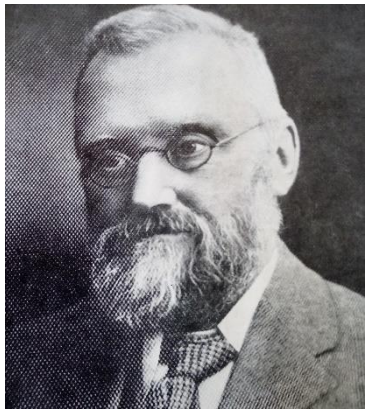
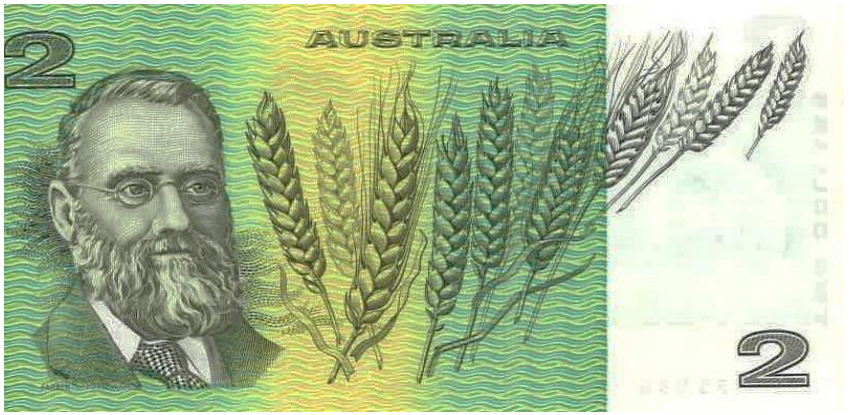




Farrer Memorial Oration : The wheat plant and its genome

Rudi Appels, University of Melbourne

Wheat Breeders Assembly

Narrabri August 28 -31

Images kindly provided by Lindsay O'Brien

One mechanism by which we remember the debt we owe to colleagues who especially contributed to our well-being is by way of an annual event to focus on the particular achievement or achievements … the "lest we forget" mind set

The Farrer Memorial oration is such an event to remind us of our debt to William James Farrer and I am honoured to be contributing to the Farrer Memorial heritage

TITLE: 'LAMBRIGG'. THE HOME OF WM.[WILLIAM] FARRER WITH THE MURRUMBIDGEE IN THE FOREGROUND 1939 [PICTURE]
DATE: [between 1929-1939]. National Library of Australia , REQUEST NO.: CDC-10797335

Most of Farrer's wheat studies, from 1886 onwards, were carried out at Lambrigg just south of the CSIRO labs in Canberra

Farrer was apparently considered a "woolly headed crank" when he explained, in the 1880's, his plans to carry out cross-breeding for improving wheat agronomic and quality attributes

Farrer's first release in 1889, Blount's Lambrigg, was actually just a selection out-of wheat lines sent to him by AE Blount who was based in Colorado (USA)

The parents for his cross-breds included Indian wheats from the British Empire collection in the UK and cv Fife from Canada aiming to combine

- **drought resistance,**
- **early maturity,**
- **quality**

Indian wheat/Canadian Fife germplasm provided selections for the release of Yandila in 1895

In 1894 the Indian wheat/Canadian Fife germplasm selections were crossed to cv Purple Straw from the USA

wheat cv Federation

released to farmers in 1903
(Farrer died 1906)

considered one of Farrer's greatest achievements

was the most popular variety rom 1910 to 1925

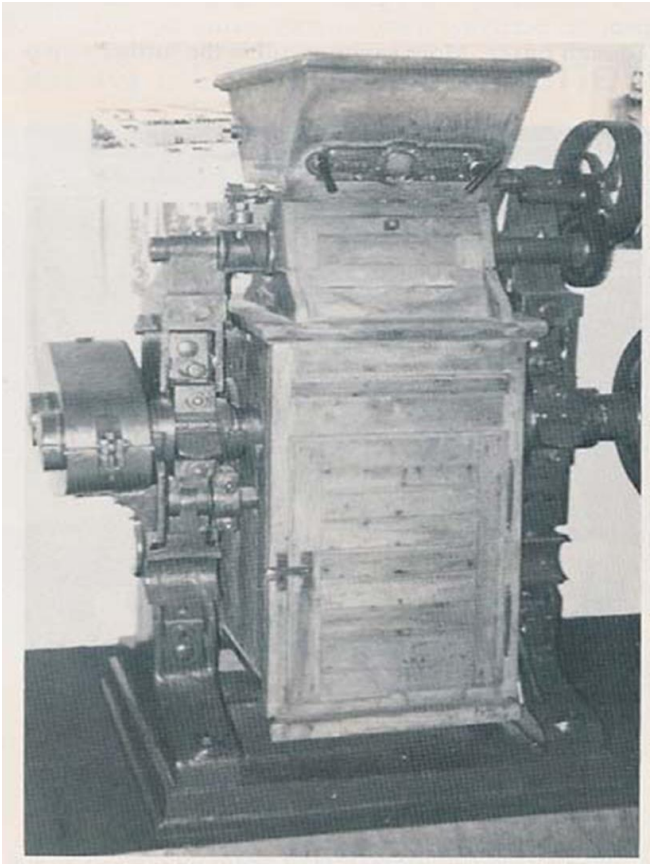
So in the 20 years, starting "from scratch", Farrer achieved the output of the highly successful variety Federation [1]

In reading extracts from reports written at the time it is clear Farrer also influenced a range of agricultural practises and importantly brought grain quality into sharp focus

[1] Russell A (1949) William James Farrer: a biography. Publisher FW Cheshire Melbourne & London

In 1894 he developed his contact with Frederick Guthrie the cereal chemist in the Dept of Agriculture in Sydney to test relatively small samples of wheat grain for their milling and baking quality

In 1898 the collaboration with Guthrie was formalised when Farrer was appointed to the Dept of Agriculture (but still based at Lambrigg)

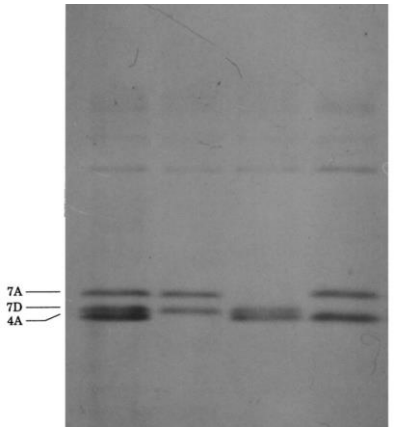


Photograph of Guthrie mill for assessing relatively small grain samples from Farrer (photograph kindly supplied by Colin Wrigley)

If we now fast forward to 1996, it turns out one of the quality traits that Farrer selected for was UDON noodle quality (although he did not know it!)

In terms of illustrating the value of Farrer's focus on capturing new genetic diversity we can see, based on the work of a student of Peter Sharp's in Cobbitty that Federation was missing one of three Granule Bound Starch Synthases (GBSS)

Absence of the GBSS on chromosome 4A (GBSS-4A null) matches differences in starch swelling properties in UDON noodle (Graham Crosbie}

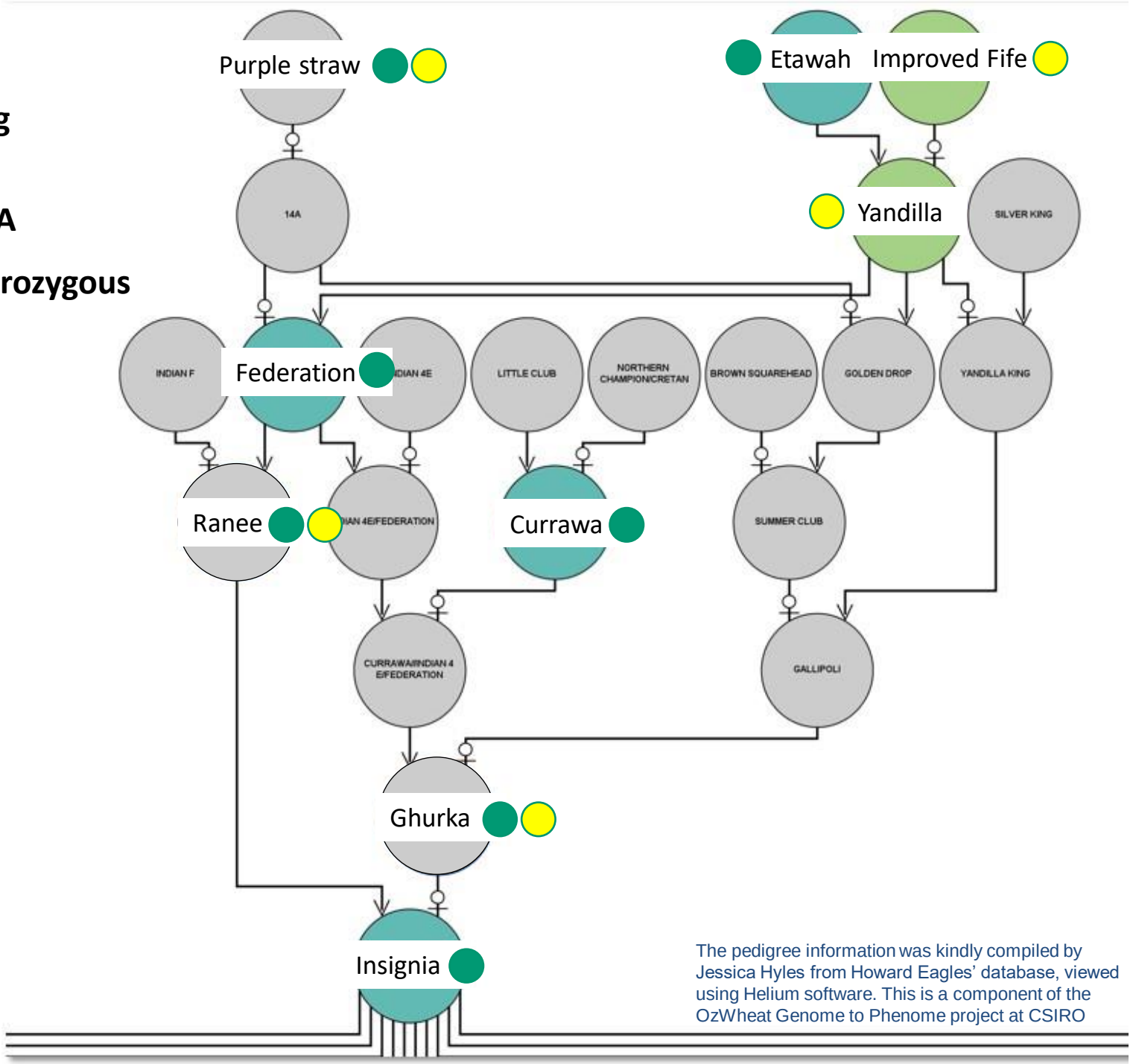


Zhao and Sharp 1996

Zhao, Xiaochun (1999). Molecular and functional studies of granule-bound starch synthase in wheat. PhD thesis, University of Sydney, Plant Breeding Institute

Portion of early Australian wheat heritage including Federation

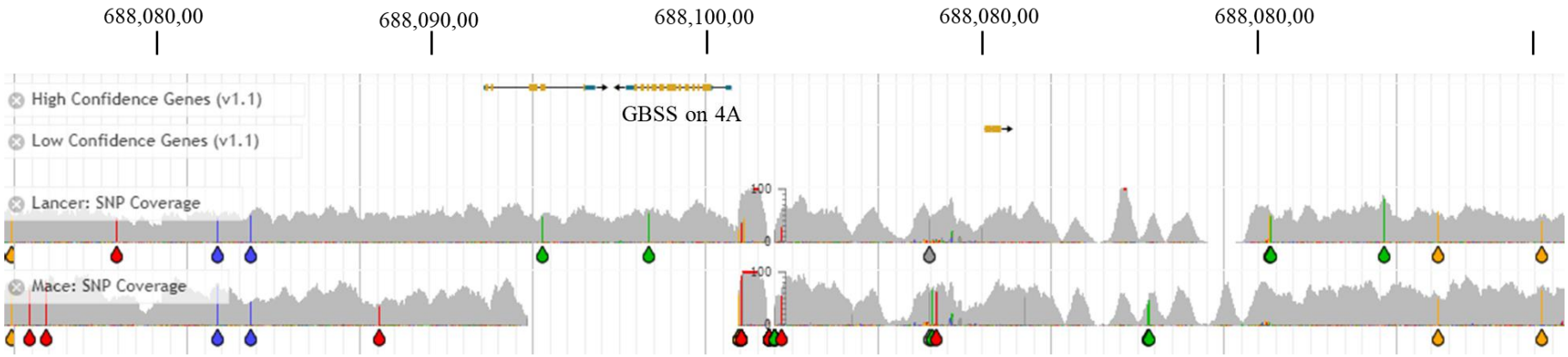
- GBSS-null 4A
- Mixed/heterozygous
- Wild type



The pedigree information was kindly compiled by Jessica Hyles from Howard Eagles' database, viewed using Helium software. This is a component of the OzWheat Genome to Phenome project at CSIRO

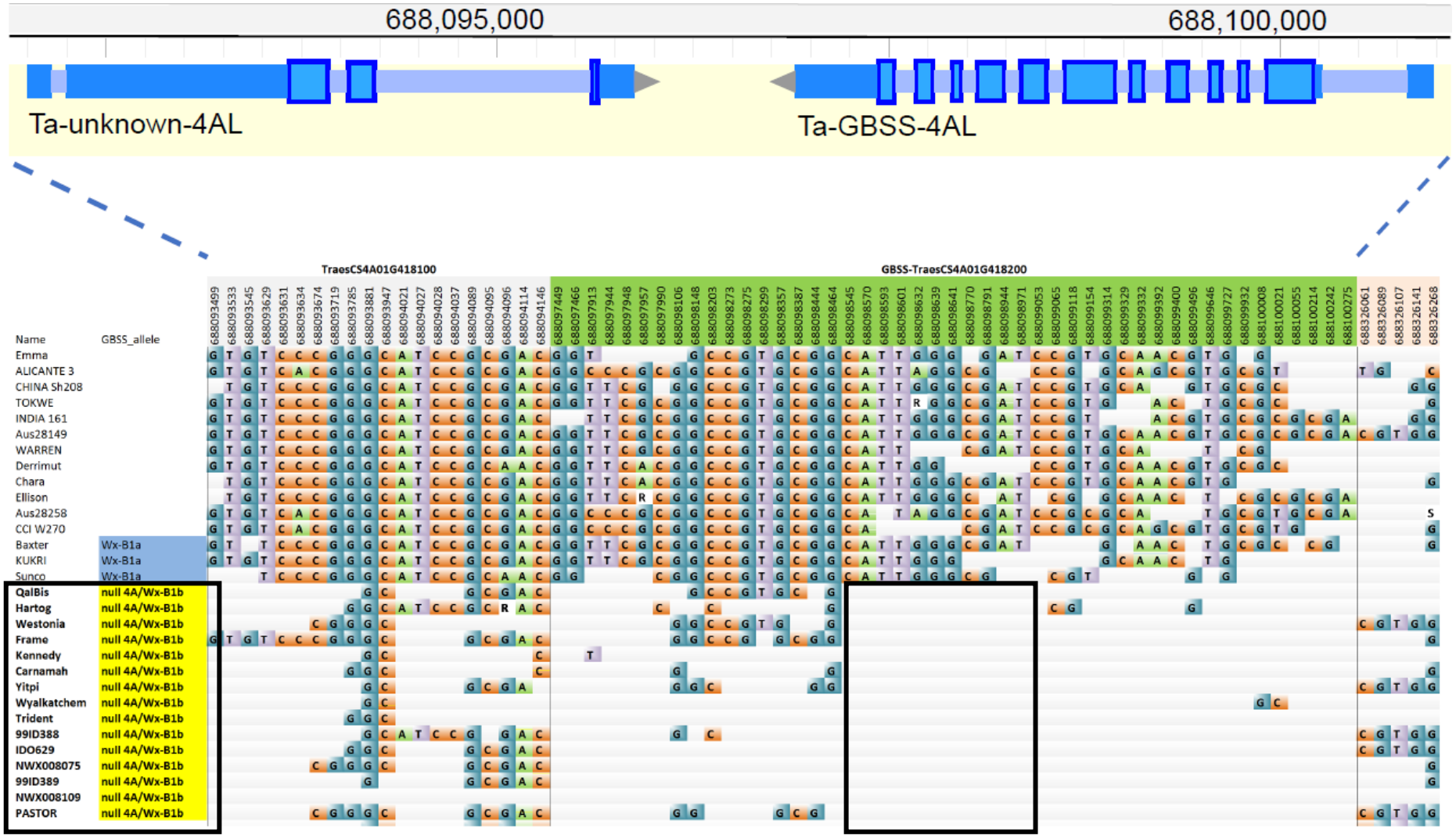
GBSS-4A null was one of the early DNA markers for our Japanese UDON noodle exports based on Graham Crosbie's extensive work within the Japanese production chain and accounted for 80% of the variation perceived by the Japanese consumers

If we now fast forward again, this time to 2017 we can see how the GBSS-4A provides a good example of the contribution that DNA/genome level analyses make to the breeding space



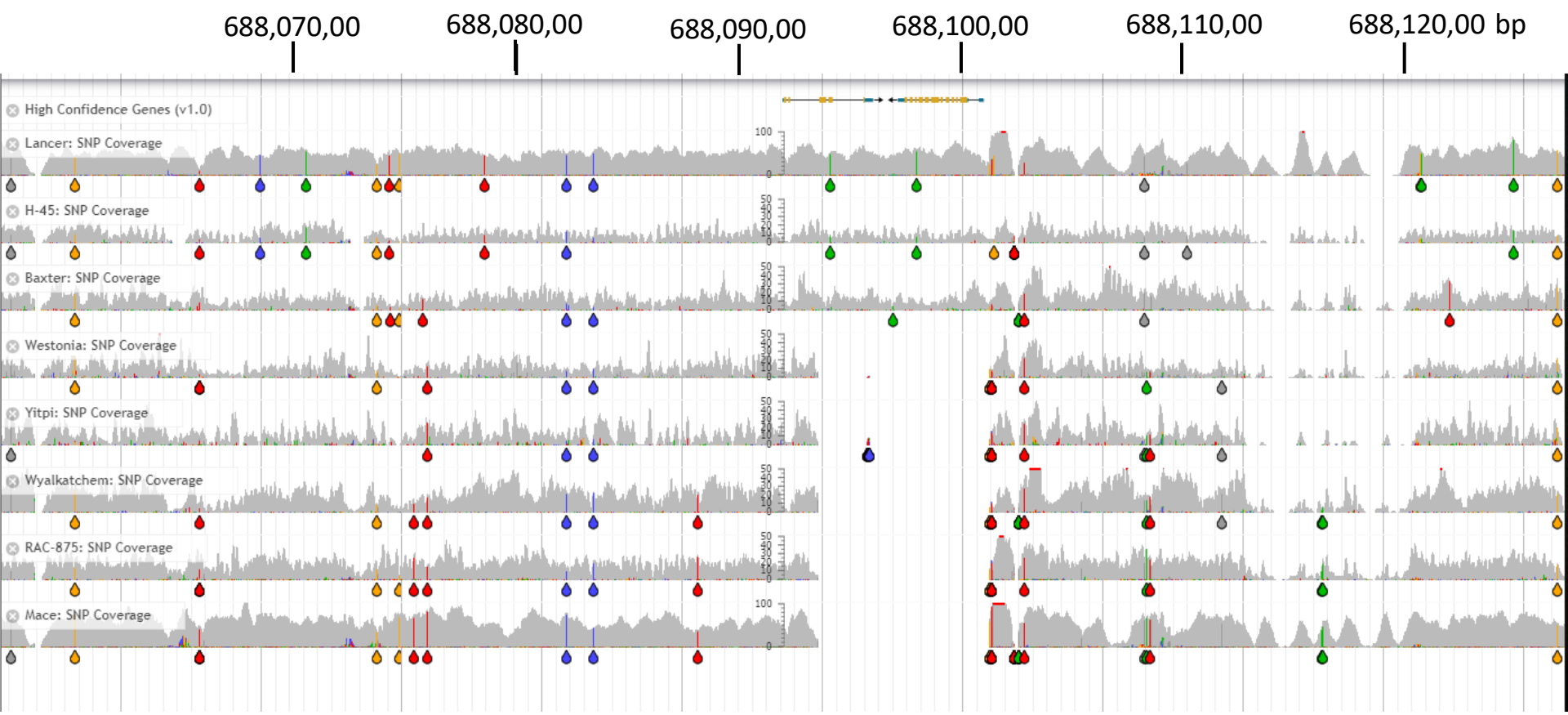
This illustration shows how have we have moved from a detailed sequence level analysis of a single gene region of about 5000 bp to whole chromosomes about 700 million bp (700 Mb)

The yellow highlighted lines were independently confirmed to be genetically GBSS 4A null



The large base of genome-level sequences now available (AgriBio) resource fast searches for parents with the DNA fingerprint or haplotype associated with UDON noodle quality

Haplotype concept to provide fingerprints for genome segments



Haplotypes are characterized by a particular order of markers (SNPs) in segments of the genome generally more that 10,000 bp in length.

Can also apply to short genome segment with a specific gene

Genome/DNA level information can also make multiple gene copies less ambiguous to work with since the genome assembly covers essentially all the genes

For example in 2007 we worked with Iain Barclay, wheat breeder at DAFWA, to characterise a new mutation in the acetohydroxyacid synthase (AHAS) gene

The AHAS^{mut} gene confers resistance to imidazolinone, a herbicide that normally kills plants/weeds by stopping the production of the amino acids Leu, Isoleu, Val.

The new A122T mutation in wheat AHAS on 6D conferred a significantly better resistance to imidazolinone than the more commonly used S635N mutation on 6A.

Li D, Barclay I, Jose K, Stefanova K, **Appels R**. (2008) A mutation at the Ala122 position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: improved resistance to imidazolinone and a faster assay for marker assisted selection. Mol Breeding 22: 217 - 225

So, with the more effective PCR based assays we (Dora Li) developed, the new A122T mutation could be rapidly deployed and combined with the standard S635N mutation to develop new cv Clearfield STL lines for use in managing weeds in cropping fields.

Li D, Barclay I, Jose K, Stefanova K, Appels R. (2008) A mutation at the Ala122 position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: improved resistance to imidazolinone and a faster assay for marker assisted selection. Mol Breeding 22: 217 - 225

In 2007 we did not have a high quality genome assembly for wheat

The AHAS project required several months of primer design and resequencing the PCR products to confirm we were targeting the correct AHAS gene

Today this type of specific problem solving comes down to a few weeks with the availability of genome sequence and faster DNA sequencing turn-around

So how did we get here today with the high level of genome sequence detail for wheat and the close engagement of groups in Australia in a large international effort?

1977: I was among the first in Australia to isolate DNA from wheat with Jim Peacock

- **the main purpose was to identify rye-specific DNA sequences for the registration of new Triticales (Colin Driscoll)**
- **this was successful and attracted colleagues who ended up being significant in wheat genome sequencing, to work in the Canberra labs**
- **the wheat and barley genome commitments in Adelaide University also attracted colleagues and students into the genome sequencing space**

1980's-1990's: The genome sequencing technology was established in eukaryotes including yeast, human, rice and Arabidopis but activity in wheat was focused on specific genes and specific anonymous markers genetically linked to agronomic traits.

- **GRDC investments in Molecular Marker programs were matched by significant institutional investments (CSIRO, CRCs, AgriBio, Adelaide University, Sydney University)**

- **disease resistance, starch biosynthesis, grain protein, agronomic (eg Rht) by map based/candidate gene cloning**

- **new anonymous molecular markers linked to complex agronomic traits by linkage to QTLs in molecular maps**

2002: ITMI meeting in Winnipeg, Canada, Bikram Gill (Kansas) and myself were very vocal about the need for establishing a wheat reference genome sequence

2004: A group of interested colleagues and funding agencies met in Washington DC (USA) in November of that year

- **Wheat enthusiasts like me argued for funding to use available resources to start building the genome sequence**
- **Others at the meeting considered the suggestion crazy, basically impossible because of the large polyploid nature of the genome**

2005: We formed the International Wheat Genome Sequencing Consortium with Kellye Eversole as chairman and a leadership group including Rudi Appels, Jan Dvorak, Bikram Gill, Beat Keller

2005 -2006: We engaged with Evans Lagudah and Wolfgang Spielmeyer to assemble the sequence information for the Sr2 region in chromosome 3B that the INRA group in France had assigned to us

2014: Group in INRA France (Catherine Feuillet) completed the sequencing of the flow-sorted (physical purification, Jaroslav Dolezel, Czech Republic) chromosome 3B

2011-2015: GRDC /Bioplatforms Australia investment in assembling chromosome 7A sequences (Rudi Appels, Gabriel Keeble-Gagnere, in combination with Delphine Fleury based in Adelaide University) on flow-sorted 7AS and 7AL chromosome arms, Dolezel lab

2016: The IWGSC engaged the Israel group NRGene to use their DeNovoMAGIC software for an assembly of the wheat genome without sorting individual chromosomes

2015 - 2018: The IWGSC had two genome assemblies happening in parallel

- **For Australia's 7A project, the final assembly was moved to AgriBio and was able to draw on world-class resources to complete the assembly of the minimum tiling path of 11,451 genome segments covering 755 Mb (sequence data generated by Australia Genome Resource Facility).**

- **Outputs from new technologies such DNA optical mapping on flow sorted 7AS, 7AL (Dolezel lab) were incorporated as they became available**

- **The Israel NRGene group assembling the 21 chromosomes de novo using DeNovoMAGIC software for an assembly of the wheat genome without sorting individual chromosomes**

At AgriBio the unique bioinformatic skills of Gabriel Keeble-Gagnère, Philippe Rigault and Josquin Tibbits were engaged to complete chromosome 7A

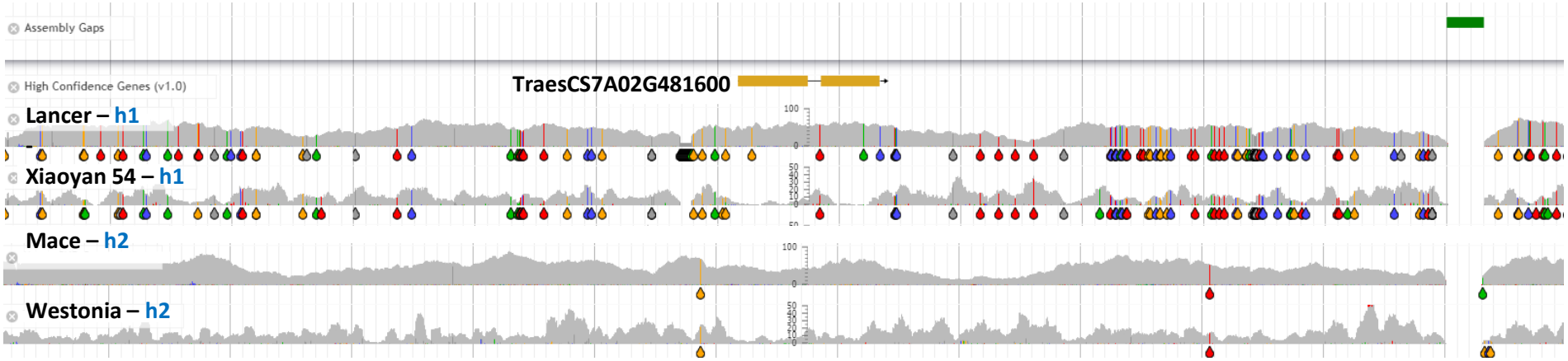
Importantly the AgriBio chromosome 7A aligned well with the DeNovoMAGIC chromosome 7A and the agreement remains one of the few independent validations of the DeNovoMAGIC assembly process

The AgriBio group of Gabriel Keeble-Gagnère, Josquin Tibbits and Matt Hayden were also engaged in identifying the *APO1* gene, together with Delphine Fleury, as a key component of the yield QTL on 7AL - a key milestone in the GRDC investment

https://maswheat.ucdavis.edu/protocols/WAPO1

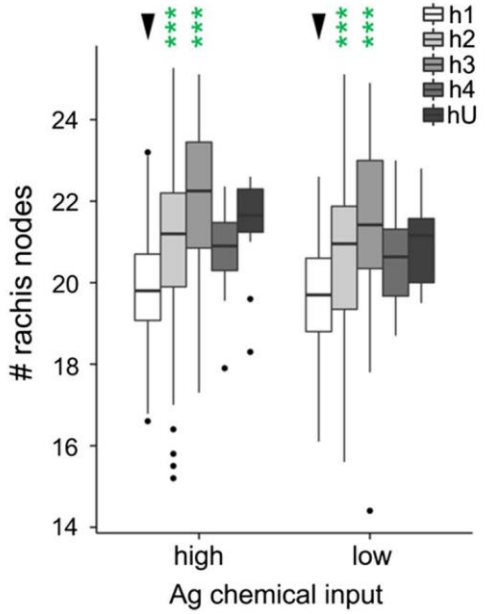
To wrap up this presentation

Haplotype association for a component of yield (grain number per spike)



In a large analysis of 699 wheat lines in annual yield trials:

- 2 major haplotypes in the yield region, h1, h2
- In all yield trials h2 was significantly associated with increased grain yield relative to h1
- Presents a clear value proposition for selecting h2 over h1 in Australian breeding



To wrap up this presentation

The Farrer-Guthrie drive to ensure grain quality associates with grain yield lives on

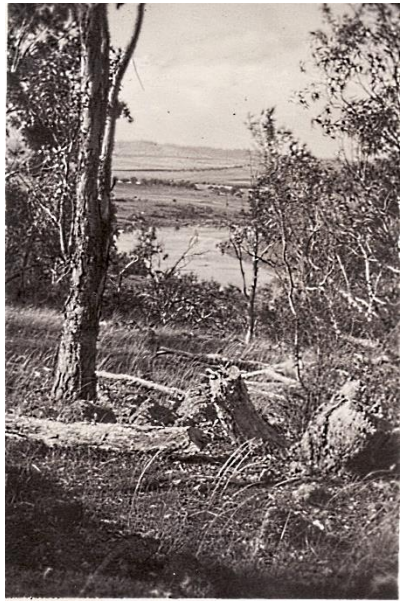
grain quality	flour quality	bread/noodle dough quality	processing
• test weight (TKW)	• ash content	• water absorption	• long fermentation
• hardness	• colour	• stability	• rapid dough
• total protein	• starch damage	• dough breakdown	
• falling number		• extensibility	
• screenings		• maximum resistance	
		• paste viscosity	

Nutrients, allergens, starch, protein components

Acknowledgements

- **Evans Lagudah, David Appels, GRDC funding/patience,**
- **colleagues in the Molecular Marker programs in Australia (especially Peter Langridge, Delphine Fleury),**
- **International Wheat Genome Sequencing Consortium (IWGSC), Kellye Eversole, Jaroslav Dolezel, Jan Dvorak, Bikram Gill,**
- **colleagues in AgriBio especially Philippe Rigault, Matt Hayden, Josquin Tibbits, Gabriel Keeble-Gagnere,**
- **colleagues at Dept of Agriculture Western Australia,**
- **colleagues at CSIRO Division of Plant Industry (especially Jim Peacock),**
- **colleagues in Chinese Academy of Agricultural Sciences (CAAS, Zhang Xueyong, He Zhonghu, Jia Jizeng)**

FARRER MEMORIAL TRUST



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