



CAMPER

Calculation of Amplification Efficiencies for RT-PCR experiments

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Overview

- Motivation
- CAmpER features
- Summary
- Outlook


Motivation

- High throughput qPCR experiments generate huge amounts of data
- Standardized storage of experiment and results
- Standardized experiment annotation system
- Manual efficiency calculation using e.g. Excel spreadsheets time consuming
- Calculation of amplification efficiencies needed


Technical overview

- Implemented in Perl
- Object-oriented data model
- Based on relational database (MySQL)
- Web-frontend as user-interface

Upload and annotation



CAMPeR

 You are about to upload an Lightcycler experiment. Proceed or choose another option from the sidebar.

Upload Experiments

Lightcycler Upload
Opticon Upload

Calculation

Calculate efficiency - DART
Calculate efficiency - FPLM

Gene expression

Geneexpression (DART-Effis)
Geneexpression (FPLM-Effis)
Geneexpression (all Effis)

Overview


Experiment Details
RNA Details

Camper_test

Upload an Lightcycler experiment:

Step 1: Please insert the files to upload.
The following files are needed by CAMPeR:

- ◆ **.FLO-file** The .FLO-file given by the Lightcycler-Machine;
- ◆ **.ABT-file** The .ABT-file given by the Lightcycler-Machine;
- ◆ **Protocol-file** A protocol-file defining the terms of the Lightcycler-runs.

 **.FLO-file and .ABT-file are mandatory and must have the same name except for the file-ending. The protocol-file is also mandatory, its recommended to use a defined format for the protocol-files.**

You can find a set of sample-files at `'/vol/bioinfo/share/camper_data_set'` if you don't have access to lightcycler experiments.

.FLO-file

.ABT-file

Protocol-file

Supported systems

- Adjusted for different qPCR–Systems:
 - Lightcycler® (Roche Diagnostics)
 - parsing output files (.ABT, .FLO)
 - Opticon® (Bio–Rad Laboratories, Inc.)
 - Opticon MONITOR™ exports TAB separated values
 - parsing these TAB separated values
- Experimental data parsed automatically
- Software extendable to support more systems

Experiment annotation

- Annotation of RNA
- Annotation for each sample of an experiment
- Comparable annotation of experiment setup

http://gimli:1155/software/camper/cgi-bin/camper.cgi

You are actually uploading experiments, please finish the upload-process before doing anything else.

Annotate the used RNA

Please be sure to have filled out all fields marked by "*" before submitting

Unique name: name of the RNA
 *

Creation-date: date of the RNA-preparation (dd.mm.yyyy)
 *

Operator: the person who performed the purification
 *

Organism: the organism from which the RNA is extracted
 *

Tissue: the tissue from which the RNA is extracted

Growth-conditions: the growth conditions in the experiment
 *


Volume: the volume used in µl
 *


Concentration: the concentration in ng/µl
 *

Username: jblom
 Role: Chief
 Project: Camper_test

[Change project.](#)
[Report a bug \(Bugzilla\).](#)
[Logout.](#)

Efficiency calculation



 You can upload real-time PCR experiments to the CAmpER-DB.

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Amplification efficiencies for the DART method

Experiment 5_samples_dilution_series:

Mean efficiency: 1.8043
Standard deviation: 0.0787

Efficiencies deviating more than the standard deviation from the mean are colored red.

Sample number:	Sample name:	Crossing point:	Efficiency:
6	230_0	21.717	1.652
7	230_-2	21.071	1.823
8	230_-4	21.875	1.840
9	230_-6	23.446	1.827
10	230_-8	24.779	1.880

You can export the efficiencies as TAB separated values to a .tsv file

You can view plots of the sample data

Efficiency calculation

- Amplification efficiency = ratio of DNA amplification
- Theoretical amplification efficiency should be 2
- In practice mostly between 1.5 and 1.9
- High influence on calculation of gene expression

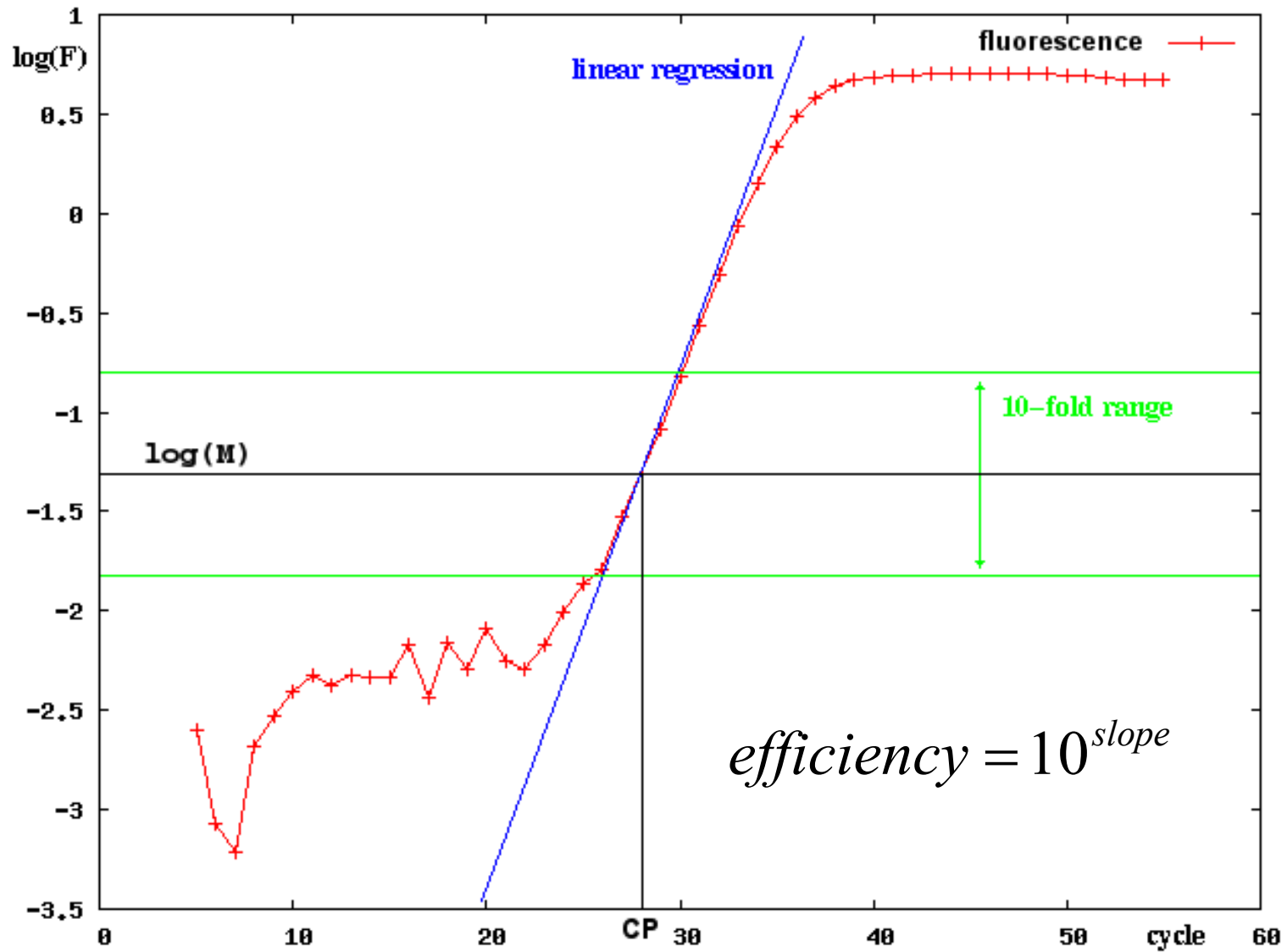
Efficiency calculation

- Several algorithms published
- Two algorithms used in CAmpER:
 - DART-PCR¹ (Data Analysis RT-PCR)
 - Slope of the log-linear phase
 - FPLM method²
 - Four Parametric Logistic Model
 - Modelling of the exponential phase
- Efficiency calculation for each sample

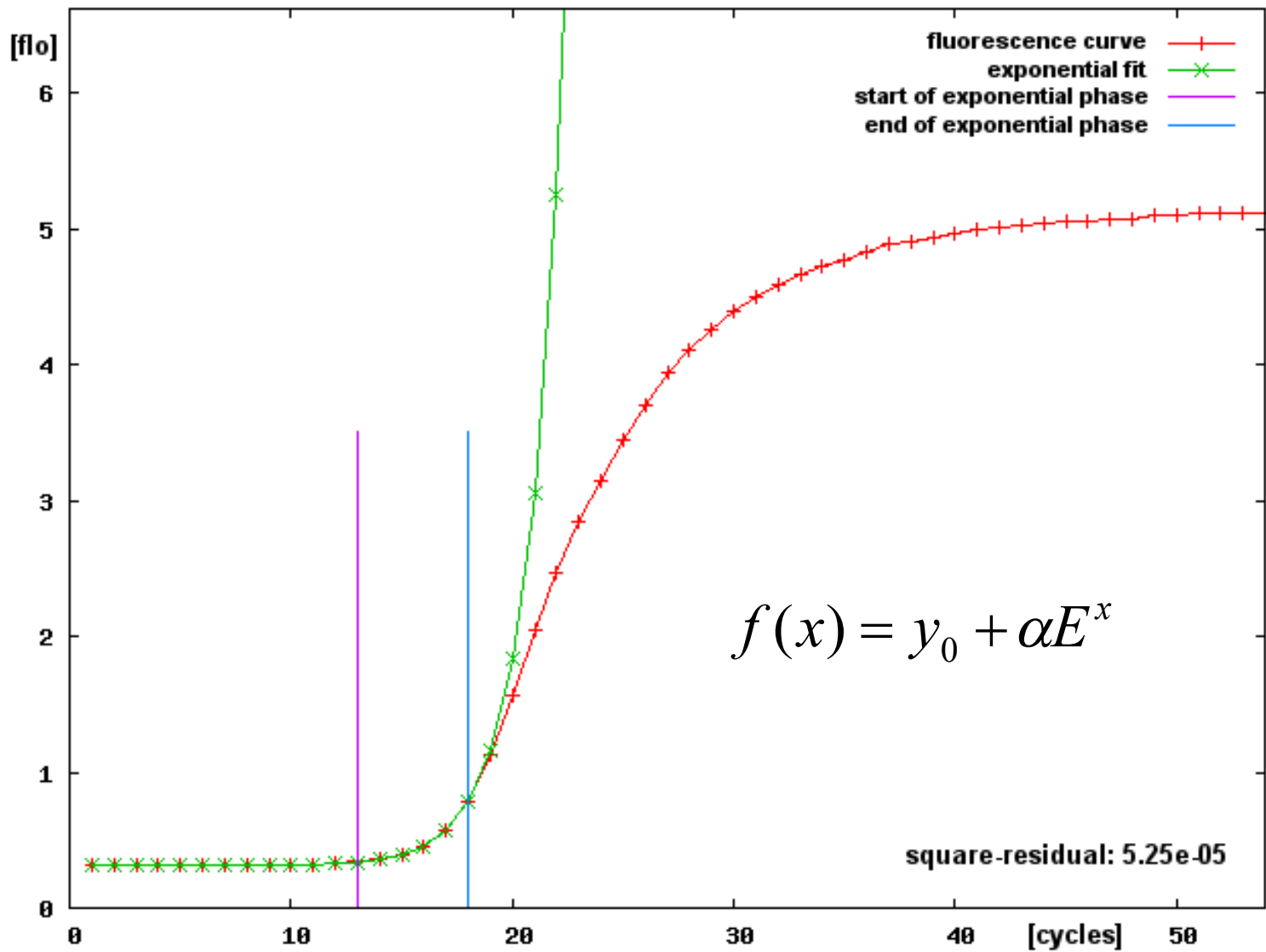
¹ Peirson et al., 2003

² Tichopad et al., 2003


DART-PCR




FPLM method



Gene expression calculation



CAmpER

 You are about to calculate relative gene expression ratios.

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Relative gene expression ratio for the DART efficiencies

Experiment 5_samples_dilution_series:

Gene expression ratios of the listed samples compared to reference sample230_-8

Reference efficiency: 1.8798
 Reference CP: 24.7793
 Reference cCP: 22.5643

Sample number:	Sample name:	Efficiency:	corrected CP:	Expression ratio:
6	230_0	1.652	15.729	114.160
7	230_-2	1.823	18.254	19.841
8	230_-4	1.840	19.238	10.028
9	230_-6	1.827	20.391	4.510
10	230_-8	1.880	22.564	1.000


You can export the expression ratios as TAB separated values to a .tsv-file.



Relative gene expression ratios

- Expression in relation to a reference sample
 - Calculation using the CP difference: $Ratio = 2^{(CP_{reference} - CP_{sample})}$
- > Inaccurate if amplification efficiencies vary
- Normalization to an efficiency of 2 : $cCP = \log_2(E^{CP})$
 - Calculation using the cCP difference: $Ratio = 2^{(cCP_{reference} - cCP_{sample})}$

Visualization



[Get a general overview of the experiments.](#)

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Amplification efficiencies for Experiment "5_samples_dilution_series".

Click on the RNA name to view the RNA annotation details.
Efficiencies marked red deviate more than the standard deviation from the mean efficiency.

LC_DART:
Mean efficiency: 1.804, SD: 0.079

FPLM_modified:
Mean efficiency: 1.783, SD: 0.086

Sample number:	Sample name:	Used RNA:	CP FPLM_modified:	Efficiency FPLM_modified:	CP LC_DART:	Efficiency LC_DART:
6	230_0	5_sams_dil	24.771	1.612	21.717	1.652
7	230_-2	5_sams_dil	23.882	1.816	21.071	1.823
8	230_-4	5_sams_dil	25.067	1.841	21.875	1.840
9	230_-6	5_sams_dil	26.205	1.812	23.446	1.827
10	230_-8	5_sams_dil	27.929	1.832	24.779	1.880

You can view plots for the samples of the experiment.

[Plot data for the FPLM_modified method](#)

[Plot data for the LC_DART method](#)

You can download the files this Experiment was uploadet from.

[Download FLO-file](#) [Download ABT-file](#)

Plotting experiment data

View plots for the chosen experiment.

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Experiment Details

RNA Details

Username: jblom

Role: Chief

Project: Camper_test

Change project.

Report a bug (Bugzilla).

Logout.

Please use the plot Buttons in the table.

FLO = Fluorescence curve

MELT = Melting curve

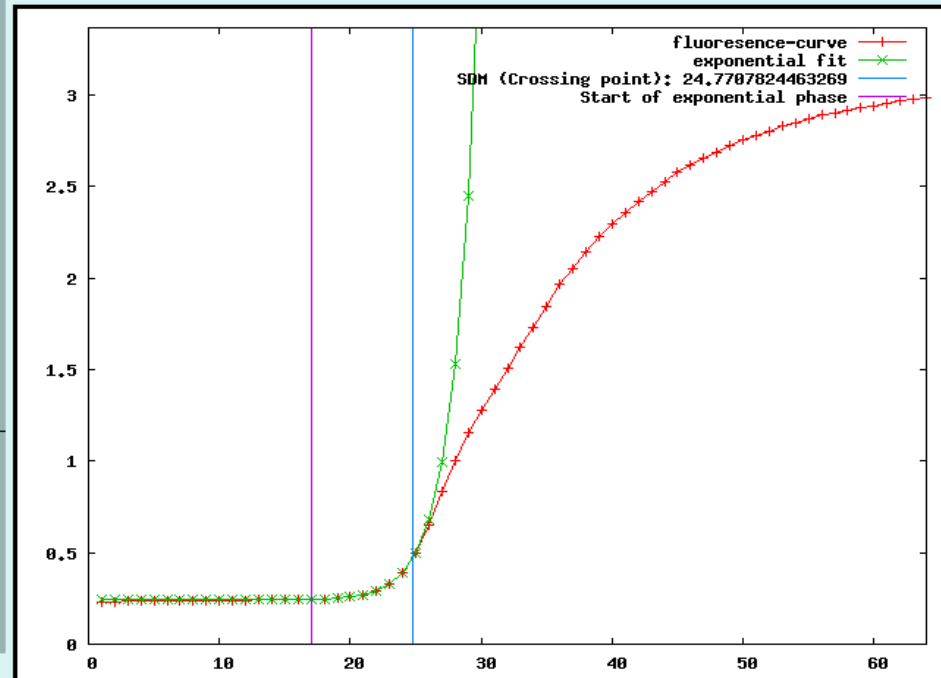
LOG = Logistic Fit

EXP = Exponential Fit

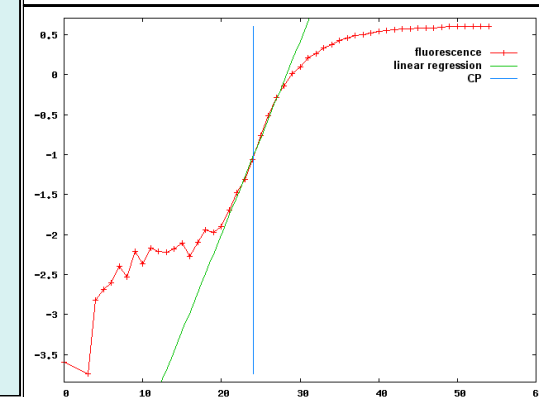
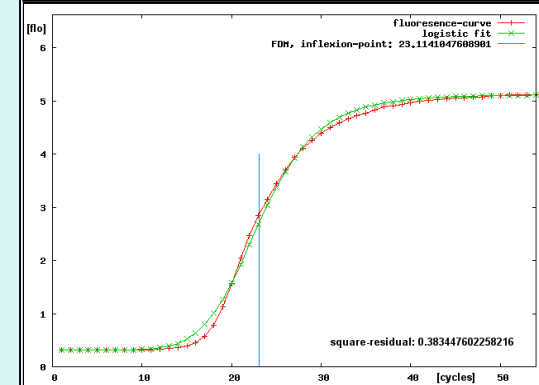
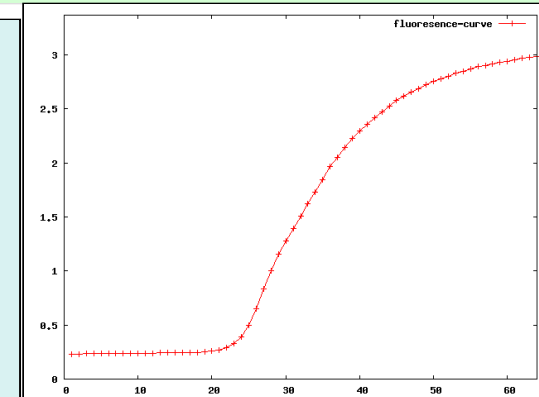
If one button is missing this plot is not available for the used efficiency calculation method.

Sample number:	Sample name:	Crossing point:	Efficiency:	Plots:			
6	230_0	24.771	1.710	FLO	MELT	LOG	EXP
7	230_-2	23.882	1.878	FLO	MELT	LOG	EXP
8	230_-4	25.067	1.817	FLO	MELT	LOG	EXP
9	230_-6	26.205	1.860	FLO	MELT	LOG	EXP
10	230_-8	27.929	1.887	FLO	MELT	LOG	EXP

Sample: 230_0



Square residual : 6.796e-06

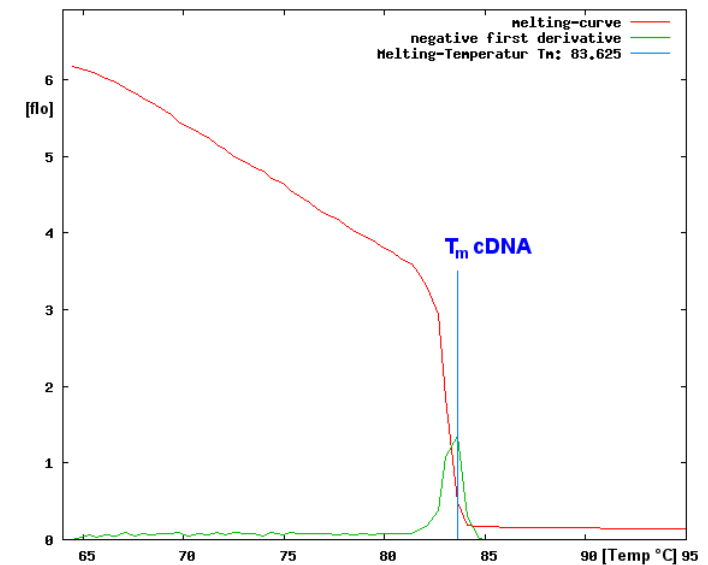
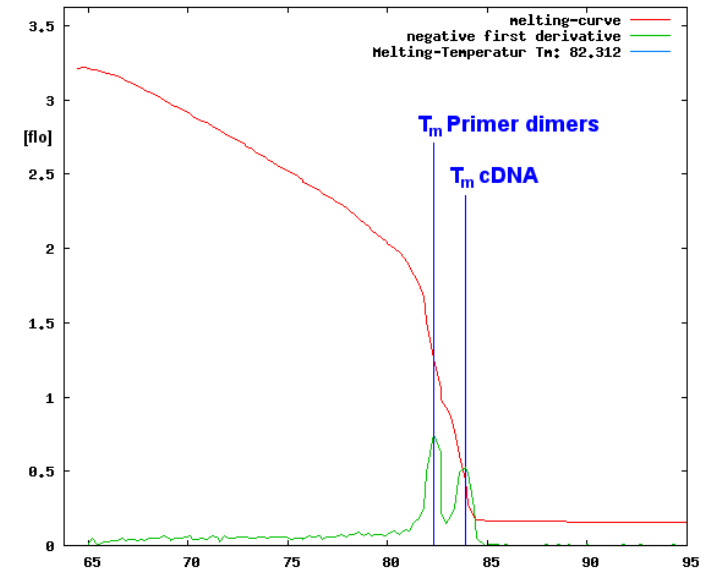


Melting curves

- Quality control
- Some dyes (e.g. Sybr Green I) detect double-stranded DNA
- Primer dimers lead to rising fluorescence without cDNA amplification
- Melting curves used to detect primer dimerisation

Melting curves:

- Primer dimers are short
-> low melting point
- cDNAs are longer
-> higher melting point
- Two melting points indicate primer dimerisation



Data export

- All calculated data can be exported
- Export as TAB separated values

```
Camper version : 1.01
.tsv-file created at: 20.03.2007
Experiment: 5_samples_dilution_series
Uploaded at: 16.03.2007
Uploaded by: jblom
Analysis method: LC_DART
Mean efficiency: 1.80437184426568
Standard deviation: 0.078748394497607
```

Amplification efficiencies:

samples: number:	name:	CP:	efficiency:
6	230_0	21,7172305175423	1,65208110974444
7	230_-2	21,0714068567886	1,82296761395418
8	230_-4	21,8750192197381	1,83969645622661
9	230_-6	23,4464669214745	1,82726932076248
10	230_-8	24,7793071860244	1,87984472064067

User management

- User management integrated

- Including project management system

- User roles with different rights
 - Admin (all rights)
 - Developer (extended rights, e.g. delete)
 - User (basic read/write rights)
 - Guest (read only)

Summary of CAmpER features



- Parse qPCR data of different systems
- Basic LIMS functionality
- Calculate amplification efficiencies & gene expression
- Plot fluorescence, fitted models and melting curves
- User and project management
- Consistent storage of uploaded and calculated data
 - MySQL database
 - File storage system for uploaded files

Outlook:

- Launch of web-application (april 2007)
www.cebitec.uni-bielefeld.de/groups/brf/software/
- Multiple reference genes in gene expression calculation
- Database search functionality
- Include more qPCR systems

Acknowledgements

- Bioinformatics Resource Facility
 - Alexander Goesmann
 - Burkhardt Linke
 - The whole team
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