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An optimised approach to sequence and assemble BACs: application to bread wheat chromosome arm 7DS

David Edwards
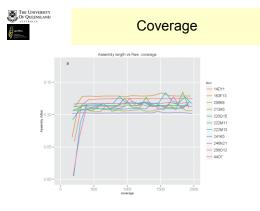University of Queensland, Australia
Dave.Edwards@uq.edu.au

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Outline

- Sequencing coverage requirements
- Integrity of pooled assemblies
- Assembly validation

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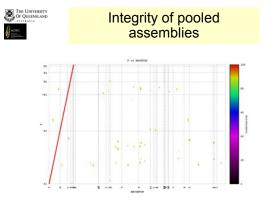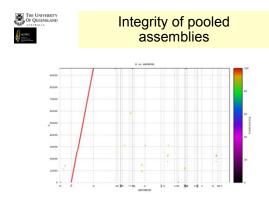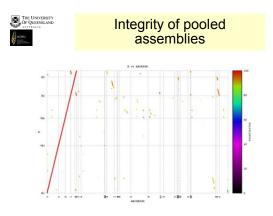OF QUEENSLAND
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Coverage

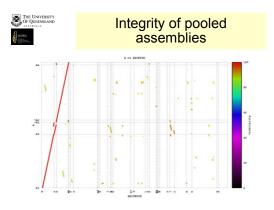Sassy assembler requires high physical coverage

- We determined optimum sequencing depth for assembly to be around 500x

- Need to pool BACs to sequence to above coverage cost effectively

- Assemblies of BACs generated using a variety of kits showed Truseq kits performed better
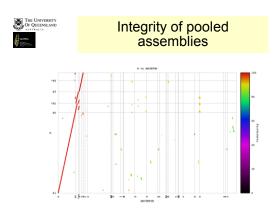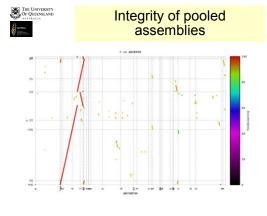

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Integrity of pooled assemblies

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Integrity of pooled assemblies

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Sequencing

- Hiseq

  - Four non overlapping BACs are sequenced in a pool.

  - 96 pools indexed on one Hiseq lane (384 BACs)

  - 27 Gbs

  - ~ 633x coverage per BAC with 100bp PE reads

Assembly

- Results update per lane:

  - Approx. 2.7 contigs per BAC, ~11 contigs per pool

  - Average N50 67.8 Kbp

  - 68/384 BACs were fully assembled

  - Scaffolding of remaining BACs underway with MP, PacBIO and BES

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Assembly validation

BES mappings example pool: A2-C2-E2-G2

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Assembly validation

PE coverage plots

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