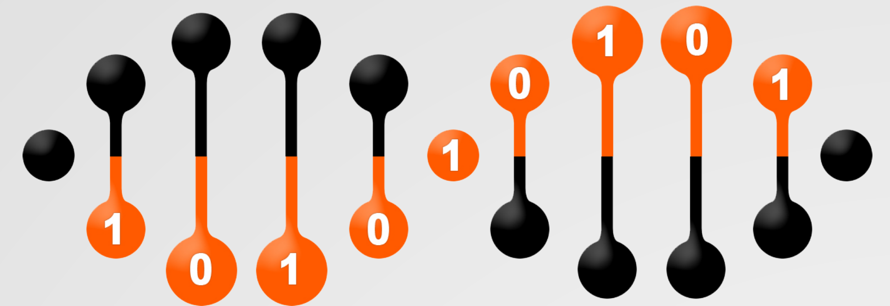
CBC Data Therapy

RADSeq Discussion



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Traditional RADSeq

RAD-Seq Considerations

- Library preparation
- Traditional RAD
  - Uses standard restriction enzymes
  - Random fragments
    - 96 individuals ligated with RAD adapters then split into two separate library preps for sonication and Illumina prep – sequenced across 1-2 lanes generally
    - Might be better to create more libraries rather than deeper sequencing to content with PCR duplicates in the data (increases homozygosity)
  - http://www.molecularecologist.com/2016/08/the-trouble-with-pcr-duplicates/
    - Degenerate adaptor sequences to identify duplicates (identical at the 5'end)
    - Approach: http://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12314/abstract;jsessionid=64DBB9EA713E765363F48A676F948B42.f04t01

Modifications (2013)

- EzRAD
  - Uses standard TruSeq libraries
  - https://peerj.com/articles/203/
  - *Allows selection of specific enzymes (or combinations of enzymes) that cut frequently enough to generate fragments of the desired size range, without requiring the purchase of separate adapters for each enzyme*
    - Less expensive, more control
  - Tools: Rainbow, VarScan2, VCFTools (no strand specificity)
- ddRAD
  - 2 specific enzymes
  - Multiple rounds of size selection
  - Improved coverage at selected loci

More Targeted

- NextRAD (SNPSaurus) -> HyRAD (http://www.molecularecologist.com/2016/04/hyrad-and-museum-genomics/)
- Fragments generated from a standard double-digest RAD protocol as biotinylated probes.
  - Probes are hybridized with fragments from shotgun genomic libraries, capturing homologous sequences for PCR enrichment.
  - By sequencing both the original ddRAD library and the enriched shotgun library, short reads from the latter can be assembled into contigs and mapped onto the former
    - Phylogenomics (sequence capture)
    - Adapted for degraded DNA
    - Better coverage per loci

RADSeq (Hybrid approaches)

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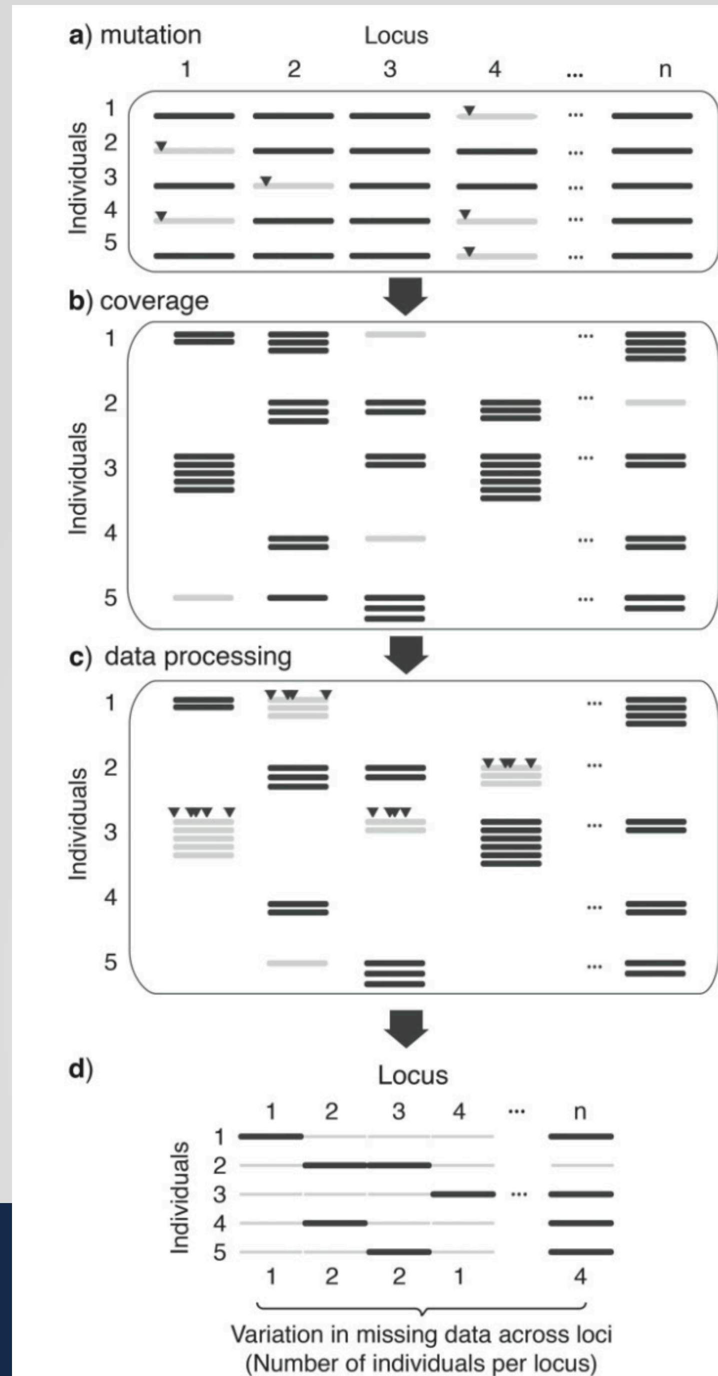
RAPTURE (RAD-Capture

- MISSING DATA!
  - http://www.genetics.org/content/202/2/389
  - The new RAD protocol improves versatility by separating RAD tag isolation and sequencing library preparation into two distinct steps. P
  - Protocol also recovers more unique (nonclonal) RAD fragments
    - In-solution capture of chosen RAD tags to target sequencing reads to desired loci.
    - Rapture combines the benefits of both RAD and sequence capture, *i.e.*, very inexpensive and rapid library preparation for many individuals as well as high specificity in the number and location of genomic loci analyzed.
  - Modified protocol for the bioinformatics

How to handle that missing data…



Institute for Systems Genomics:
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Bioinformatics

- http://www.nature.com/nrg/journal/v17/n2/full/nrg.2015.28.html?WT.feed_name=subjects_dna-sequencing
  - Stacks (PopGen) -> supported in Galaxy
    - http://catchenlab.life.illinois.edu/stacks/
  - PyRAD (Phylogenetics)
    - https://github.com/dereneaton/pyrad
  - UNEAK – Tassel for GWAS
  - Hybrid approaches – Rainbow, Variant Callers (VarScan2, GATK)
    - https://sourceforge.net/projects/bio-rainbow/files/
- Nice Review on NGS approaches for non-models:
    - http://www.sciencedirect.com/science/article/pii/S1874778716300368

- Questions:
  - Approaches for Assembly (Modification of Genome Assembly – specialized such as Rainbow)
  - How to annotate the assembled contigs – BLAST databases to approach the problem
  - When do we need to worry about phasing?