Genome Variant Analysis 2016

General Introduction

Background

- Key research interest identifying rare variants in variety of systems and determining how to leverage that information.
- Self-taught computational biologist
- TA two years
- 3rd year teaching this course

A nod to the past …

- Scott Hunicke-Smith
- Former Director of Genome Sequencing Analysis Facility

- Jeffrey Barrick
- Assistant Professor MBS & ICMB

Disclaimers up front

- Royal “we” – Tutorials written without clean tense and pronouns. We likely means me, so don’t blame Sean for any problems you find, but feel free to assume its only working because of his work.
- Spelling – Has never been a skill I possess, hopefully will only be noticeable if I write on board.
- Typos – Will likely be your biggest problem in using the commands we provide, and the biggest problem in your own work. Try to type the commands out to get practice, but remember you can copy paste.
- Names – I usually use this opportunity to apologize for my inability to remember people’s names with a funny anecdote, with small class might not be an issue.

Where to start

- Many say “don’t know where to start” their data analysis once they have their data files.
- Ideally should have “started” weeks-months ago in planning experiments.
  - If you fall in this category don’t worry you are in a common situation
- Not all libraries are created the same, and can drastically effect analysis.

Standard Library Prep

1. Fragment DNA
    1. Enzymatic, sonication, acoustic, nebulization
2. Blunt DNA
3. “A”-Tail DNA
4. Ligate adapters
5. PCR

Standard library prep sufficient

- Clonal samples
  - Each base 0 or 100%
  - 50% possible in diploids
- Low-moderate coverage depth populations (<100)
  - Standard Illumina error rate 1%
  - Much more on this later
- Good reference
  - Typical insert sizes 250 – 700 bp

Standard library prep lacking

- High coverage populations (>100)
  - Error rate of 1% sets limit of detection at 1% regardless of depth
    - Error rate reduction
- Non-model organism
  - Difficult to generate good reference assemblies using 200 - 700bp
    - Mate-pair libraries several kb inserts
    - Combine with other long read sequencing solutions
- Repeat-mediated rearrangements
  - Repeats often 1.5kb+ long, difficult to get reads on both sides
    - Mate-pair libraries again

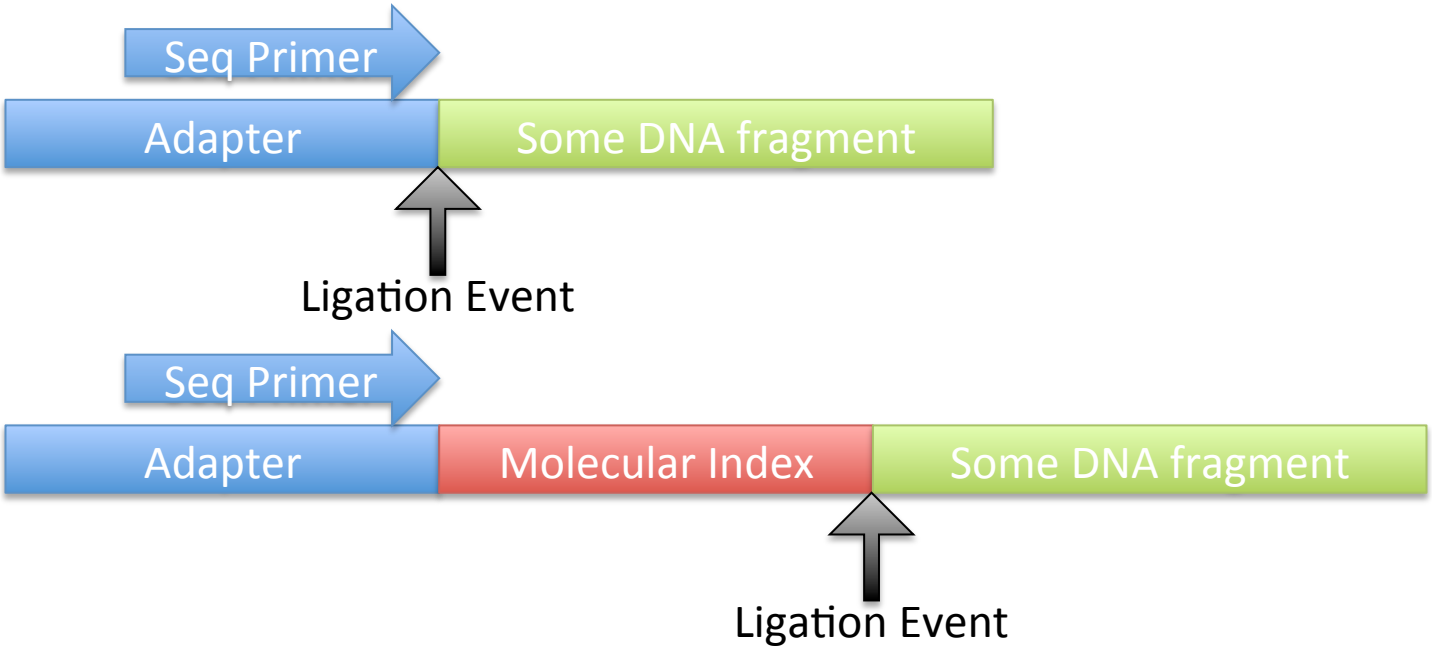
Error rate reduction

- Key = reading the same fragment of DNA multiple times independently. 2 main ways.

1. Molecular indexing
2. Circle sequncing

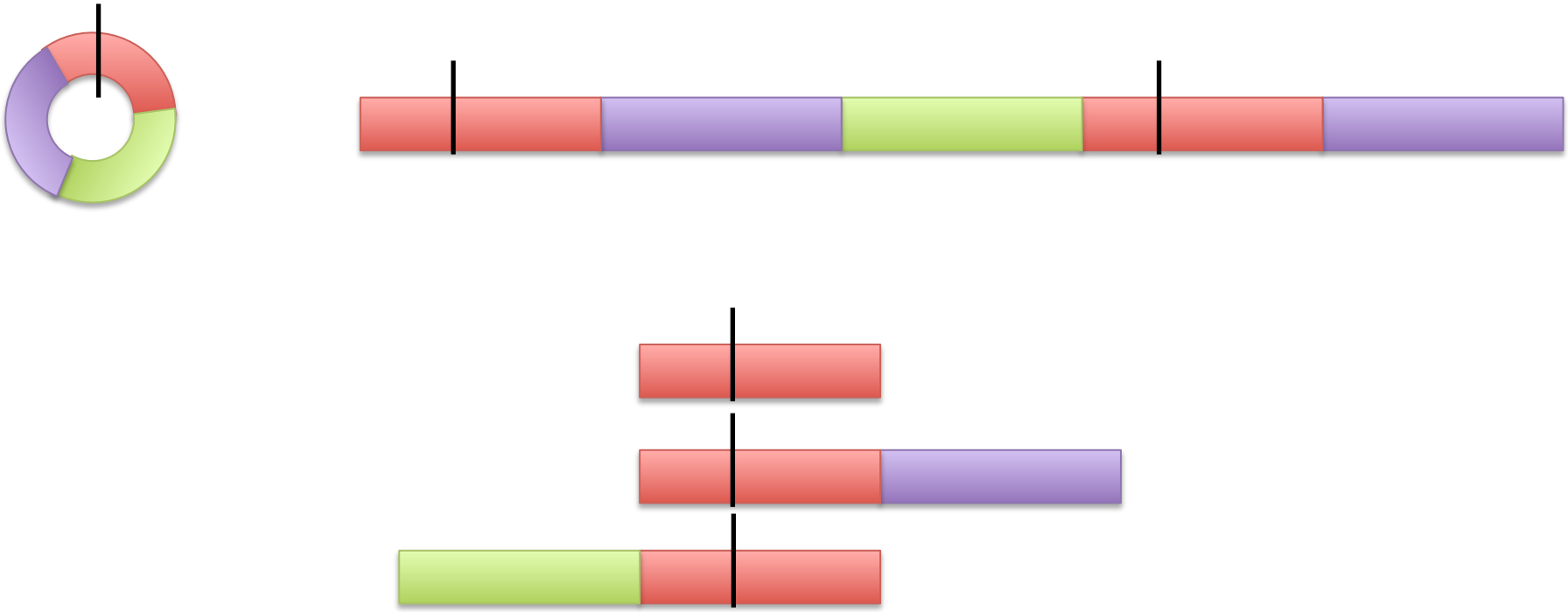
Molecular Indexing

- Ligating adapters with degenerate sequences in the sequence read to fragmented DNA.
  - Schmitt et al PNAS 2012



Circle Sequencing

- Circularize DNA fragments, rolling circle amplification, standard library prep.
  - Lou et al, PNAS 2013



Mate Pair Library

- Generates 2 outwardly facing reads separated by up to 25kb

Computers Computers Computers

- Millions of reads, 100s of bp long, mapping to millions-billions of base long references.
- Windows is your enemy, linux/Mac is your friend.
- TACC is a time machine that lets you get stuff done much faster
  - This is where we will start the class.