Analyzing Digital PCR Data in R

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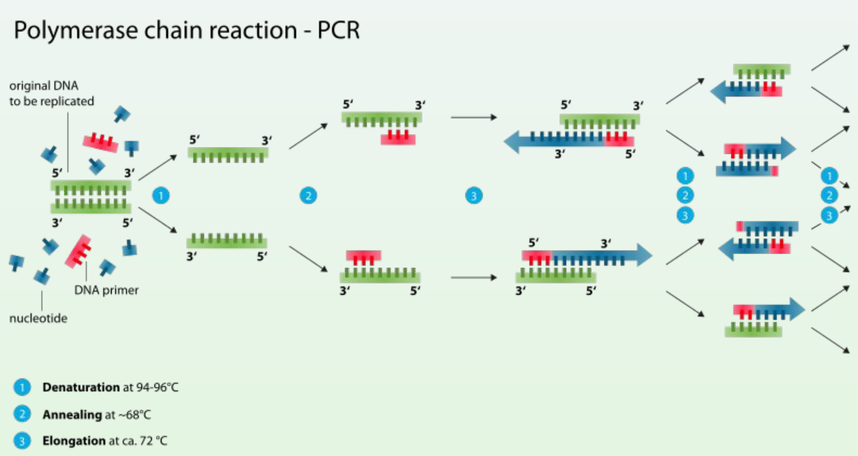
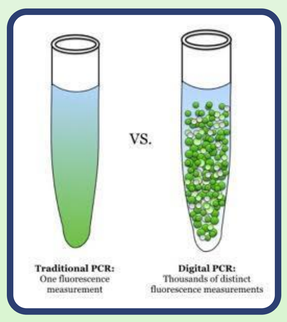
RESEARCH QUESTION

Basic Polymerase Chain Reaction (PCR) consists of 3 steps



quantitative PCR (qPCR)

- aka real-time PCR
- A fluorescent dye or probe is added such that during each PCR cycle the fluorescent signal becomes stronger
- The original number is estimated from the number of cycles before a pre-defined fluorescent signal threshold is exceeded



digital PCR (dPCR)

- Partitioning into large number of subreactions (e.g. 20 000)
- Each subreaction is amplified using typical qPCR protocols

→ Some amplify (positive)
→ Others not (negative)

- Ratio of positive versus negative subreactions determines the original number of target molecules

Multiple analysis methods for estimating the number of target DNA using dPCR

ESTIMATE CONCENTRATION

A positive subreaction may include multiple target molecules:
Given **Poisson distribution** with λ, average number of copies per partition *j*

$$\lambda = -\log P(Y_j = 0) = -\log(1 - P(Y_j = 1))$$

where Y_j (j = 1, …, J partitions) is 1 if subreaction was positive and 0 else
Concentration is estimated by $c = \lambda / V_{partition}$ given volume of the partitions

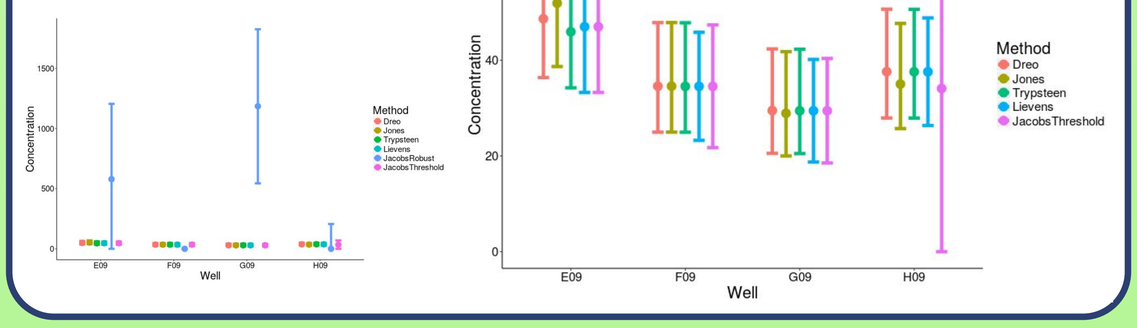
ANALYSIS RESULTS

```
> library(dpcrAnalysis)
> performAnalysis(dpcrData = dpcrData, method = "dreo")
```

Method choices:
- dreo
- jones
- trypsteen
- lievens
- jacobs
  (robust & threshold)

Well	# Positive	# Negative	# Valid	Concentration	LowerBound Conc.	UpperBound Conc.	# Rain
E09	45	10138	10183	48.67	36.37	65.11	362
F09	36	11427	11463	34.57	24.97	47.85	7
G09	29	10788	10817	29.50	20.54	42.36	20
H09	43	12543	12586	37.61	27.92	50.65	5

```
> summaryPlotConcentration(
    results = methodResults)
```

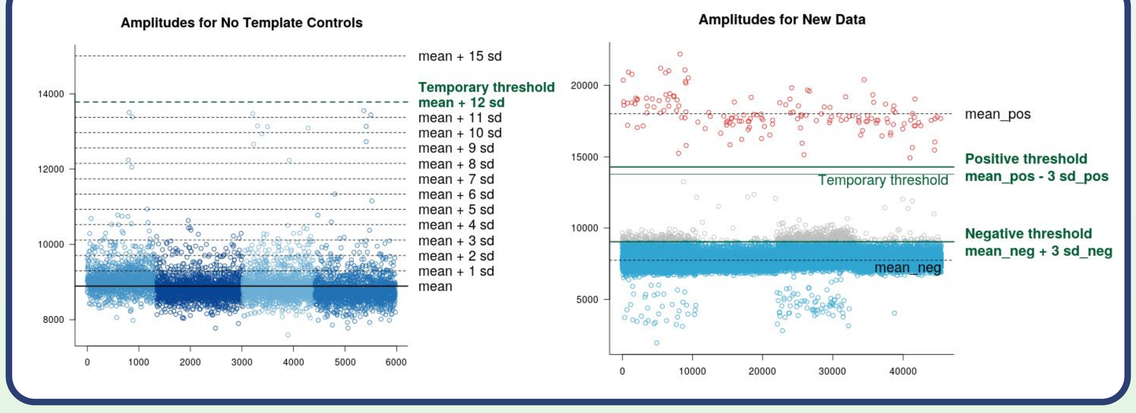


REFERENCES

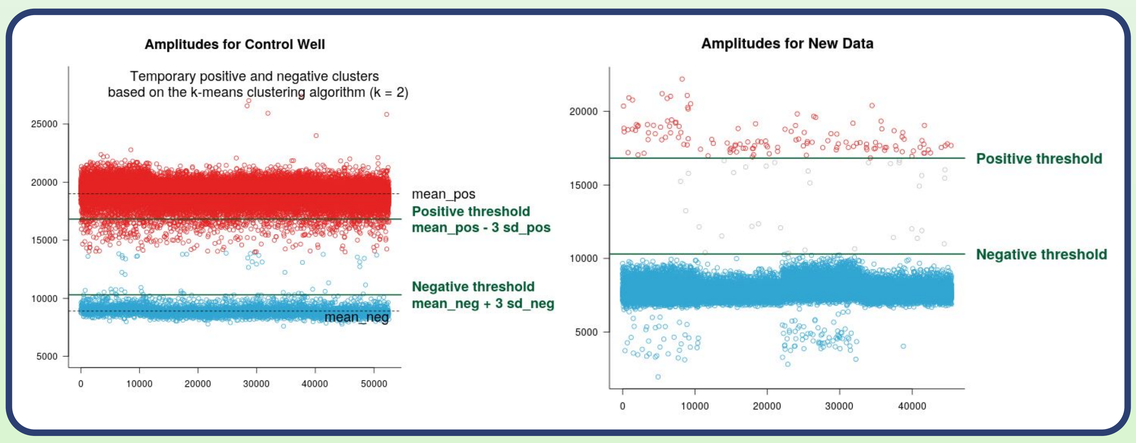
- Dreo, Tanja, et al. "Optimising droplet digital PCR analysis approaches for detection and quantification of bacteria: a case study of fire blight and potato brown rot." *Analytical and bioanalytical chemistry* 406.26 (2014): 6513-6528.
- Jones, Mathew, et al. "Low copy target detection by Droplet Digital PCR through application of a novel open access bioinformatic pipeline,'definetherain'." Journal of virological methods 202 (2014): 46-53.
- Trypsteen, Wim, et al. "ddpcRquant: threshold determination for single channel droplet digital PCR experiments." Analytical and bioanalytical chemistry 407.19 (2015): 5827-5834.
- Lievens, A., et al. "Measuring digital PCR quality: performance parameters and their optimization." *PloS one* 11.5 (2016): e0153317.
- Jacobs, Bart KM, et al. "Model-Based Classification for Digital PCR: Your Umbrella for Rain." Analytical Chemistry 89.8 (2017): 4461-4467.

METHODS

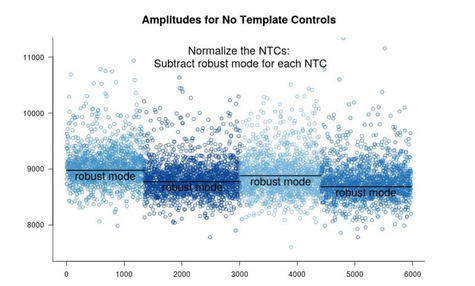
Dreo's Method — thresholdDreo(); plotDreo()

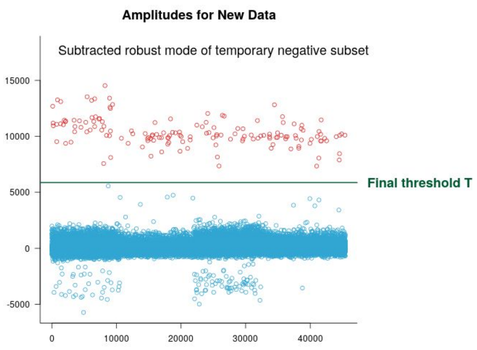


Jones's Method — thresholdJones(); plotJones()

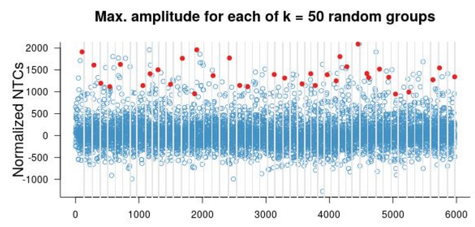


Trypsteen's Method — thresholdTrypsteen(); plotTrypsteen()





Repeat *n* times



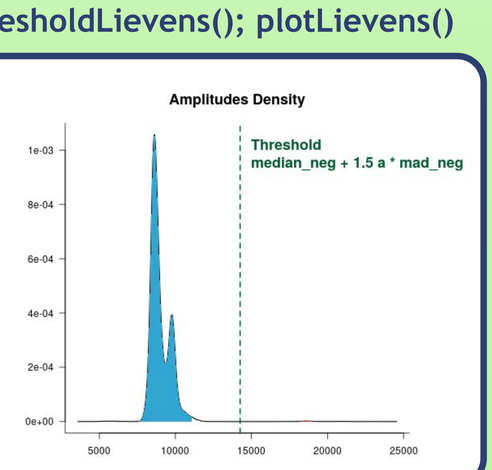
- Fit a generalized extreme value model to the *k* block maxima
- Consider the *(1 - a)* percentile to be the threshold

→ A temporary threshold is set at the average of the NTCs' robust modes + the average of the *n* thresholds (*T*)

(defines temporary negative subset)

Lievens's Method — thresholdLievens(); plotLievens()

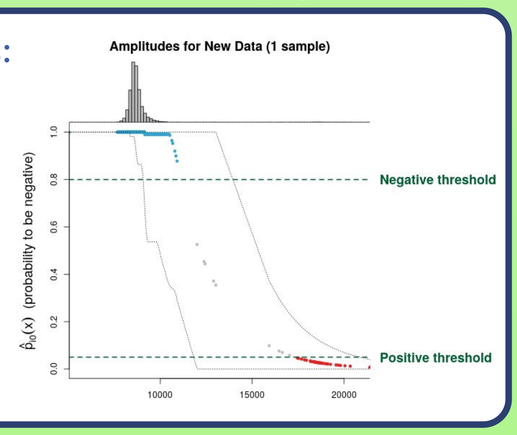
- Amplitudes' kernel density: Outermost two peaks give initial estimates for median (μ) and median absolute deviation (σ) of negative and positive population
- Re-evaluate population parameters in range $a \cdot \sigma$, where $a = f(kurtosis)$
- Final thresholds: above $\mu_n + 1.5\ a \cdot \sigma_n$ → positive else → negative



Jacobs's Method — thresholdJacobs(); plotJacobs()

Assuming mixture density for amplitudes:
$f(x) = p_{neg}\, f_{neg}(x) + (1 - p_{neg})\, f_{pos}(x)$,
with $f_{\cdot}(x)$ density neg/pos partitions,
p_{neg} proportion of negative partitions

Estimating concentration:
- Using p_0 → Robust estimator
- Using thresholds 0.8 and 0.05 → Threshold estimator



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