Pink salmon sequencing and marker development

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Outline

- Why do we need new genetic markers?
- How will we find them?
- Proposed study design
- Next steps

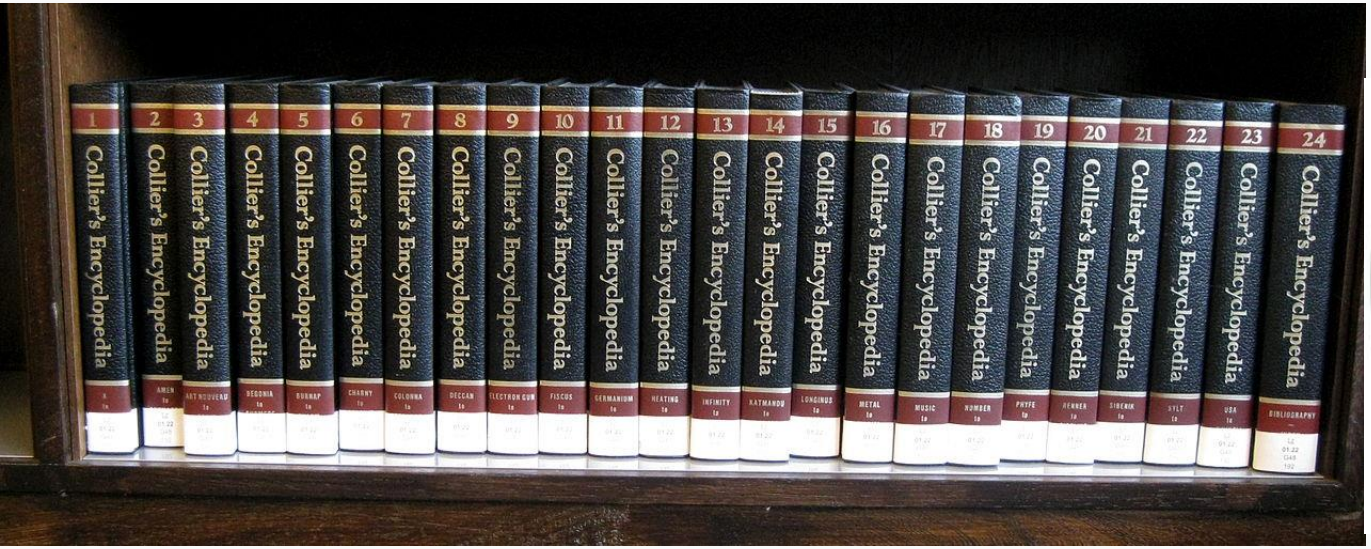
Marker choice for fitness study

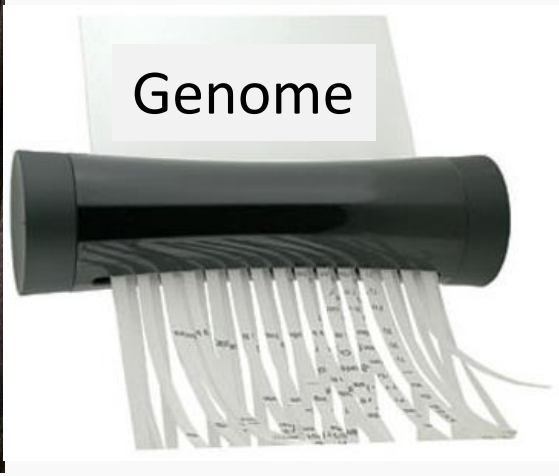
- Fitness study needs
  - Many alleles
  - MAF > 0.3
  - High throughput
- ADF&G current pink salmon markers
  - 16 microsatellites
  - Good for population structure with many alleles, but,
  - Low MAFs and low throughput
- Single nucleotide polymorphisms (SNPs) are a solution

Marker development

- Methods for genetic marker discovery have rapidly evolved
- Current methods based upon **r**estriction-site **a**ssociated **D**NA sequencing (RAD sequencing)
- What is RAD sequencing?

RAD sequencing





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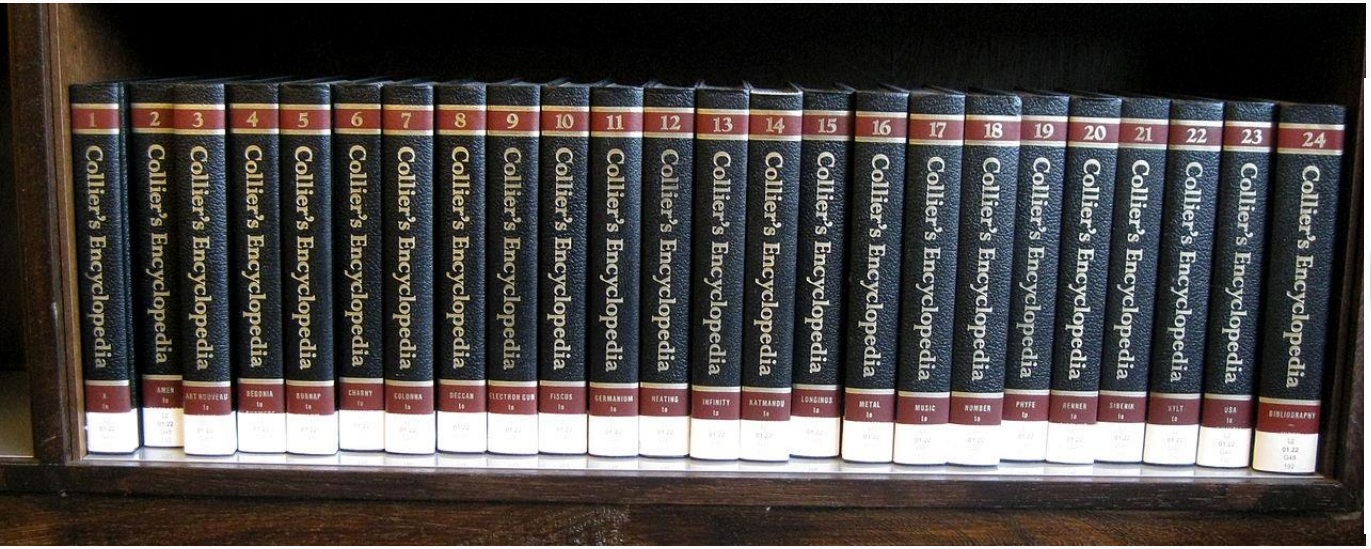
Restriction enzyme = cutter

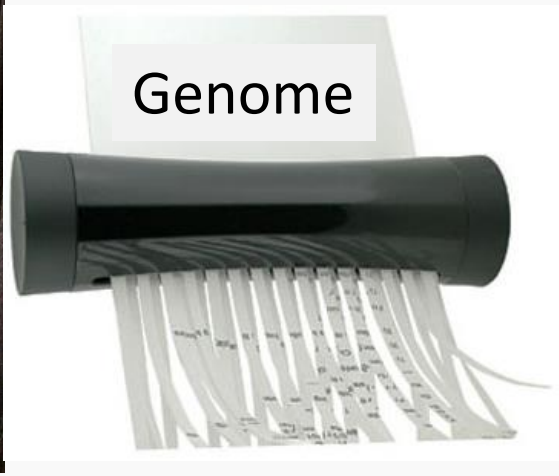
" the " = cut site

"**The** Alaska Department of Fish and Game (ADF&G) is a department within **the** government of Alaska. **The** Department of Fish and Game manages Alaska's fish, game, and aquatic plant resources."

Individual 1: he Alaska Department of **Fish** and

RAD sequencing





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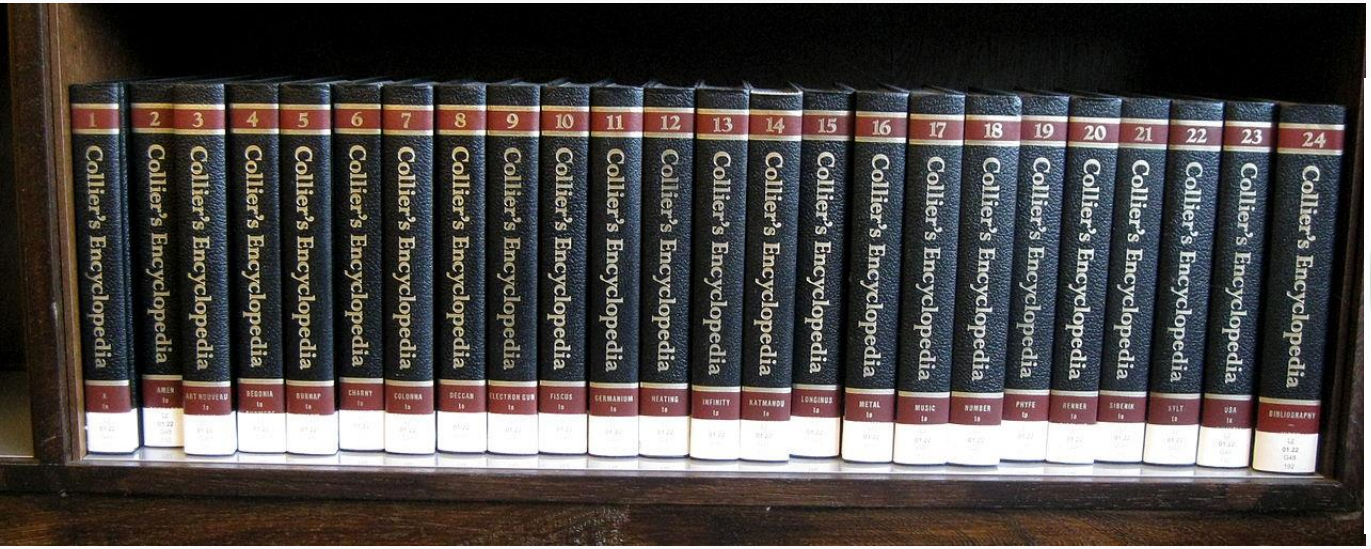
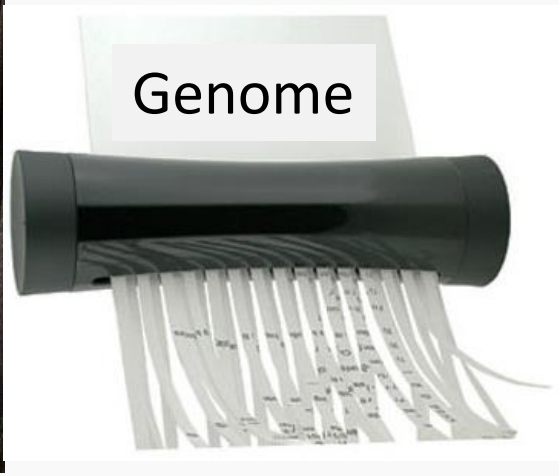
SWP= Fish/Fisheries

RAD sequencing





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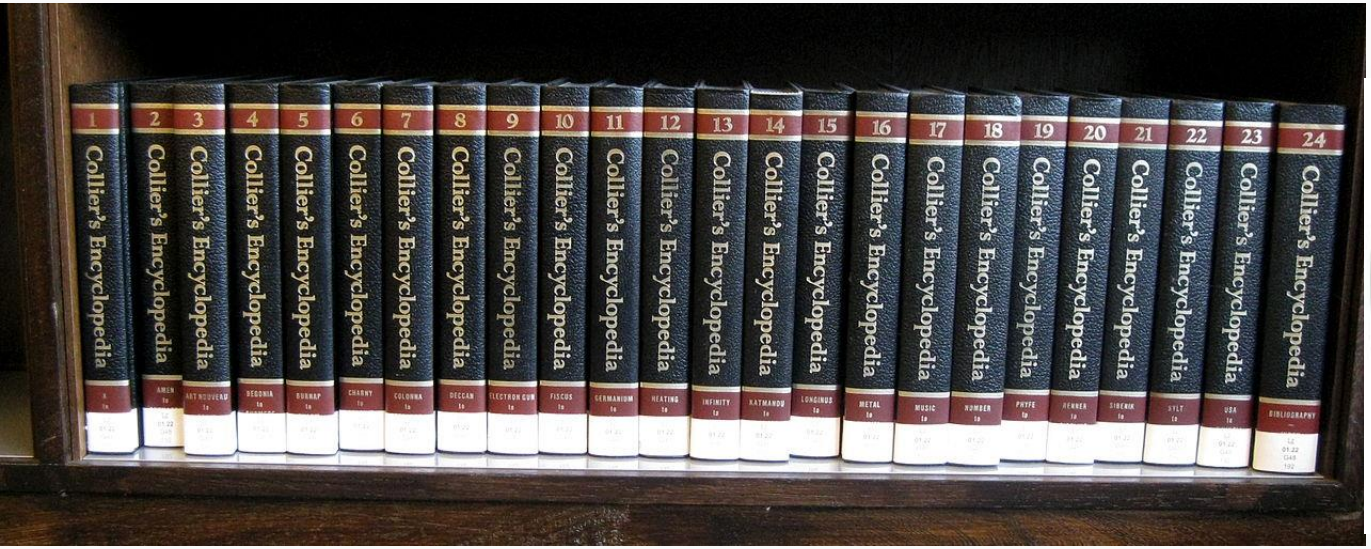

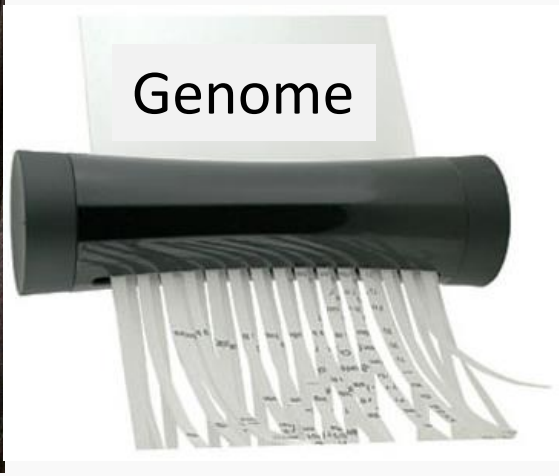

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Restriction enzyme = cutter

"CCTGCAGG" = cut site

TATACGAGT**CCTGCAGG**GCATTAGCCGTACGATCAGTAC

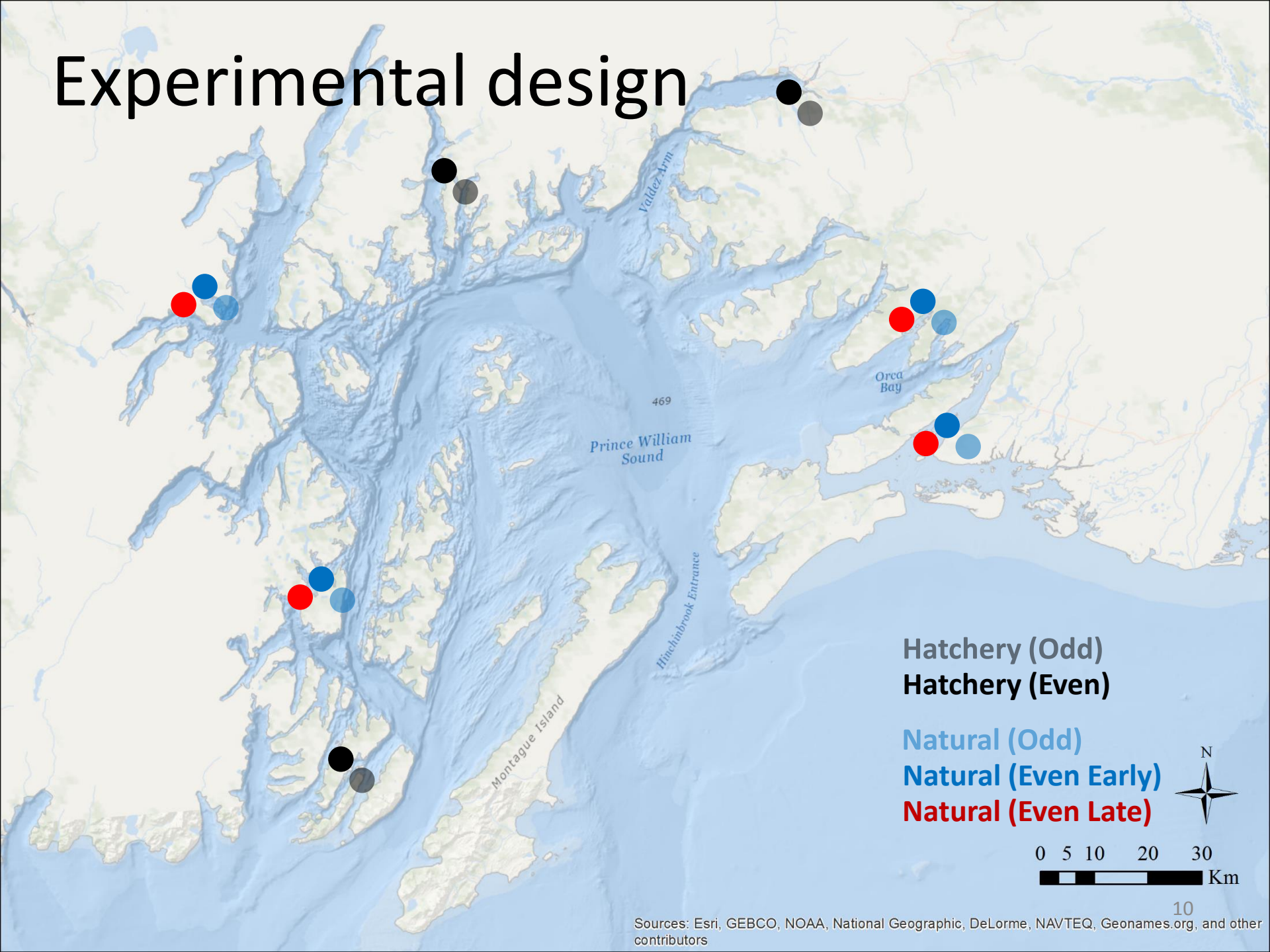
Individual 1:	**TGCAGG**GCATTAGCCGT**A**CGATCAGTAC
Individual 2:	**TGCAGG**GCATTAGCCGT**G**CGATCAGTAC
Individual 3:	**TGCAGG**GCATTAGCCGT**A**CGATCAGTAC

SNP = A/G

Proposed study design

- Apply RAD sequencing to samples from PWS
- Which populations?
  - Include samples of hatcheries potentially straying into pedigree streams
  - Pedigree streams make the most sense, but DNA quality is likely poor (carcass sampling)
  - Choose populations representative of pedigree stream populations (geography and abundance)
  - If possible, use new high quality tissue samples with paired sex data to allow for identification of sex-linked SNPs (useful for parentage and quality control)

Experimental design



Next steps

- Select natural-origin fish using otoliths
- Extract DNA from natural-origin and hatchery collections (Winter-Spring 2015)
- Prepare samples for sequencing (Spring 2015)
- RAD sequence (Summer 2015)
- Identify SNPs useful for parentage analysis (Fall 2015)

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- VFDA: Mike Wells and staff for Solomon Gulch samples

Questions?