Primer Design for Microbial Genomes

How to focus on getting enough of what you want.

J. Craig Venter™ INSTITUTE

Where we are

- **13:30-14:00 – Primer Design to Amplify Microbial Genomes for Sequencing**
- 14:00-14:15 – Primer Design Exercise
- 14:15-14:45 – Molecular Barcoding to Allow Multiplexed NGS
- 14:45-15:15 – Processing NGS Data – de novo and mapping assembly
- 15:15-15:30 – Break
- 15:30-15:45 – Assembly Exercise
- 15:45-16:15 – Annotation
- 16:15-16:30 – Annotation Exercise
- 16:30-17:00 – Submitting Data to GenBank

J. Craig Venter™ INSTITUTE

What is PCR?

- Polymerase Chain Reaction
- In vitro amplification of DNA
- Basic Ingredients for PCR
  - DNA Sample
  - Forward/Reverse primers (short oligonucleotides)
  - dNTPs (ie. dATPs, dCTPs, dGTPs, and dTTPs)
  - Polymerase (e.g., Taq)
  - Other reaction additives (eg. cations, Tm reducing agents)
- Thermocycler
  - Each cycle should theoretically double the DNA present (for exponential amplification of original targeted sequence)
- What changes for RT-PCR? What about sequencing reactions?

Melting

1.) Donor dsDNA

```
CATCGATCGACTGATACCAGTGATCGATGCATC
||||||||||||||||||||||||||||||||
GTAGCTAGCTGACTATGGTCACTAGCTACGTAG
```

2.) Melt dsDNA into ssDNA

```
CATCGATCGACTGATACCAGTGATCGATGCATC
||||||||||||||||||||||||||||||||

                  ||||||||||||||||||||||||||||||||
                  GTAGCTAGCTGACTATGGTCACTAGCTACGTAG
```

Annealing and Extension

3.) Cool ssDNA in presence of **forward primers** and **reverse primers**

```
CATCGATCGACTGATACCAGTGATCGATGCATC
||||||||||||||||||||||||||||||||
                        GCTACGT

                         ATCGATC
                        |||||||||||||||||||||||||||||||
                        GTAGCTAGCTGACTATGGTCACTAGCTACGTAG
```

4.) Polymerase extends only from 5' to 3' ends with **dNTPs**

```
CATCGATCGACTGATACCAGTGATCGATGCATC
||||||||||||||||||||||||||||||||
GTAGCTAGCTGACTATGGTCACTAGCTACGT

                        ATCGATCGACTGATACCAGTGATCGATGCATC
                        ||||||||||||||||||||||||||||||||
                        GTAGCTAGCTGACTATGGTCACTAGCTACGTAG
```

J. Craig Venter INSTITUTE

Amplicon is Formed

5.) After many cycles, exponential quantities of dsDNA product (Amplicon) is formed

```
ATCGATCGACTGATACCAGTGATCGATGCA
|||||||||||||||||||||||||||||
TAGCTAGCTGACTATGGTCACTAGCTACGT
```

PCR For Viral Genomics

- Testing for virus positive samples
- Whole segment amplification
- Tiling amplicons across an unsegmented virus – smaller amplicons for Sanger sequencing, larger amplicons for NGS
- Closure reactions to finish regions in viruses after NGS

PCR for Bacterial Genomics

- Amplifying 16S ribosomal RNA or hypervariable regions of 16S
- Sequencing O antigen regions in E. coli isolates
- Sequencing “housekeeping” genes
- Closing gaps in bacterial genomes

PCR for Next Generation Sequencing

- Roche/454 and LifeTechnologies Ion use emulsion PCR (PCR in tiny oil/water micelles)
- Illumina HiSeq/MiSeq use bridge PCR on glass slides
- We'll talk about these in NGS technologies session

Software for Designing Primers

- **Primer3** – probably most popular, Perl&C, http://sourceforge.net/projects/primer3/
- Many tools are wrappers for Primer3
  - **Primer3web** - http://primer3.wi.mit.edu/
  - **Primer3plus** - http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi
  - **Primer-BLAST** - http://www.ncbi.nlm.nih.gov/tools/primer-blast/
  - **JCVI Primer Designer** - http://sourceforge.net/projects/primerdesigner/