Model-Based Clustering of Digital PCR Droplets using Expectation Maximization

A Thesis Proposal
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Master of Science in Statistics

by

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Abstract

From 150 to 200 words of short, direct and complete sentences, the abstract should be informative enough to serve as a substitute for reading the thesis document itself. It states the rationale and the objectives of the research.

In the final thesis document (i.e., the document you'll submit for your final thesis defense), the abstract should also contain a description of your research results, findings, and contribution(s).

Keywords can be found at `http://www.acm.org/about/class/class/2012?pageIndex=0`. Click the link "HTML" in the paragraph that starts with "The **full CCS classification tree**...".

Keywords: Keyword 1, keyword 2, keyword 3, keyword 4, etc.

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Research Description

1.1 Introduction

1.2 Background of the Study

Quantification of Nucleic acids (NA) is a developing research field in molecular biology for the detection and quantification expression levels of genes (Huggett, O'Grady, & Bustin, 2015). These NA molecules are found in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which carries genetic information and is used as biomarkers for the detection of diseases (Cao et al., 2017). Additionally, along with the rise of bioinformatics tools, NA quantification methods are also utilized in rare mutation detection, copy number variation detection, single-cell gene and microRNA expression analysis, and next-generation sequencing (Quan, Sauzade, & Brouzes, 2018). Outside the scope of molecular biology, its application has also found its way in forensic research (Whale, Cowen, Foy, & Huggett, 2013), medical diagnosis, environmental monitoring, and food safety analysis (Cao et al., 2017).

To be able to determine the concentration of target NAs, NA detection is naturally a pre-requisite. There are, however, NAs of interests that have very low concentrations to the point that it becomes undetectable in existing detection technologies. This problem is solved by amplifying the NA sequences using Polymerase Chain Reaction (PCR), a widely-used method for NA amplification since its invention in the 1980s (Cao et al., 2017). PCR can multiply specific NA sequences in DNA or RNA from low concentrations to millions of copies. This method exposes the NA sequences mixed with chemical components in a series of 20 to 40 temperature cycles. In each cycle, PCR doubles the NA molecule;

theoretically producing 2^n molecules after n cycles (Quan et al., 2018).

After PCR amplification, absolute NA quantification is achieved using digital Polymerase Chain Reaction (dPCR) technique. This equally divides the NA samples into thousands of partitions; each of these partitions is evaluated as either off or on, or in this context, labeled as positive or negative, hence the term "digital" (Cao et al., 2017).

The dPCR workflow, as illustrated in Figure 1.1, is typically a sequential procedure of extracting the sample from an organism, preparing the sample, distribution of the sample to partitions, PCR amplification and detection, and finally is estimated using a Poisson correction factor. In (Jacobs, Goetghebeur, & Clement, 2014), it was emphasized that every step of the dPCR workflow inevitably allows for the introduction of different sources of variation. These variance components within the dPCR workflow is shown in Figure 1.2.

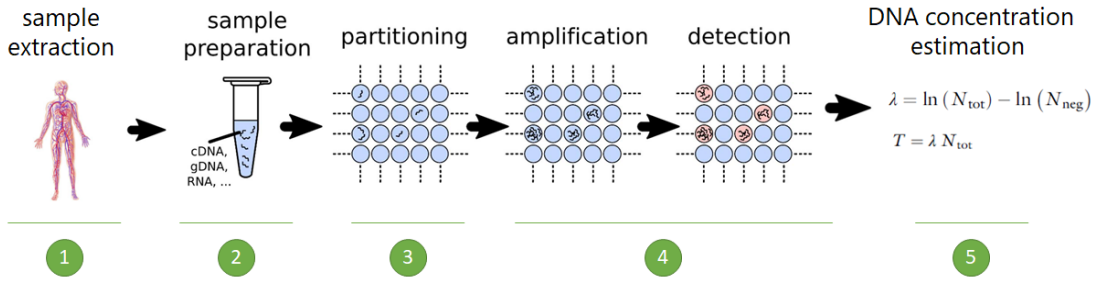

Figure 1.1: The dPCR workflow

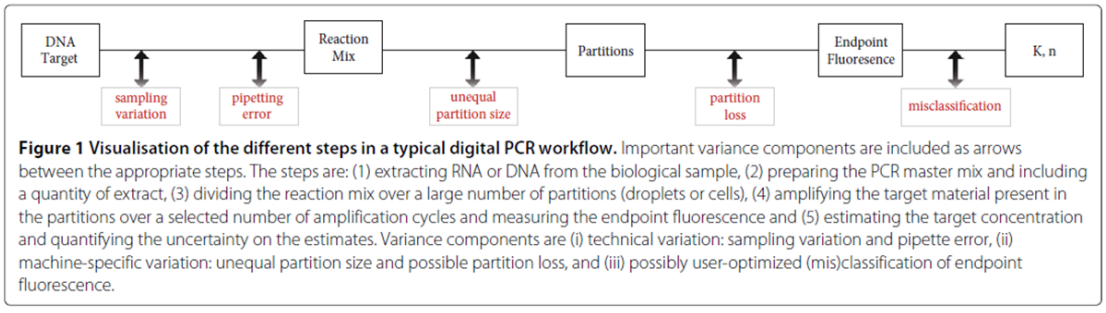

Figure 1 Visualisation of the different steps in a typical digital PCR workflow. Important variance components are included as arrows between the appropriate steps. The steps are: (1) extracting RNA or DNA from the biological sample, (2) preparing the PCR master mix and including a quantity of extract, (3) dividing the reaction mix over a large number of partitions (droplets or cells), (4) amplifying the target material present in the partitions over a selected number of amplification cycles and measuring the endpoint fluorescence and (5) estimating the target concentration and quantifying the uncertainty on the estimates. Variance components are (i) technical variation: sampling variation and pipette error, (ii) machine-specific variation: unequal partition size and possible partition loss, and (iii) possibly user-optimized (mis)classification of endpoint fluorescence.

Figure 1.2: Potential variation components between steps of the dPCR workflow

When

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Albumin

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Appendix A

Diagrams and Other Documentation Tools

This appendix may consist of proposed architectural design, algorithms, scientific formula for MSCS and Data Flow Diagrams, Fishbone for MSIT.

Appendix B

Theoretical and/or Conceptual Framework

Discusses the basic framework/foundation the thesis is based on. This section is normally referred to when discussing Scope and Limitations, and Research Methodology

Appendix C

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References

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