

#### **Document information**

Software: realplex 2.2

File Name: EPPENDORF\Lorenzo\QPCR09.05.2

Printed by: EPPENDORF
Created: May/09/2018 17:47

Serial No. Thermo Module: 6325 30387 Serial No. realplex Module.: 630011465

Acquisition Start Time: EPPENDORF May/09/2018 17:51
Acquisition End Time: EPPENDORF May/09/2018 19:20
Last updated: EPPENDORF Apr/06/2018 12:28

Background: Sarstedt-20µl Sep/12/2011 10:28 Color Calibration: SYBR Mar/12/2018 15:31

QPCR09.05.2018 Quantification May/09/2018 19:43

Inverted Data: OFF

Comment:

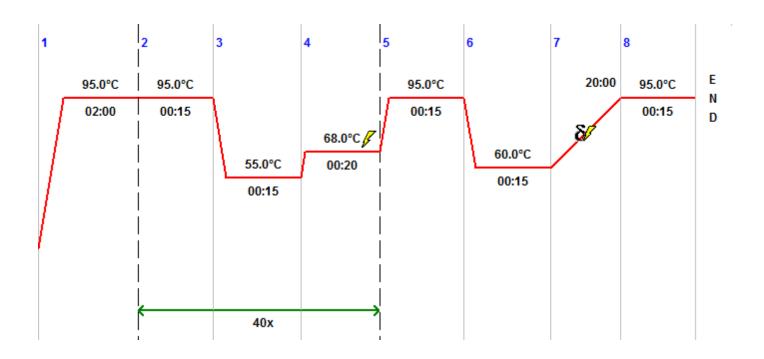


# **Plate layout**

|   | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      |
|---|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Α | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| В | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| С | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| D | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| E | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| F | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| G | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | CEWE    | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  | ILWE_A  |
|   | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 | 1: 1.00 |
| Н | NTC     | NTC     | NTC     | NTC     | NTC     | NTC     | water   | water   | water   | water   | water   | water   |



# **PCR Program**



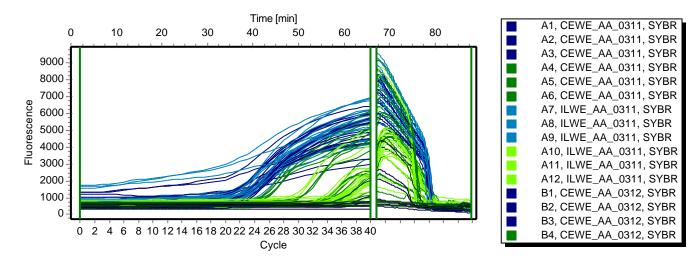
## **Program Header**

| Lid Temp   | 105 °C   | TSP Heated Lid                   | Yes |
|------------|----------|----------------------------------|-----|
| Temp. Mode | Standard | Switch off lid at low block temp | No  |
| Impulse    | No       | Simulate Mastercycler gradient   | No  |

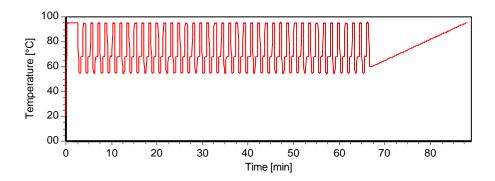


#### **Raw Data SYBR**

#### Fluorescence Profile



### **Temperature Profile**





## **Quantification SYBR**

| Pos             | Name         | Ct SYBR | Ct Mean<br>SYBR | Ct Dev.<br>SYBR | Amount<br>SYBR<br>[Copies] | Amount<br>Mean SYBR | Amount<br>Dev. SYBR | Target SYBR |
|-----------------|--------------|---------|-----------------|-----------------|----------------------------|---------------------|---------------------|-------------|
| ! <b></b> ■ A1  | CEWE_AA_0311 | 20.60   | 18.87           | 1.97            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> A2   | CEWE_AA_0311 | 16.73   | 18.87           | 1.97            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ A3   | CEWE_AA_0311 | 19.26   | 18.87           | 1.97            | 1.00                       |                     |                     | mouse       |
| ! <b></b> ■ A4  | CEWE_AA_0311 |         | 34.47           | 1.49            | 1.00                       |                     |                     | eimeria     |
| ! <b>□</b> ■A5  | CEWE_AA_0311 | 35.52   | 34.47           | 1.49            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ A6   | CEWE_AA_0311 | 33.42   | 34.47           | 1.49            | 1.00                       |                     |                     | eimeria     |
| ! <b>□</b> ►A7  | ILWE_AA_0311 | 20.75   | 20.68           | 0.07            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ A8   | ILWE_AA_0311 | 20.62   | 20.68           | 0.07            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ A9   | ILWE_AA_0311 | 20.66   | 20.68           | 0.07            | 1.00                       |                     |                     | mouse       |
| ! <b> </b>      | ILWE_AA_0311 | 36.00   | 36.16           | 0.37            | 1.00                       |                     |                     | eimeria     |
| ! <b>□</b>      | ILWE_AA_0311 | 36.59   | 36.16           | 0.37            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | ILWE_AA_0311 | 35.90   | 36.16           | 0.37            | 1.00                       |                     |                     | eimeria     |
| ! <b></b> ■ B1  | CEWE_AA_0312 | 23.17   | 21.58           | 1.39            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> B2   | CEWE_AA_0312 | 20.68   | 21.58           | 1.39            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> B3   | CEWE_AA_0312 | 20.88   | 21.58           | 1.39            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ B4   | CEWE_AA_0312 |         | 32.99           | 0.35            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> B5   | CEWE_AA_0312 | 33.24   | 32.99           | 0.35            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ B6   | CEWE_AA_0312 | 32.74   | 32.99           | 0.35            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ B7   | ILWE_AA_0312 | 23.83   | 23.39           | 0.48            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> ■B8  | ILWE_AA_0312 | 23.48   | 23.39           | 0.48            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> ■B9  | ILWE_AA_0312 | 22.88   | 23.39           | 0.48            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ B10  | ILWE_AA_0312 | 34.18   | 34.95           | 0.94            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | ILWE_AA_0312 | 34.68   | 34.95           | 0.94            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ B12  | ILWE_AA_0312 | 35.99   | 34.95           | 0.94            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> C1   | CEWE_AA_0313 | 22.22   | 21.29           | 0.81            | 1.00                       |                     |                     | mouse       |
| ! C2            | CEWE_AA_0313 | 20.74   | 21.29           | 0.81            | 1.00                       |                     |                     | mouse       |
| <b>i</b>        | CEWE_AA_0313 | 20.92   | 21.29           | 0.81            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> C4   | CEWE_AA_0313 |         | 33.38           | 0.64            | 1.00                       |                     |                     | eimeria     |
| ! C5            | CEWE_AA_0313 | 33.83   | 33.38           | 0.64            | 1.00                       |                     |                     | eimeria     |
| <b>i</b>        | CEWE_AA_0313 | 32.93   | 33.38           | 0.64            | 1.00                       |                     |                     | eimeria     |
| ! <b>[ C</b> 7  | ILWE_AA_0313 | 14.61   | 16.51           | 2.01            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0313 | 16.31   | 16.51           | 2.01            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0313 | 18.60   | 16.51           | 2.01            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> C10  | ILWE_AA_0313 | 34.90   | 34.41           | 0.58            | 1.00                       |                     |                     | eimeria     |
| ! <b>□</b> □C11 | ILWE_AA_0313 | 34.56   | 34.41           | 0.58            | 1.00                       |                     |                     | eimeria     |



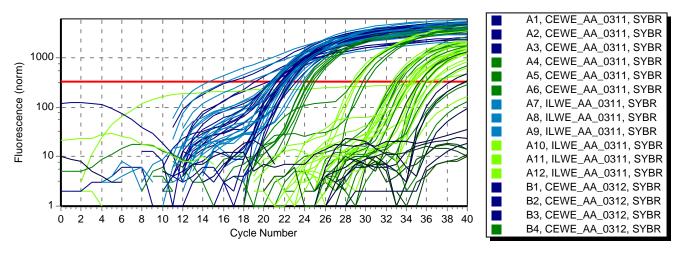
| Pos             | Name         | Ct SYBR | Ct Mean<br>SYBR | Ct Dev.<br>SYBR | Amount<br>SYBR<br>[Copies] | Amount<br>Mean SYBR | Amount<br>Dev. SYBR | Target SYBR |
|-----------------|--------------|---------|-----------------|-----------------|----------------------------|---------------------|---------------------|-------------|
| ! <b>∏</b> □C12 | ILWE_AA_0313 | 33.76   | 34.41           | 0.58            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> D1   | CEWE_AA_0314 | 21.72   | 21.43           | 0.25            | 1.00                       |                     |                     | mouse       |
| . □ D2          | CEWE_AA_0314 | 21.28   | 21.43           | 0.25            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> D3   | CEWE_AA_0314 | 21.29   | 21.43           | 0.25            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ D4   | CEWE_AA_0314 | 24.54   | 24.25           | 0.27            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> D5   | CEWE_AA_0314 | 24.02   | 24.25           | 0.27            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ D6   | CEWE_AA_0314 | 24.17   | 24.25           | 0.27            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ D7   | ILWE_AA_0314 | 22.16   | 21.98           | 0.16            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ D8   | ILWE_AA_0314 | 21.93   | 21.98           | 0.16            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■ D9   | ILWE_AA_0314 | 21.85   | 21.98           | 0.16            | 1.00                       |                     |                     | mouse       |
| <b>!</b> □ D10  | ILWE_AA_0314 | 33.02   | 32.88           | 0.26            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> □ D11  | ILWE_AA_0314 | 33.04   | 32.88           | 0.26            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> □ D12  | ILWE_AA_0314 | 32.59   | 32.88           | 0.26            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> E1   | CEWE_AA_0315 | 22.04   | 21.58           | 0.48            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> E2   | CEWE_AA_0315 | 21.62   | 21.58           | 0.48            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> E3   | CEWE_AA_0315 | 21.08   | 21.58           | 0.48            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> E4   | CEWE_AA_0315 | 35.99   | 35.91           | 1.46            | 1.00                       |                     |                     | eimeria     |
| ! <b>■</b> E5   | CEWE_AA_0315 | 37.32   | 35.91           | 1.46            | 1.00                       |                     |                     | eimeria     |
| ■E6             | CEWE_AA_0315 | 34.41   | 35.91           | 1.46            | 1.00                       |                     |                     | eimeria     |
| _<br>!∏         | ILWE_AA_0315 | 22.07   | 21.33           | 0.66            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> E8   | ILWE_AA_0315 | 20.84   | 21.33           | 0.66            | 1.00                       |                     |                     | mouse       |
| ! <b>■</b> E9   | ILWE_AA_0315 | 21.06   | 21.33           | 0.66            | 1.00                       |                     |                     | mouse       |
| <b>!</b> ■E10   | ILWE_AA_0315 | 37.52   | 36.13           | 1.21            | 1.00                       |                     |                     | eimeria     |
| ! <b>∏</b> □E11 | ILWE_AA_0315 | 35.42   | 36.13           | 1.21            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ■ E12  | ILWE_AA_0315 | 35.44   | 36.13           | 1.21            | 1.00                       |                     |                     | eimeria     |
| ! <b></b>       | CEWE_AA_0316 | 21.86   | 21.78           | 0.09            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | CEWE_AA_0316 | 21.80   | 21.78           | 0.09            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | CEWE_AA_0316 | 21.68   | 21.78           | 0.09            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | CEWE_AA_0316 | 25.06   | 24.84           | 0.20            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | CEWE_AA_0316 | 24.83   | 24.84           | 0.20            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | CEWE_AA_0316 | 24.65   | 24.84           | 0.20            | 1.00                       |                     |                     | eimeria     |
| ! <b>∏ □</b> F7 | ILWE_AA_0316 | 22.79   | 22.42           | 0.50            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0316 | 22.61   | 22.42           | 0.50            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0316 | 21.85   | 22.42           | 0.50            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0316 | 29.06   | 29.01           | 0.29            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | ILWE_AA_0316 | 29.28   | 29.01           | 0.29            | 1.00                       |                     |                     | eimeria     |
| -<br>!∏         | ILWE_AA_0316 | 28.71   | 29.01           | 0.29            | 1.00                       |                     |                     | eimeria     |
|                 | CEWE_AA_0317 | 22.68   | 22.61           | 0.16            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | CEWE_AA_0317 | 22.71   | 22.61           | 0.16            | 1.00                       |                     |                     | mouse       |



| Pos             | Name         | Ct SYBR | Ct Mean<br>SYBR | Ct Dev.<br>SYBR | Amount<br>SYBR<br>[Copies] | Amount<br>Mean SYBR | Amount<br>Dev. SYBR | Target SYBR |
|-----------------|--------------|---------|-----------------|-----------------|----------------------------|---------------------|---------------------|-------------|
| <b>!</b>        | CEWE_AA_0317 | 22.43   | 22.61           | 0.16            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | CEWE_AA_0317 | 30.15   | 29.59           | 0.67            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | CEWE_AA_0317 | 29.76   | 29.59           | 0.67            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | CEWE_AA_0317 | 28.85   | 29.59           | 0.67            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | ILWE_AA_0317 | 22.25   | 21.34           | 0.80            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0317 | 20.77   | 21.34           | 0.80            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0317 | 21.00   | 21.34           | 0.80            | 1.00                       |                     |                     | mouse       |
| <b>!</b>        | ILWE_AA_0317 | 36.67   | 35.55           | 1.58            | 1.00                       |                     |                     | eimeria     |
| <b>!</b> ☐ G11  | ILWE_AA_0317 |         | 35.55           | 1.58            | 1.00                       |                     |                     | eimeria     |
| <b>!</b>        | ILWE_AA_0317 | 34.43   | 35.55           | 1.58            | 1.00                       |                     |                     | eimeria     |
| <b>-</b> ■ H1   | NTC          | 39.92   |                 |                 | -                          |                     |                     | mouse       |
| <b>-</b> □ ■H2  | NTC          | -       |                 |                 | -                          |                     |                     | mouse       |
| <b>-</b> ■H3    | NTC          | -       |                 |                 | -                          |                     |                     | mouse       |
| <b>-□</b> ■H4   | NTC          | 35.36   |                 |                 | -                          |                     |                     | eimeria     |
| <b>-</b> □ ■H5  | NTC          | -       |                 |                 | -                          |                     |                     | eimeria     |
| <b>-</b> □ ■H6  | NTC          | -       |                 |                 | -                          |                     |                     | eimeria     |
| <b>-</b> □ ■H7  | water        | -       |                 |                 | -                          |                     |                     | mouse       |
| <b>-■</b> H8    | water        | 38.42   |                 |                 | -                          |                     |                     | mouse       |
| <b>-□</b> ■H9   | water        | -       |                 |                 | -                          |                     |                     | mouse       |
| <b>-</b> □ ■H10 | water        | -       |                 |                 | -                          |                     |                     | eimeria     |
| <b>-</b> □ ■H11 | water        | -       |                 |                 | -                          |                     |                     | eimeria     |
| <b>-</b> □ ■H12 | water        | -       |                 |                 | -                          |                     |                     | eimeria     |



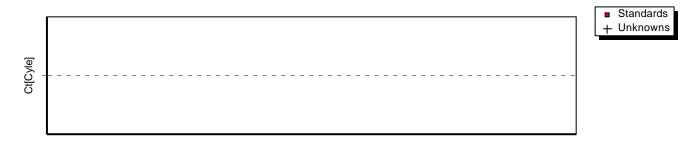
### **Amplification Plot**



Threshold 336 (Noiseband)

Baseline automatic, Drift correction OFF

#### Standard curve



Amount[Copies]

Slope - R^2 -Y-Intercept - Efficiency -