

Best Practices for Diploid Assembly of Complex Genomes Using PacBio: A Case Study of Cascade Hops

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Summary

A high quality reference genome is an essential resource for plant and animal breeding and functional and evolutionary studies. The common hop (Humulus lupulus, Cannabaceae) is an economically important crop plant used to flavor and preserve beer. Its genome is large (flow cytometrybased estimates of diploid length >5.4Gb¹), highly repetitive, and individual plants display high levels of heterozygosity, which make assembly of an accurate and contiguous reference genome challenging with conventional short-read methods. We present a contig assembly of Cascade Hops using PacBio long reads and the diploid genome assembler, FALCON-Unzip². The assembly has dramatically improved contiguity and completeness over earlier short-read assemblies^{3,4}. The genome is primarily assembled as haplotypes due to the outbred nature of the organism. We explore patterns of haplotype divergence across the assembly and present strategies to deduplicate haplotypes prior to scaffolding.

Assembly Stages and Compute Resources



 Table 3. Wall times and number of jobs for five
major stages of assembly.

Haplotype Divergence

Haplotypes may be assembled as single primary contigs, primary-haplotig pairs, or primary contig pairs, depending on the level of heterozygosity in the genomic region.





Sample Prep and Sequencing



- Cascade hops widely used in American craft brewing.
- Backcross hybrid of English and Russian varieties.



intensive stages. Job was run on SGE-managed cluster with 30 nodes, each with 512 Gb of RAM distributed over 64 slots. Up to 384 jobs were scheduled at a time.

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Haplotype Deduplication

Purge haplotigs⁶ was run on FALCON-Unzip primary contigs with the goal of generating haploid version of the assembly prior to scaffolding. Contigs with >80% haploid coverage depth by length were flagged as "candidate haplotigs". Six iterations of homology assignment and duplicate haplotig removal was performed with BLAST and LASTZ alignments. Curated assembly length and BUSCO duplicate gene percentage indicate that highly divergent haplotypes have been retained.

C. Assembly Output			
"Collapsed Haplotype"	"Unzipped Ha	plotypes"	"Homologous Primary Contigs"
Modified from Chin et al. 2016			

Figure 4. Impact of heterozygosity on assembly output. A. Low (green), moderate (purple), and high heterozygosity regions (red). SNPs: tic marks, structural variants (SVs): triangles. B. Overlap graph for contig assembly. C. Resulting contigs.

Haplotype divergence was compared for primary-haplotig pairs ("unzipped haplotypes") and homologous primary contigs. Haplotypes were aligned with nucmer⁸ and analyzed with showcoords and Assemblytics⁹. Homologous primary contig pairs identified with purge haplotigs⁶.

Table 8. Summary statistics for classes of assembled haplotypes.Mean alignment proportion: ratio of total alignment length to length of haplotig or shorter primary contig.	Statistic	Unzipped Haplotypes	Homol. Prim. Contigs
	Number Contig Pairs	31,654	2,491
	Mean Alignment Proportion	0.952	0.873

			🗖 Unzi	pped Haplotypes	s 🗖 Homol. Prim.	Contigs	
	A. Si	NPs, small l	nDels	B. Inde	els (2–50bp)	C. S	Vs (>51bp)
ses	8.0 -		-	1.5 -		0.25 -	
Bas						0.20 -	

Figure 1. Genetic background of Cascade Hops⁵.

- Cascade clones grown in greenhouse under controlled conditions. ~100 ug young leaves harvested and placed immediately on ice.
- DNA extracted with modified Qiagen DNAeasy mini-prep kit. Chemical precipitation and glass hooks substituted for spin columns to prevent shearing.
- 2 SMRTbell libraries constructed from unsheared DNA with 10kb size selection (BluePippin). Quality assessed with pulse field gels.

Table 1. PacBio SMRT Sequence Data. Data were generated from Nov. 2016-July 2017 on PacBio RS II with P6-C4 chemistry, and Sequel 1.2.1 – 2.0 chemistry.	PacBio SMRT Sequence Data		
	Sequel Cells	83	
	PacBio RS II Cells	126	
	Number Subreads	50,416,927	
	Total Subread Bases	342,733,627,375	
	Prop. Sequel Data (bp)	74 %	

Pre-Assembly Results

- Pre-assembly ("error-correction") generates high accuracy preassembled reads ("preads") by raw read overlap and consensus (Fig 2A).

Statistic	Draft PacBio Primary	Draft PacBio Haplotigs	Curated PacBio Primary	Teamaker Short Read Asm
Total Length	4.24 Gb	1.35 Gb	3.79 Gb	1.77 Gb
No. Contigs	11,705	38,060	8,652	861,745
Contig NG50	867 kb	NA	866 kb	1.4 kb
Longest Contig	8.25 Mb	1.77 Mb	8.25 Mb	69 kb

 Table 5. Genome Assembly Stats for PacBio assembly before and after Purge
Haplotigs. The Illumina-based Teamaker v1.1⁴ assembly is shown for comparison. Flow cytometry 1N size is 2.7 Gb¹ and is the genome size used for NG50 statistics.

BUSCO Status	Draft Assembly	Draft Primary Only	Curated Assembly
Complete	89.3 %	86.7 %	85.0 %
Duplicated	55.3 %	37.7 %	30.1 %
Missing	8.8 %	10.9 %	12.8 %
Fragmented	1.9 %	2.2 %	2.2 %

Table 6. Genome completeness assessment for PacBio assembly with BUSCO. Embryophyta gene set (N=1440) run with BUSCO37.

Assembled Genome Size Estimate

Purge haplotigs pipeline defines four coverage bins from raw read coverage histogram for the full draft assembly. The proportion of each contig with haploid and diploid coverage was used to estimate assembled genome length.

ow	haploid	diploid	h
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Figure 5. Unzipped haplotypes display lower variation than homologous primary contigs. A. Percent divergence in SNPs and small insertion-deletions. B. Percentage of bases in small indels (2-50 bp). C. Percentage of bases in SVs (>50 bp).

Conclusion

- The PacBio FALCON-Unzip diploid assembly is both more complete (>2-fold increase in assembly length) and more contiguous (>600-fold increase in contig NG50) than previous short-read assemblies.
- Most assembled contigs are haplotypes due to high heterozygosity, with haplotype divergence ranging from 1-5% for SNPs and small indels.
- Haplotype deduplication removed ~450 Mb of haplotigs prior to scaffolding.

Pre-assembled yield = 75% = (total length preads / total length seed reads), used as metric for data quality and coverage.

Statistic	Raw Reads	Seed Reads	PReads
Read Count	35.2 M	12.1 M	13.8 M
Total Bases	288 Gb	182 Gb	135 Gb
Coverage	107 fold	67 fold	50 fold
Mean Length	8 kb	15 kb	12 kb
N50 Length	12 kb	15 kb	12 kb

Table 2. Summary Statistics of Reads. Raw reads: filtering: subreads >500 bp, single subread from each ZMW. Seed reads: Raw reads longer than 10 kb (manual cut- off). **Preads** may be shorter than seed reads due to low coverage regions that break or truncate seed reads. Coverage based on 2.7 Gb 1N genome size.



Figure 3. Coverage of raw PacBio reads for draft FALCON-Unzip assembly. Lack of diploid coverage peak indicates genome largely assembled as haplotypes (not collapsed diploid haplotypes, Fig 4). Raw reads >2500 bp were mapped to draft FALCON-Unzip assembly as part of phased polishing (Fig 2E).

Coverage Bin	Total Length	Table 7. Length of diploid- and haploid-
Diploid	297 Mb	assembled genome.
Haploid	4.16 Gb	1N length calculated as:
1N Length	2.38 Gb	

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