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Supplemental Information

Fast Quantitative Real-Time PCR-Based Screening for Common Chromosomal Aneuploidies in Mouse Embryonic Stem Cells

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Inventory of Supplemental Information

1. **Figure S1: Non-normalized chromosome copy numbers. Related to Figure 1.** A. Non-normalized absolute chromosome copy number calculated using chrX as a reference. B. Non-normalized absolute chromosome copy number calculated using chr7 as a reference. For the autosomes a copy number of 2 reflects normal ploidy and a copy number of 3 reflects trisomy. For the sex chromosomes a copy number of 1 reflects normal ploidy (since we are using XY ESCs), a copy number of 0 reflects loss of the chromosome and a copy number of 2 reflects duplication of the chromosome. The dotted line marks the border between mESCs with previously determined (known) and unknown karyotypes at the time of qPCR analysis.

Figure S1 relates to Figure 1. It shows the absolute copy numbers per mESC line before normalization to our endogenous control genes.

2. **Figure S2: Calibration curves for the primer pairs used in the qPCR, related to Table 2.** To determine the qPCR amplification efficiency of the different primer sets, a standard curve was prepared for each primer pair in which serial diluted *EcoRI*-digested ESC DNA samples (2, 4, 8, and 16-fold dilutions) were included. PCR efficiencies were calculated using the slopes of the standard curves according to the following formula: PCR E = $10^{-1/\text{slope}}$ -1. Slopes, PCR efficiencies, r^2 values and limit of detection (LOD) are shown in Table 2. B. Results of the amplicon-size verification using gel electrophoresis. Single bands prove specificity of the primers. Shown on the left is the 100bp ladder. Different amplicons are indicated on the ethidium bromide gel. Each data point represents the average of 6 measurements and the standard error of the mean is indicated on the graph.

Figure S2 relates to Table 2. It further shows the empirical validation of the qPCR

primer sets.

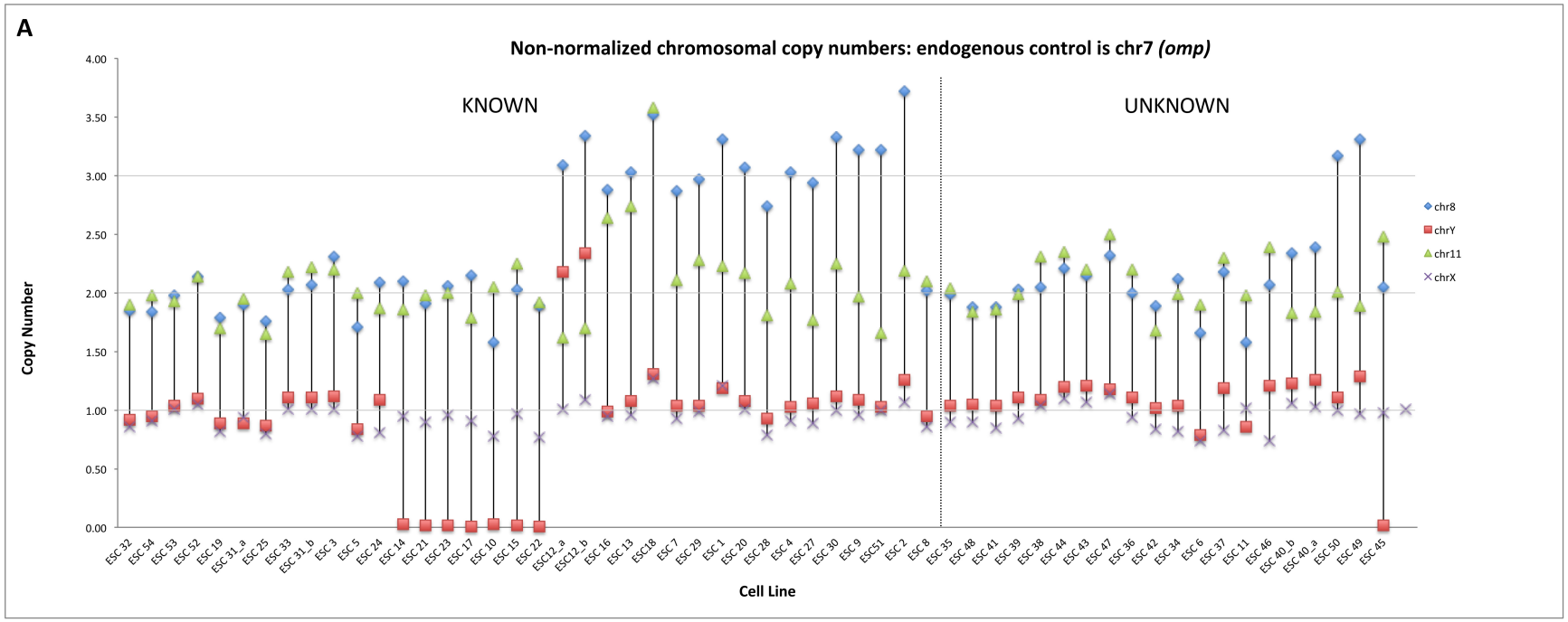
3. **Table S1: Summary of cytogenetic findings (DAPI-banded karyotyping) of mouse ESC lines (N=141), related to Table 1.**

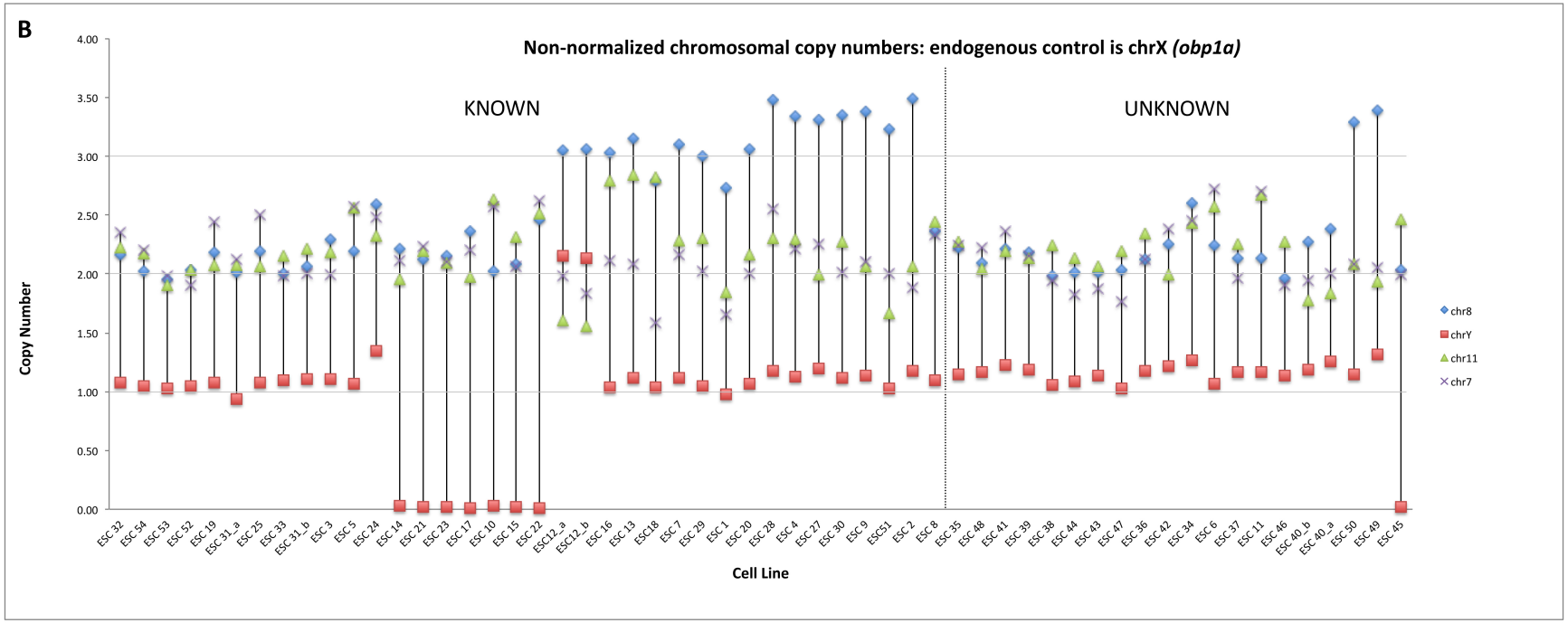
Table S1 relates to Table 1. It gives a detailed overview of the karyotyping analysis of our 141 mESC lines.

4. **Table S2: MIQE Guidelines Checklist, related to Experimental Procedures.** *The Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines (MIQE) checklist shows that our qPCRs are carried out in compliance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines.*

5. **Table S3, S4, S5 and S6** show the Excel data analysis, related to Experimental Procedures.

Supplemental Figures and Legends, Tables





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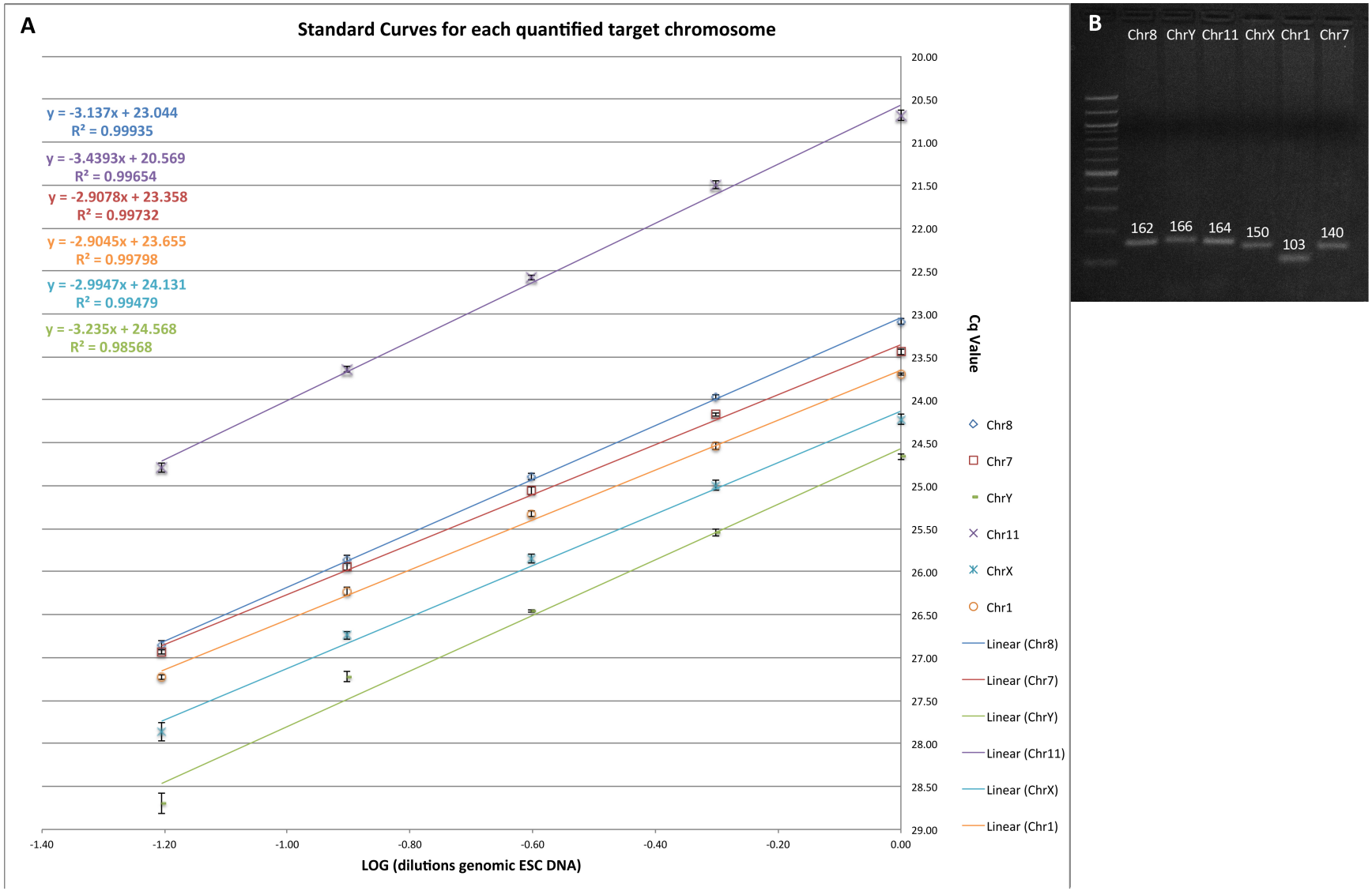

Figure S2: Calibration curves for the primer pairs used in the qPCR, Related to Table 2. To determine the qPCR amplification efficiency of the different primer sets, a standard curve was prepared for each primer pair in which serial diluted *EcoRI*-digested ESC DNA samples (2, 4, 8, and 16-fold dilutions) were included. PCR efficiencies were calculated using the slopes of the standard curves according to the following formula: PCR E = $10^{-1/slope}$ -1. Slopes, PCR efficiencies, r^2 values and limit of detection (LOD) are shown in Table 2. B. Results of the amplicon-size verification using gel electrophoresis. Single bands prove specificity of the primers. Shown on the left is the 100bp ladder. Different amplicons are indicated on the ethidium bromide gel. Each data point represents the average of 6 measurements and the standard error of the mean is indicated on the graph.

Table S1: Summary of cytogenetic findings (DAPI-banded karyotyping) of mouse ESC lines (N=141), Related to Table 1.

Karyotype*	ISCN	# cell lines	%
ESC Normal Karyotype	40, XY	**68**	**48.2**
ESC Abnormal Karyotype		**73**	**51.8**
Numerical changes		*53*	
Trisomy 8	41,XY,+8	34	
30% Trisomy 8	40,XY[10]/ 41,XY,+8[4]	1	
Partial Monosomy 8	40,XY [25]/ 39,XY,-8 [3]	1	
Trisomy 8 & 11	42,XY,+8, +11	3	
Trisomy 8 & gain of chrY	42,XYY,+8	1	
Loss of chrY	39,X,-Y	10	
<50% of chrY	39,X,-Y[3] /40, XY[12]; 39,X,-Y[3] /40, XY[12]; 39,X,-Y [3]/ 40,XY [10]	3	
Structural changes		*16*	
Aberrant chr1	40,XY,?del(1)(~qH4qH6)[20]	1	
Aberrant chr3	40,XY,?del(3)(~qE2qF1)[20]; 39,X,Y,?der(3)[4]/ 40,XY,?der(3)(?QF/G)[12]	2	
Partial Trisomy 8	40,XY,invdup(8)(qA1qD1)[13]; 40,XY,invdup(8)(qC1qE1)[16]	2	
Short chr19	40,XY,?del(19)(qD)[18]/ 40,XY?[2]	1	
Interstitial16q deletion/ short chromosome	40,XY,?del(16)(~qC1qC2)[15]	1	
Interstitial deletion of chr1	40,XY,dup(1)(~qE2qE4)[5]/ 40,XY[8]	1	
Interstitial deletion of chr6	40,XY,del(6)(?qB1qB3)[16]	1	
High chromosomal breakage	40,XY	2	
Translocation	40,XY,t(4;17)(qA2;qE5)[14]	1	
Balanced translocation	40,XY,t(6;8)(~qB3;qA4)[14];	1	
Balanced translocation chr1-11	40,XY,t(1;10)(qF;qD)[15]	1	

Low mitotic index	40,XY[5 cells only]; 38~40,XY[13]	2	
Both Structural and numerical changes		*4*	
This sample has a mix of populations, with trisomy 1 present in approximately 31% of cells	1**. 40-41, XY, +1 [cp3]/39-40, XY, rob(1;1) [cp2], 40, XY [cp13] 2. 40~41,XY,+1 [cp14]/ 42,XY,+1,+8[cp2]/ 40~41,XY,+11[cp2]/39~40,XY [cp18] (ESC 8)	1	
The Y chromosome has a prominent secondary constriction. It is not clear if this represents a local structural change (fragility) or an unbalanced rearrangement with another chromosome	40,X?Y[15]	1	
One additional cell was 39,X,-Y. ~30% had one chromosome 15 with reduced pericentromeric heterochromatin	40,XY[14]	1	
14 metaphases were 40,XY. 3 additional cells had structural aberrations: 40,XY,?dup(10)(qCqD); 40,X,der(Y), acer(Y); 40,XY,del(5)(~qC),-14,-17,+2mar. 1 additional cell had trisomy 8: 41,XY,+8.	37~40,XY[20] (ESC 40)	1	

*140 mESC lines emanate from a variety of sources including 19 different electroporations.

** An initial count of 18 metaphases (1) was followed by an extra count of 36 metaphases (2) for ESC 8.

Table S2: MIQE Guidelines Checklist

Project: chromosomal aneuploidies
Opened on: 07/01/2013
Project type: review the status of my own experiment - qPCR
Journal: Stem Cell Reports
Field of work: olfaction

Global advancement : 89.90 %
All essential items validated : YES

Nomenclature : 100.00 %
All essential items validated : YES

Experimental Design : 100.00 %
All essential items validated : YES

Sample : 100.00 %
All essential items validated : YES

Nucleic Acid Extraction : 81.80 %
All essential items validated : YES

qPCR Target Information : 88.90 %
All essential items validated : YES

qPCR Oligonucleotides : 83.30 %
All essential items validated : YES

qPCR Protocol : 90.90 %
All essential items validated : YES

qPCR Validation : 91.70 %
All essential items validated : YES

Data Analysis : 85.70 %
All essential items validated : YES

Nomenclature

100.00 %

- [x] Reference gene(s) not Housekeeping gene
- [x] Quantification not quantitation
- [x] Quantify preferable to quantitate
- [x] Hydrolysis probes not TaqMan®
- [x] Dual Hydrolysis Probes not FRET
- [x] Quantification cycle Cq not Ct, Cp, TOP

Experimental Design

100.00 %

- [x] Definition of experimental and control groups
- [x] Number within each group
- [x] Assay carried out by core lab or investigator's lab
- [x] Acknowledgement of authors' contributions

Sample

100.00 %

- [x] Description
- [x] Volume/mass of sample processed
- [x] Microdissection or macrodissection
- [x] Processing procedure
- [x] If frozen - how and how quickly
- [x] Sample storage conditions and duration (especially for FFPE samples)

Nucleic Acid Extraction

81.80 %

- [x] Procedure and/or instrumentation
- [x] Name of kit and details of any modifications
- [ ] Source of additional reagents used Details
- [x] of DNase or RNAse treatment
- [x] Contamination assessment (DNA or RNA)
- [x] Nucleic acid quantification
- [x] Instrument and method
- [x] Purity (A260/A280)
- [x] Yield
- [ ] Electrophoresis traces
- [x] Inhibition testing (Cq dilutions, spike or other)

qPCR Target Information

88.90 %

- [x] If multiplex, efficiency and LOD of each assay
- [x] Sequence accession number
- [x] Location of amplicon
- [x] Amplicon length
- [x] In silico specificity screen (BLAST, etc)
- [ ] Pseudogenes, retropseudogenes or other homologs
- [x] Sequence alignment
- [x] Secondary structure analysis of amplicon
- [x] Location of each primer by exon or intron (if applicable)

qPCR Oligonucleotides

83.30 %

- [x] Primer sequences
- [x] RTPrimerDB Identification Number
- [ ] Probe sequences
- [x] Location and identity of any modifications
- [x] Manufacturer of oligonucleotides
- [x] Purification method

qPCR Protocol

90.90 %

- [x] Complete reaction conditions
- [x] Reaction volume and amount of cDNA/DNA
- [x] Primer, (probe), Mg++ and dNTP concentrations
- [x] Polymerase identity and concentration
- [x] Buffer/kit identity and manufacturer
- [ ] Exact chemical constitution of the buffer
- [x] Additives (SYBR Green I, DMSO, etc.)
- [x] Manufacturer of plates/tubes and catalog number
- [x] Complete thermocycling parameters
- [x] Reaction setup (manual/robotic)
- [x] Manufacturer of qPCR instrument

qPCR Validation

91.70 %

- [x] Evidence of optimisation (from gradients)
- [x] Specificity (gel, sequence, melt, or digest)
- [x] For SYBR Green I, Cq of the NTC
- [x] Standard curves with slope and y-intercept
- [x] PCR efficiency calculated from slope
- [x] Confidence interval for PCR efficiency or standard error
- [x] r2 of standard curve
- [x] Linear dynamic range
- [x] Cq variation at lower limit
- [ ] Confidence intervals throughout range
- [x] Evidence for limit of detection
- [x] If multiplex, efficiency and LOD of each assay

Data Analysis

85

- [x] qPCR analysis program (source, version)
- [x] Cq method determination
- [x] Outlier identification and disposition
- [x] Results of NTCs
- [x] Justification of number and choice of reference genes
- [x] Description of normalisation method
- [x] Number and concordance of biological replicates
- [x] Number and stage (RT or qPCR) of technical replicates
- [x] Repeatability (intra-assay variation)
- [x] Reproducibility (inter-assay variation, %CV)
- [ ] Power analysis
- [x] Statistical methods for result significance
- [x] Software (source, version)
- [ ] Cq or raw data submission using RDML