Analysis of Bias during PCR and Whole Genome Amplification

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Introduction

The GC content of DNA, i.e. the percent guanine and cytosine, is not uniform along the genome. Sections of genomes can be very high, neutral or low in %GC. Amplification technologies, such as Polymerase Chain Reaction (PCR) and Whole Genome Amplification (WGA), are potentially sensitive to base composition. This means that some sections are not copied as well as the more GC-neutral sections of the genome. This is a problem when PCR is used to amplify genomic DNA for genomic sequencing. Due to the bias during PCR, specifically in the Illumina library prep protocol, the genome of the organism being sequenced will have holes that are either under represented, incomplete or nonexistent in the sequencing reads.

Example of a hole in sequence coverage

Goals

• Test and compare bias between different thermal cyclers.
• Test if slower ramp (1.5°C/sec) speed decreases bias.
• Test the standard PCR protocol and the optimized PCR protocol.
• Test GC Bias during Whole Genome Amplification (WGA)

Results: Polymerase Chain Reaction (PCR)

• Bias introduced after gel extraction by subsequent PCR amplification.
• Standard PCR protocol is sensitive to the model of the PCR machine used.
• Optimized PCR protocol gives a relatively flat GC profile from ~15% to ~90% GC.

Results: Whole Genome Amplification (WGA)

The WGA Process:

• Random hexamer primers bind to denatured input DNA.
• Primers are extended by Phi29 DNA polymerase.
• Strands are displaced by proceeding primers.
• Primers bind to newly copied strands as well.

The Project Goal:

WGA is often used to amplify small quantities of genomic DNA prior to sequencing. We used the same mix of genomes and qPCR assays to study bias during WGA. Amounts of input DNA was varied from 1pg to 100ng.

Materials and Methods

Illumina Library Preparation

• DNA input amount can vary from 1pg to 100ng.
• Amplification reaction can proceed for >30min.
• All reactions were done in triplicate.

PCR Protocols

| Standard | Phusion DNA Polymerase |
| 1x | 30/58°C |
| 1x | 72/72°C |
| 1x | 5min/98°C |

Optimized: AccuPrime Taq

| HF DNA polymerase |
| 1x | 72/72°C |
| 1x | 5min/98°C |
| 1x | 12min/98°C |
| 1x | 10min/72°C |

PCR Machines

Analysis of Bias via qPCR

Conclusions

• PCR introduces bias in the Illumina Library Preparation protocol.
• Standard PCR protocol (Phusion High Fidelity with short denaturation) can have varying degree of bias, depending on the thermocycler used. Bias on poorly performing fast-ramping instruments can be slightly improved by slowing ramping speed to 1.5°C/sec (further optimization is needed for better performance).
• Optimized PCR protocol (AccuPrime Taq High Fidelity) has very little bias regardless of thermocycler. Slowing the ramping speed to 1.5°C/sec has little to no effect on performance.
• WGA has bias too, however it appears to depend on the GC content of the organism’s genome, rather than the %GC of each individual locus.

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