

# Manual to perform a qPCR with the ABI 7500Fast



Required material:


Product name	REF Nummer / Order No.	costs
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode	4360954	20 Stk. 183.00 Fr. (Lubio).
MicroAmp® Optical Adhesive Film	4360954	25 Stk. 149.00 Fr. (Lubio)
LightCycler® 480 SYBR Green I Master <sup>1</sup>	04887352001	5ml ca. 200.00 Fr.
Nuclease-free water		
Primer (4.5 µM) <sup>2</sup>		
One channel pipette (10, 100, 1000 µl)		Tips from tick-list
8-channel pipette (10 µl)		Tips from tick-list
Stepper + 0.2 ml tips		Tips from tick-list
Centrifuge in G62 (with plate rotor)		
1.5 ml tubes		Tick-list
qPCR protocol	See next page (pipetting template) – Settings for the protocol are in the following pages (Example of a pipetting template see last page)	

<sup>1</sup>) buy via UTOX – 30% discount on Roche products

<sup>2</sup>) Stock conc. = 100µM ; 4,5µM is „working conc.“

Tips:

- Pipette neat and tidy (qPCR is very sensitive)
- Dispense MasterMix with stepper
- First pipette samples, than negative and positive control(s) –and at the end the standards
- Pipette standards with the 8-channel pipette
- If pipetting the same many times, it is worth distributing all the samples in a 96 well plate in advance so you can use a multi-channel pipette
- Stick the adhesive cover foil with Kleenex and not with your gloves or your bare fingers, **foil has to be clean!**
- Pipette the dilutions from A1 to H1 and in duplicate (e.g. in A1 pipette standard  $10^7$  and in H1 place standard  $10^0$ . The duplicates should be pipetted next to A1 to H1 → A2 to H2. Replicates with the same dilution should be pipetted next to each other, i. e. A1 is duplicated in A2 and not in B1

	Aphids quantitative PCR <b>Lab-Form Primer testing: Analysis</b>		<b>Aquatic Ecology</b>
	Created by Marco Thali	created on 17.03.2016	Version 2016.01  <i>updated : 17.03.2016</i>

### Primer testing with 2x SYBR Green I Master

Reagents	Primer names and/or Aliquot / Lot No	Concentration	Date of preparation/dilution
SYBR Green I Master (Roche)	Product No. 04887352001	2 x	
ddH <sub>2</sub> O			
Forward Primer		4.5 µM	
Reverse Primer		4.5 µM	

Sample names	Concentration	Date / Date of dilution
Sample DNA	see sample sheet file:	see sample sheet from:
	ng/µl	

#### Procedure:

1. Fill in name of PCR program, annealing temperature, no of PCR cycles, name of PCR machine, no of primer pairs and total no of PCR reactions below

<b>PCR program name</b> 2-Step	<b>Annealing temp (°C)</b>	<b>Nr of cycles</b> 50	<b>PCR machine</b> Cycler 11 - ABI 7500Fast
<b>Nr of primer pairs</b> 1	<b>Total Nr of PCR reactions</b> 96	<b>Plus volume (%)</b> 5	<b>Total volume of SYBR mix (µl)</b> 630.00

- Prepare Mix by adding ddH<sub>2</sub>O (I) and 2x SYBR Green I Master (II) in separate tube
- Add primers (III) / (IV) and mix well
- Distribute 10 µl of mix into PCR wells
- Add 2.5 µl of sample DNA or positive/negative control into corresponding PCR well(s)

	Mix per primer	Mix per reaction	Final concentr.
ddH <sub>2</sub> O	126.0 µl (I)	1.25 µl	
SYBR Green I Master (Roche)	630.0 µl (II)	6.25 µl	1 x
Forward Primer	126.0 µl (III)	1.25 µl	0.450 µM
Reverse Primer	126.0 µl (IV)	1.25 µl	0.450 µM
<b>Total:</b>	<b>1008.0 µl</b>	<b>10.00 µl</b>	
Sample DNA / controls		2.50 µl	0 ng/µl
Final volume		12.50 µl	

#### PCR program 2-Step:

Heated lid	105 °C	
50 °C	2 min	
95 °C	10 min	
95 °C	15 sec	50x
0 °C	30 sec	
<b>PCR program melting curve:</b>		
95 °C	15 sec	
60 °C	60 sec	30 min
95 °C		
10 °C	∞	

#### Sample order:

	Primer Fwd												Primer Rev	Species		
A																A
B																B
C																C
D																D
E																E
F																F
G																G
H																H
	1	2	3	4	5	6	7	8	9	10	11	12				

Notes:

<b>Project Group:</b>	<b>Organism(s):</b>	<b>Date:</b>	<b>Person in charge:</b>

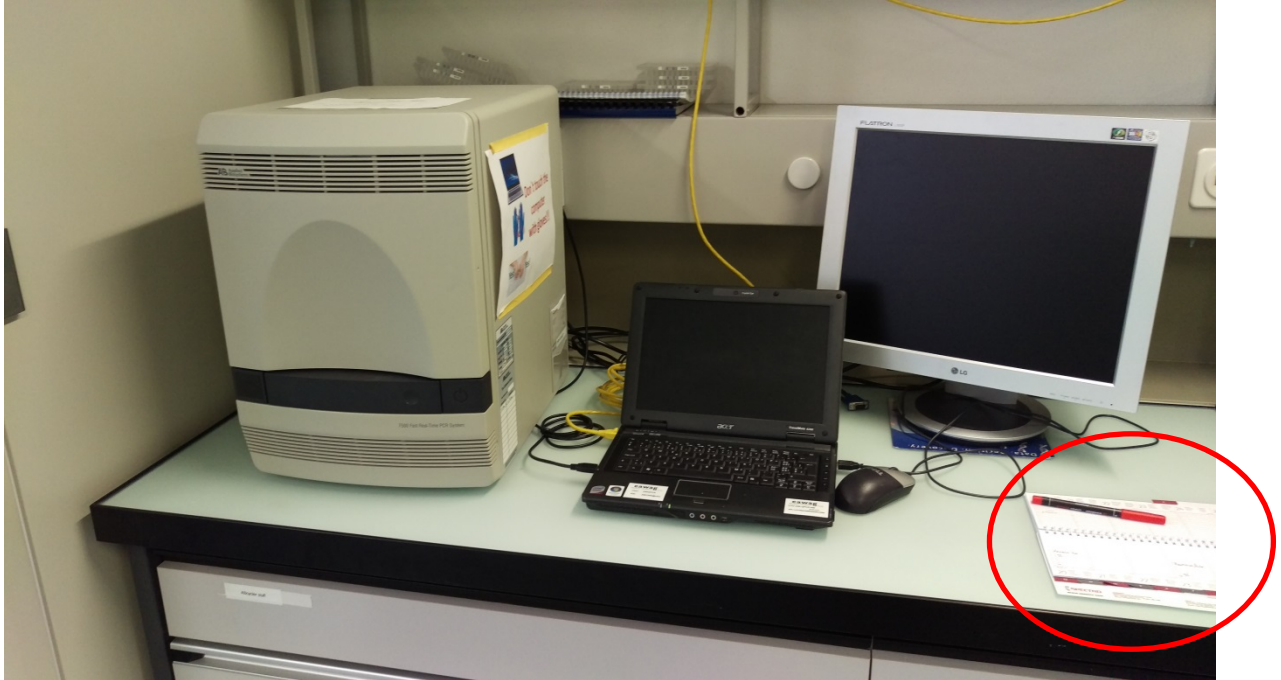
Site of machine: LA-E76 ; right to the -80°C freezer

Person in charge: Smitha Pillai – BU-E09 (Utox) ☎ 5255

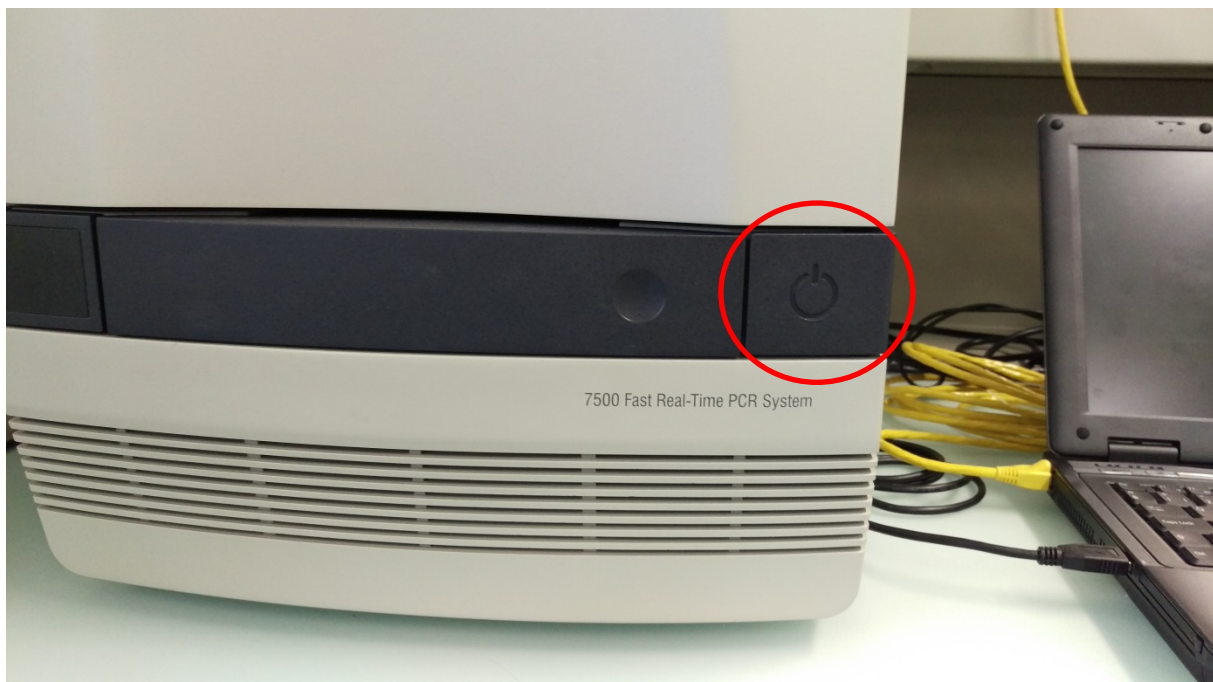
Stephan Fischer – BU-E09 (Utox) ☎ 5567

**If you use the cycler for the first time, you have to be introduced by one of the persons in charge!**

Book machine in advance with name, time and length of use (calendar is next to the notebook)



Start machine (the best is to start the machine 30 minutes before use to heat up)



Start computer

Username: ABIQPCR (should already be written in)

Password: ABI7500@eaw

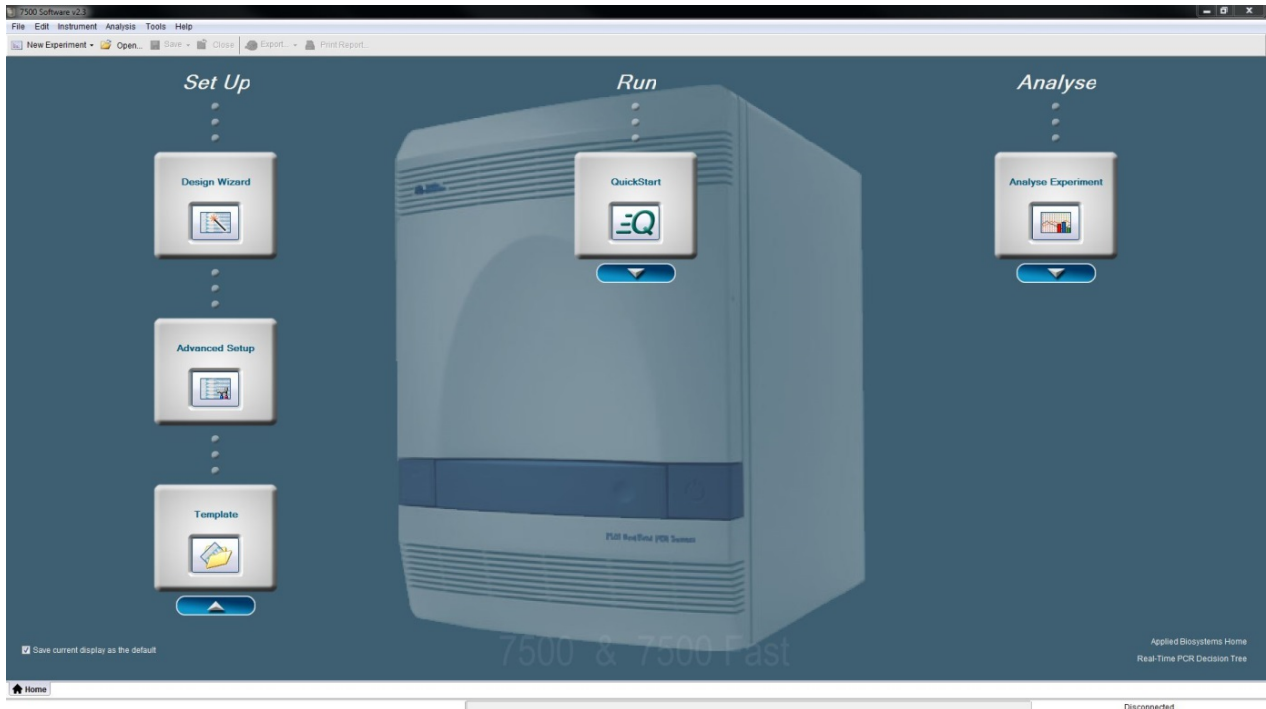
Open software

Software name is **7500 Software v2.3**



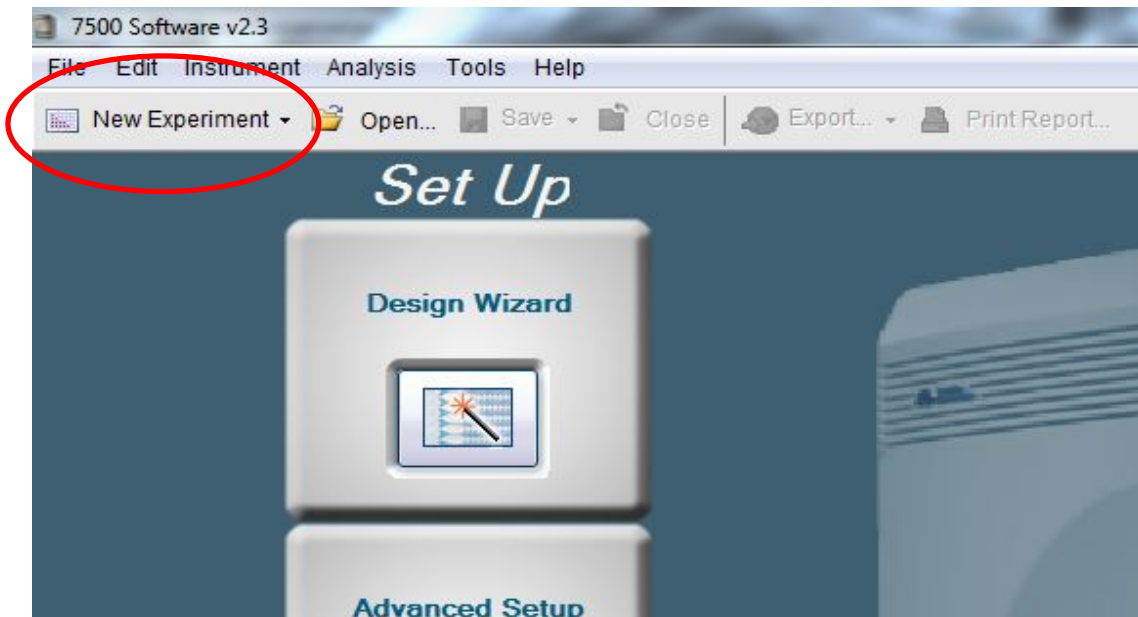
[If error message appears saying a calibration is required contact Smitha or Stephan]

This window should open

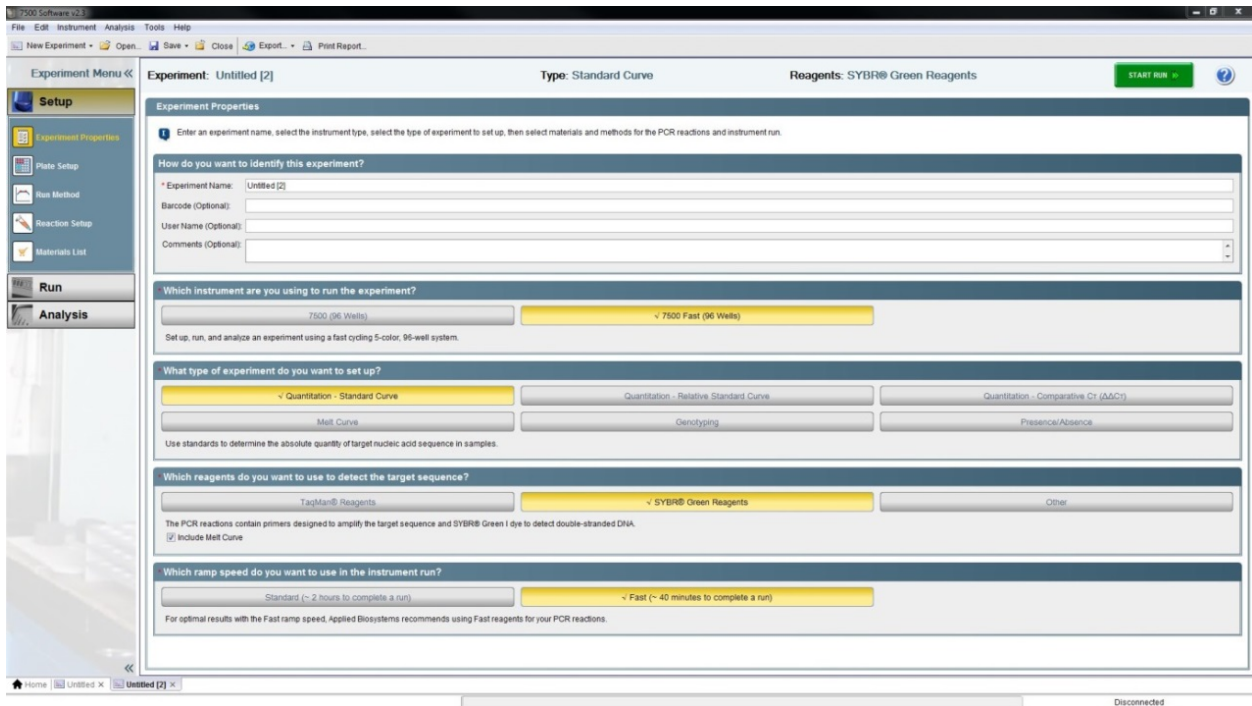




Start new Experiment Click on field **New Experiment** but **not on the arrow**



This window should open



1. Write in experiment name
2. Which Instrument you use: 7500 Fast (96 Wells) If changing the Cycler you must click yes on the message popping up
3. What type of Experiment: Quantitation- Standard Curve
4. Which reagents to detect: SYBR® Green Reagents
5. Which ramp speed: Fast (~ 40 Minutes to complete a run)

The screenshot shows the 'Experiment Properties' configuration window. The following options are circled in red:

- Experiment Name:** Untitled
- Which instrument are you using to run the experiment?:** 7500 Fast (96 Wells)
- What type of experiment do you want to set up?:** Quantitation - Standard Curve
- Which reagents do you want to use to detect the target sequence?:** SYBR® Green Reagents
- Which ramp speed do you want to use in the instrument run?:** Fast (~ 40 minutes to complete a run)

Select plate setup (2<sup>nd</sup> element in the dark blue box left on the screen)



This window get open

Target Name	Reporter	Quencher	Colour
Target 1	SYBR	None	

Add New Target

For each gene amplified you have to create a new target. Then fill in Target Name, Reporter is SYBR and Quencher is none (Target one is filled in automatically)

Target Name	Reporter	Quencher	Colour
Target 1	SYBR	None	

Define Samples:

Therefore select **add new sample** and fill in names (find on the right of “define targets) \_\_\_\_\_

Sample Name	Color
Sample 1	

Here, all samples (incl. standards and controls) should be assigned. So for each used „well“ on the plate, a sample must be defined.



Select Assign Targets and Samples: Each well must be assigned to the corresponding sample

Define Targets and Samples **Assign Targets and Samples**

To set up standards: Click 'Define and Set Up Standards'.  
To set up unknowns: Select wells, assign target(s), select 'U' (Unknown) as the task for each target assignment, then assign a sample.  
To set up negative controls: Select wells, assign target(s), then select 'N' (Negative Control) as the task for each target assignment.

**Assign target(s) to the selected wells.**

Assign	Target	Task	Quantity
<input type="checkbox"/>	Target 1	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/>	

Mixed  Unknown  Standard  Negative Control

**Define and Set Up Standards**

**Assign sample(s) to the selected wells.**

Assign	Sample
<input type="checkbox"/>	Sample 1

**Assign sample(s) of selected well(s) to biological group.**

Assign	Biological Group
--------	------------------

Select the dye to use as the passive reference.

ROX

View Plate Layout View Well Table

Select Wells With: - Select Item - - Select Item -

Show in Wells View Legend

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Wells:  Unknown  Standard  Negative Control

96 Empty

First the standard curve must be defined

Click **Define and Set Up Standards**

**Assign target(s) to the selected wells.**

Assign	Target	Task	Quantity
<input type="checkbox"/>	Target 1	<input type="checkbox"/> <input checked="" type="checkbox"/> <input type="checkbox"/>	

Mixed  Unknown  Standard  Negative Control

**Define and Set Up Standards**

**Assign sample(s) to the selected wells.**

Assign	Sample
<input type="checkbox"/>	Sample 1

The following window opens

**Select a target** \* = Required

\* Select a target for the standards Target 1

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**Define the standard curve** \* = Required

**Standard Curve Preview**

\* # of Points:  5 Recommended

\* # of Replicates:  3 Recommended

\* Starting Quantity:  Enter the highest or lowest standard quantity for the standard curve.

\* Serial Factor:  Select a value from 1:10 to 10×

8 Points X 2 Replicates = 16 Required Wells

Click here to see preview.

---

**Select and arrange wells for the standards**

Use Wells:  Automatically Select Wells for Me  **Let Me Select Wells**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

16 Required Wells / 16 Selected Wells

A1,A2,B1,B2,C1,C2,D1,D2,E1,E2,F1,F2,G1,G2,H1,H2

Arrange standards in:  Columns  Rows

Apply

Reset Fields

Close

- Select Target:            Select respective target
  - # of Points:             Number of dilutions you made
  - # of Replicates:         Number of replicates for each dilution
  - Starting Quantity:       Highest standard concentration (e.g. 100'000)
  - Serial Factor:            select a dilution factor (1:2 up to 1:10 are possible)
- Select **Let Me Select Wells**, then mark the desired wells. Afterward select **Apply** (if field "close" is selected before selecting apply, the standard curve is not saved and you have to do everything again)
- Select **Close** to close the windows


Afterward define the passive-reference:

Make sure that in **Select the Dye to use as the passive Reference** you mark „None“  
(This setting is below the field where the standards can be defined)

**Assign target(s) to the selected wells.**

Assign	Target	Task	Quantity
<input type="checkbox"/>	Target 1	<input type="checkbox"/> U <input type="checkbox"/> S <input type="checkbox"/> N	

Mixed  U Unknown  S Standard  N Negative Control

 Define and Set Up Standards

**Assign sample(s) to the selected wells.**

Assign	Sample
<input type="checkbox"/>	Sample 1

**Assign sample(s) of selected well(s) to biological group.**

Assign	Biological Group
--------	------------------

**Select the dye to use as the passive reference.**

None ▾

After that, all samples will be defined:

- Define Samples:
- Mark desired well on the plate
  - Set a "check mark" for the right target in the "Assign"-column
  - Choose if the sample is unknown(U) or a negative control (N)
  - Set a "check mark" to choose the right sample

The screenshot shows three main components of the software interface:

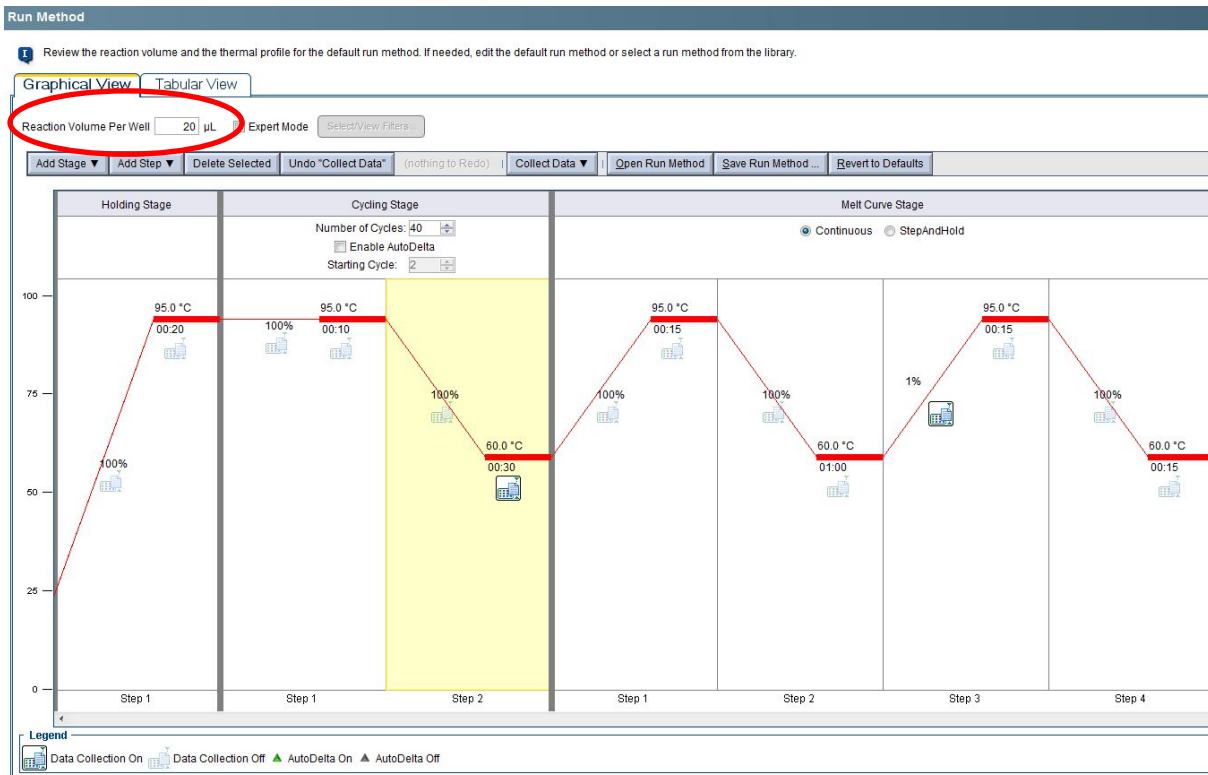
- Assign target(s) to the selected wells:** A table with columns 'Assign', 'Target', 'Task', and 'Quantity'. The 'Assign' column has a checked box. The 'Target' column contains 'Target 1'. The 'Task' column contains three buttons: 'U' (Unknown), 'S' (Standard), and 'N' (Negative Control). Below the table is a legend: Mixed (asterisk icon), Unknown (U), Standard (S), Negative Control (N). A yellow button labeled 'Define and Set Up Standards' is at the bottom.
- Assign sample(s) to the selected wells:** A table with columns 'Assign' and 'Sample'. The 'Assign' column has a checked box. The 'Sample' column contains 'Sample 1'.
- View Plate Layout:** A grid showing a 5x3 plate layout (rows A-E, columns 1-3). Each cell contains a target assignment (e.g., 'S Target 1 1E6') or a sample assignment ('U Target 1'). The cell at row A, column 3 is circled in red and contains 'Sample 1' and 'U Target 1'.

Select run method (3<sup>rd</sup> element in the dark blue box left of the screen)

The screenshot shows the 'Experiment Menu' with the following options:

- Setup
- Experiment Properties
- Plate Setup
- Run Method** (highlighted with a red circle)
- Reaction Setup
- Materials List

## Fill in the right reaction volume





## Add Stage and Steps:


**Add Stage:** Here it is possible to add a new stage (holding and cycling stages are possible). The melt curve stage should be in the run method automatically, if not then control if TaqMan® is selected in **which reagents to detect**. If there is not **SYBR® Green** marked, no melt curve is possible.

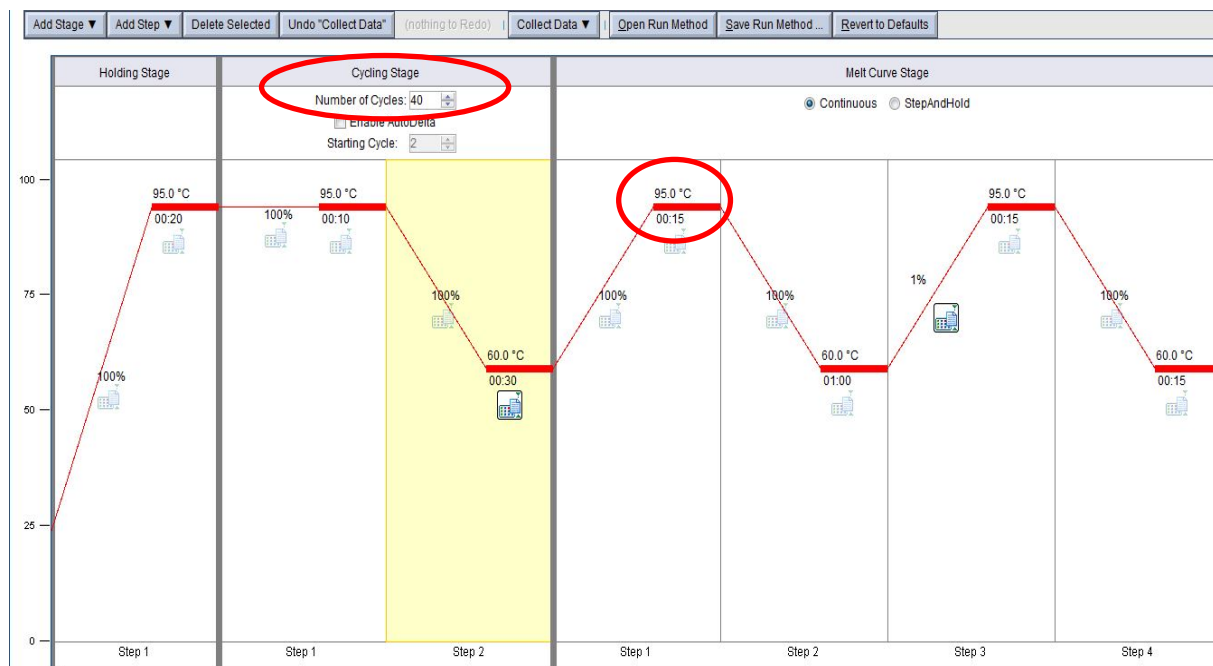
**Add Step:** Define if the step should be added before or after the marked step (yellow)

**Delete Selected:** The yellow marked stage or step will be deleted

Fill in the right values for temperature and time (cycling conditions)

Fill in the cycle number

This sign  stands for the measuring point (must be in the elongation step in the cycling stage and in the melt curve stage it should be set during the increasing of the temperature). If a yellow field appears called **The number of data collection points is not valid**, then you must control if there is a measuring point set somewhere wrong. This can be seen through controlling if the sign is slightly transparent (point inactive) or not (point active)



1% slope in the melt curve means, that the temperature will be increased from 65°C to 95°C within ~30 minutes.

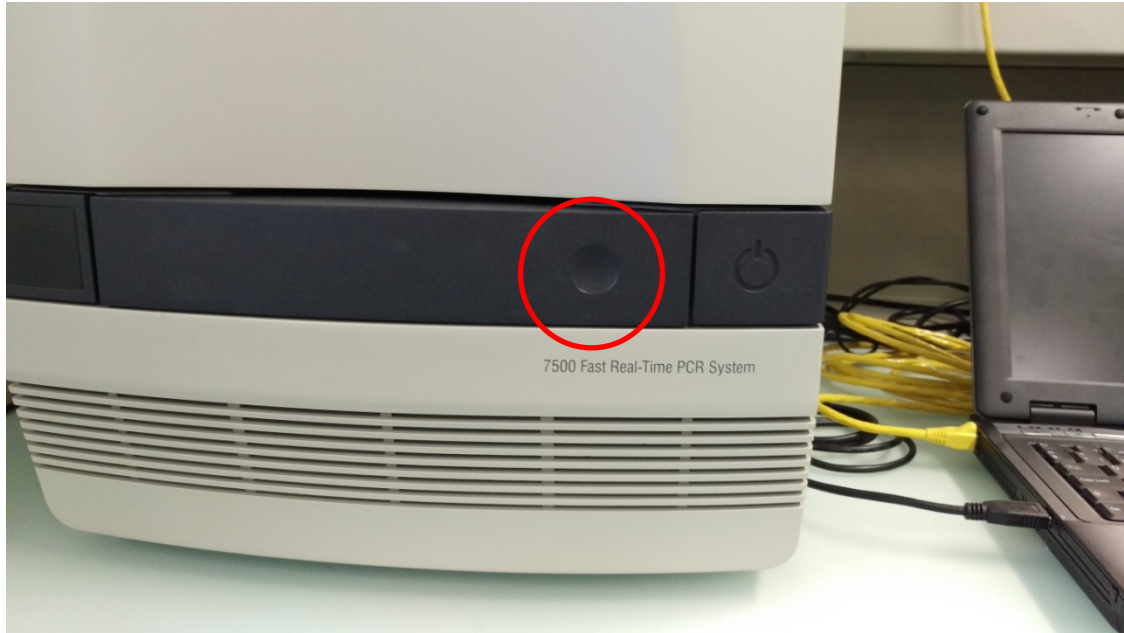
**A Holding stage always consists of 1 step (hold of temperature)**

**A Cycling stage always consists of 2 steps (denaturation + annealing/elongation)**

**A Melt curve stage always consists of 4 steps (4x hold temperature)**

Put plate in ABI7500:

In the recess of the tray press firmly until it „clicks“ so that the tray comes out by **itself**



Put plate in ABI cycler

**Well A1 must be on top left!**

Then press the tray back (press firmly again) until it „clicks“ again



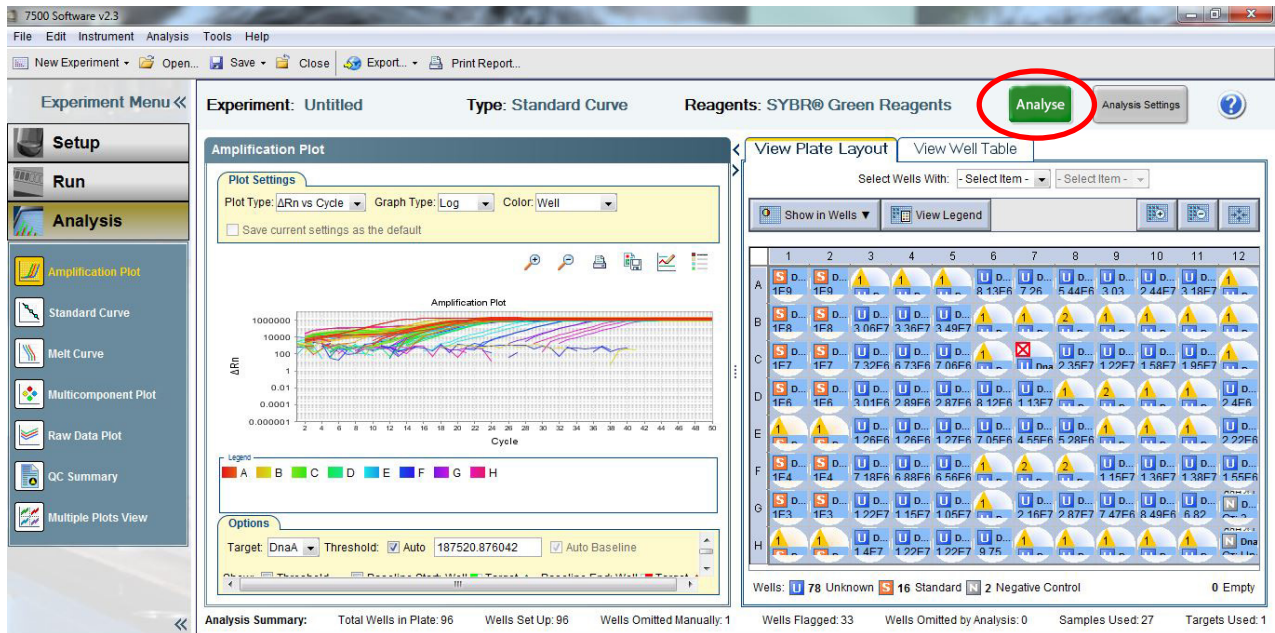


When everything is correct, press

**Important:** Stay near the Cyclor until the first or second cycle is finished. Sometimes an error message appears that the Cyclor has a problem and does not start!

After the run's finished:

To receive data press the green button **ANALYSE** (top right). After that it says **Reanalyse** on the button. This way you can check, if you have pressed "analyse" already in case you are not sure anymore.

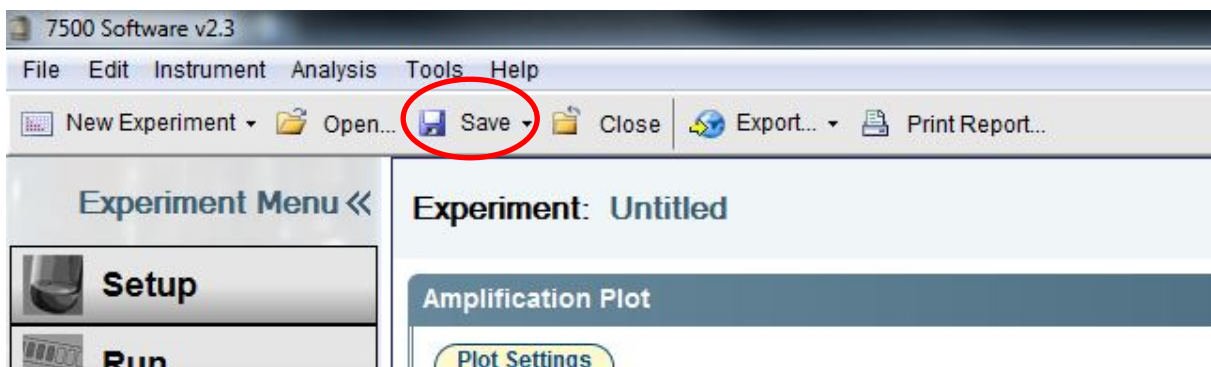


Save run:

Press on the arrow next to the diskette symbol and then on **save as** to select the save location.

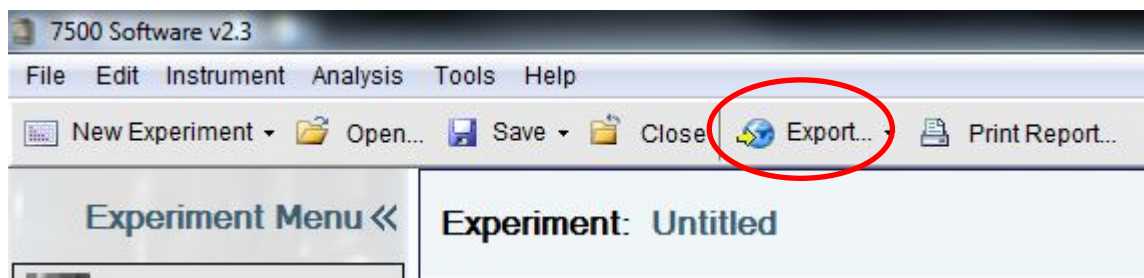
If repeating the same experiment more than once, it can be saved as template.

**Never save your run as a template before you've selected "Save As". If you do, your data will be lost!!!**

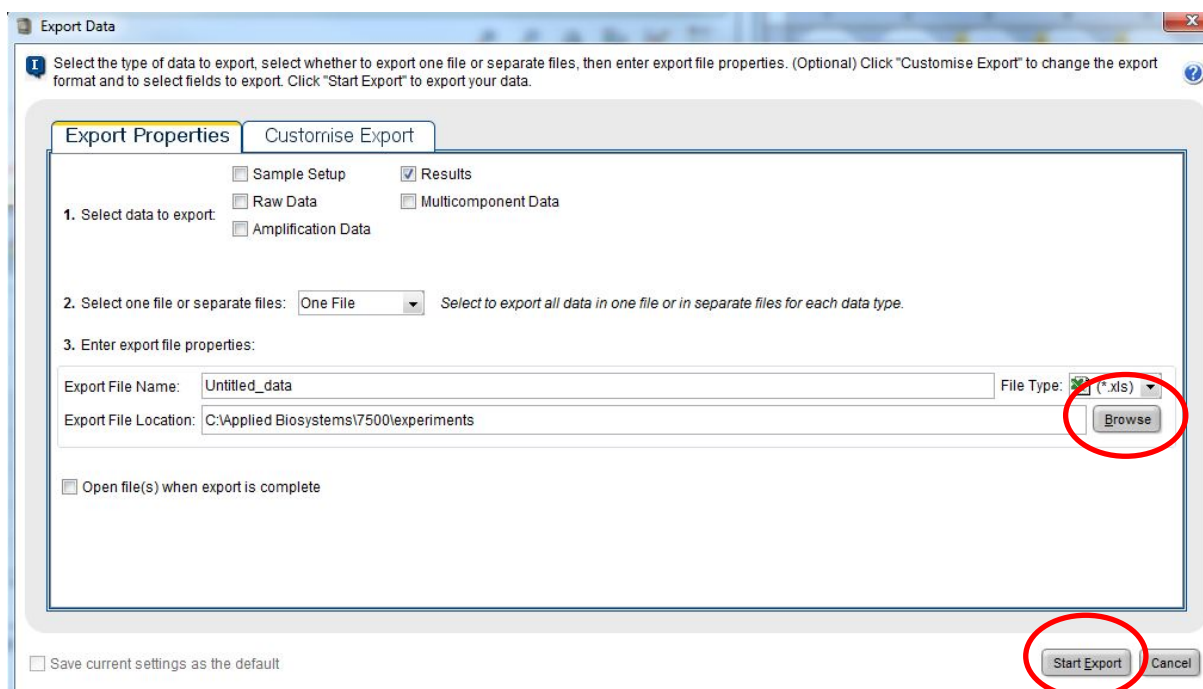


Export data:

Press on **Export**



This window should open



In 1. select which data you want to export (important: make sure you check “results”)

If you select more than one checkbox in 1. Then in 2. You can choose if all the data should be exported in one file or in separate files (samples setup, raw date, etc.).

In 3. choose a file name and a saving location (press browse).

Then press **Start Export**

After exporting all the data a window pops up asking whether you want to export more data or close the window. This window can be closed.

**Now remove plate from cyclor and throw away if not needed anymore!**

At the end turn off the Cyclor and the notebook.



Example of a qPCR pipette template

<b>eawag</b> aquatic research	Aphids quantitative PCR <b>Lab-Form Primer testing: Analysis</b>	<b>Aquatic Ecology</b>	
Created by Marco Thali	created on 17.03.2016	Version 2016.01	updated : 17.03.2016

**Primer testing with 2x SYBR Green I Master**

Reagents	Primer names and/or Aliquot / Lot No	Concentration	Date of preparation/dilution
SYBR Green I Master (Roche)	Product No. 04887352001	2 x	
Ap_DnaA_Fwd	5'-AAT GCT TGG ATC ATA ATT TAA AGA C-3' - 2558179	4.5 µM	05.04.2016
Ap_DnaA_Rev	5'-GTT TTG AAG AAA GAA ATG TTT CAA G-3' - 2558180	4.5 µM	05.04.2016
Ap_EF1a_Fwd	5'-TAG CAG TTA CAT CAA GAA AAT CGG-3' - 2558177	4.5 µM	05.04.2016
Ap_EF1a_Rev	5'-ATG TTG TCT CCA TTC CAT CCA G-3' - 2558178	4.5 µM	05.04.2016

Sample names	Concentration	Date / Date of dilution
see sample sheet file:		see sample sheet from:
	ng/µl	05.04.2016

**Procedure:**

1. Fill in name of PCR program, annealing temperature, no of PCR cycles, name of PCR machine, no of primer pairs and total no of PCR reactions below

<b>PCR program name</b> 2-Step	<b>Annealing temp (°C)</b> 57	<b>Nr of cycles</b> 50	<b>PCR machine</b> Cyder 11 - ABI 7500Fast
<b>Nr of primer pairs</b> 1	<b>Total Nr of PCR reactions</b> 96	<b>Plus volume (%)</b> 5	<b>Total volume of SYBR mix (µl)</b> 630.00

2. Prepare Mix by adding ddH<sub>2</sub>O (I) and 2x SYBR Green I Master (II) in separate tube
3. Add primers (III) / (IV) and Probe (V) and mix well
4. Distribute 10 µl of mix into PCR wells
5. Add 2.5 µl of sample DNA or positive/negative control into corresponding PCR well(s)

	Mix per primer	Mix per reaction	Final concentr.
ddH <sub>2</sub> O	126.0 µl (I)	1.25 µl	
SYBR Green I Master (Roche)	630.0 µl (II)	6.25 µl	1 x
Forward Primer	126.0 µl (III)	1.25 µl	0.450 µM
Reverse Primer	126.0 µl (IV)	1.25 µl	0.450 µM
<b>Total:</b>	<b>882.0 µl</b>	<b>10.00 µl</b>	
Sample DNA / controls		2.50 µl	0 ng/µl
Final volume		12.50 µl	

**PCR program 2-Step:**

Heated lid	105°C	
50 °C	2 min	
95 °C	10 min	
95 °C	15 sec	50x
57 °C	30 sec	
<b>PCR program mekting curve:</b>		
95 °C	15 sec	↓ 15 min
60 °C	60 sec	
95 °C		
10 °C	∞	

**Sample order:**

	Primer Fwd	Standardreihe	Proben										Primer Rev	Species	
A	Ap_DnaA_Fwd	10*9	10*9	A15-10	A15-10	A15-10	A15-383	A15-383					Ap_DnaA_Rev	Spiroplasma	A
B		10*8	10*8	A15-11	A15-11	A15-11	A15-385pl	A15-385pl							B
C		10*7	10*7	A15-17	A15-17	A15-17	A15-392gr	A15-392gr							C
D		10*6	10*6	A15-27	A15-27	A15-27	OX-C161	OX-C161							D
E		10*5	10*5	A15-198bis	A15-198bis	A15-198bis	A15-383	dtH2O							E
F		10*4	10*4	A15-316	A15-316	A15-316	A15-385pl	dtH2O							F
G		10*3	10*3	A15-364	A15-364	A15-364	A15-392gr								G
H		10*2	10*2	A15-370	A15-370	A15-370	OX-C161								H
		1	2	3	4	5	6	7	8	9	10	11	12		

Notes:

<b>Project Group:</b>	<b>Organism(s):</b> <i>Spiroplasma sp.</i>	<b>Date:</b>	<b>Person in charge:</b>
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