Project 2: "On-chip" Real-time Polymerase Chain Reaction – Week 1

BE/EE189 Design and Construction of Biodevices

Spring 2017

Learning Objectives

1. Understand principles of real-time polymerase chain reaction (qPCR).
2. Understand how qPCR can be used to detect specific DNA sequences (qualitative analysis).
3. Through a literature search and critical assessment, develop understanding of basic design considerations of on-chip PCR based detection device.
4. Attempt design of readout component of a semi-on-chip qPCR and evaluate design.
5. Develop the ability to evaluate merits and limitations current technologies (including your own design) and identify design/re-design opportunities.

Prelab exercise instructions

1. To be completed individually. Discussions are allowed and encouraged.
2. This lab assumes pre-knowledge of the basic principles of DNA replication and polymerase chain reaction (PCR) and fluorescence probes. Please refer to the following resources for an overview of PCR:
    - *Molecular Cell Biology*, 6th ed., Lodish et. al., p. 188-189 'PCR' video: `http://www.youtube.com/watch?v=_YgXcJ4n-kQ`
    - Wikipedia 'PCR': `http://en.wikipedia.org/wiki/Pcr`
3. Please refer to the following resource for a basic idea of fluorescence detection:
    - "Fluorescence Fundamentals" from ThermoFisher. `http://www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook/introduction-to-fluorescence-tec` `html`

qPCR Lab Overview and Prelab questions

What is qPCR?

Real time PCR (qPCR) shares the same basic working principle as that of PCR. "Real time" refers to the ability of the method to read out the amount of amplification product in real time. This is achieved through fluorescence chemistry. The fluorescence emitted by the fluorescence probes of the reaction increases as more DNA products are produced. Using optical detection, the presence of the target DNA can be detected. In a processes with high accuracy (you will discover that this means both detection accuracy and biology techniques of the user), the amount of target DNA present can be detected. So, how do these fluorescence probes work?

qPCR probes

Two of the more common probes are TaqMan and SYBR Green. The understanding of the working principles of these two probes will equip you with understanding of the other qPCR probes available, should you be interested to find out more.

TaqMan - A TaqMan probe is an example of sequence specific probes. A short strand of DNA that has a fluorophore conjugated to its $5'$ end and a fluorescence quencher on its $3'$ end. Thus, in its native state, the probe does not fluoresce under the excitation light (the quencher quenches the fluorophore). These probes are usually specifically designed to be complimentary to a DNA sequence in the internal region of the target amplicon (sequence to be amplified, usually a gene). DNA polymerase, the enzyme used to extend a growing DNA strand, has $5'$-nuclease activity. This means that when the DNA polymerase arrives at the internal region of the target amplicon template, as it grows the DNA strand, it cleaves the TaqMan probe and allows the fluorophore to be separated from its quencher. Fluorescence, which is related to the amount of DNA copies made, thus results and accumulates as the qPCR proceeds.

SYBR Green – The principle of SYBR Green is relatively simple. SYBR Green simply binds indiscriminately to double-stranded DNA (dsDNA). SYBR Green fluoresces quite minimally in the unbound state. Once bound to a dsDNA strand, its fluorescence will dramatically increase (up to 1000 fold).

Prelab Q1: What are the advantages and disadvantages of each of the two qPCR probes introduced above?

Prelab Q2: What is a melting curve analysis? Why is it important? What else do biologists routinely do to check the results of a PCR run?

Typical qPCR instrumentation

Prelab Q3: Draw a block diagram (on paper) of a typical qPCR system available on the market. Hint: Things to consider/include – temperature cycling (detection and feedback), components that enable real-time readout, melt curve analysis, etc.

Prelab Q4: We demonstrated in class how a typical PCR machine works and what its interface looks like. List the design features of a typical PCR interface. Pay special attention to the following:

- Temperature tolerance (i.e. how close to the target temperature before time counts down)

- Time calculation
- User controllable features
- Options to start/stop/pause
- Information displayed on front panel

This should eventually guide your LabVIEW VI design. Note that your LabVIEW VI should include fluorescence signal detection and display, although the PCR machine shown in class does not have that capability.

Project Instrumentation Design

You will be asked to build a heating circuit for qPCR system and control it using LabVIEW. A typical PCR heating/cooling routine looks like this:

Temperature (°C)	Time	Action
94	2 min	Initial denaturation of DNA
94	30 sec	Denaturation
52	30 sec	Annealing of probe to DNA template strand
72	1 min	Extension
Repeat preceding 3 steps for specified number of cycles		
10	∞	Final cool down and hold until user stops program

Prelab Q5: Sketch out the front panel design including these parameters' information so that the user can easily change the settings and check the status of the thermocycler.

You'll also be asked to build the optical setup for fluorescence detection at the end of each cycle. You'll be given the following:

- Thermistor
- Thermoelectric cooler (TEC). Hint: can be used for heating
- LED (broad bandwidth blue light source)
- Fan (for cooling)
- Photodiode
- Optical filters, lenses etc.
- A chamber to hold your sample. Note: we're only performing PCR on one sample (i.e. no multiplexing involved)

Prelab Q6: Write a detailed experimental plan. Include:

1. Schematics of setup (Both heating and optical detection, but don't worry about the circuit design)
2. LabVIEW algorithm (a sketch of algorithm will suffice, you do not have to include specific sub-VIs/functions) and front panel feature design

Note:

1. You do not have to worry about mixing the reaction mixture. Assume the DNA samples, probes required etc. are all put together and given to you.
2. Your VI should display the fluorescence signal acquired over number of cycles.