

ReadqPCR: Functions to load RT-qPCR data into R

James Perkins
University of Malaga

Matthias Kohl
Furtwangen University

July 3, 2024

Contents

1	Introduction	1
2	read.LC480	2
3	read.qPCR	7
4	read.taqman	8
5	qPCRBatch	9

1 Introduction

The package "ReadqPCR" contains different functions for reading qPCR data into R.

As well as the functions to read in the data, "ReadqPCR" contains the `qPCRBatch` and `CyclesSet` class definition. The data output by these RT-qPCR systems is in the form of fluorescence values or quantification cycle (Cq values), which represents the number of cycles of amplification needed in order to detect the expression of a given gene from a sample.

"ReadqPCR" is designed to be complementary to another R package, "NormqPCR", which is intended for the normalisation of qPCR data. It must be installed before the other module.

We load the "ReadqPCR" package.

```
> library(ReadqPCR)
```

2 read.LC480

With function `read.LC480` raw fluorescence data of Roche LightCycler 480 may be read in. The result is saved in an object of class `CyclesSet`. At the moment the function works only with the data exported to a txt-file, but we plan to add support also for the xml-export.

```
> path <- system.file("exData", package = "ReadqPCR")
> LC480.example <- file.path(path, "LC480_Example.txt")
> cycData <- read.LC480(file = LC480.example)
> ## Fluorescence data
> head(exprs(cycData))
```

	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1	15.58	16.93	27.32	16.60	16.88	27.93	15.37	17.27	27.26	15.91	41594.00	25.74
2	15.53	16.96	27.69	16.68	16.91	28.23	15.47	17.35	27.59	15.87	16.17	25.76
3	15.49	16.93	27.56	16.67	16.95	28.27	15.41	17.32	27.60	15.83	16.23	25.77
4	15.47	16.97	27.62	16.63	16.87	28.22	15.42	17.28	27.62	15.83	16.17	25.70
5	15.47	16.88	27.56	16.65	16.92	28.22	15.38	17.31	27.52	15.74	16.18	25.64
6	15.41	16.90	27.55	16.65	16.87	28.20	15.41	17.27	27.57	15.76	16.23	25.70
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	
1	16.61	17.74	27.15	17.57	17.67	29.30	41322	41625.00	26.84	16.93	16.64	
2	16.67	17.80	27.42	17.61	17.71	29.76	41322	17.19	27.58	41625.00	16.68	
3	16.57	17.78	27.47	17.64	17.73	29.74	41322	17.16	27.58	41350.00	16.64	
4	16.56	17.72	27.49	17.57	17.64	29.87	41442	17.22	27.61	17.14	16.57	
5	16.52	17.68	27.44	17.59	17.62	29.78	17	41625.00	27.56	41472.00	16.57	
6	16.59	17.69	27.50	17.54	17.62	29.90	41564	17.22	27.61	17.14	16.56	
	B12	C1	C2	C3	C4	C5	C6	C7	C8	C9		
1	41512.00	41322.00	16.60	27.13	41292.00	16.77	26.88	17.43	16.96	27.86		
2	26.35	16.89	16.55	27.43	18.14	16.77	27.19	17.50	41442.00	28.19		
3	26.21	16.86	16.54	27.46	41473.00	16.81	41574.00	17.50	16.99	28.22		
4	26.27	16.81	16.51	27.38	41504.00	16.83	27.17	17.43	41564.00	41606.00		
5	26.22	16.79	16.43	27.48	41443.00	16.87	27.16	17.47	41322.00	28.15		
6	26.15	16.84	16.44	27.47	41626.00	16.86	27.14	17.51	41291.00	28.17		
	C10	C11	C12	D1	D2	D3	D4	D5	D6	D7	D8	
1	41443.00	16.49	25.92	17.22	16.80	27.25	17.66	17.18	27.41	17.42	17.22	
2	41535.00	16.53	26.22	17.28	16.76	27.62	17.69	17.26	27.59	17.49	17.14	
3	18.00	16.57	26.30	41564.00	16.72	27.51	17.71	17.26	27.55	17.40	17.26	
4	41443.00	16.55	26.24	41595.00	16.76	27.61	17.62	17.30	27.72	17.50	17.27	
5	18.00	16.54	41390.00	41534.00	16.72	27.45	17.73	17.27	27.66	17.49	17.27	
6	17.98	16.49	41451.00	41291.00	16.71	27.47	17.64	17.29	27.69	17.45	17.20	
	D9	D10	D11	D12	E1	E2	E3	E4	E5	E6	E7	
1	27.28	17.47	16.63	41332.00	27.52	17.80	26.51	28.69	17.62	27.48	27.64	

2	27.74	17.46	16.70	27.38	27.78	17.79	26.84	29.00	17.67	27.79	28.00	
3	27.74	17.40	16.66	27.38	27.69	17.75	26.88	28.99	17.71	27.89	27.99	
4	27.91	17.51	16.76	27.42	27.61	17.71	26.90	41423.00	17.72	27.93	41361.00	
5	27.82	17.43	16.69	27.33	27.63	17.79	26.82	41393.00	17.74	27.95	41483.00	
6	27.87	17.46	16.69	27.41	27.59	17.76	26.84	41362.00	17.77	41302.00	28.14	
	E8	E9	E10	E11	E12	F1	F2	F3	F4	F5		
1	16.97	41361.00	27.17	16.69	26.82	41605.00	41473.00	28.28	28.24	41442.00		
2	41442.00	28.53	27.41	16.88	27.20	27.25	18.15	28.65	28.65	41472.00		
3	41442.00	28.51	27.33	16.83	27.19	26.99	41535.00	28.61	28.64	41381.00		
4	41534.00	28.54	27.37	16.85	27.19	41544.00	18.19	28.61	28.60	17.16		
5	41411.00	28.52	27.33	16.81	27.19	26.99	41412.00	28.53	28.61	17.18		
6	41350.00	28.58	27.41	16.80	27.18	27.00	41412.00	28.48	28.66	17.18		
	F6	F7	F8	F9	F10	F11	F12	G1	G2	G3	G4	
1	27.99	27.50	16.67	27.65	41422.00	16.68	26.61	27.91	44743	15189	27.7	
2	28.29	27.79	16.74	27.95	28.39	16.83	26.97	41453.00	45108	17380	41392.0	
3	28.25	27.84	16.74	27.98	28.35	16.78	26.95	27.99	46204	16285	28.0	
4	28.30	27.88	16.84	41333.00	28.38	16.82	26.99	27.89	44743	15554	41453.0	
5	28.33	27.94	16.92	41392.00	28.31	16.86	26.96	27.84	44743	15554	41422.0	
6	28.28	27.95	16.82	41361.00	28.39	16.86	26.97	27.74	41456	12997	41514.0	
	G5	G6	G7	G8	G9	G10	G11	G12	H1	H2	H3	H4
1	31929	22433	27.79	34121	29738	28.32	20668	32295	15.97	41410.00	45108	15158
2	33756	23894	41361.00	35582	31929	28.58	24685	35582	15.85	15.97	46204	17715
3	33025	22433	41422.00	34851	30834	28.46	23590	33756	15.76	15.88	46569	16984
4	32660	24259	41514.00	35217	31199	28.42	22859	34121	15.72	15.88	45474	15523
5	33025	21702	41392.00	33756	28642	28.38	20302	33025	15.75	15.84	44378	16619
6	32660	20972	27.97	32295	29738	28.41	21398	33756	15.75	15.82	42552	13697
	H5	H6	H7	H8	H9	H10	H11	H12				
1	26.70	41391.00	14427	21002	24624	24624	41371	17746				
2	41391.00	27.28	18810	24289	28277	26816	41554	21763				
3	26.90	27.17	17349	22098	25720	24259	41312	19572				
4	26.85	27.21	15158	21732	26816	25355	41432	19572				
5	26.82	27.21	14427	21002	26451	23894	41312	17746				
6	26.80	27.26	14793	22098	25720	23163	36312	15554				

```
> ## Pheno data
> head(pData(cycData))
```

Sample position	Sample name	Program number	Segment number
A1	A1	Sample_1	2
A2	A2	Sample_2	2
A3	A3	Sample_2	2

```

A4          A4   Sample_3          2          3
A5          A5   Sample_4          2          3
A6          A6   Sample_4          2          3

```

```

> ## Feature data
> head(fData(cycData))

```

```

  Cycle number Acquisition time Acquisition temperature
1           1           691600           71.79
2           2           770133           71.64
3           3           848716           71.64
4           4           927233           71.67
5           5          1005816           71.67
6           6          1084316           71.67

```

The class `CyclesSet` is an extension of class `eSet`.

```

> cycData

```

```

CyclesSet (storageMode: lockedEnvironment)
assayData: 45 features, 96 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: A1 A2 ... H12 (96 total)
  varLabels: Sample position Sample name Program number Segment number
  varMetadata: labelDescription
featureData
  featureNames: 1 2 ... 45 (45 total)
  fvarLabels: Cycle number Acquisition time Acquisition temperature
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

```

```

> ## Information about class CyclesSet
> getClass("CyclesSet")

```

```

Class "CyclesSet" [package "ReadqPCR"]

```

```

Slots:

```

```

Name:          assayData          phenoData          featureData

```

Class: AssayData AnnotatedDataFrame AnnotatedDataFrame

Name: experimentData annotation protocolData
Class: MIAxE character AnnotatedDataFrame

Name: `.__classVersion__`
Class: Versions

Extends:

Class "eSet", directly

Class "VersionedBiobase", by class "eSet", distance 2

Class "Versioned", by class "eSet", distance 3

The additional sample information file can be read in with function `read.LC480SampleInfo` and is saved in an object of class `AnnotatedDataFrame`.

```
> LC480.SamInfo <- file.path(path, "LC480_Example_SampleInfo.txt")
> samInfo <- read.LC480SampleInfo(file = LC480.SamInfo)
> ## Additional sample information
> head(pData(samInfo))
```

Sample	position	Sample name	Replicate	of Filter combination	Target name
1	A1	Sample_1		465-510	negativ Kontrolle
2	A2	Sample_2		465-510	goi
3	A3	Sample_2		465-510	hk
4	A4	Sample_3		465-510	negativ Kontrolle
5	A5	Sample_4		465-510	goi
6	A6	Sample_4		465-510	hk

Sample	Pref color	Efficiency	Combined sample and target type
1	\$00FF8000	2	Target Negative
2	c1Red	2	Target Unknown
3	\$0030D700	2	Ref Unknown
4	c1Fuchsia	2	Target Negative
5	c1Gray	2	Target Unknown
6	\$0012D7FA	2	Ref Unknown

For adding this additional information to the `CyclesSet` we provide a method for function `merge`.

```
> cycData1 <- merge(cycData, samInfo)
> ## Extended pheno data
> phenoData(cycData1)
```

```
An object of class 'AnnotatedDataFrame'
 sampleNames: 1 2 ... 96 (96 total)
 varLabels: Sample position Sample name ... Combined sample and target
 type (10 total)
 varMetadata: labelDescription
```

```
> head(pData(cycData1))
```

	Sample position	Sample name	Program number	Segment number	Replicate of
1	A1	Sample_1	2	3	
2	A2	Sample_2	2	3	
3	A3	Sample_2	2	3	
4	A4	Sample_3	2	3	
5	A5	Sample_4	2	3	
6	A6	Sample_4	2	3	

	Filter combination	Target name	Sample Pref color	Efficiency
1	465-510	negativ Kontrolle	\$00FF8000	2
2	465-510	goi	clRed	2
3	465-510	hk	\$0030D700	2
4	465-510	negativ Kontrolle	clFuchsia	2
5	465-510	goi	clGray	2
6	465-510	hk	\$0012D7FA	2

	Combined sample and target type
1	Target Negative
2	Target Unknown
3	Ref Unknown
4	Target Negative
5	Target Unknown
6	Ref Unknown

```
> varMetadata(phenoData(cycData1))
```

	labelDescription
Sample position	Sample position
Sample name	Sample name
Program number	Program number
Segment number	Segment number
Replicate of	Replicate of
Filter combination	Filter combination
Target name	Target name
Sample Pref color	Sample Pref color
Efficiency	Efficiency
Combined sample and target type	Combined sample and target type

3 read.qPCR

`read.qPCR` allows the user to read in qPCR data and populate a `qPCRBatch` R object (see section `qPCRBatch`) using their own data matrix. The format of the data file should be tab delimited and have the following columns, the first two of which are optional (although they should either be provided together, or not at all):

Well Optional, this represents the position of the detector on a plate. This information, if given, will be used to check the plates are of the same size and will also be used in order to plot a representation of the card to look for spatial effects and other potential problems. Both Well number and Plate ID must be present to enable a plate to be plotted.

Plate Optional, this is an identifier for the plate on which an experiment was performed. It is not possible to have duplicate plate IDs with the same Well number. Neither is it possible to have Plate Ids without Well numbers. Both Well number and Plate ID must be present to enable a plate to be plotted.

Sample The sample being analysed. Each sample must contain the same detectors in order to combine and compare samples effectively and to form a valid expression set matrix.

Detector This is the identifier for the gene being investigated. The Detectors must be identical for each sample.

Cq This is the quantification cycle value for a given detector in the corresponding sample.

The generic function `read.qPCR` is called to read in the qPCR file. It is similar to the `read.affybatch` function of the "affy" package, in that it reads a file and automatically populates an R object, `qPCRBatch` described below. However it is different in that the file is user formatted. In addition, unlike `read.affybatch`, and also unlike the `read.taqman` function detailed below, only one file may be read in at a time.

If `Well` and `Plate ID` information are given, then these are used to populate the `exprs.well.order`, a new `assayData` slot introduced in the `qPCRBatch` object, as detailed below in section `qPCRBatch`.

So for the `qPCR.example.txt` file, in directory `exData` of this library, which contains `Well` and `Plate ID` information, as well as the mandatory `Sample`, `Detector` and `Cq` information, we can read in the data as follows.

```
> path <- system.file("exData", package = "ReadqPCR")
> qPCR.example <- file.path(path, "qPCR.example.txt")
> qPCRBatch.qPCR <- read.qPCR(qPCR.example)
```

`qPCRBatch.qPCR` will be a `qPCRBatch` object with an `exprs` and `exprs.well.order`, as well as a `phenoData` slot which gets automatically populated in the same way as when using `read.affybatch`. More detail is given in the `qPCRBatch` section below.

`read.qPCR` can deal with technical replicates. If the same detector and sample identifier occurs more than once, the suffix `_TechReps.n` is concatenated to the detector name, where n in $\{1, 2, \dots, N\}$ is the number of the replication in the total number of replicates, N , based on order of appearance in the qPCR data file. So for a qPCR file with 2 technical replicates and 8 detectors per replicate, with one replicate per plate, the detector names would be amended as follows:

```
> qPCR.example.techReps <- file.path(path, "qPCR.techReps.txt")
> qPCRBatch.qPCR.techReps <- read.qPCR(qPCR.example.techReps)
> rownames(exprs(qPCRBatch.qPCR.techReps))[1:8]

[1] "gene_aj_TechReps.1" "gene_aj_TechReps.2" "gene_al_TechReps.1"
[4] "gene_al_TechReps.2" "gene_ax_TechReps.1" "gene_ax_TechReps.2"
[7] "gene_bo_TechReps.1" "gene_bo_TechReps.2"
```

The reason for appending the suffix when technical replicates are encountered is in order to populate the `exprs` and `exprs.well.order` slots correctly and keep them to the `assayData` format. It also allows the decisions on how to deal with the analysis and combination of technical replicates to be controlled by the user, either using the `"NormqPCR"` package, or potentially some other function that takes `assayData` format R objects as input.

4 read.taqman

`read.taqman` allows the user to read in the data output by the Sequence Detection Systems (SDS) software which is the software used to analyse the Taqman Low Density Arrays. This data consists of the header section, which gives some general information about the experiment, run date etc., followed by the raw Cq values detected by the software, followed by summary data about the experiment. `read.taqman` is a generic function, and is called in a way similar to the `read.affybatch` function of the `"affy"` package.

```
> taqman.example <- file.path(path, "example.txt")
> qPCRBatch.taq <- read.taqman(taqman.example)
```

Currently the SDS software only allows up to 10 plates to be output onto one file. `read.taqman` allows any number of SDS output files to be combined to make a single `qPCRBatch`, as long as they have matching detector identifiers.

```
> path <- system.file("exData", package = "ReadqPCR")
> taqman.example <- file.path(path, "example.txt")
```

```

> taqman.example.second.file <- file.path(path, "example2.txt")
> qPCRBatch.taq.two.files <- read.taqman(taqman.example,
+                                       taqman.example.second.file)

```

SDS output will not necessarily contain plate identifiers, in which case a numeric identifier will be generated, which will increment for each plate, depending on the order of the plates within the SDS files. This is important for filling the `exprs.well.order` slot of the `qPCRBatch`.

`read.taqman` can also deal with technical replicates. If the same detector and sample identifier occurs more than once, the suffix `_TechRep.n` will be concatenated to the detector name, where n in $\{1, 2, \dots, N\}$ is the number of the replication in the total number of replicates N , based on the order of occurrence in the taqman data file. So for a taqman file with 4 technical replicates of 96 detectors per sample, with one sample per plate, the detector names would be amended as follows:

```

> taqman.example.tech.reps <- file.path(path, "exampleTechReps.txt")
> qPCRBatch.taq.tech.reps <- read.taqman(taqman.example.tech.reps)
> rownames(exprs(qPCRBatch.taq.tech.reps))[1:8]

```

```

[1] "ACE.Hs00174179_m1_TechReps.1"
[2] "ACE.Hs00174179_m1_TechReps.2"
[3] "ACE.Hs00174179_m1_TechReps.3"
[4] "ACE.Hs00174179_m1_TechReps.4"
[5] "AT1R.AGTR1..Hs00241341_m1_TechReps.1"
[6] "AT1R.AGTR1..Hs00241341_m1_TechReps.2"
[7] "AT1R.AGTR1..Hs00241341_m1_TechReps.3"
[8] "AT1R.AGTR1..Hs00241341_m1_TechReps.4"

```

As with `read.qPCR`, the motivation for appending the suffix when technical replicates are encountered is in order to populate the `exprs` and `exprs.well.order` slots correctly and keep them to the `assayData` format. Again it allows the decisions on how to deal with the analysis of technical replicates to be controlled by the user, either using the `"NormqPCR"` package, or otherwise.

5 qPCRBatch

`qPCRBatch` is an S4 class, designed to store information on the raw Cq values which represents the relative gene expression for a given sample, phenotypic information on the different samples which enable the user to compare expression across different conditions or cell lines, and information on the spatial location of the different detectors used to measure Cq. This is achieved by making `qPCRBatch` an extension of `eSet`, which means we

can recycle slots such as `exprs` and `pData`, and by introducing a new `assayData` slot. Here is an example of what a `qPCRBatch` looks like. note the similarity to `eSet`:

```
> qPCRBatch.taq
```

```
qPCRBatch (storageMode: lockedEnvironment)
assayData: 96 features, 8 samples
  element names: exprs, exprs.well.order
protocolData: none
phenoData
  sampleNames: fp1.day3.v fp2.day3.v ... fp.8.day.3.mia (8 total)
  varLabels: sample
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation:
```

`pData` will be filled automatically if no data is given, in a way analagous to `read.affybatch`:

```
> pData(qPCRBatch.taq)
```

	sample
fp1.day3.v	1
fp2.day3.v	2
fp5.day3.mia	3
fp6.day3.mia	4
fp.3.day.3.v	5
fp.4.day.3.v	6
fp.7.day.3.mia	7
fp.8.day.3.mia	8

In addition there is a new slot, `exprs.well.order` which extends the `assayData` slot used for `exprs()`. It has the same dimensions as `exprs` (as every instance of `assayData` must). The cells contain further details on the position on the arrays where the different measurements were taken.

The data provided by this slot can be used in order to identify certain problems with arrays, perhaps due to spatial effects and other problems with the microfluidics technology that is used by many of these systems.

This is conceptually similar to the `cdf` file information being stored in the `AffyBatch` class, which contains information on the spatial layout of features on an affy chip. However it differs since it allows for different arrays within the same `affyBatch` object to have different

layouts to each other. This information can be viewed using the `exprs.well.order()` function.

When using `read.taqman`, if the input file includes identifiers for the different arrays in the experiment, the identifiers will be of the format `<plate.id>-<plate.position>`. However if no names are given for the different plates, "ReadqPCR" will assign them a numeric identifier, which increments depending on the order of plates in the original file. When several input files are given, as in the case of SDS files, the order in which they are supplied as arguments to the `read.taqman` function will be mirrored in the order of the numeric identifiers for the different plates. However, to minimise confusion, we recommend the user giving the plates their own unique identifiers where possible.

Without plate names:

```
> head(exprs.well.order(qPCRBatch.taq))
```

	fp1.day3.v	fp2.day3.v	fp5.day3.mia	fp6.day3.mia
Actb.Rn00667869_m1	"1-38"	"1-134"	"1-230"	"1-326"
Adipoq.Rn00595250_m1	"1-61"	"1-157"	"1-253"	"1-349"
Adrbk1.Rn00562822_m1	"1-66"	"1-162"	"1-258"	"1-354"
Agtrl1.Rn00580252_s1	"1-91"	"1-187"	"1-283"	"1-379"
Alpl.Rn00564931_m1	"1-10"	"1-106"	"1-202"	"1-298"
B2m.Rn00560865_m1	"1-37"	"1-133"	"1-229"	"1-325"

	fp.3.day.3.v	fp.4.day.3.v	fp.7.day.3.mia	fp.8.day.3.mia
Actb.Rn00667869_m1	"2-38"	"2-134"	"2-230"	"2-326"
Adipoq.Rn00595250_m1	"2-61"	"2-157"	"2-253"	"2-349"
Adrbk1.Rn00562822_m1	"2-66"	"2-162"	"2-258"	"2-354"
Agtrl1.Rn00580252_s1	"2-91"	"2-187"	"2-283"	"2-379"
Alpl.Rn00564931_m1	"2-10"	"2-106"	"2-202"	"2-298"
B2m.Rn00560865_m1	"2-37"	"2-133"	"2-229"	"2-325"

With plate names:

```
> taqman.example.plateNames <- file.path(path, "exampleWithPlateNames.txt")
> qPCRBatch.taq.plateNames <- read.taqman(taqman.example.plateNames)
> head(exprs.well.order(qPCRBatch.taq.plateNames))
```

	gp.1.day.3.v	gp.2.day.3.v	gp.5.day.3.mia	gp.6.day.3.mia
Actb.Rn00667869_m1	"PlateA-38"	"PlateA-134"	"PlateA-230"	"PlateA-326"
Adipoq.Rn00595250_m1	"PlateA-61"	"PlateA-157"	"PlateA-253"	"PlateA-349"
Adrbk1.Rn00562822_m1	"PlateA-66"	"PlateA-162"	"PlateA-258"	"PlateA-354"
Agtrl1.Rn00580252_s1	"PlateA-91"	"PlateA-187"	"PlateA-283"	"PlateA-379"
Alpl.Rn00564931_m1	"PlateA-10"	"PlateA-106"	"PlateA-202"	"PlateA-298"
B2m.Rn00560865_m1	"PlateA-37"	"PlateA-133"	"PlateA-229"	"PlateA-325"

```

gp.3.day.3.v gp.4.day.3.v gp.7.day.3.mia gp.8.day.3.mia
Actb.Rn00667869_m1 "PlateB-38" "PlateB-134" "PlateB-230" "PlateB-326"
Adipoq.Rn00595250_m1 "PlateB-61" "PlateB-157" "PlateB-253" "PlateB-349"
Adrbk1.Rn00562822_m1 "PlateB-66" "PlateB-162" "PlateB-258" "PlateB-354"
Agtrl1.Rn00580252_s1 "PlateB-91" "PlateB-187" "PlateB-283" "PlateB-379"
Alpl.Rn00564931_m1 "PlateB-10" "PlateB-106" "PlateB-202" "PlateB-298"
B2m.Rn00560865_m1 "PlateB-37" "PlateB-133" "PlateB-229" "PlateB-325"

```

In addition, a mixture of files with and without plate identifiers is possible.

```

> taqman.example <- file.path(path, "example.txt")
> taqman.example.plateNames <- file.path(path, "exampleWithPlateNames.txt")
> qPCRBatch.taq.mixedPlateNames <- read.taqman(taqman.example,
+                                             taqman.example.plateNames)
> head(exprs.well.order(qPCRBatch.taq.mixedPlateNames))

```

```

fp1.day3.v fp2.day3.v fp5.day3.mia fp6.day3.mia
Actb.Rn00667869_m1 "1-38" "1-134" "1-230" "1-326"
Adipoq.Rn00595250_m1 "1-61" "1-157" "1-253" "1-349"
Adrbk1.Rn00562822_m1 "1-66" "1-162" "1-258" "1-354"
Agtrl1.Rn00580252_s1 "1-91" "1-187" "1-283" "1-379"
Alpl.Rn00564931_m1 "1-10" "1-106" "1-202" "1-298"
B2m.Rn00560865_m1 "1-37" "1-133" "1-229" "1-325"

fp.3.day.3.v fp.4.day.3.v fp.7.day.3.mia fp.8.day.3.mia
Actb.Rn00667869_m1 "2-38" "2-134" "2-230" "2-326"
Adipoq.Rn00595250_m1 "2-61" "2-157" "2-253" "2-349"
Adrbk1.Rn00562822_m1 "2-66" "2-162" "2-258" "2-354"
Agtrl1.Rn00580252_s1 "2-91" "2-187" "2-283" "2-379"
Alpl.Rn00564931_m1 "2-10" "2-106" "2-202" "2-298"
B2m.Rn00560865_m1 "2-37" "2-133" "2-229" "2-325"

gp.1.day.3.v gp.2.day.3.v gp.5.day.3.mia gp.6.day.3.mia
Actb.Rn00667869_m1 "PlateA-38" "PlateA-134" "PlateA-230" "PlateA-326"
Adipoq.Rn00595250_m1 "PlateA-61" "PlateA-157" "PlateA-253" "PlateA-349"
Adrbk1.Rn00562822_m1 "PlateA-66" "PlateA-162" "PlateA-258" "PlateA-354"
Agtrl1.Rn00580252_s1 "PlateA-91" "PlateA-187" "PlateA-283" "PlateA-379"
Alpl.Rn00564931_m1 "PlateA-10" "PlateA-106" "PlateA-202" "PlateA-298"
B2m.Rn00560865_m1 "PlateA-37" "PlateA-133" "PlateA-229" "PlateA-325"

gp.3.day.3.v gp.4.day.3.v gp.7.day.3.mia gp.8.day.3.mia
Actb.Rn00667869_m1 "PlateB-38" "PlateB-134" "PlateB-230" "PlateB-326"
Adipoq.Rn00595250_m1 "PlateB-61" "PlateB-157" "PlateB-253" "PlateB-349"
Adrbk1.Rn00562822_m1 "PlateB-66" "PlateB-162" "PlateB-258" "PlateB-354"
Agtrl1.Rn00580252_s1 "PlateB-91" "PlateB-187" "PlateB-283" "PlateB-379"

```

```
Alpl.Rn00564931_m1    "PlateB-10"  "PlateB-106" "PlateB-202"  "PlateB-298"
B2m.Rn00560865_m1    "PlateB-37"  "PlateB-133" "PlateB-229"  "PlateB-325"
```

If the files to be combined do not have matching detector names, or if duplicate sample or plate names are given, `read.taqman` will stop and give an error message.

When reading in `qPCR` files with `read.qPCR`, `exprs.well.order` will be populated as long as `Well` and `Plate ID` columns are given in the input file, otherwise the `exprs.well.order` slot will be `NULL`.

So when plate ID and Well data are given:

```
> head(exprs.well.order(qPCRBatch.qPCR))

              caseA caseB controlA controlB
gene_ai_TechReps.1 A-18  A-43      B-18      B-43
gene_ai_TechReps.2 C-18  C-43      D-18      D-43
gene_az_TechReps.1 A-23  A-48      B-23      B-48
gene_az_TechReps.2 C-23  C-48      D-23      D-48
gene_bc_TechReps.1 A-6   A-31      B-6       B-31
gene_bc_TechReps.2 C-6   C-31      D-6       D-31
```

And when they are not:

```
> qPCR.example.noPlateOrWell <- file.path(path, "qPCR.noPlateOrWell.txt")
> qPCRBatch.qPCR.noPlateOrWell <- read.qPCR(qPCR.example.noPlateOrWell)
> exprs.well.order(qPCRBatch.qPCR.noPlateOrWell)
```

`NULL`

Once a `qPCRBatch` has been populated it is theoretically possible to use any tool which takes as it's input an `exprs` set matrix. However it is important to bear in mind the values are not raw expression values but `Cq` values, and a lower `Cq` will indicate a higher expression level for a given transcript in the sample. Also it is important to note that when normalising, the amount is relative and is intended to be compared to another condition or tissue type in order to look for differential expression between condition; the technology is not designed to give absolute quantification.