

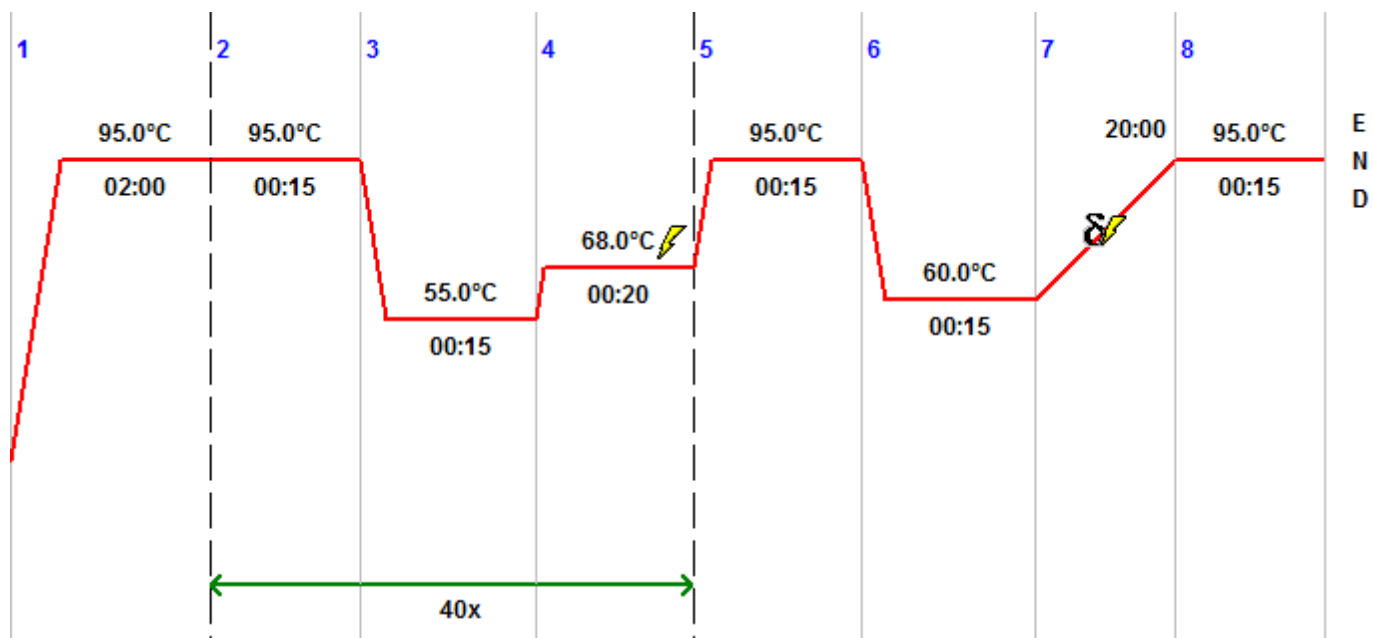
## Document information

|                              |                               |                   |
|------------------------------|-------------------------------|-------------------|
| Software:                    | realplex 2.2                  |                   |
| File Name:                   | EPPENDORF\Lorenzo\QPCR20.06.2 |                   |
| Printed by:                  | EPPENDORF                     |                   |
| Created:                     | Jun/20/2018 12:43             |                   |
| Serial No. Thermo Module:    | 6325 30387                    |                   |
| Serial No. realplex Module.: | 630011465                     |                   |
| Acquisition Start Time:      | EPPENDORF                     | Jun/20/2018 12:44 |
| Acquisition End Time:        | EPPENDORF                     | Jun/20/2018 14:12 |
| Last updated:                | EPPENDORF                     | Apr/06/2018 12:28 |
| Background:                  | Sarstedt-20µl                 | Sep/12/2011 10:28 |
| Color Calibration:           | SYBR                          | Mar/12/2018 15:31 |
| QPCR20.06.2018part2          | Quantification                | Jun/20/2018 14:13 |
| Inverted Data:               | OFF                           |                   |
| Comment:                     |                               |                   |

## Plate layout

|   | 1                   | 2                   | 3                   | 4                   | 5                   | 6                   | 7                   | 8                   | 9                   | 10                  | 11                  | 12                  |
|---|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| A | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| B | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| C | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| D | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| E | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| F | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| G | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 | CEWE_...<br>1: 1.00 |
| H | NTC                 | NTC                 | NTC                 | NTC                 | NTC                 | NTC                 | water               | water               | water               | water               | water               | water               |

## PCR Program

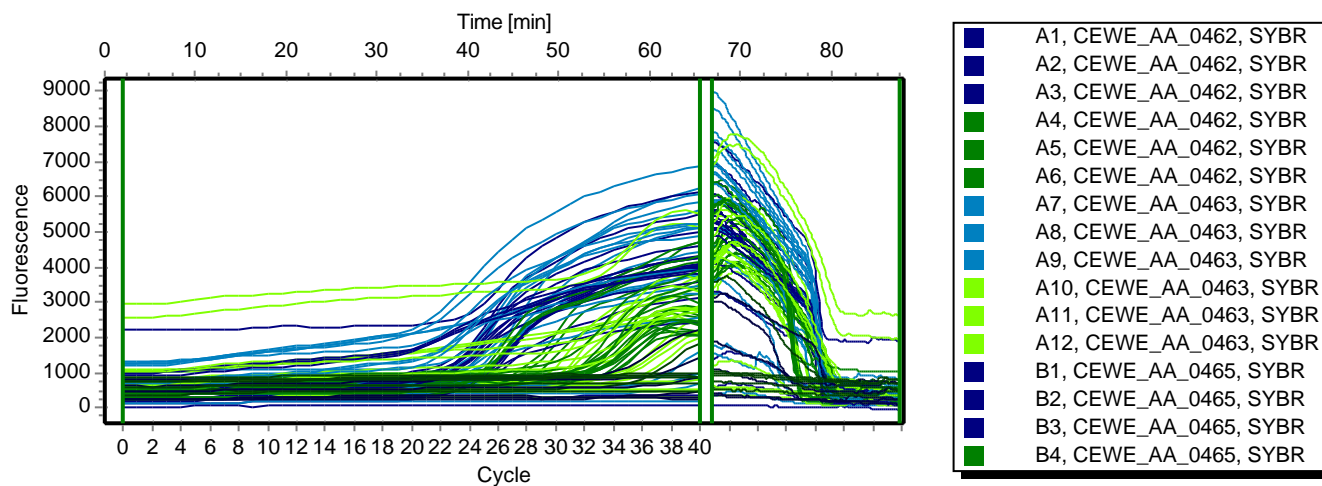


## Program Header

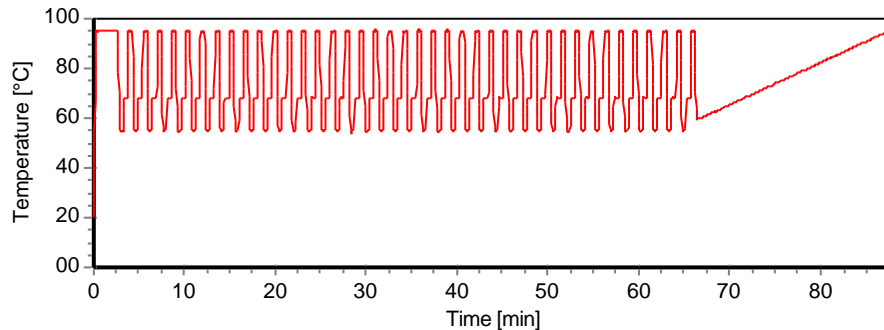
|            |          |                                  |     |
|------------|----------|----------------------------------|-----|
| Lid Temp   | 105 °C   | TSP Heated Lid                   | Yes |
| Temp. Mode | Standard | Switch off lid at low block temp | No  |
| Impulse    | No       | Simulate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

## Raw Data SYBR







































































### Fluorescence Profile







































































































### Temperature Profile



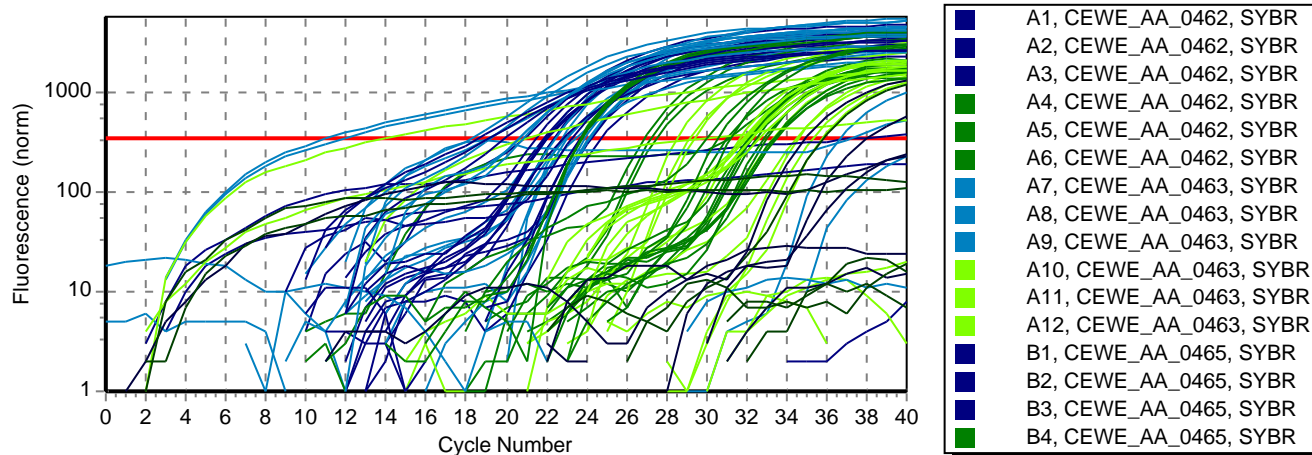
## Quantification SYBR

| Pos  | Name          | Ct SYBR | Ct Mean SYBR | Ct Dev. SYBR | Amount SYBR [Copies] | Amount Mean SYBR | Amount Dev. SYBR | Target SYBR |
|--|---------------|---------|--------------|--------------|----------------------|------------------|------------------|-------------|
|   A1      | CEWE_AA_0462  | 22.38   | 21.85        | 0.88         | 1.00                 |                  |                  | mouse       |
|   A2      | CEWE_AA_0462  | 22.35   | 21.85        | 0.88         | 1.00                 |                  |                  | mouse       |
|   A3      | CEWE_AA_0462  | 20.83   | 21.85        | 0.88         | 1.00                 |                  |                  | mouse       |
|   A4      | CEWE_AA_0462  | 32.28   | 31.97        | 0.74         | 1.00                 |                  |                  | eimeria     |
|   A5      | CEWE_AA_0462  | 31.13   | 31.97        | 0.74         | 1.00                 |                  |                  | eimeria     |
|   A6      | CEWE_AA_0462  | 32.50   | 31.97        | 0.74         | 1.00                 |                  |                  | eimeria     |
|   A7      | CEWE_AA_0463  | 22.43   | 22.72        | 0.28         | 1.00                 |                  |                  | mouse       |
|   A8      | CEWE_AA_0463  | 22.77   | 22.72        | 0.28         | 1.00                 |                  |                  | mouse       |
|   A9      | CEWE_AA_0463  | 22.98   | 22.72        | 0.28         | 1.00                 |                  |                  | mouse       |
|   A10     | CEWE_AA_0463  | 35.33   | 35.24        | 0.59         | 1.00                 |                  |                  | eimeria     |
|   A11     | CEWE_AA_0463  | 34.61   | 35.24        | 0.59         | 1.00                 |                  |                  | eimeria     |
|   A12     | CEWE_AA_0463  | 35.78   | 35.24        | 0.59         | 1.00                 |                  |                  | eimeria     |
|   B1  | CEWE_AA_0465  | 24.11   | 24.35        | 0.33         | 1.00                 |                  |                  | mouse       |
|   B2  | CEWE_AA_0465  | 24.21   | 24.35        | 0.33         | 1.00                 |                  |                  | mouse       |
|   B3  | CEWE_AA_0465  | 24.72   | 24.35        | 0.33         | 1.00                 |                  |                  | mouse       |
|   B4  | CEWE_AA_0465  | 26.86   | 27.19        | 0.32         | 1.00                 |                  |                  | eimeria     |
|   B5  | CEWE_AA_0465  | 27.50   | 27.19        | 0.32         | 1.00                 |                  |                  | eimeria     |
|   B6  | CEWE_AA_0465  | 27.21   | 27.19        | 0.32         | 1.00                 |                  |                  | eimeria     |
|   B7  | CEWE_AA_04666 | 21.90   | 22.76        | 0.79         | 1.00                 |                  |                  | mouse       |
|   B8  | CEWE_AA_04666 | 22.92   | 22.76        | 0.79         | 1.00                 |                  |                  | mouse       |
|   B9  | CEWE_AA_04666 | 23.45   | 22.76        | 0.79         | 1.00                 |                  |                  | mouse       |
|   B10 | CEWE_AA_0466  | 33.32   | 33.16        | 1.45         | 1.00                 |                  |                  | eimeria     |
|   B11 | CEWE_AA_0466  | 31.65   | 33.16        | 1.45         | 1.00                 |                  |                  | eimeria     |
|   B12 | CEWE_AA_0466  | 34.52   | 33.16        | 1.45         | 1.00                 |                  |                  | eimeria     |
|   C1  | CEWE_AA_0467  | 37.29   |              |              | 1.00                 |                  |                  | mouse       |
|   C2  | CEWE_AA_0467  |         |              |              | 1.00                 |                  |                  | mouse       |
|   C3  | CEWE_AA_0467  |         |              |              | 1.00                 |                  |                  | mouse       |
|   C4  | CEWE_AA_0467  | 33.42   | 32.47        | 1.29         | 1.00                 |                  |                  | eimeria     |
|   C5  | CEWE_AA_0467  | 31.00   | 32.47        | 1.29         | 1.00                 |                  |                  | eimeria     |
|   C6  | CEWE_AA_0467  | 33.00   | 32.47        | 1.29         | 1.00                 |                  |                  | eimeria     |
|   C7  | CEWE_AA_0469  | 23.90   | 24.02        | 0.11         | 1.00                 |                  |                  | mouse       |
|   C8  | CEWE_AA_0469  | 24.12   | 24.02        | 0.11         | 1.00                 |                  |                  | mouse       |
|   C9  | CEWE_AA_0469  | 24.06   | 24.02        | 0.11         | 1.00                 |                  |                  | mouse       |
|   C10 | CEWE_AA_0469  | 28.52   | 29.02        | 0.45         | 1.00                 |                  |                  | eimeria     |
|   C11 | CEWE_AA_0469  | 29.37   | 29.02        | 0.45         | 1.00                 |                  |                  | eimeria     |

| Pos  | Name         | Ct SYBR | Ct Mean SYBR | Ct Dev. SYBR | Amount SYBR [Copies] | Amount Mean SYBR | Amount Dev. SYBR | Target SYBR |
|--|--------------|---------|--------------|--------------|----------------------|------------------|------------------|-------------|
|   C12     | CEWE_AA_0469 | 29.16   | 29.02        | 0.45         | 1.00                 |                  |                  | eimeria     |
|   D1      | CEWE_AA_0471 | 21.81   | 22.21        | 0.51         | 1.00                 |                  |                  | mouse       |
|   D2      | CEWE_AA_0471 | 22.02   | 22.21        | 0.51         | 1.00                 |                  |                  | mouse       |
|   D3      | CEWE_AA_0471 | 22.78   | 22.21        | 0.51         | 1.00                 |                  |                  | mouse       |
|   D4      | CEWE_AA_0471 | 32.85   | 33.23        | 0.33         | 1.00                 |                  |                  | eimeria     |
|   D5      | CEWE_AA_0471 | 33.38   | 33.23        | 0.33         | 1.00                 |                  |                  | eimeria     |
|   D6      | CEWE_AA_0471 | 33.46   | 33.23        | 0.33         | 1.00                 |                  |                  | eimeria     |
|   D7      | CEWE_AA_0470 | 36.66   | 36.88        | 0.31         | 1.00                 |                  |                  | mouse       |
|   D8      | CEWE_AA_0470 |         | 36.88        | 0.31         | 1.00                 |                  |                  | mouse       |
|   D9      | CEWE_AA_0470 | 37.10   | 36.88        | 0.31         | 1.00                 |                  |                  | mouse       |
|   D10     | CEWE_AA_0470 |         |              |              | 1.00                 |                  |                  | eimeria     |
|   D11     | CEWE_AA_0470 |         |              |              | 1.00                 |                  |                  | eimeria     |
|   D12     | CEWE_AA_0470 |         |              |              | 1.00                 |                  |                  | eimeria     |
|   E1      | CEWE_AA_0490 | 21.43   | 21.79        | 0.32         | 1.00                 |                  |                  | mouse       |
|   E2    | CEWE_AA_0490 | 21.94   | 21.79        | 0.32         | 1.00                 |                  |                  | mouse       |
|   E3  | CEWE_AA_0490 | 22.01   | 21.79        | 0.32         | 1.00                 |                  |                  | mouse       |
|   E4  | CEWE_AA_0490 | 32.08   | 32.13        | 0.05         | 1.00                 |                  |                  | eimeria     |
|   E5  | CEWE_AA_0490 | 32.13   | 32.13        | 0.05         | 1.00                 |                  |                  | eimeria     |
|   E6  | CEWE_AA_0490 | 32.19   | 32.13        | 0.05         | 1.00                 |                  |                  | eimeria     |
|   E7  | CEWE_AA_0489 | 11.09   | 11.50        | 0.58         | 1.00                 |                  |                  | mouse       |
|   E8  | CEWE_AA_0489 | 11.91   | 11.50        | 0.58         | 1.00                 |                  |                  | mouse       |
|   E9  | CEWE_AA_0489 |         | 11.50        | 0.58         | 1.00                 |                  |                  | mouse       |
|   E10 | CEWE_AA_0489 | 29.54   | 21.54        | 7.76         | 1.00                 |                  |                  | eimeria     |
|   E11 | CEWE_AA_0489 | 14.03   | 21.54        | 7.76         | 1.00                 |                  |                  | eimeria     |
|   E12 | CEWE_AA_0489 | 21.04   | 21.54        | 7.76         | 1.00                 |                  |                  | eimeria     |
|   F1  | CEWE_AA_0497 | 23.69   | 23.54        | 0.23         | 1.00                 |                  |                  | mouse       |
|   F2  | CEWE_AA_0497 | 23.65   | 23.54        | 0.23         | 1.00                 |                  |                  | mouse       |
|   F3  | CEWE_AA_0497 | 23.27   | 23.54        | 0.23         | 1.00                 |                  |                  | mouse       |
|   F4  | CEWE_AA_0497 | 23.73   | 23.72        | 0.02         | 1.00                 |                  |                  | eimeria     |
|   F5  | CEWE_AA_0497 | 23.71   | 23.72        | 0.02         | 1.00                 |                  |                  | eimeria     |
|   F6  | CEWE_AA_0497 | 23.70   | 23.72        | 0.02         | 1.00                 |                  |                  | eimeria     |
|   F7  | CEWE_AA_0491 | 18.32   | 18.92        | 0.52         | 1.00                 |                  |                  | mouse       |
|   F8  | CEWE_AA_0491 | 19.28   | 18.92        | 0.52         | 1.00                 |                  |                  | mouse       |
|   F9  | CEWE_AA_0491 | 19.17   | 18.92        | 0.52         | 1.00                 |                  |                  | mouse       |
|   F10 | CEWE_AA_0491 | 31.79   | 32.00        | 0.22         | 1.00                 |                  |                  | eimeria     |
|   F11 | CEWE_AA_0491 | 31.99   | 32.00        | 0.22         | 1.00                 |                  |                  | eimeria     |
|   F12 | CEWE_AA_0491 | 32.24   | 32.00        | 0.22         | 1.00                 |                  |                  | eimeria     |
|   G1  | CEWE_AA_0499 | 18.65   | 19.24        | 0.63         | 1.00                 |                  |                  | mouse       |
|   G2  | CEWE_AA_0499 | 19.91   | 19.24        | 0.63         | 1.00                 |                  |                  | mouse       |

| Pos  | Name         | Ct SYBR | Ct Mean SYBR | Ct Dev. SYBR | Amount SYBR [Copies] | Amount Mean SYBR | Amount Dev. SYBR | Target SYBR |
|--|--------------|---------|--------------|--------------|----------------------|------------------|------------------|-------------|
|  G3    | CEWE_AA_0499 | 19.15   | 19.24        | 0.63         | 1.00                 |                  |                  | mouse       |
|  G4    | CEWE_AA_0499 | 30.56   | 30.58        | 0.61         | 1.00                 |                  |                  | eimeria     |
|  G5    | CEWE_AA_0499 | 29.99   | 30.58        | 0.61         | 1.00                 |                  |                  | eimeria     |
|  G6    | CEWE_AA_0499 | 31.20   | 30.58        | 0.61         | 1.00                 |                  |                  | eimeria     |
|  G7    | CEWE_AA_0498 | 20.76   | 20.66        | 0.09         | 1.00                 |                  |                  | mouse       |
|  G8    | CEWE_AA_0498 | 20.61   | 20.66        | 0.09         | 1.00                 |                  |                  | mouse       |
|  G9    | CEWE_AA_0498 | 20.61   | 20.66        | 0.09         | 1.00                 |                  |                  | mouse       |
|  G10   | CEWE_AA_0498 | 32.22   | 32.25        | 0.34         | 1.00                 |                  |                  | eimeria     |
|  G11   | CEWE_AA_0498 | 31.93   | 32.25        | 0.34         | 1.00                 |                  |                  | eimeria     |
|  G12   | CEWE_AA_0498 | 32.61   | 32.25        | 0.34         | 1.00                 |                  |                  | eimeria     |
|  H1    | NTC          | -       |              |              | -                    |                  |                  | mouse       |
|  H2    | NTC          | -       |              |              | -                    |                  |                  | mouse       |
|  H3    | NTC          | -       |              |              | -                    |                  |                  | mouse       |
|  H4    | NTC          | -       |              |              | -                    |                  |                  | eimeria     |
|  H5    | NTC          | 35.84   |              |              | -                    |                  |                  | eimeria     |
|  H6   | NTC          | -       |              |              | -                    |                  |                  | eimeria     |
|  H7  | water        | -       | 36.44        | 2.15         | -                    |                  |                  | mouse       |
|  H8  | water        | 37.96   | 36.44        | 2.15         | -                    |                  |                  | mouse       |
|  H9  | water        | 34.92   | 36.44        | 2.15         | -                    |                  |                  | mouse       |
|  H10 | water        | -       |              |              | -                    |                  |                  | eimeria     |
|  H11 | water        | -       |              |              | -                    |                  |                  | eimeria     |
|  H12 | water        | -       |              |              | -                    |                  |                  | eimeria     |

## Amplification Plot



## Standard curve



Slope - R<sup>2</sup> -

Y-Intercept - Efficiency -