629	Yellow: G Green: A	0,03	on	15%	0
S01191	HFE	His63Asp rs1799945	Yellow: C Green: G	0,03	on	15%	5
S01192	HFE	Ser65Cys rs1800730	Yellow: A Green: T	0,03	on	15%	5
S01193	HFE	Cys282Tyr rs1800562	Yellow: G Green: A	0,03	on	20%	10

NOTE for Rotorgene 6000/Q (Corbett Research, Qiagen): if a Ct value is higher than 37 the sample is considered invalid and must be repeated.If there is no Ct value in both channels the sample is invalid and must be repeated starting from the extraction.

Interpretation of results for CFX-96/iQ5 (Bio-rad):

Principle of interpretation:

- **Signal only in allele 1 (channel HEX) : homozygous "***wild type"*
- **Signal only in allele 2 (channel FAM) : homozygous "***mutant"*
- **Signal in both allele 1 and allele 2 (channels HEX and FAM) : heterozygous**

Set **Baseline Cycles** at 5-15 and **Crossing Threshold** value at 100.

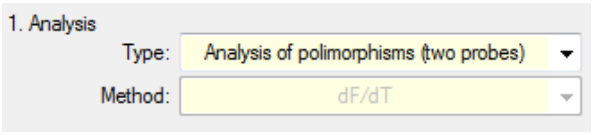
NOTE FOR CFX-96/iQ5 (Bio-rad): if a Ct value is higher than 32 the sample is considered invalid and must be repeated. If there is no Ct value in both channels the sample is invalid and must be repeated starting from the extraction.

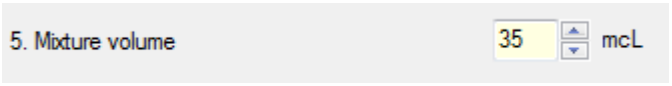
Interpretation of results for SaCycler-96 (Sacace Biotechnologies):

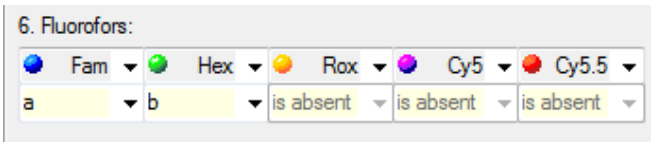
Principle of interpretation:

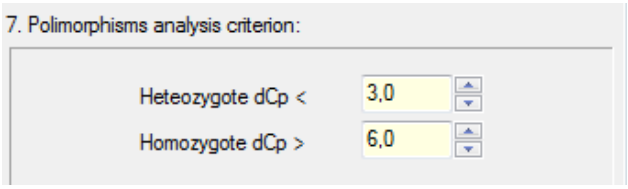
- **Signal only in allele 1 (channel HEX) : homozygous "***wild type***"**
- **Signal only in allele 2 (channel FAM) : homozygous** *"mutant"*
- **Signal in both allele 1 and allele 2 (channels HEX and FAM) : heterozygous**

NOTE: when creating new test for Sacace Molecular Genetics, select **"Analysis of polimorphisms (two probes)"**, name "a" on FAM channel and name "b" on HEX channel. Set **Heterozygote dCp < 3,0** and **Homozygote dCp > 6** (see pictures below).

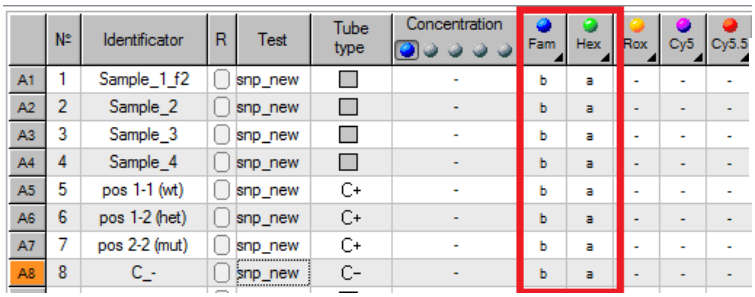




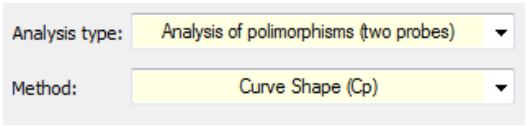




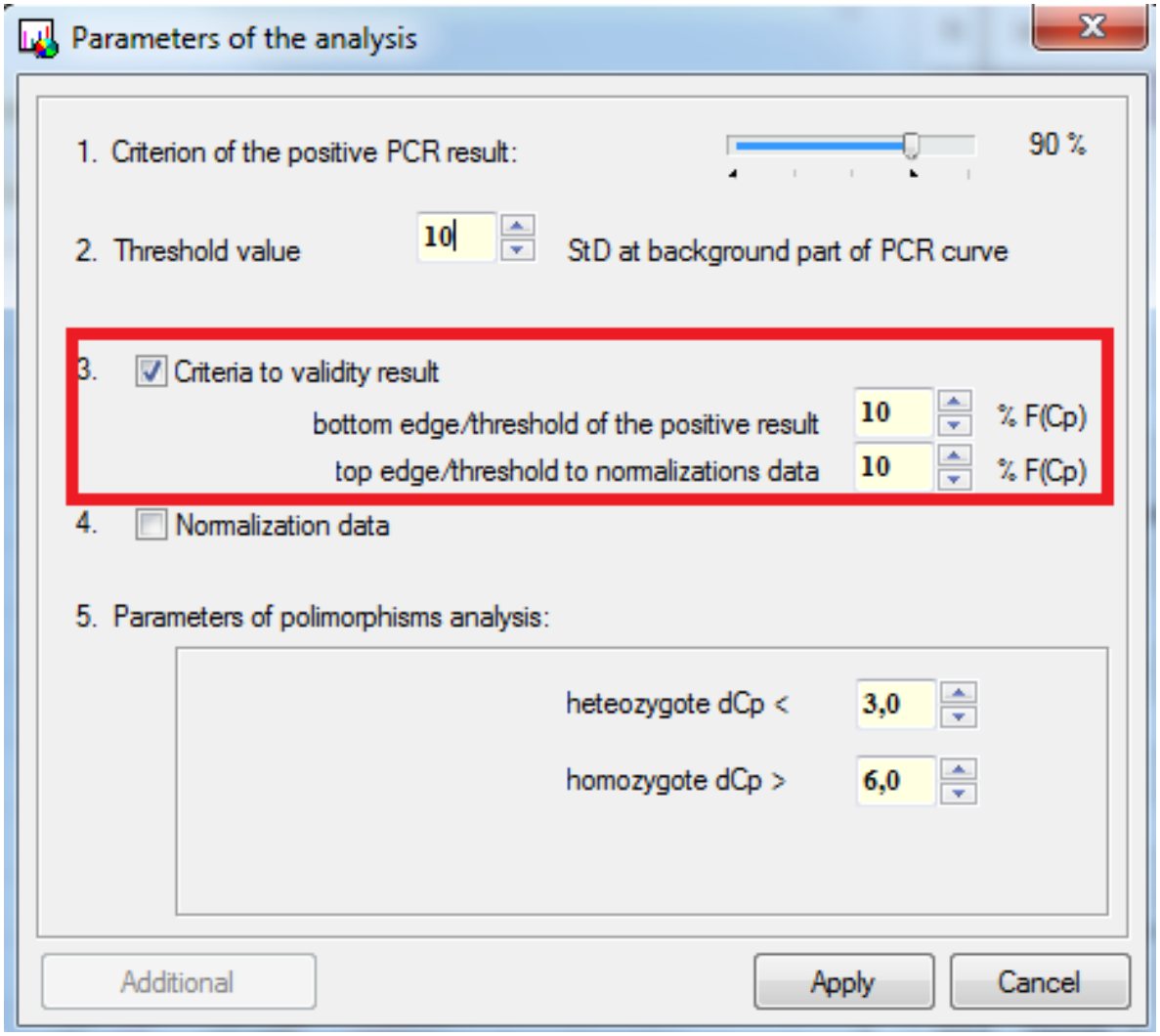
To analyse results, be sure to select **"Analysis of polimorphisms (two probes)"** as Analysis type and **"Curve Shape (Cp)"** as Method.



	№	Identificator	R	Test	Tube type	Concentration	Fam	Hex	Rox	Cy5	Cy5.5
A1	1	Sample_1_f2		snp_new		-	b	a	-	-	-
A2	2	Sample_2		snp_new		-	b	a	-	-	-
A3	3	Sample_3		snp_new		-	b	a	-	-	-
A4	4	Sample_4		snp_new		-	b	a	-	-	-
A5	5	pos 1-1 (wt)		snp_new	C+	-	b	a	-	-	-
A6	6	pos 1-2 (het)		snp_new	C+	-	b	a	-	-	-
A7	7	pos 2-2 (mut)		snp_new	C+	-	b	a	-	-	-
A8	8	C_-		snp_new	C-	-	b	a	-	-	-



Click on the icon for changing the parameter of data analysis , a new window will show up.



Set **90%** as *"Criterion of the positive PCR result"*; *"Normalization data"* checkbox must be **deselected.**
Select checkbox **"Criteria to validity result"** and insert between **10-20% F(Cp)** for *"bottom edge/threshold of the positive result"* and insert between **10-20% F(Cp)** for *"top edge/threshold to normalizations data",* then click "**Apply**".

The results will be displayed in the table on the right (see below pictures as reference).

Example of results:

Results | Statistics

N	Identificator	Polimorphism		dCp	Cp Fam	Cp Hex
A1	Sample_1_f2	a	b	0,2	19,2	19,1
A2	Sample_2	b	b	>17		17,7
A3	Sample_3	b	b	>16		18,3
A4	Sample_4	b	b	>16		18,8
A5	K+	b	b	>15		19,4
A6	K+	a	b	0,1	18,9	19,0
A7	K+	a	a	>16	18,4	
A8	C_-	-	-			

D1	Sample_1_mthfr	a	b	0,1	18,6	18,7
D2	Sample_2	a	a	>18	17,0	
D3	Sample_3	b	b	>17		17,5
D4	Sample_4	b	b	>16		18,1
D5	K+	b	b	>19		15,7
D6	K+	a	b	0,4	13,9	13,5
D7	K+	a	a	>21	13,0	
D8	C_-	-	-			

a = FAM (allele2) b = HEX (allele1)

a | b **= sample heterozygous (<u>both allele 1 and allele 2 present</u>)**

b | b **= sample homozygous with <u>only allele 1</u> present** *("wild type")*

a | a **= sample homozygous with <u>only allele 2</u> present** *("mutant")*

NOTE FOR SaCycler-96 (Sacace Biotechnologies): if a Ct value is higher than 32 the sample is considered invalid and must be repeated. If there is no Ct value in both channels the sample is invalid and must be repeated starting from the extraction.

KEY TO SYMBOLS USED

REF	List Number		Caution!
RUO	For Research Use Only		Contains sufficient for <n> tests
	Expiration Date	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**IC**	Internal Control
LOT	Lot Number		

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* iQ5™ is a registered trademark of Bio-Rad Laboratories
* Rotor-Gene™ Technology is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
*ABI® is a registered trademark of Applied Biosystems
* LightCycler® 96 is trademark of Roche

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