

How we do GBS...

And what's next?

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MapNet GBS workshop

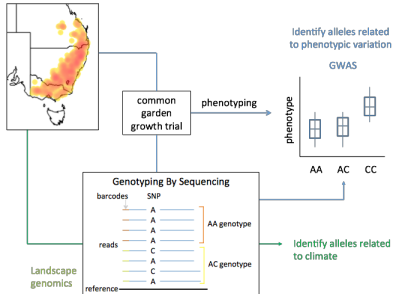
23 Oct 2014

- ▶ Bioinformatics R/A
 - ▶ TraitCapture Project Developer
 - ▶ Genomics (Low level sequence analysis)
 - ▶ Phenomics (Image analysis)
 - ▶ Sample tracking, data standards, HPC.
- ▶ Starting PhD in Bioinformatics of Evolutionary Genomics next year
- ▶ *"Data Intensive Biologist"*

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- ▶ *“Data Intensive Biologist”*
- ▶ A **very** lapsed kiwi (from Napier)

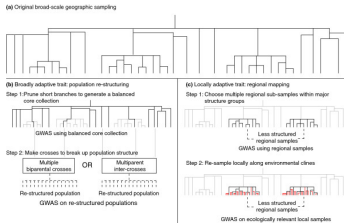
What do we use GBS for?

- ▶ Population and Landscape Genomics
- ▶ Population re-sampling
- ▶ Genotype verification
- ▶ Epigenomics - HpaII/Mspl



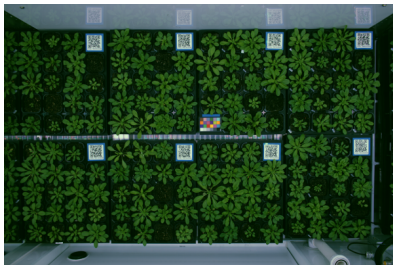
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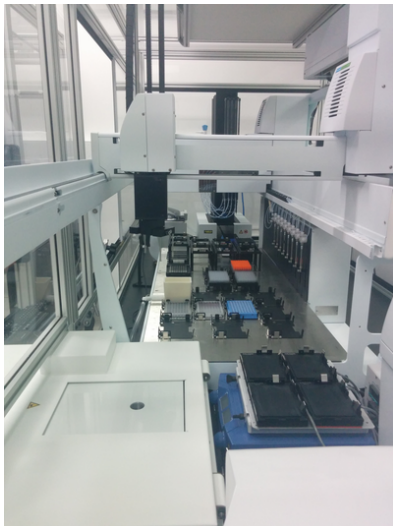
Library preparation

- ▶ We do all our library prep. in house
- ▶ Cost \approx \$10/sample including extraction
- ▶ Done in lots of 96
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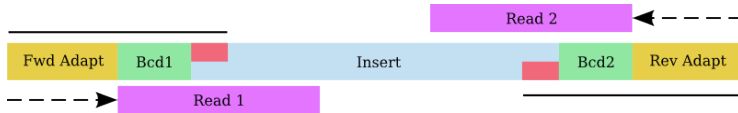
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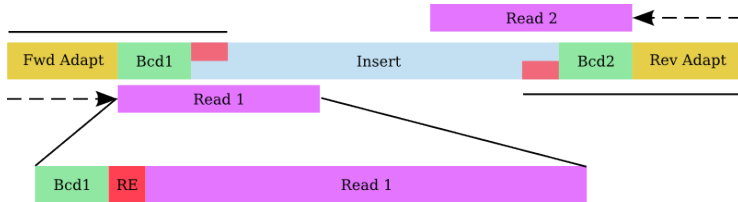
Library preparation protocol

- ▶ Modified protocol from Elshire et al. (2011)
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- ▶ We use in read barcodes
- ▶ Sequence 576-1152 samples/lane
- ▶ Need “combinatorial” barcoding (next slide)



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Combinatorial Barcoding

- ▶ We use dual in read barcodes
- ▶ Independent barcodes for R1 & R2
- ▶ Set of $96 \times 12 = 1152/\text{lane}$
- ▶ Must be balanced & staggered
- ▶ Hamming distance > 2

TGCG
AGGAT
TTCAGA
CGCGGT
GAATTCA
CTACGGA
....
CCGGATAT
TTCCTGGA

X

TGCG
AGGAT
TTCAGA
CGCGGT
GAATTCA
CTACGGA
....
CCGGATAT
TTCCTGGA

=

CTCG	ATGAAAG	1_A1
TGCA	ATGAAAG	1_A2
ACTA	ATGAAAG	1_A3
AACT	ATGAAAG	1_A5
.....		
GCTGTGGA	CTTGCTT	12_H8
GTGAGGGT	CTTGCTT	12_H10
TATCGGGA	CTTGCTT	12_H11
TTCCTGGA	CTTGCTT	12_H12

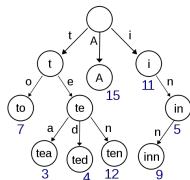
96 R1

12 R2

1152 pairs

De-multiplex with AXE

- ▶ Barcoding scheme requires advanced de-multiplexing
- ▶ Trie-based lookup algorithm
- ▶ Fast (PE lane in 5-10 mins)
- ▶ Highly accurate (99.6%TPR, 100%TNR)
- ▶ Open source at <http://git.io/kIhEZA>
- ▶ IPython notebook “torture-test” at <http://bit.ly/1sXrx4E>

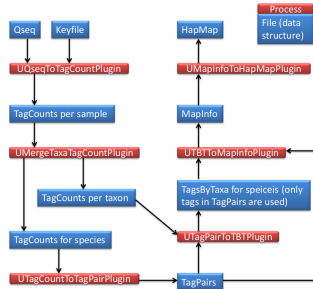


- ▶ Sample-tracking system manages FASTQs after demux:
 - ▶ QC reads (scythe, sickle, seqqs)
 - ▶ After QC, 16-mer globally unique ID re-added to reads
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- ▶ DB links FASTQs to real world
 - ▶ Integrate w/ ALA
 - ▶ Provide user-friendly names to downstream analysis

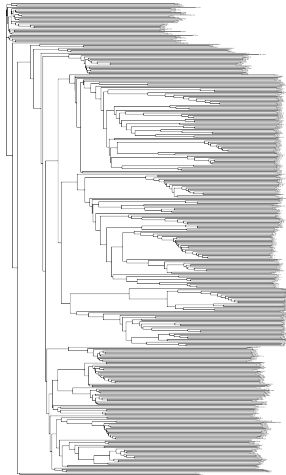
Downstream Analysis

- ▶ Variant calling:
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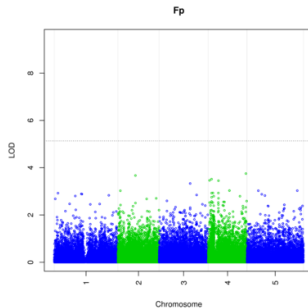
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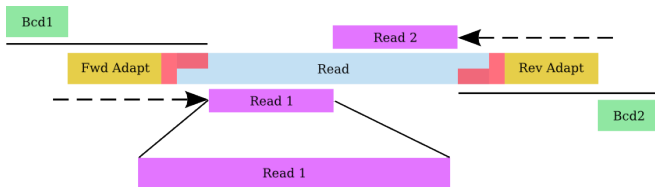


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- ▶ Downstream:
 - ▶ Structure
 - ▶ BayENV
 - ▶ QTLRel

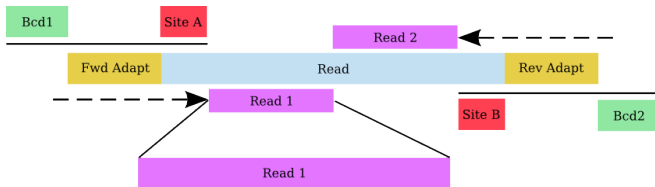


► Better adaptors



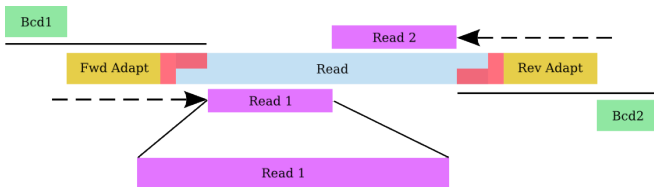
The Future!

- ▶ Better adaptors
- ▶ Home-brew NexTera
 - ▶ NextRAD
 - ▶ Low coverage WGS
 - ▶ Long Pseudo-reads



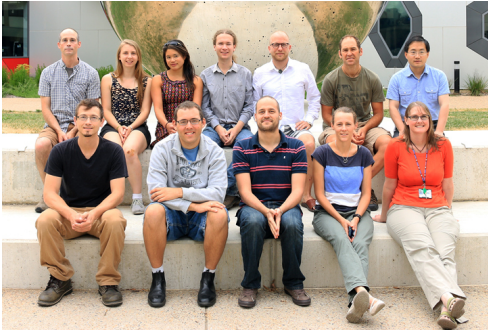
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- ▶ Informatics:
 - ▶ Imputation?
 - ▶ Paralog/Ploidy detection
 - ▶ Streaming Variant Calling (my PhD)



Thanks

- ▶ Justin Borevitz
- ▶ Comrades in Informatics
 - ▶ Aaron Chuah
 - ▶ Riyan Chen
 - ▶ Jared Streich
- ▶ Wet-lab Wizardry
 - ▶ Niccy Aitken
 - ▶ Norman Warthmann
- ▶ Rob for the invitation
- ▶ You all for listening!!



`git.io/TYrIFw`