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bAPP - Custom recipe setup

version 1.1

Hyris Ltd

HYRIS Headquarters
Lower Ground Floor, One George Yard,
EC3V 9DF, London, UK
Phone: +44.2036082968
Mail: office@hyris.net

HYRIS Research Center
Corso Garibaldi 60,
Milano 20121, Italy
Phone: +39.02.82951302
Mail: administrator@hyris.net

HYRIS Asia Pac
Block 998 Toa Payoh North #06-06
Toa Payoh North Industrial Estate
Singapore 318993, Singapore
Phone: +65.8160.7207
Mail: office@hyris.net

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Introduction

Establishment of a new recipe includes 6 steps:

- 1- General information;
- 2- Detection channels;
- 3- Process steps;
- 4- Wells types;
- 5- Targets;
- 6- Cartridge

Step 1 to 3 are mandatory, whereas step 4 to 6 are optional.

Basic recipe setup

This section describes how to setup a basic protocol for bCUBE.

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

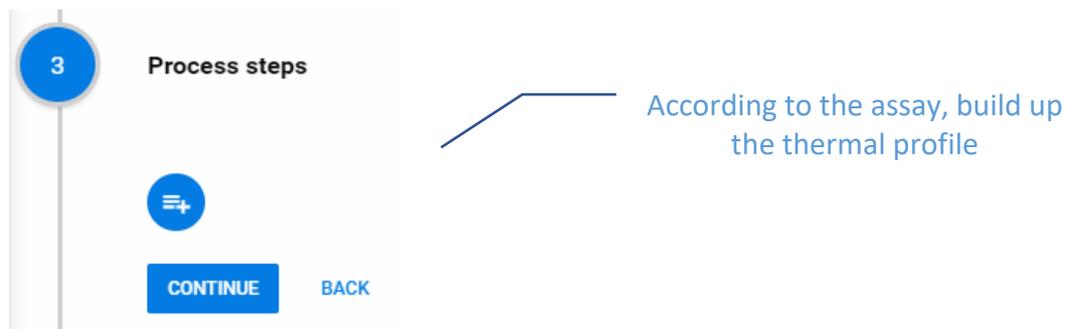
VIC

 According to the assay, select at least one detection channel

CONTINUE **BACK**

3- Process step

This section allows the user to establish the thermal profile of the analysis. Using four different steps, it is possible to set-up a Real-time PCR or LAMP protocol, both with or without melting curve and preliminary activation/denaturation step.



Process step type

Four kinds of steps can be used:

- Constant temperature step;
- PCR Amplification;
- Isothermal Amplification;
- Melting Curve

Constant temperature step



PCR amplification

PCR Amplification

Number of cycles (N)
- 40 +

CycleSteps ADD STEP

1 - Temperature: 95 Length: 30		
2 - Temperature: 60 Length: 30		
3 - Temperature: 72 Length: 30		

AcquisitionSubStep #3

SAVE CANCEL

Use this option to set-up a PCR amplification step. Set the conditions for:

- the number of cycles;
- the temperature and the length for the denaturation, annealing and extension steps
- the fluorescence acquisition step.

Isothermal amplification

Isothermal Amplification

Amplification temperature (°C)
- 65 +

Amplification duration (s)
- 1200 +

Acquisition Delay (s)
- 10 +

ADD CANCEL

Use this option for LAMP (Loop-mediated isothermal amplification) assays. Set the conditions for:

- Amplification temperature;
- Amplification duration;
- Acquisition delay

Melting curve

Melting Curve

Starting temperature (°C)
- 95 +

End temperature (°C)
- 75 +

Ramp speed (°C/s)
- 0,05 +

ADD CANCEL

Use this option to add a melting curve step, defining:

- starting temperature;
- end temperature;
- ramp speed

Editing of parameters

For each step previously discussed, there are default parameters. The following icon allows the user to edit them:

One of the editable parameters that allow to get more robust results is the “Threshold” value. In fact, depending on the characteristics of each assay, the background noise could be different. This phenomenon could affect Cq/TTS determination; to face it, it could be useful to decrease/increase the “Threshold” value. If considering background noise in “PCR amplification” step, it is possible to edit the “Threshold” value clicking on the “edit parameter” icon (). When the following window appear, click on the pencil icon () to edit it.

CT	Quantification cycle (Cq)		
Description: Quantification cycle (Cq)			
Threshold: 3000			
XLabel: Counter			
YLabel: Intensity			
DecimalDigits: 2 digit			

The same considerations/operations apply to TTS determination on “Isothermal amplification” step. If the automatic data elaboration is desired, go to step 4. Otherwise go directly to step 6.

Advanced recipe setup

These steps are optional, and must be filled up to get automatic results interpretation. If you don't want to add an automatic results interpretation to your recipe, you can skip this section.

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.

4- Well types

In this section it is possible to define the wells types. It will be possible to define different results interpretation rules for each of these types. The default option showed below includes three wells types: a positive control, a negative control, and a sample. However, each well type can be edited or removed, and new ones can be added.

4

Wells Types

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

+

+

CONTINUE

BACK

5- Targets

This section allows the user to define the target(s). For each target is possible to define different labels that, in order to be assigned to a sample, require the fulfillment of a list of defined rules. Here, we show the essential steps required to get automatic data elaboration.

Define the target

Add target

Target name

Insert here the name of the target

CANCEL OK

Define the labels for each target

Many “labels” can be defined for each target. Here, an example with 5 different labels.

5

Targets

Target 1

LABELS	RULES	+
<div style="display: flex; align-items: center;"> <div style="width: 15px; height: 15px; background-color: #007bff; margin-right: 5px;"></div> OK <i>ok</i> </div>		✎ 🗑
<div style="display: flex; align-items: center;"> <div style="width: 15px; height: 15px; background-color: #9900cc; margin-right: 5px;"></div> KO <i>ko</i> </div>		✎ 🗑
<div style="display: flex; align-items: center;"> <div style="width: 15px; height: 15px; background-color: #90ee90; margin-right: 5px;"></div> Present <i>P</i> </div>		✎ 🗑
<div style="display: flex; align-items: center;"> <div style="width: 15px; height: 15px; background-color: #ff0000; margin-right: 5px;"></div> Absent <i>A</i> </div>		✎ 🗑
<div style="display: flex; align-items: center;"> <div style="width: 15px; height: 15px; background-color: #ffff00; margin-right: 5px;"></div> Indeterminate <i>I</i> </div>		✎ 🗑

✎ 🗑

Define the labels for each Well Type

Each of the “Well Types” defined at step (4) will have a different set of possible labels and specific rules for their assignment.

For each “Well Type”, the user must define which label apply, choosing among the labels that were previously defined. In the example below, two labels for the controls types and three for the sample type have been included.

5

Targets

Target 1				
LABELS	RULES			
Well Type	Default Label	Conditional Labels	Add Label	
PosCtrl	■ KO	■ OK	+	
NegCtrl	■ KO	■ OK	+	
Sample	■ Indeterminate	■ Absent	■ Present	+

✎ 🗑

Define the rules for labels assignment

Each well type will have a “Default Label” a custom number of “Conditional Labels”.

For the “Default Label” no rules have to be defined; this label is assigned to the well type under examination if none of the “Conditionals Labels” can be assigned.

For each “Conditional Label” of each “Well Type”, the user should 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”.

Value conditions

A “value condition” will check that one of the calculated parameters of a step (e.g. ‘CT’ for PCR amplification) stays within defined limits. The user will have to choose the target step, channel, and parameter.

The following symbols can be used to set-up the limits.

Symbols	Meaning
<	Less than
>	Higher than

==	equal to
<=	Less than or equal to
>=	greater than or equal to
!=	not equal to

Example: if a Ct less than 30 is required to assign the label “OK” to the “PosCtrl”, the rule must be written as follow:

Label OK add Value Condition

Target Step
PCR Amplification

Target Channel
VIC

Parameter Type
CT

Condition Formulae
x < 30

CANCEL SAVE

More than one value can be included in the “Condition Formulae” using the functions “and”, “or”.

For samples that don’t show amplification curves, no Ct values are calculated, showing N/A in the results.

In order to consider valid the case of N/A samples, the “None” value must be included in the “Condition Formulae” string. For example, a possible “Condition Formulae” for a Negative control could be: “x == None or x > 31”

Label conditions

This option allows to bind the rules for Labels assignment of the Well Type under examination to the results of other Well Types. It could be useful to assign a defined label to the sample type in relation to the results of the controls types; as in the following example, which refer to the label “Present” for the well type “sample”:

Label Present rules

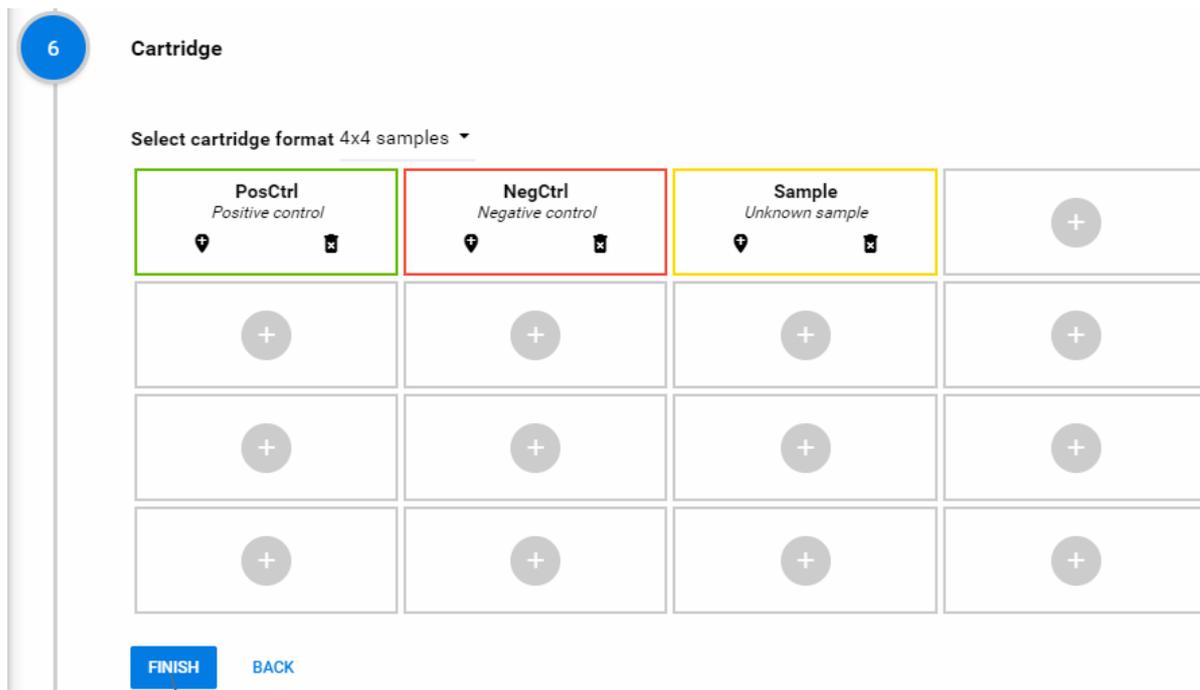


This “Label condition” means that both the controls (“PosCtrl” and “NegCtrl”) must be satisfied (“OK”), in order to assign the label “Present” to the sample.

Cartridge template setup

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 the ones available, the name and the color an in the example below.



Click here to save the recipe