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Automatic data elaboration set-up

SYBR Green based Real-time PCR assay - version 1.0

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Aim

The Hyris platform is able to perform an automatic interpretation of the results by defining appropriate conditions on the bAPP. The aim of this guide is to provide the instructions to get automatic results interpretation for a SYBR Green based Real-time PCR assay. As an example, it has been considered a qualitative assay for the detection of one target, using one positive control and one negative control.

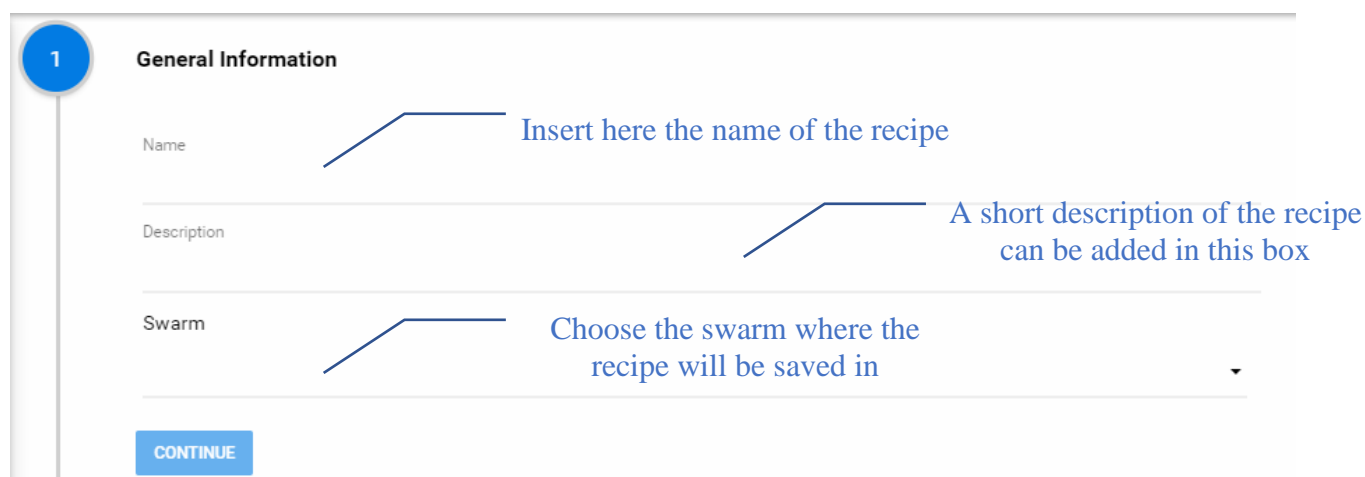
Important note

The possibility to setup an automatic data interpretation recipe is reserved to ‘Developer’ users. If you wish to exploit this possibility, ask to have the ‘Developer’ flag enabled writing at support@hyris.net. Without this flag enabled, step 4 (“Well Types”) and step 5 (“Targets”) described in this document won’t be available.

Recipe setup

The following paragraphs provide the information to set-up a recipe for a SYBR Green based Real-time PCR assay with automatic results interpretation. To this purpose, the guide focus on the required input to perform the automatic data elaboration and obtain the consequent automatic interpretation of the results. For details about each step see, also, the “Quick start guide for bAPP users - Recipe set-up - version 1.1”.

1- General information



1

General Information

Name

Insert here the name of the recipe

Description

A short description of the recipe can be added in this box

Swarm

Choose the swarm where the recipe will be saved in

CONTINUE

2- Detection channels



2 **Detection channels**

Select at least one detection channel

☐ **FAM**

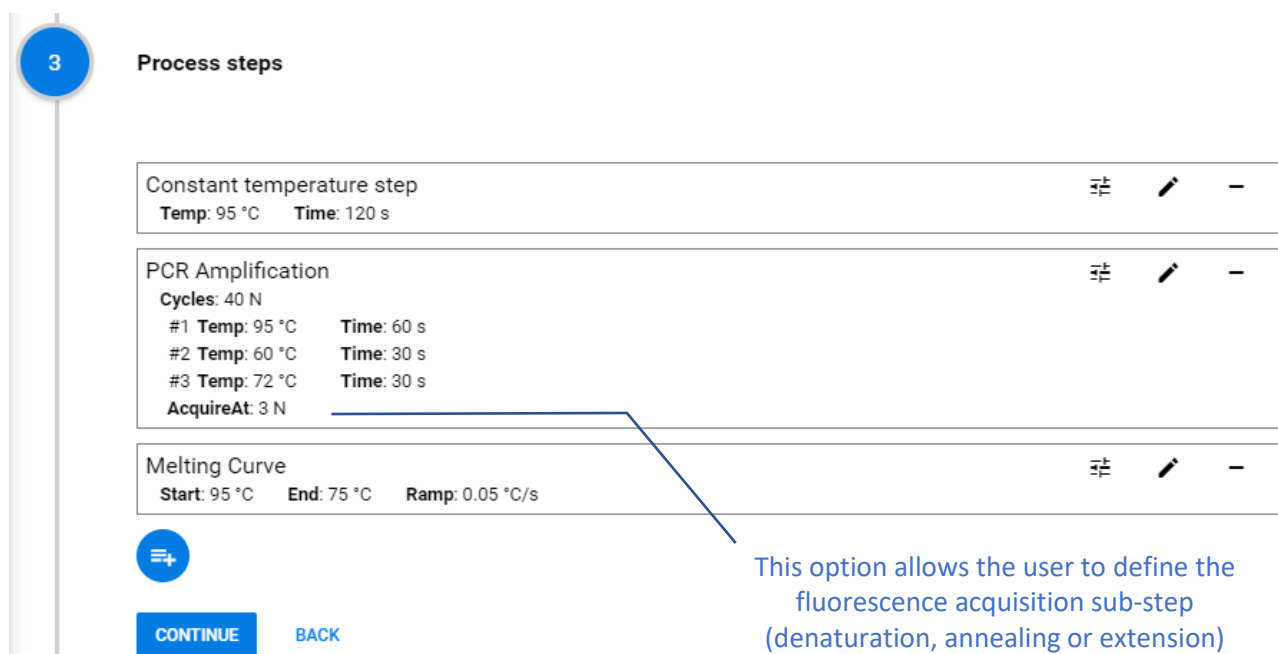
☐ **VIC**

CONTINUE **BACK**

According to the assay, select at least one detection channel

3- Process step

This section allows the user to establish the thermal profile of the analysis. Considering a typical SYBR Green based Real-time PCR assay, a preliminary activation/denaturation step, cycles of denaturation, annealing and extension and a final validation step are required. To this purpose include the “Constant temperature step”, the “PCR amplification” and the “Melting Curve” steps in the recipe, defining the conditions for each step as illustrated in “Quick start guide for bAPP users – Recipe set-up - version 1.1”. Here an example of a thermal profile for a SYBR Green based Real-time PCR assay.



3 **Process steps**

Constant temperature step
Temp: 95 °C Time: 120 s

PCR Amplification
Cycles: 40 N
#1 Temp: 95 °C Time: 60 s
#2 Temp: 60 °C Time: 30 s
#3 Temp: 72 °C Time: 30 s
AcquireAt: 3 N

Melting Curve
Start: 95 °C End: 75 °C Ramp: 0.05 °C/s

CONTINUE BACK

This option allows the user to define the fluorescence acquisition sub-step (denaturation, annealing or extension)

4- Wells types

In this section the user must define the wells types planned by the assay. Considering an assay with: a positive control, a negative control, and a sample type the following default option can be used.

4

Wells Types

PosCtrl	Positive control sample	✎	–	↑	↓
NegCtrl	Negative control sample	✎	–	↑	↓
Sample	Sample to be tested	✎	–	↑	↓

≡+

CONTINUE

BACK

If needed, each well type can be edited or removed, and new ones can be added.

5- Target

This is the essential step to set-up the automatic results interpretation. As an example, it has been considered a SYBR Green based Real-time PCR assay for the qualitative detection of one sample type (Target), using two controls types: one negative and one positive. As first step, the user must define the target as follows:

Add target

Target name

Insert here the name of the target (e.g. "YFT")

CANCEL

OK

Define the labels

This step allows the user to define all the possible "labels" that can be assigned to the wells types defined in step (4). Considering an assay for the detection of one target (e.g. "YFT"), different labels can be defined depending on the possible expected results. For example, the target can be "Present", "Absent" or it couldn't be determinate on the basis of the results ("Indeterminate"); whereas two labels can be considered for each control used: work/doesn't work ("OK"/"KO"). For each label included, the user must define the name and the color. Here an example of 5 different labels for a SYBR Green based Real-time PCR assay for the qualitative detection of one target (e.g. "YFT").

5

Targets

YFT

+

LABELS

RULES

OK <i>ok</i>	✎ 🗑
KO <i>ko</i>	✎ 🗑
Present <i>P</i>	✎ 🗑
Absent <i>A</i>	✎ 🗑
Indeterminate <i>I</i>	✎ 🗑

✎ 🗑

CONTINUE

BACK

Define the labels for each Well Type

In the section “Rules”, according to the “Wells Types” and the “Labels” previously set-up, define the allowed “Labels” for each “Well Type”. For each “Well Type”, a “Default Label” and the “Conditional Labels” must be defined. The first one is assigned when no one of the “Conditional Labels” is verified. For a full description of the details about the “Default Label” and the “Conditional Labels” see the “Quick start guide for bAPP users - Recipe set-up - version 1.1”. Considering the “YFT” target, the following approach can be used.

5

Targets

YFT

LABELS

RULES

Well Type	Default Label	Conditional Labels	Add Label
PosCtrl	🟪 KO	🟢 OK	🗑
NegCtrl	🟪 KO	🟢 OK	🗑
Sample	🟡 Indeterminate	🟢 Present 🟥 Absent	🗑

✎ 🗑

CONTINUE

BACK

For each “Conditional Label” of each “Well Type”, the user has to define the conditions that must be satisfied in order to assign it. Two kinds of conditions can be set-up: “Value conditions” and “Label conditions”. (for details about “Value conditions” and “Label conditions”, refer to “Quick start guide for bAPP users - Recipe set-up - version 1.1”).

Setting-up automatic results interpretation

As an example of setting-up an automatic results interpretation for a typical SYBR Green based Real-time PCR assay, consider an assay for the qualitative detection of a target (e.g. “YFT”) where the following requirements must be satisfied:

- 1- presence of a PCR amplification curve and a melting curve within a defined temperature range for the positive control;
- 2- no amplification curve and no melting curve within the defined temperature range for the negative control;
- 3- If both the controls are satisfied, the presence of a PCR amplification curve and the specific melting curve for the sample under investigation indicates the presence of the target;
- 4- If both the controls are satisfied, the absence of a PCR amplification curve and a specific melting curve for the sample under investigation indicates the absence of the target.

The analysis of these four points can be automatized defining the rules for the “Conditional label” for each “Well Type”. The steps required for each of the four points are discussed below.

1- Presence of a PCR amplification curve and a melting curve within a defined temperature range for the positive control

Referring to the “PosCtrl”, edit the “Conditional Label” named “OK” in the section “Value condition” as follows.

Label OK add Value Condition

Target Step

PCR Amplification

Target Channel

VIC

Parameter Type

CT

Condition Formulae

$x < 40$

CANCEL

SAVE



The “PosCtrl” is satisfied if an amplification curve is present. This can be set by defining the requirement for a Ct value lower than the number of cycles planned in the analysis (e.g. 40 cycles).

Label OK add Value Condition

Target Step
Melting Curve

Target Channel
VIC

Parameter Type
PEAKS

Condition Formulae
 $88 \leq x \leq 90$



CANCEL SAVE

The “PosCtrl” is satisfied if a specific melting curve is present. This can be set by defining the requirement for a peak within a specific temperature range (e.g. between 88°C and 90°C).

2- No amplification curve and no melting curve within the defined temperature range for the negative control

Referring to the “NegCtrl”, edit the “Conditional Label” named “OK” in the section “Value condition”.

Label OK add Value Condition

Target Step
PCR Amplification

Target Channel
VIC

Parameter Type
CT

Condition Formulae
 $x == \text{None}$



CANCEL SAVE

The “NegCtrl” is satisfied if an amplification curve is absent. This can be set by defining the requirement for the absence of a Ct value.

Label OK add Value Condition

Target Step
Melting Curve

Target Channel
VIC

Parameter Type
PEAKS

Condition Formulae
 $x == \text{None or } x < 88 \text{ or } x > 90$



CANCEL SAVE







The “NegCtrl” is satisfied if a specific melting curve is absent. This can be set by defining the requirement for the absence of a peak within a specific temperature range.

3- If both the controls are satisfied, the presence of a PCR amplification curve and the specific melting curve for the sample under investigation indicates the presence of the target

In order to assign the label “Present” to the sample type, the controls types must be satisfied. To this purpose, referring to the “Sample” type, edit the “Conditional label” named “Present” in the section “Label conditions” as follows:

Label Present rules

VALUE CONDITIONS
 LABEL CONDITIONS
+

PosCtrl:  OK	 
NegCtrl:  OK	 

OK
 DELETE

Define, also, the Ct and Peaks parameters in the section “Value condition” as follows:

Label Present add Value Condition

Target Step
 PCR Amplification

Target Channel
 VIC

Parameter Type
 CT

Condition Formulae
 $x < 40$



CANCEL
 SAVE

The presence of the target is defined by the presence of an amplification curve. It can be set by defining a Ct value lower than the number of cycles planned in the analysis or, according to the assay, a different Ct value.


Label Present add Value Condition

Target Step
 Melting Curve

Target Channel
 VIC

Parameter Type
 PEAKS

Condition Formulae
 $88 \leq x \leq 90$



CANCEL
 SAVE

The presence of the target is confirmed by the presence of a specific melting curve. This can be set by defining the requirement for a peak within a specific temperature range.

- 4- *If both the controls are satisfied, the absence of a PCR amplification curve and a specific melting curve for the sample under investigation indicates the absence of the target*

In order to assign the label “Absent” to the sample type, the controls types must be satisfied. To this purpose, referring to the “Sample” type, edit the “Conditional label” named “Absent” in the section “Label conditions” as follows:

Label Absent rules

VALUE CONDITIONS	LABEL CONDITIONS	
PosCtrl: OK		
NegCtrl: OK		

OK DELETE

Define, also, the Ct and Peaks parameters in the section “Value condition” as follows:

Label Absent add Value Condition

Target Step
PCR Amplification

Target Channel
VIC

Parameter Type
CT

Condition Formulae
x == None

CANCEL SAVE

The absence of the target is defined by the absence of an amplification curve. It can be set by defining the requirement for the absence of a Ct value.

Label Absent add Value Condition

Target Step
Melting Curve

Target Channel
VIC

Parameter Type
PEAKS

Condition Formulae
x == None or x < 88 or x > 90

CANCEL SAVE

The absence of the target is confirmed by the absence of a specific melting curve. This can be set by defining the requirement for the absence of a peak within a specific temperature range.

6- Cartridge

This last section is optional, since it can be filled out (and/or edited) in the initialization phase of a new analysis.

Two kinds of cartridge can be selected: 3x3 or 4x4. For each well define the “Well Type” (among that defined in “Step 4”), the name and the color as in the example below.


6

Cartridge

Select cartridge format 4x4 samples ▼

PosCtrl <i>Positive control</i>	Sample <i>Unknown sample</i>	NegCtrl <i>Negative control</i>	
+	+	+	+
+	+	+	+
+	+	+	+
+	+	+	+

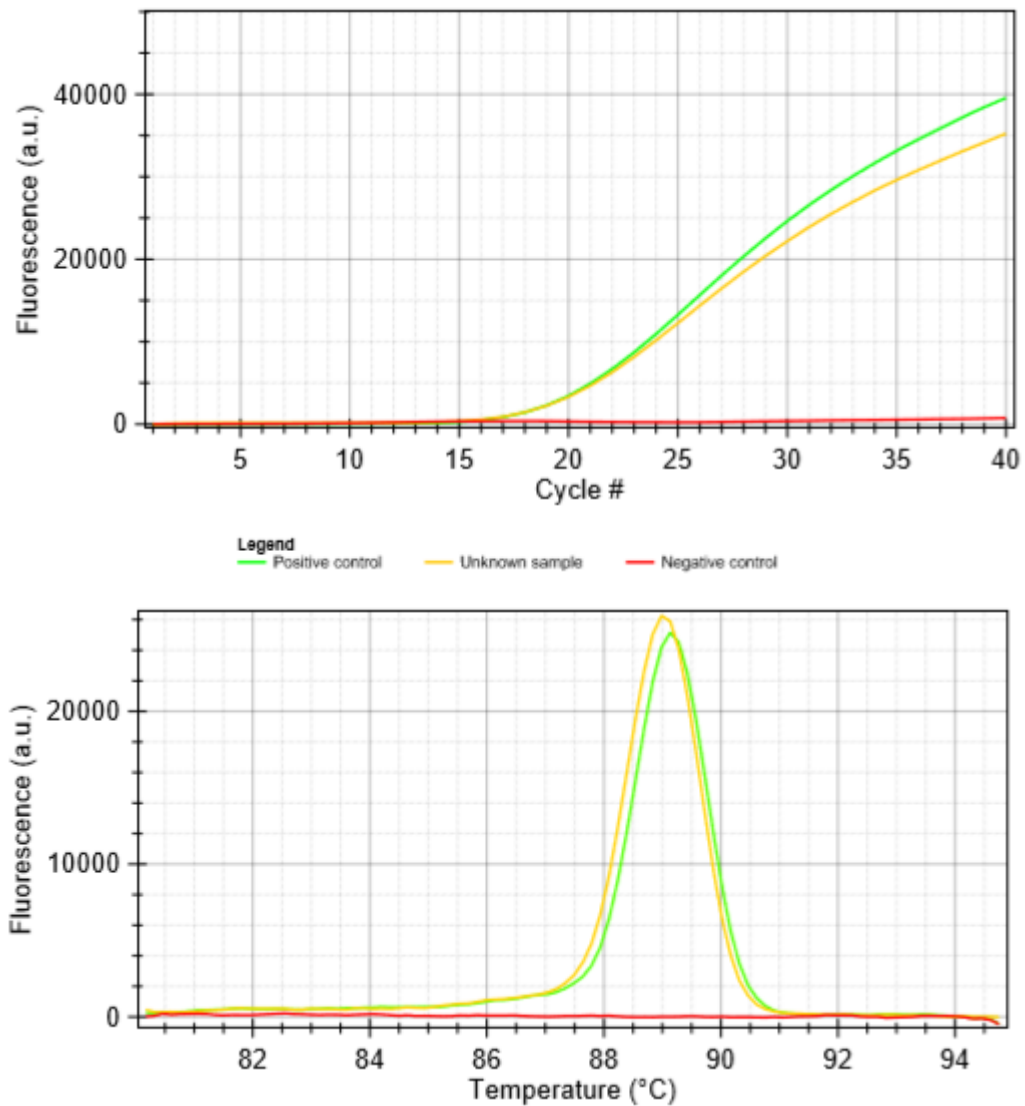
FINISH
BACK



Click here to save the recipe

Example result

The procedure described allows to get an automatic interpretation of the results for a SYBR Green based Real-time PCR assay. As an example, the figures below represent the trend of the fluorescence, during the “PCR amplification” and the “Melting Curve” steps respectively, of a SYBR Green based Real-time PCR analysis for the qualitative detection of a target (e.g. “YFT”). The three lines represent the “Positive control” (green line), the “Negative control” (red line) and one “Unknown sample” (yellow line).



Taking into account the instructions defined in the recipe, the Hyris platform is able to automatically interpret the analysis and release the results for the sample(s) under investigation, as follows:

Results for target YFT		
Positive control	(PosCtrl)	OK
Unknown sample	(Sample)	Present
Negative control	(NegCtrl)	OK