Seminar 5

What do you want to know?

Data Analysis and interpretation

Lectures 1 and 2, seminar 1 and 2

Application of qPCR

Lecture 2, seminar 2, practical part

Statistical analysis of qPCR

Basics

Lectures 2, 4 and 5

How qPCR works

Lectures 6 and seminar 3

Normalization

Seminar 3, today

Troubleshooting

practical part, seminar 3

Everything

Lecture 7, seminar 4

More

Lectures 3 and 4, today

Results of the quiz for the weimar 4 (statistics)

1. Did you learn anything new from the lecture

yes
100%

2. If yes, do you think you will remember it tomorrow?

No	yes, after revising it	yes
12.5%	25%	62.5%

3. Would you prefer to have more information in the lecture, keep it the same, reduce it?

Reduce	Keep the same
10%	90%

4. Were the tasks too easy, ok, or too hard?

too hard	ok
25%	75%

Results of the quiz

8. Do you think statistical analysis should be included in the qPCR course at all?

Yes

100%

qPCR data analysis

Biological problem

Planning the experiment

this will be very tricky to plan

Publication

if you do not know what is required here

Biological problem

Planning the experiment

1. …
2. …
3. …
4. …
5. …

and this

Analysing the results

you still need to know how to do this

Publication

1. …
2. …
3. …
4. …
5. …

if you know this

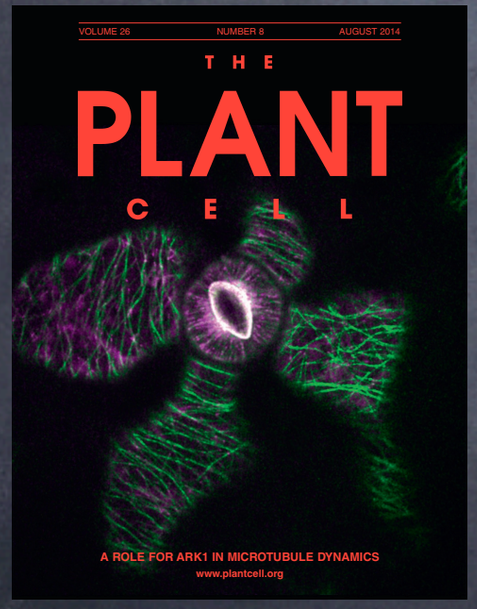
- what is ROX
- why didn't we use it?
- if all your samples do not fit into one plate, how will you distribute them on two plates:
  - you have 10 samples
  - you are detecting 3 genes of reference and one GOI
  - each reaction is represented by three technical replicates

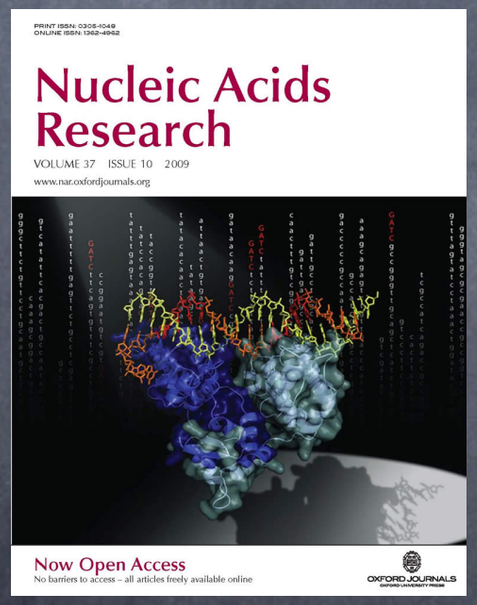
Group 1:
Reza
Jun
Anna
Shirin

Group 2:
Martin
Zaenab
Tina

Group 3:
Jule
Maite
Enrique
Kiran

Group 4:
Enid
Mohammad
Anders





Please discuss the assigned questions and summarise your conclusions

Table 1. MIQE checklist for authors, reviewers, and editors.[a]

Item to check	Importance	Item to check	Importance
Experimental design		qPCR oligonucleotides	
Definition of experimental and control groups	E	Primer sequences	E
Number within each group	E	RTPrimerDB identification number	D
Assay carried out by the core or investigator's laboratory?	D	Probe sequences	D[d]
Acknowledgment of authors' contributions	D	Location and identity of any modifications	E
Sample		Manufacturer of oligonucleotides	D
Description	E	Purification method	D
Volume/mass of sample processed	D	qPCR protocol	
Microdissection or macrodissection	E	Complete reaction conditions	E
Processing procedure	E	Reaction volume and amount of cDNA/DNA	E
If frozen, how and how quickly?	E	Primer, (probe), Mg^{2+}, and dNTP concentrations	E
If fixed, with what and how quickly?	E	Polymerase identity and concentration	E
Sample storage conditions and duration (especially for FFPE[b] samples)	E	Buffer/kit identity and manufacturer	E
Nucleic acid extraction		Exact chemical composition of the buffer	D
Procedure and/or instrumentation	E	Additives (SYBR Green I, DMSO, and so forth)	E
Name of kit and details of any modifications	E	Manufacturer of plates/tubes and catalog number	D
Source of additional reagents used	D	Complete thermocycling parameters	E
Details of DNase or RNase treatment	E	Reaction setup (manual/robotic)	D
Contamination assessment (DNA or RNA)	E	Manufacturer of qPCR instrument	E
Nucleic acid quantification	E	qPCR validation	
Instrument and method	E	Evidence of optimization (from gradients)	D
Purity (A_{260}/A_{280})	D	Specificity (gel, sequence, melt, or digest)	E
Yield	D	For SYBR Green I, C_q of the NTC	E
RNA integrity: method/instrument	E	Calibration curves with slope and *y* intercept	E
RIN/RQI or C_q of 3′ and 5′ transcripts	E	PCR efficiency calculated from slope	E
Electrophoresis traces	D	CIs for PCR efficiency or SE	D
Inhibition testing (C_q dilutions, spike, or other)	E	r^2 of calibration curve	E
Reverse transcription		Linear dynamic range	E
Complete reaction conditions	E	C_q variation at LOD	E
Amount of RNA and reaction volume	E	CIs throughout range	D
Priming oligonucleotide (if using GSP) and concentration	E	Evidence for LOD	E
Reverse transcriptase and concentration	E	If multiplex, efficiency and LOD of each assay	E
Temperature and time	E	Data analysis	
Manufacturer of reagents and catalogue numbers	D	qPCR analysis program (source, version)	E
C_qs with and without reverse transcription	D[c]	Method of C_q determination	E
Storage conditions of cDNA	D	Outlier identification and disposition	E
qPCR target information		Results for NTCs	E
Gene symbol	E	Justification of number and choice of reference genes	E
Sequence accession number	E	Description of normalization method	E
Location of amplicon	D	Number and concordance of biological replicates	D
Amplicon length	E	Number and stage (reverse transcription or qPCR) of technical replicates	E
In silico specificity screen (BLAST, and so on)	E	Repeatability (intraassay variation)	E
Pseudogenes, retropseudogenes, or other homologs?	D	Reproducibility (interassay variation, CV)	D
Sequence alignment	D	Power analysis	D
Secondary structure analysis of amplicon	D	Statistical methods for results significance	E
Location of each primer by exon or intron (if applicable)	E	Software (source, version)	E
What splice variants are targeted?	E	C_q or raw data submission with RDML	D

[a] All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If primers are from RTPrimerDB, information on qPCR target, oligonucleotides, protocols, and validation is available from that source.

[b] FFPE, formalin-fixed, paraffin-embedded; RIN, RNA integrity number; RQI, RNA quality indicator; GSP, gene-specific priming; dNTP, deoxynucleoside triphosphate.

[c] Assessing the absence of DNA with a no–reverse transcription assay is essential when first extracting RNA. Once the sample has been validated as rDNA free, inclusion of a no–reverse transcription control is desirable but no longer essential.

[d] Disclosure of the probe sequence is highly desirable and strongly encouraged; however, because not all vendors of commercial predesigned assays provide this information, it cannot be an essential requirement. Use of such assays is discouraged.