Investigating the effect of environmental toxicants on germ cells via bisulfite sequencing
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Abstract
Bisphenol A (BPA) and arsenic are hazardous chemicals that cause reproductive dysfunctions over multiple generations. This project applies bisulfite sequencing (BS-seq) to investigate the epigenetic mechanisms underlying their transgenerational inheritance. BS-seq can comprehensively identify the cytosines that have been methylated and how these methylation events can be altered by a treatment. We compared the effectiveness of two BS-seq pipelines, wg-blimp and BSBolt, in detecting differentially methylated regions (DMRs) from untreated, BPA-exposed, and arsenic-exposed in vitro mouse primordial germ cells. Specifically, we used whole genome BS-seq (WGBS) to analyze the effects of BPA, and reduced representation BS-seq (RRBS) to analyze the effects of arsenic. While wg-blimp is designed to run a complete WGBS pipeline, BSBolt requires steps to be performed individually. I found that wg-blimp is harder to troubleshoot but that BSBolt is more time consuming. Using BSBolt, I obtained data for FastQC analysis and aligned the WGBS genomes to a reference. I was also able to complete RRBS and began to analyze the results for DMRs.

Background
- Bisphenol A (BPA) is an environmental toxicant that can cause reproductive dysfunction
- Arsenic is a naturally-occurring chemical element and a highly toxic carcinogen
- Exposure to substances such as BPA and arsenic has epigenetic effects
- Whole-genome bisulfite sequencing (WGBS) and reduced representation bisulfite sequencing (RRBS) can be used to analyze these epigenetic effects
- Identify differentially methylated regions (DMRs) by looking for cytosines in the genome that have been methylated by a treatment
- Using data from in vitro mouse primordial germ cells

Results

- Very few DMRs observed in both datasets
  - WGBS: Observed in a previous trial
  - RRBS: Observed in chromosomes 10, 11, 15, 2, 7, 9
- BSBolt was the more effective pipeline
- New roadblock: Variation in data due to segmentation
- Need to fuse files for each sample together and rerun
- Can look at differentially methylated cytosines (DMCs) instead of DMRs
- Can begin to investigate the effects of other harmful compounds, such as nicotine

Discussion & Next Steps

- WGBS pipeline
  - Completes all steps end-to-end with just one command
  - Intended to run faster and use less digital memory than existing WGBS pipelines
  - Includes visualization in Integrative Genomics Viewer (IGV) and graphing in R Shiny

Methods

BSBolt can be used for RRBS or WGBS
- Developed at UCLA
- Requires steps to be completed individually but allows for more customization
- Takes more time than wg-blimp but is easier to troubleshoot

References

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