Assembly of heterozygous Fodder beet

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Sugar beet (Beta vulgaris)

- Diploid with n = 9 chromosomes
- Estimated genome size: 731 Mbp
- Reference assembly[1] was created with a double-haploid plant

[1] Dohm et al., The genome of the recently domesticated crop plant sugar beet (Beta vulgaris), Nature, 2014

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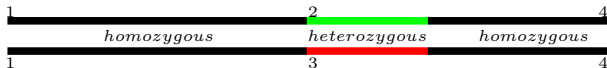**Fodder beet**
- Diploid
- Estimated genome size $\approx$ sugar beet
- Assembly size: 955 Mbp

  Assembled by J. Dohm, using Roche's Newbler

  - Highly heterozygous
    (Different alleles at one or more loci on homologous chromosomes)
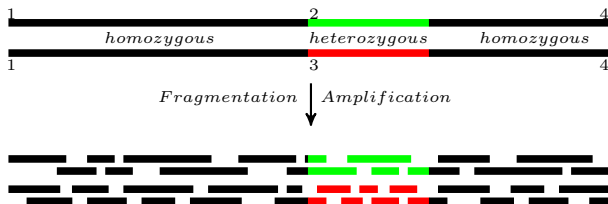- Task: Resolve the heterozygous structure of the genome
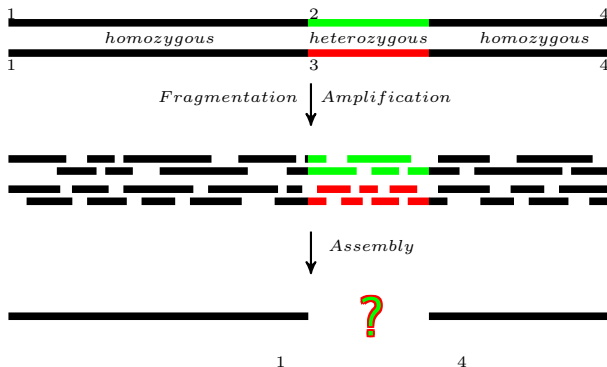
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Diploid Genome

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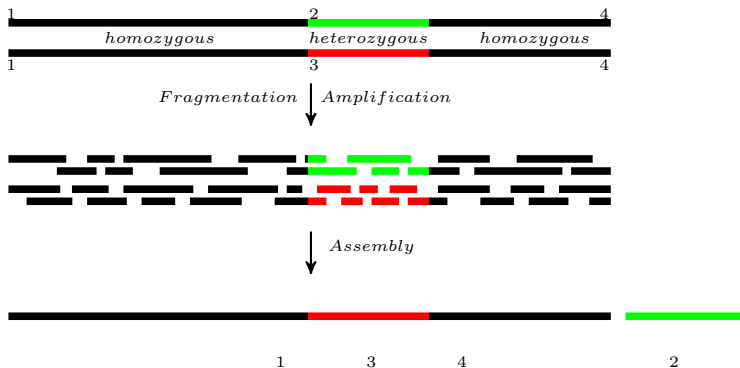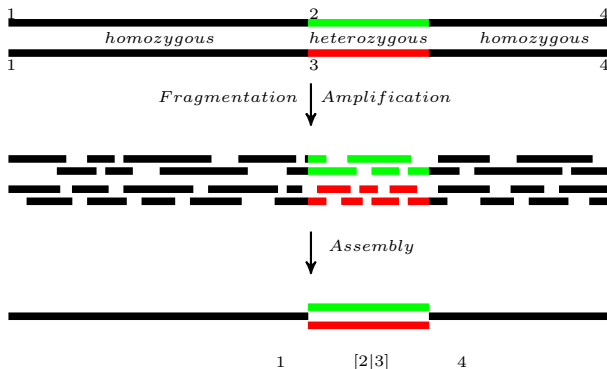
Diploid Genome

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Diploid Genome

Diploid Genome
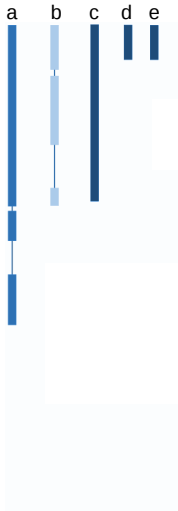
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- Raw Data
  - Moleculo reads (8-9x Coverage)
  - Illumina Mate-Pairs

- Assembled Data (Newbler)
  - Assembly (955 Mbp)
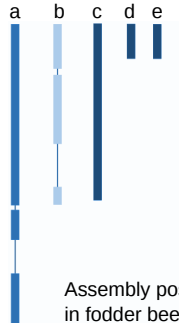 - Additional information on
    Contig connections and coverage

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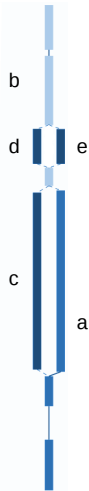assembled output, 59 kbp:

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assembled output, 59 kbp:

a b c d e

heterozygous structure:

b

d e

c

a

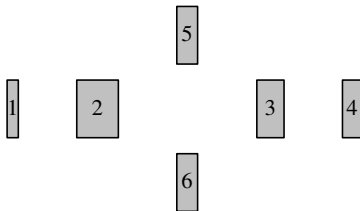Assembly postprocessing in fodder beet to resolve the heterozygous genome structure.

total length: 38 kbp

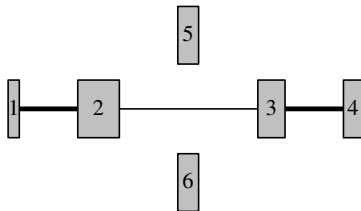# Assembly of heterozygous Fodder beet

Create Assembly Graph

▶ Contigs as vertex set

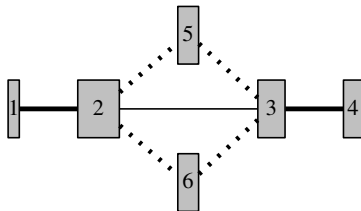# Assembly of heterozygous Fodder beet

Create Assembly Graph

- Contigs as vertex set
- Scaffold-connections of Newbler-assembly as edge set (solid)

Assembly of heterozygous Fodder beet

Create Assembly Graph

- Contigs as vertex set
- Scaffold-connections of Newbler-assembly as edge set (solid)
- Additional Links from Newbler-output as edge set (dotted)

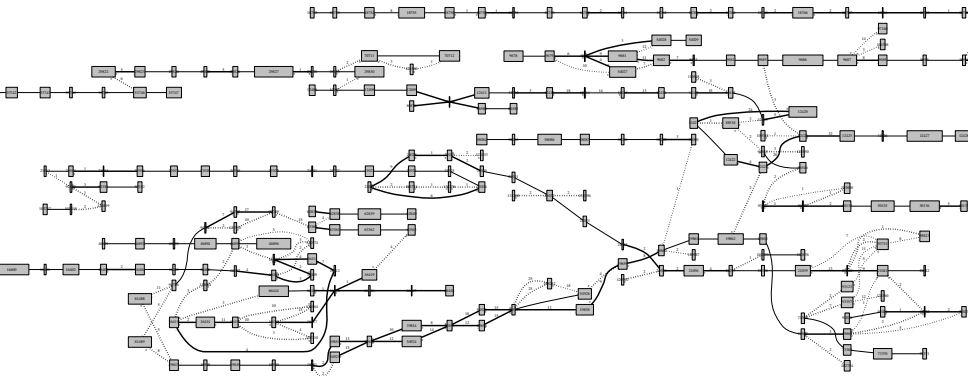# Assembly of heterozygous Fodder beet

Create Assembly Graph

- Contigs as vertex set
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Assembly of heterozygous Fodder beet

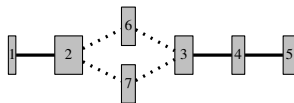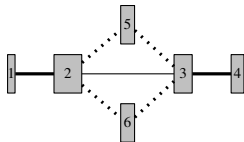**Cycles**
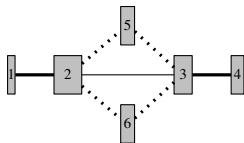
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Cycles

- Find all cycles in graph $\rightarrow$ NP-complete Problem
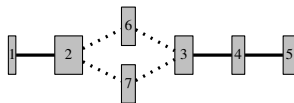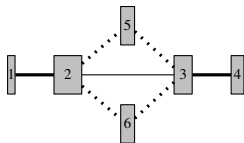
Cycles

- Find all cycles in graph $\rightarrow$ NP-complete Problem
- Which cycles are the good/correct cycles?

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Splits
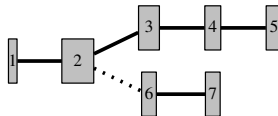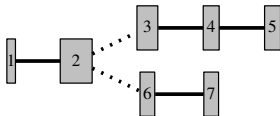
- Which splits are the good/correct splits?

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Filtering Criteria

- 3′- and 5′-ends consistent
- Distances consistent / realistic

- Structural criteria
- Coverage
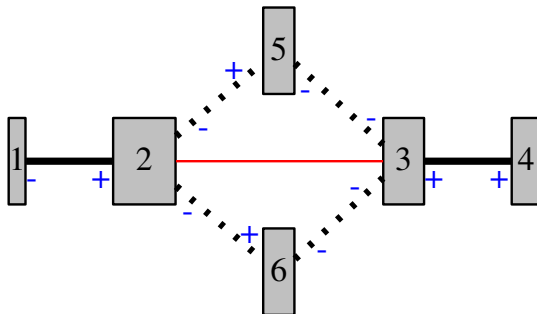
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Cycle Validation

- Mapping of Fodder beet Contigs against RefBeet 1.2
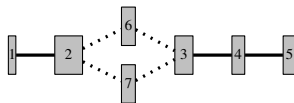- Two Contigs on same position in RefBeet
    - ⇒ **Possible heterozygous pair**
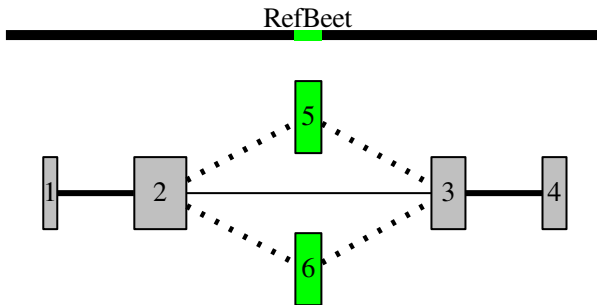
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Cycle Detection

	Scaffolds Only	
♯ **Contigs**	106732	
♯ **Cycles**	3429	
♯ **Filtered Cycles**	1733	
♯ **Validated Cycles**	1511 87.2 %	
Length Component	676.5 Mbp	
Length Filt. Cycles	34.8 Mbp 5.1 %	
Length Hetero	2×9 Mbp 2×1.33 %	
Runtime	8.4 h 1.4 GHz 4-core	

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Cycle Detection

	Scaffolds Only		Scaffolds & Contigs	
♯ **Contigs**	106732		163961	
♯ **Cycles**	3429		19452	
♯ **Filtered Cycles**	1733		3976	
♯ **Validated Cycles**	1511	87.2 %	3425	86.1 %
Length Component	676.5 Mbp		761.1 Mbp	
Length Filt. Cycles	34.8 Mbp	5.1 %	64.9 Mbp	8.5 %
Length Hetero	2×9 Mbp	2×1.33 %	2×14.2 Mbp	2×1.87 %
Runtime	8.4 h 1.4 GHz 4-core		10.8 h 1.4 GHz 4-core	

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Scaffold Reduction

	Scaffolds Only	
♯ **Scaffolds**	55093	
♯ **Scaffolds Before** (consisting of $\geq$ 4 Contigs)	27196	
♯ **Scaffolds After**	24882 (-8.5 %)	
♯ **Connected Scaffolds** (consisting of > 1 Contig)	816	

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Scaffold Reduction

	Scaffolds Only	Scaffolds & Contigs
♯ **Scaffolds**	55093	55093
♯ **Scaffolds Before** (consisting of ≥ 4 Contigs)	27196	32811
♯ **Scaffolds After**	24882 (-8.5 %)	29408 (-10.4 %)
♯ **Connected Scaffolds** (consisting of > 1 Contig)	816	1038

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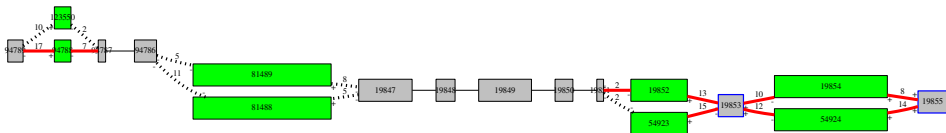
Outlook

- ▶ Find more/better criteria for Cycles and Splits
- ▶ Add more layers of information (coverage, neighborhood,...)
- ▶ Add Phasing
- ▶ Improve Validation
- ▶ Use information/output from other Scaffolders
- ▶ Data Set Simulation for Testing
- ▶ Make it usable (not only for me...)

Acknowledgements

Bioinformatics BOKU Vienna
- ▶ Heinz Himmelbauer
- ▶ Juliane Dohm

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- ▶ illumına˚
- ▶ BMBF project AnnoBeet