IWGSC Workshop and Training Session
7-8 April, 2011

Wheat Bioinformatics activities at the CCG

Matt Bellgard
Director, Centre for Comparative Genomics

MURDOCH UNIVERSITY
PERTH, WESTERN AUSTRALIA

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Outline

- Contribution to the IWGSC
  - Two specific regions of chromosome 3B
  - Chromosome 7A
- Analytical environment for analysis
- Informatics issues

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Chromosome 3B

- **The ctg506 region** Selected for detailed analysis
  - Several cell wall invertase (IVR1) genes are located in this region.
  - These genes are often important in maintaining pollen viability during early development.

- **The ctg344 region** Selected for detailed analysis
  - Carries the gwm533 marker that is widely used to track disease resistance (Sr2) located on 3B
  - Potentially a region important in several disease resistances

Core Ideas for Assembly

- We know wheat is “difficult” to assemble
  - mis-assemblies are and will be common
- If we see the same sequence assembled using different assemblers and data
  - this is more likely to be correct
- Points of divergence between different assemblers need to be analysed in detail
- Information from databases such as TREP need to be considered in compiling the detailed genome sequence.
- Genome sequence information needs to relate to a high quality molecular genetic map with traits included

Assembly approach

- Each BAC (Illumina short reads 70bp, paired-end, Sanger) assembled separately using Velvet
  - using multiple parameter combinations
- Different assemblies of the same BAC compared (mummer, freckle)
  - Potential mis-assemblies identified (eg: assemblies that disagree at certain points)
- Using the fingerprint assembly as a guide, identifying sequence present in overlapping BACs – take contigs that agree between different BACs to be “confident”. Start with large contigs and work down to shorter contigs.
- Look at paired-end alignments (BWA + genomeview, Hawkeye) and identify potential mis-assemblies
- Use paired-end information (illumina short reads 500bp insert + Sanger 4kb insert) to try to extend contigs (in general, this is difficult)
- Use LTR information from eg: TREP to order contigs

Original ctg506

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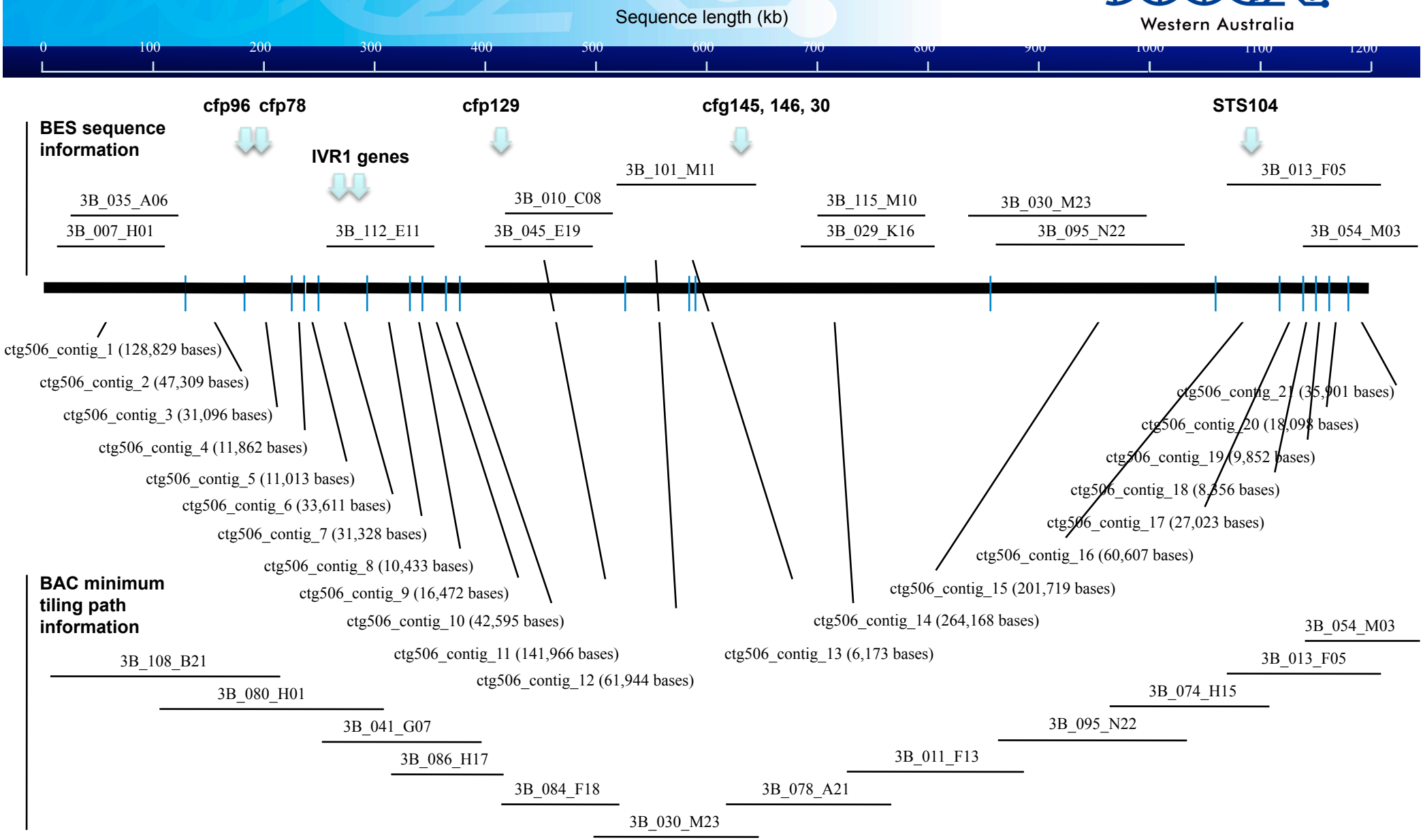
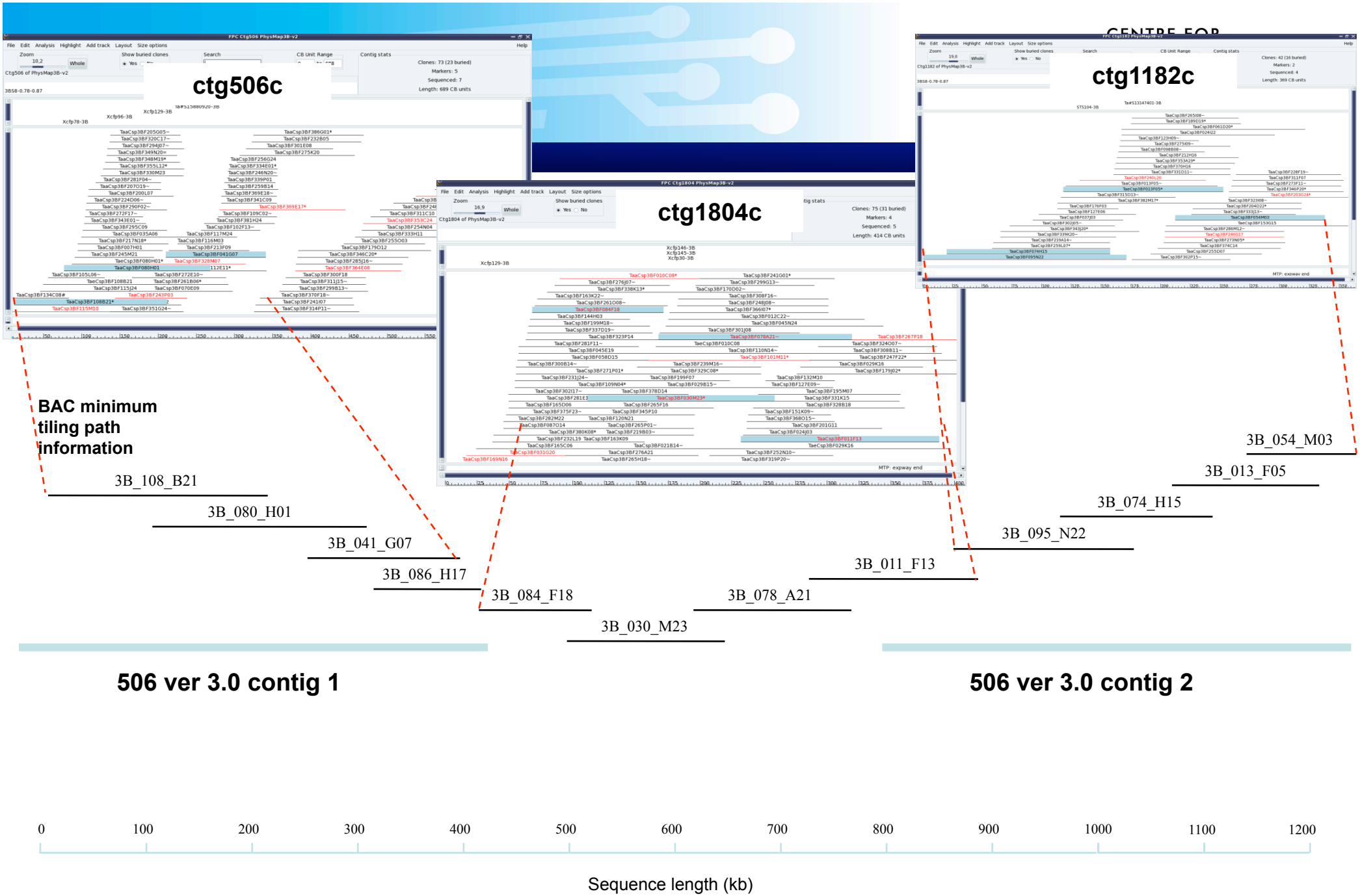


INRA 454/8kb of Ctg506

- CCG-illumina/Sanger sequencing compared to INRA 454/8kb mate pairs sequencing:
- The INRA scaffolds for ctg506
  - Closed 6 small gaps of Ns in CCG assembly
  - Improved the ordering of contigs in the CCG sequencing
  - Highlights miss-assemblies (at least 6)
- The CCG sequencing
  - Orient large INRA scaffolds
  - Closed 30/83 gaps in INRA sequencing assembly
    - Most of these gaps were in the introns of regions annotated as coding sequences.


ctg506c
ctg1804c
ctg1182c
BAC minimum tiling path information
3B_108_B21
3B_080_H01
3B_041_G07
3B_086_H17
3B_084_F18
3B_030_M23
3B_078_A21
3B_011_F13
3B_095_N22
3B_074_H15
3B_013_F05
3B_054_M03
506 ver 3.0 contig 1
506 ver 3.0 contig 2
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Sequence length (kb)

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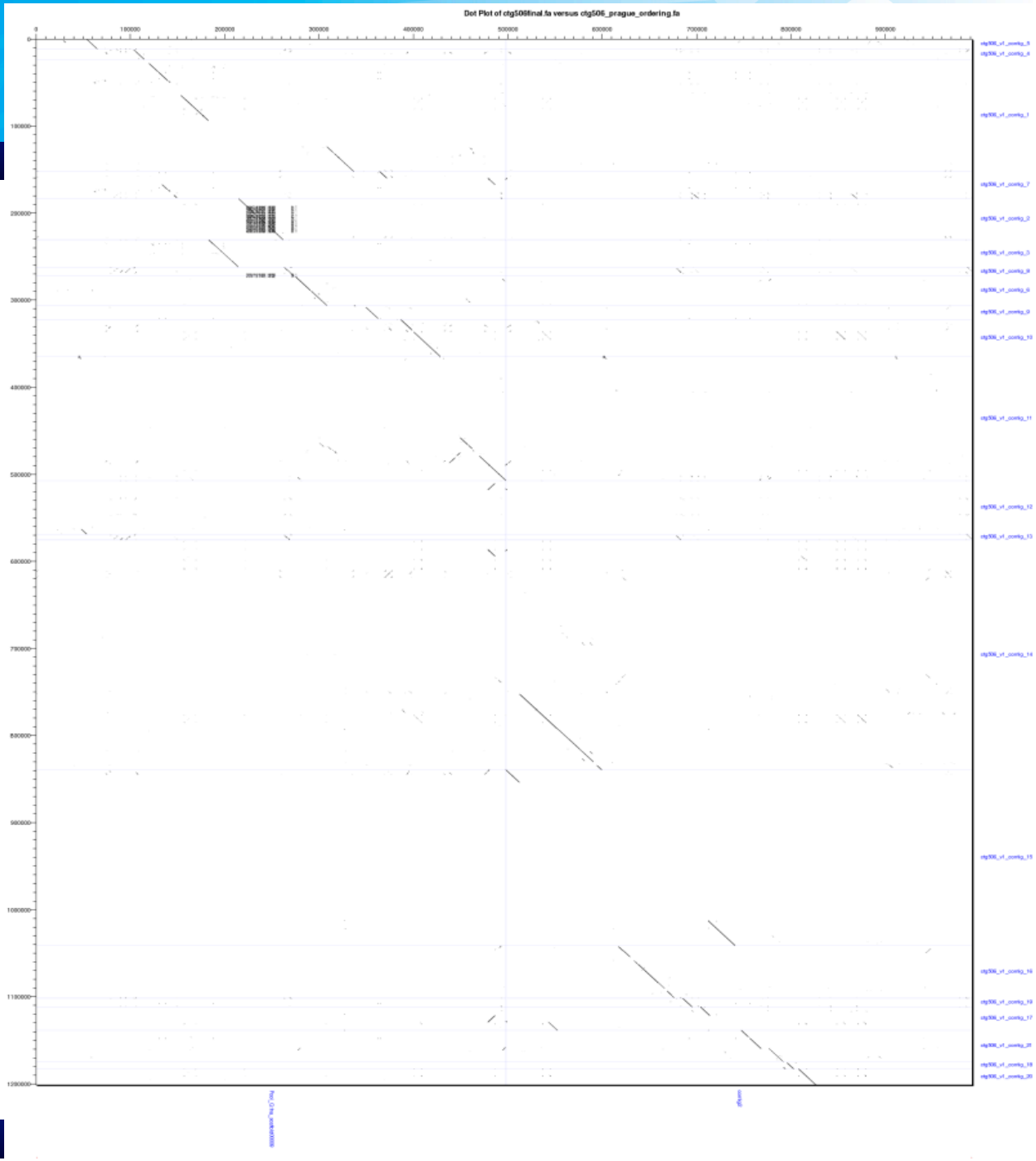

Alignment of CCG short read + Sanger assembly (y-axis) against version 3 (combined CCG + INRA) (x-axis)

The CCG contigs have been re-arranged in an attempt to fit the order revealed by INRA scaffolds

Detailed analysis of IVR gene region

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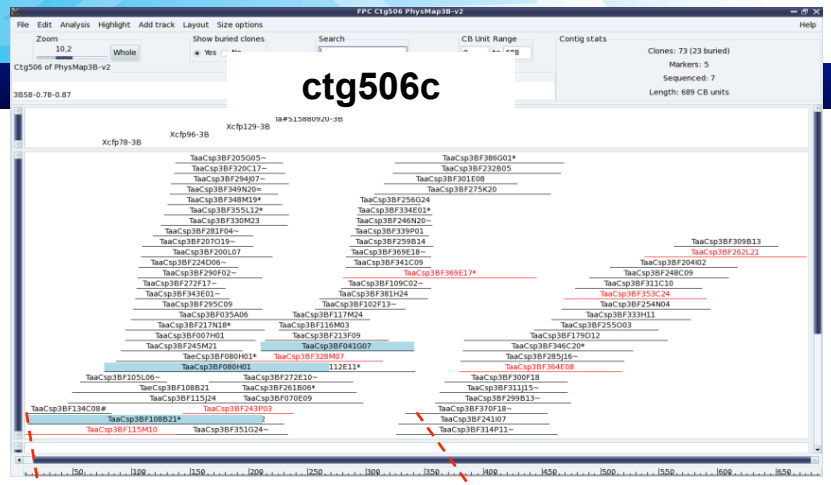
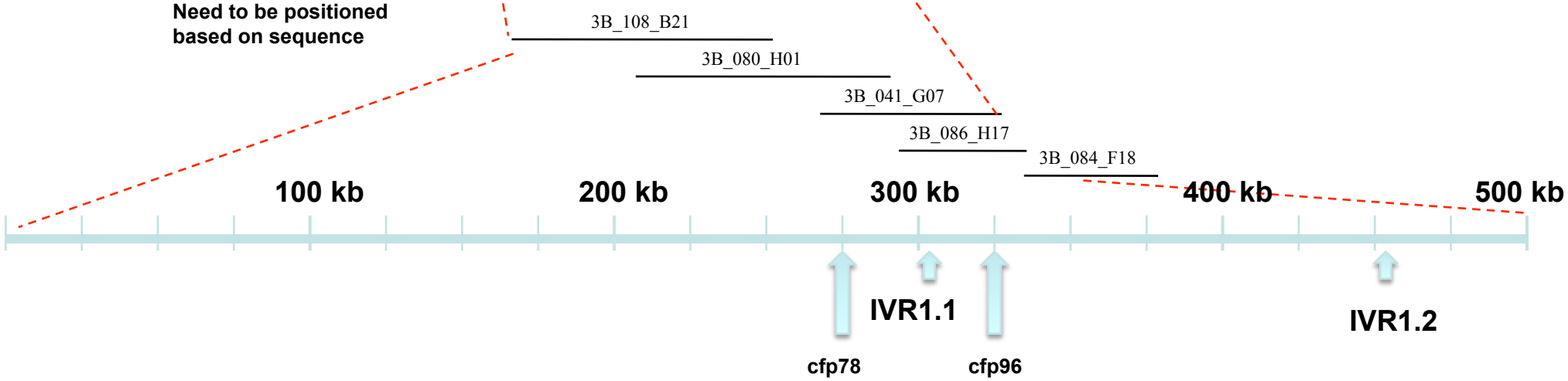


BAC minimum tiling path information

Need to be positioned based on sequence



Hollie Webster PhD thesis

Detailed analysis of IVR gene region

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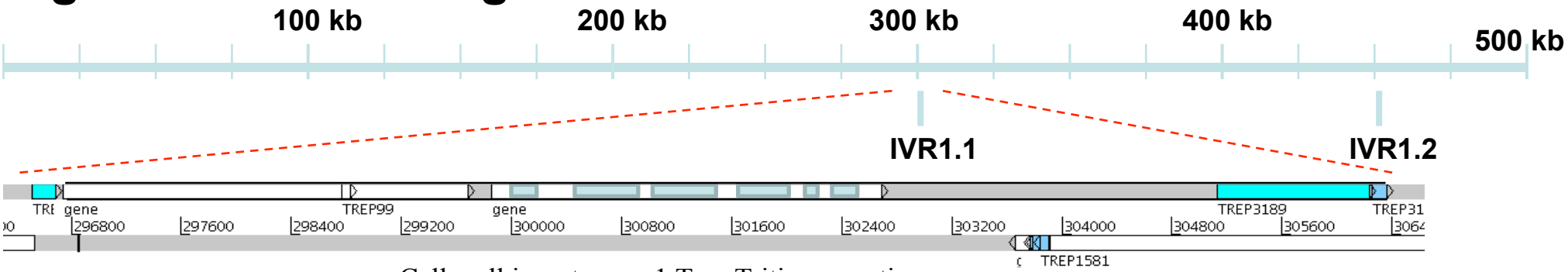
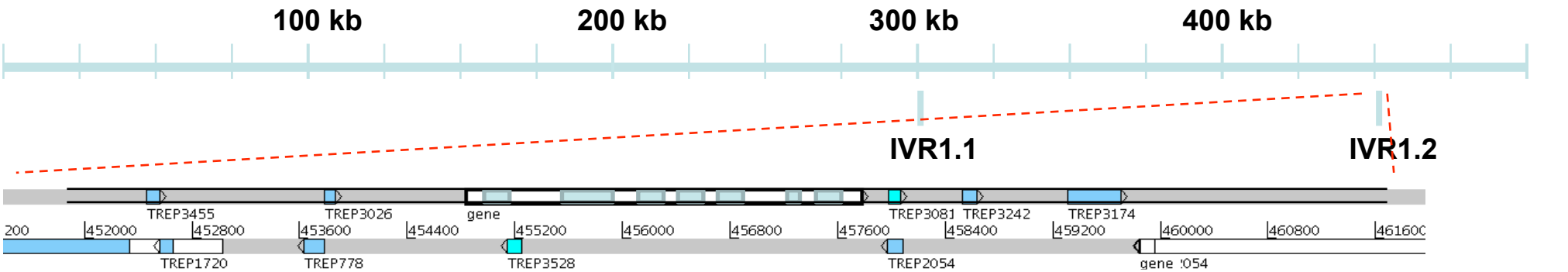
ctg506c ver3.0 contig1



Cell wall invertase n=1 Tax=Triticum aestivum RepID=O81118_WHEAT"; Evalue "0.0"; Identity "81% (476/586)";



Cell wall invertase n=1 Tax=Triticum aestivum RepID=O81118_WHEAT"; Evalue "1e-114"; Identity "54% (235/432)"

Hollie Webster PhD thesis

Summary – Chromosome 3B

- Have a good appreciation of sequence assembly issues for wheat genome
- Comparative genomics of a repetitive protein kinase locus in ctg344 and biological studies of cell wall invertase genes on ctg506
- Waiting for ctg1804c and ctg344 scaffolds from INRA

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Chromosome 7A

Overall aims

- Detailed analysis of QTL regions that are important to Australian agriculture
- Trait information linked directly to genome sequence
- ISBP identification of new markers for wheat breeding and selection for traits of interest

Tasks

- BAC-based physical map assembly of 7AS and 7AL
- BAC end sequencing
- Survey sequencing of 7A
- In-depth sequencing of QTL regions
- Anchor to genetic map

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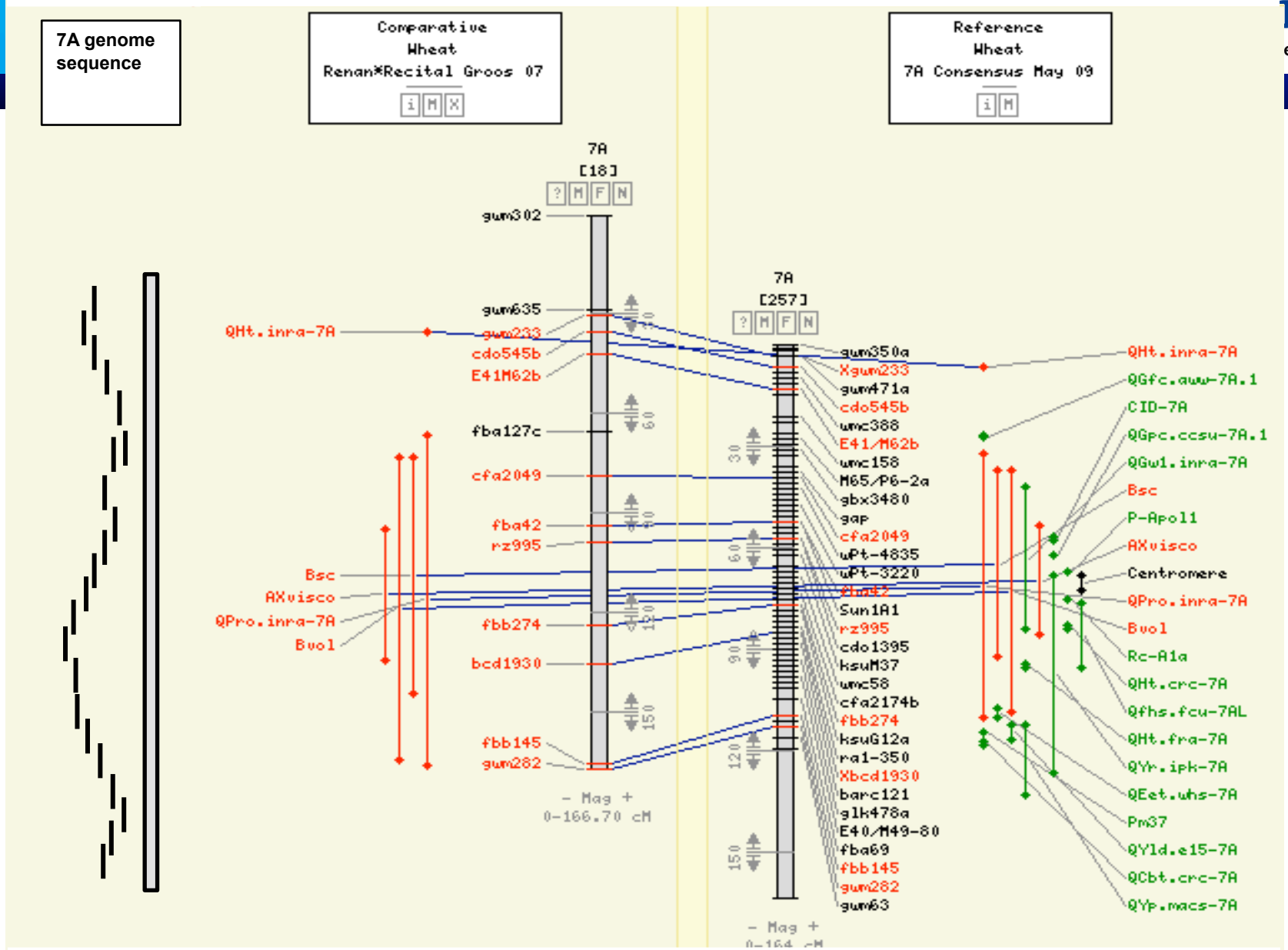


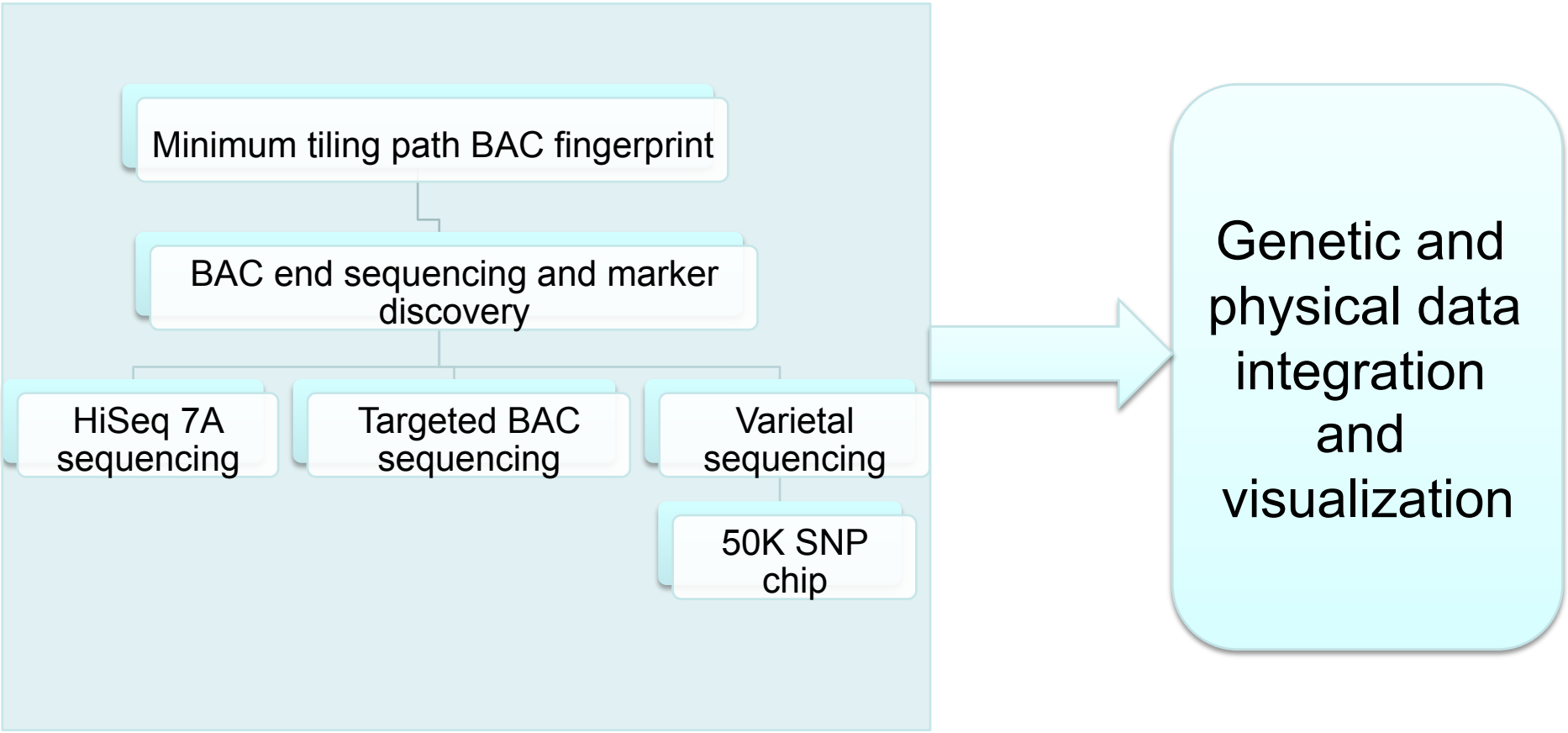
- Cmap (at CCG) compiles published QTL/trait data onto composite map.
- The QTL/trait data includes the information from the Wheat Gene Catalogue (McIntosh et al).
- The composite map is built from sections of published maps that share common markers to allow their integration into a master map. Trait data is incorporated based on flanking molecular markers. The molecular markers allow projection on to the genomic DNA sequence

Genome sequence | Molecular genetic maps | Traits

7A timeline

- **2010** Funding finalised (GRDC and BioPlatforms Australia)
  - 7A physical map, support to the IWGSC, survey sequencing, QTL region sequencing
- **March 2011** 7AS BAC library (58,000 BAC clones) produced by Dolezel lab
  - UC Davis for DNA fingerprinting (Mingcheng Luo)
- **May 2011** LTC/FPC 7AS fingerprint assembly
- **June 2011** compile BAC contigs and define minimum tiling paths (MTP) for physical map
- **May/June 2011** 7AL library from Dolezel lab (underway) shipped to UC Davis for DNA fingerprinting
- **June** commence BAC end sequencing (BACs from MTPs)
- **2011** Targeted BAC sequencing
- **2012** anchoring of BAC contigs to genetic maps

CCG: Wheat Bioinformatics 7A (Data integration)



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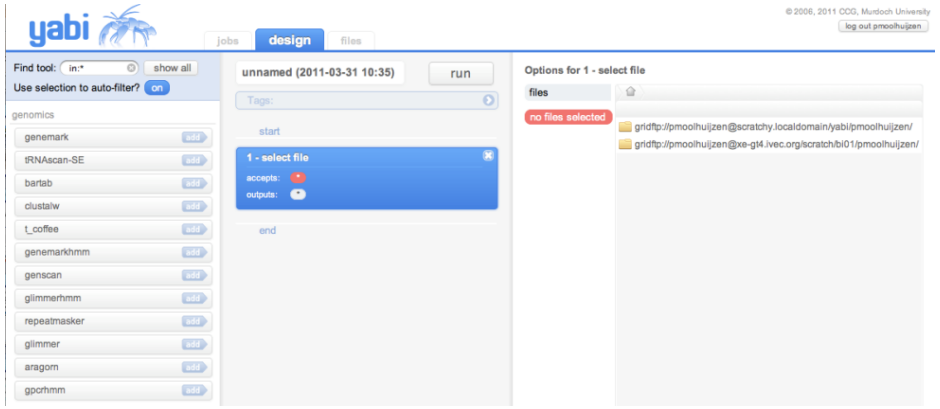
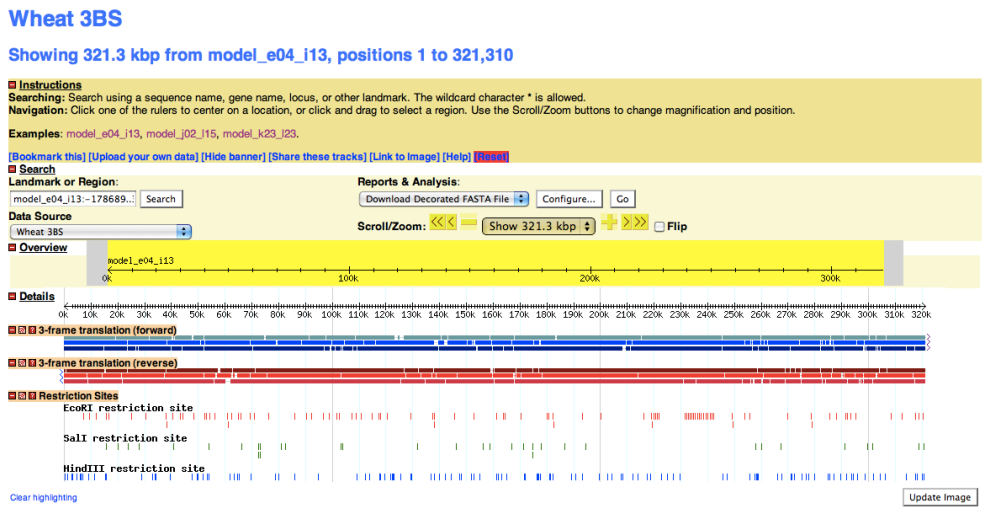
CCG: Wheat Bioinformatics activities 3B

HTP Assembly (Ctg344 and Ctg506)

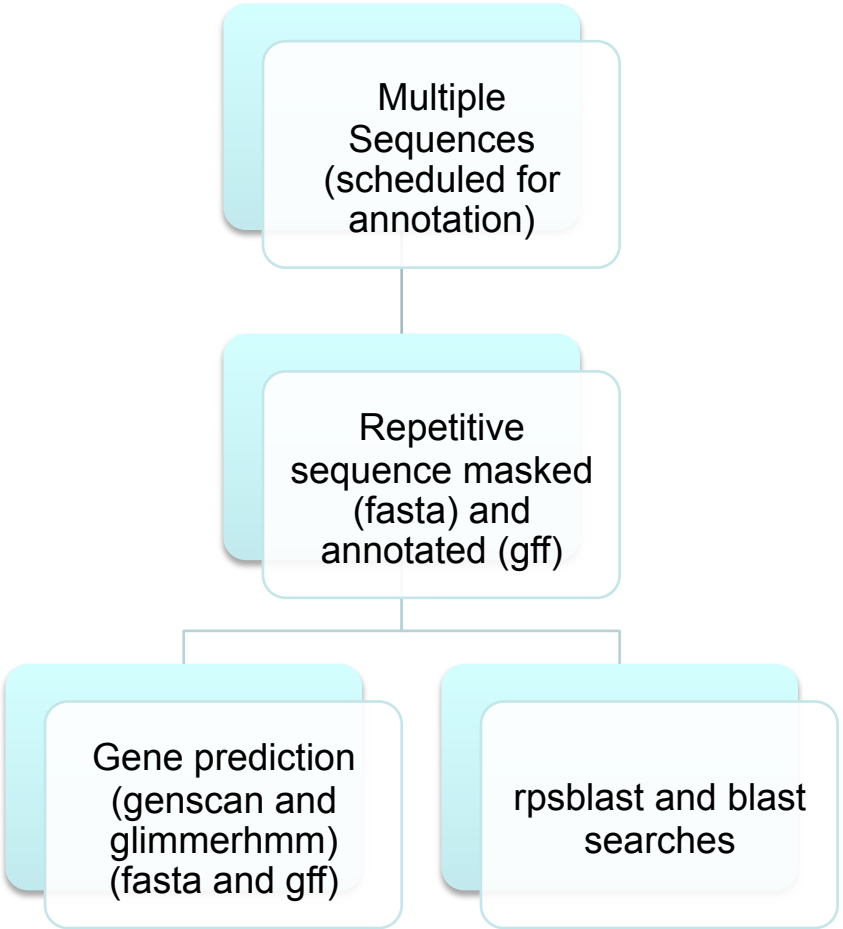
Annotation (HTP pipeline)

Visualization (Gbrowse)





Bioinformatics annotation workflows URGI (triannot) and YABI



Bioinformatics workflows - Annotate sequences (YABI and Triannot)

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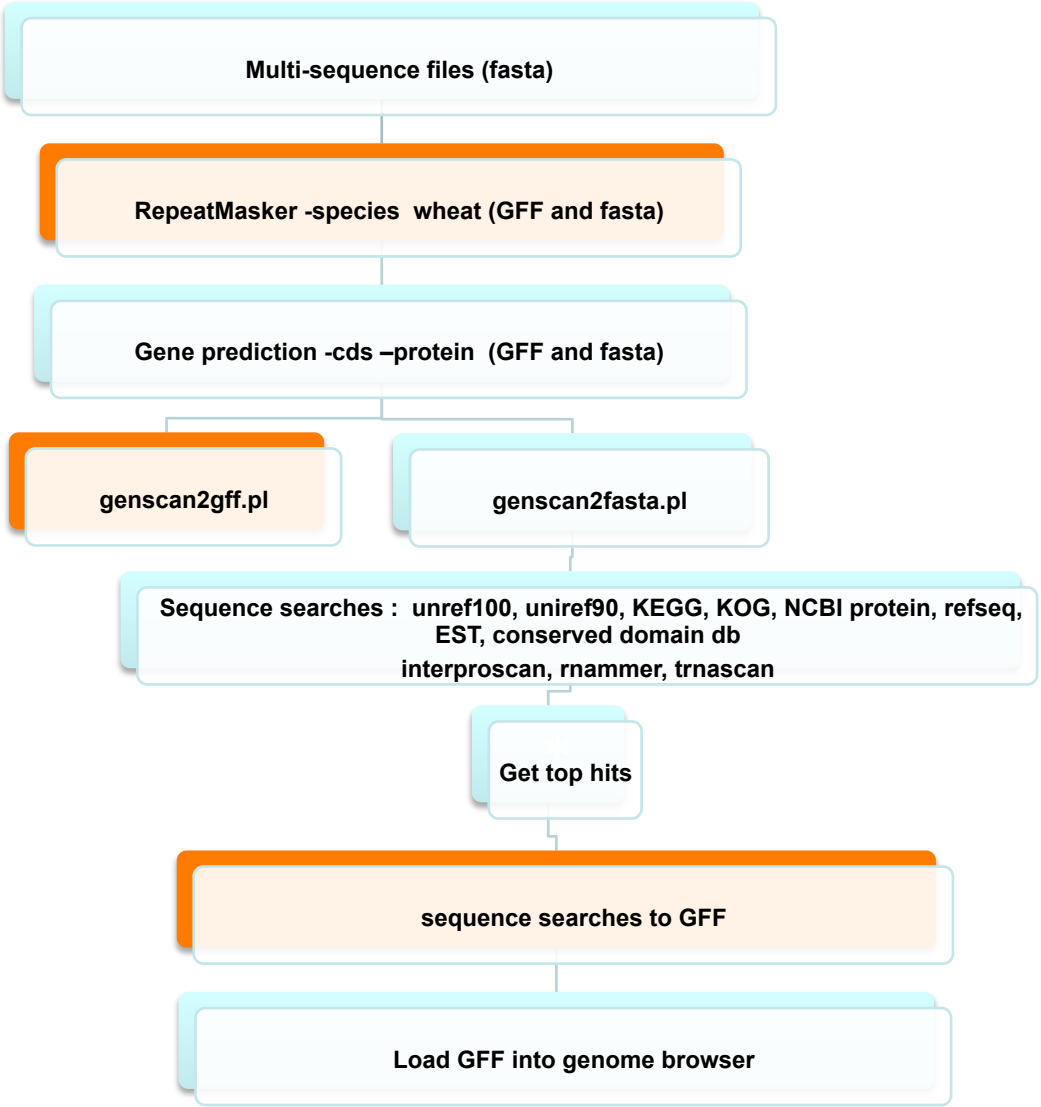


YABI - Front-end

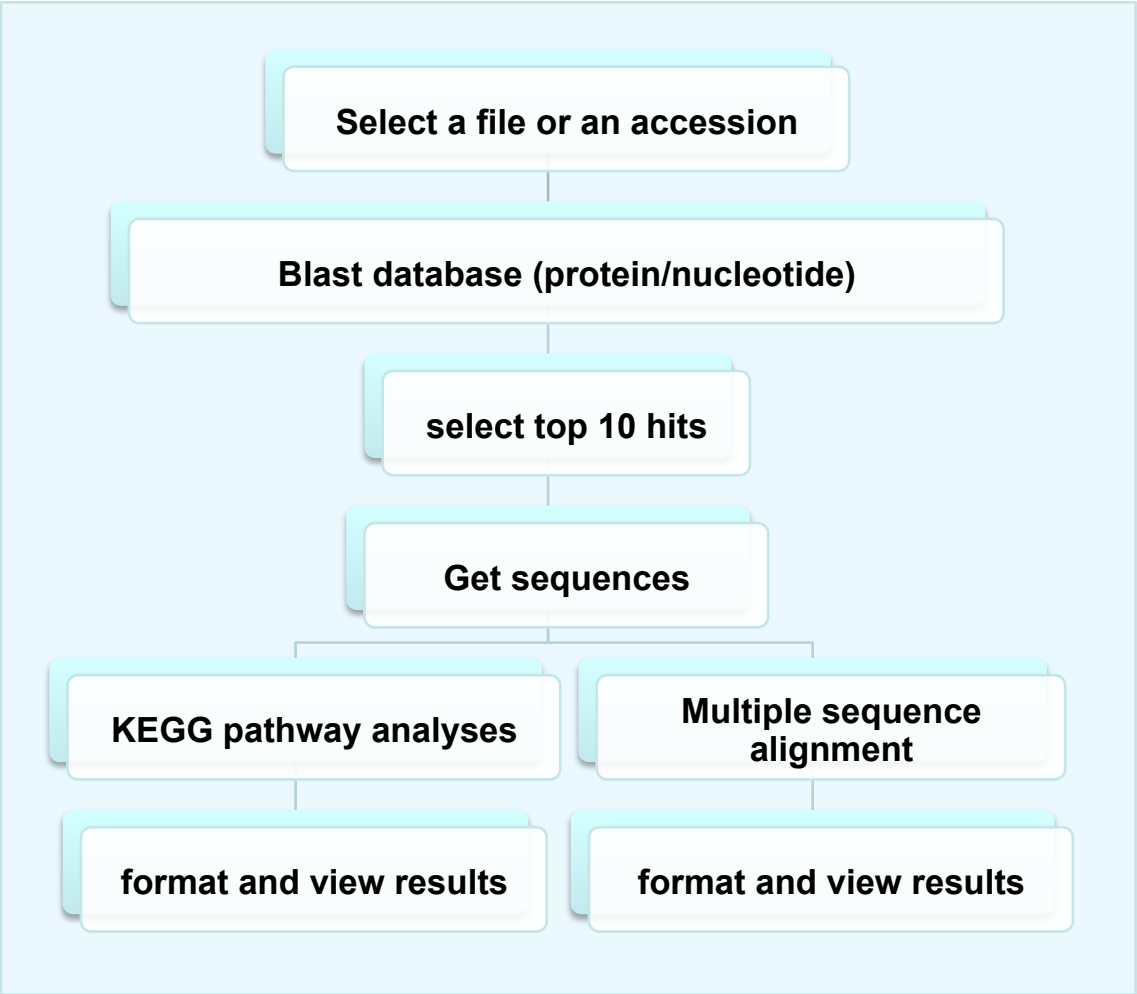
- Designed for a non-technical users
  - jobs tab, design tab, files tab
- Simple and easy to use as possible
- Reuse workflows
- User access
  - When you log in, user sees what they have access to
  - Allow scientists to work together
- Drag and drop tools

YABI - Front-end (2)

- Usability
  - Users warned if tools dragged out of place
  - Anticipate file extensions require for a given tool
    - Tools filtered
  - Tags – capture meta data
  - Errors trapped by system
- File manager
  - Drag and drop files
  - File copying via streaming
    - not via front-end
- Command-line
  - Power users

YABI demo

Simple Blast search and further analyses



CCG: Hardware

- Stage 1A Pawsey Centre
- Ranked 87 in the world
- 9600 cores

CCG: Software development

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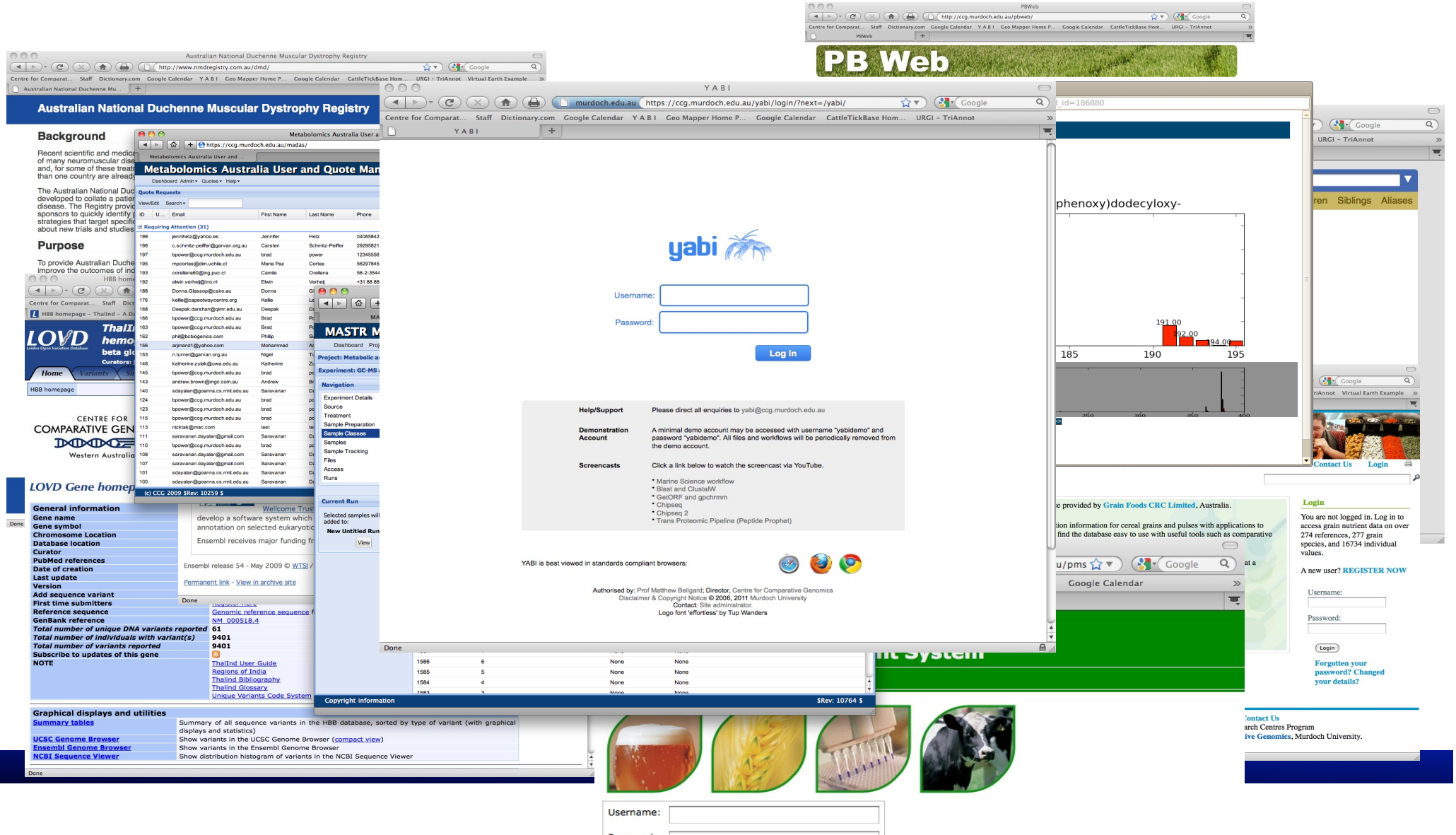

Next generation sequencing analysis projects

- De novo genome/transcriptome assembly and annotation
  - Wheat, barley, rat mutant, Wine yeast, cane toad, Rhizopertha, Campylobacter, Euphorbia, Cattle tick, dog tick, rhizobium, spirochetes
- Transcriptomics/epigenomics/metagenomics
  - microRNAs (human, Arabidopsis, cattle tick)
  - Epigenomics in Arabidopsis
  - Metagenomics of environmental samples (ancient DNA)
- Disease association
  - LPK rat mutant, human disorders, diagnosis assays

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Informatics considerations

- Process/timing to integrate 3B CCG results back to IWGSC
- Protocols for BAC physical assembly
- Process/timing to integrate 7A results back to IWGSC
  - Linkages to CCG resources
- CCG could assist in scoping LTC software porting requirements

Acknowledgements

CCG Team

- Rudi Appels
- Gabriel Keeble
- Adam Hunter
- Andrew McGreggor
- Paula Moolhuijzen

Collaborators

- Catherine Feuillet, Etienne Paux and Frederic Choulet (INRA, France)
- Jaroslav Dolezel (Czech Republic)
- Mingcheng Luo (UC Davis)

Funding

- GRDC
- BioPlatforms Australia