A Low DNA Input Protocol for High-quality PacBio *De Novo* Genome Assemblies from Single Invertebrate Individuals

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Abstract

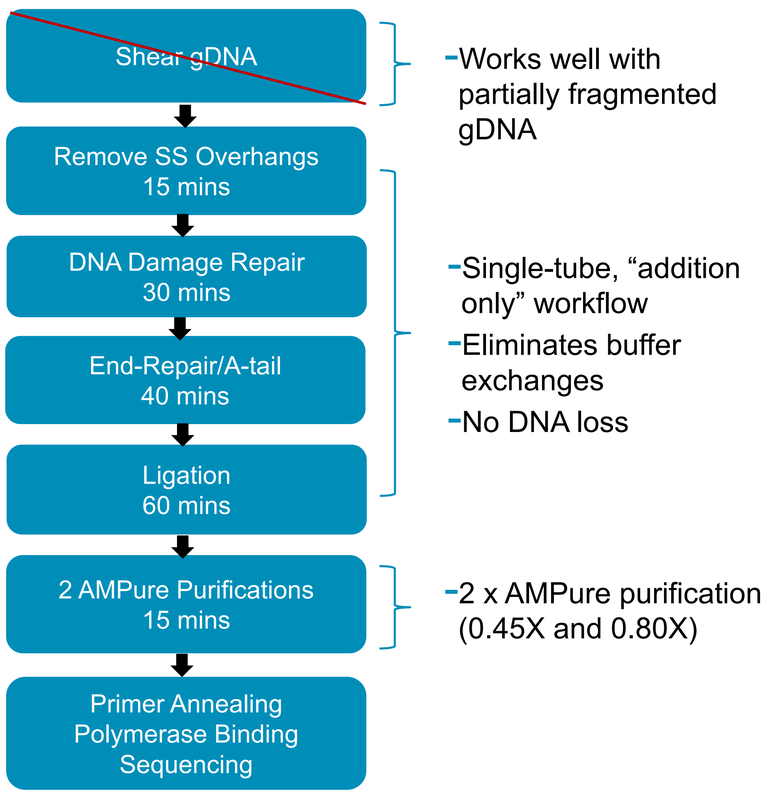
A high-quality reference genome is an essential tool for studies of plant and animal genomics. PacBio Single Molecule, Real-Time (SMRT) Sequencing generates long reads with uniform coverage and high consensus accuracy, making it a powerful technology for *de novo* genome assembly. PacBio is the core technology for many large genome initiatives, however, relatively high DNA input requirements (5 µg for standard library protocol) have placed PacBio out of reach for many projects on small, non-inbred organisms that may have lower DNA content.

Here we present high-quality *de novo* genome assemblies from single invertebrate individuals for two different species: the *Anopheles coluzzii* mosquito and the *Schistosoma mansoni* parasitic flatworm. A modified SMRTbell library construction protocol without DNA shearing and size selection was used to generate a SMRTbell library from just 50-100 ng of starting genomic DNA. The libraries were run on the Sequel System with chemistry v3.0 and software v6.0, generating a range of 21-32 Gb of sequence per SMRT Cell with 20 hour movies, and followed by diploid *de novo* genome assembly with FALCON-Unzip. The resulting assemblies had high contiguity (contig N50s over 3 Mb for both species) and completeness (as determined by conserved BUSCO gene analysis). We were also able to resolve maternal and paternal haplotypes for 1/3 of the genome in both cases.

By sequencing and assembling material from a single diploid individual, only two haplotypes are present, simplifying the assembly process compared to samples from multiple pooled individuals. This new low-input approach puts PacBio-based assemblies in reach for small ,highly heterozygous organisms that comprise much of the diversity of life. The method presented here can be applied to samples with starting DNA amounts around 100 ng per 250 Mb – 1 Gb genome size.

Library Preparation Workflow

- Partially fragmented, HMW input DNA with few fragments <20 kb is important
- QC using Femto Pulse (Agilent) due to limited DNA quantity
- No shearing
- No size selection
- Total preparation time: ~3.5 hours
- Availability of library kit: February 2019



Example: *Anopheles coluzzii*

Input DNA amount: 100 ng

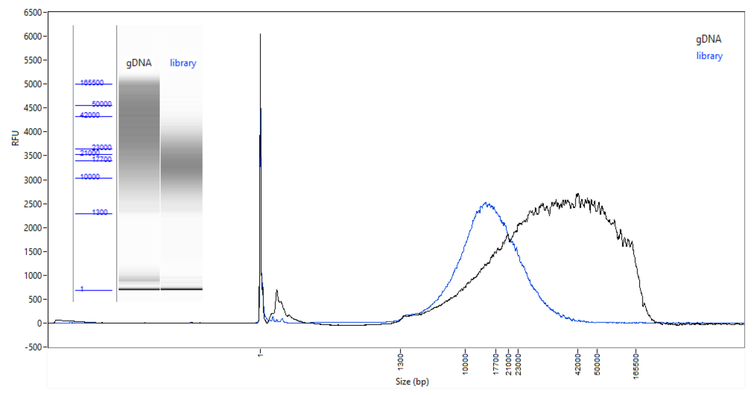


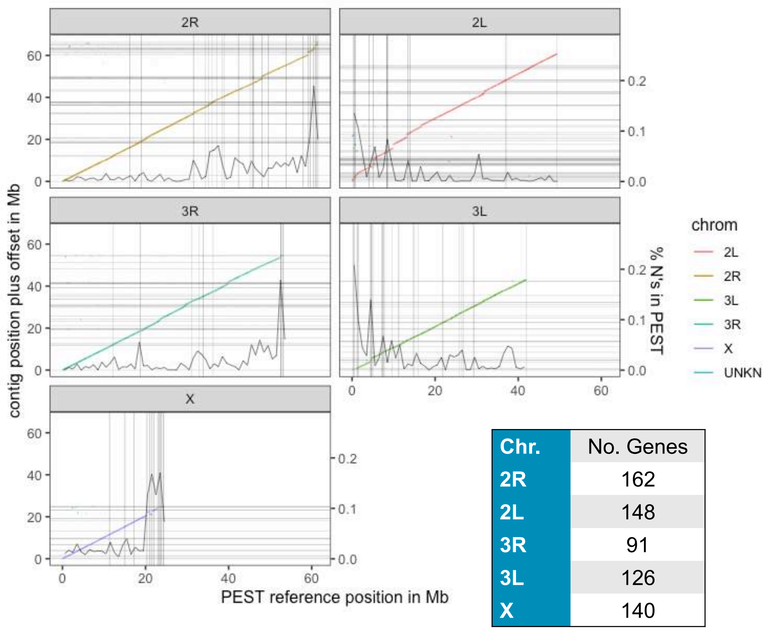
Figure 1. *Anopheles coluzzii* input DNA and resulting library. Femto Pulse QC traces and ‘gel’ images (inset) of the genomic DNA input (black) and the final library (blue) before sequencing. The library yield was 59%.

Loading Conc.	Total Yield (Gb)	Unique Mol. Yield (Gb)	N50 Polymerase Read Length	N50 Subread Length	P0	P1	P2
5 pM	24.1	4.5	116,615	12,978	26.0%	60.1%	13.9%
5 pM	23.6	4.5	114,807	13,132	27.1%	59.0%	14.0%
6 pM	25.0	3.9	122,898	12,751	35.3%	53.1%	11.7%

Table 1. Run statistics for the three Sequel SMRT Cells run from the library (v3.0 chemistry, 20 hour runs/Cell). A total of 45 X unique molecular coverage (UMC) was generated for assembly.

		PacBio	Sanger assembly
Primary Contigs	Total Length	251 Mb	224 Mb
	No. contigs	206	27,063
	Contig N50	3.47 Mb	0.025 Mb
Alternate Haplotigs	Total Length	89.2 Mb	unresolved
	No. contigs	830	N/A
	Contig N50	0.199 Mb	N/A
BUSCO (diptera N=2799)	Complete	98.1 %	87.5 %
	Duplicate	2.4 %	0.1 %
	Fragmented	0.9 %	6.8 %
	Missing	1.0 %	5.7 %

Table 2. Assembly statistics of the PacBio *Anopheles coluzzii de novo* assembly, and compared with the previous Sanger-sequence based assembly for this species from [1] (GCA_000150765.1). BUSCO [2] was run on the PacBio primary contigs after curation with Purge Haplotigs [3].



Chr.	No. Genes
2R	162
2L	148
3R	91
3L	126
X	140

Figure 2. Alignment of the curated PacBio contigs to the AgamP4 PEST reference [4]. Alignments colored by primary PEST reference chromosome to which they align. Contig ends are denoted by horizontal lines in the PacBio assembly and vertical lines in PEST. Percent Ns per megabase of PEST overlaid (right Y axis). There are no Ns in the PacBio assembly.

Table Inset: More than 90% of the 737 genes on “UNKN” PEST contigs are given chromosomal assignment by alignment to PacBio contigs. Count of genes placed to each chromosome given in table.

Example: *Schistosoma mansoni*

Input DNA amount: 45 ng

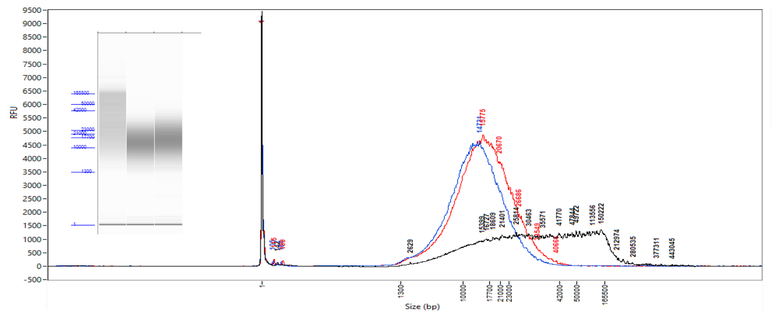


Figure 3. *Schistosoma mansoni* input DNA and two resulting libraries. Femto Pulse QC traces and ‘gel’ images (inset) of the genomic DNA input (black) and two final libraries (blue: 100 ng input, yield 50%; red: 45 ng input, yield 55%). The second library (red) was used for sequencing.

Loading Conc.	Total Yield (Gb)	Unique Mol. Yield (Gb)	N50 Polymerase Read Length	N50 Subread Length	P0	P1	P2
4 pM	32.3	3.8	161,677	13,437	47.3%	44.3%	8.5%
4 pM	26.9	3.2	157,064	13,351	54.3	36.3%	9.4%
4 pM	32.5	3.8	168,417	13,586	50.7%	39.9%	9.4%
4 pM*	20.6	3.8	78,715	12,121	34.1%	52.7%	13.2%

Table 3. Run statistics for the four Sequel SMRT Cells run from the library. (v3.0 chemistry, 20 hour runs/Cell; *10 hour run for the last Cell). A total of 41 X UMC was generated for assembly.

	PacBio	2012 reference
Total Length	382 Mb	364 Mb
No. contigs	327	9,516
Contig N50	3.8 Mb	0.077 Mb
No. scaffolds	--	885
Scaffold N50	--	32 Mb
Gap length	0	2 Mb
BUSCO (eukaryota, N=303)		
Complete	86.1 %	84.8 %
Duplicate	17.8 %	3.0 %
Fragmented	4.0 %	5.3 %
Missing	9.9 %	9.9 %

Table 4. Assembly statistics of the PacBio *Schistosoma mansoni de novo* assembly, and compared with the previous assembly for this species (GCA_000237925.2). BUSCO was run on the combined primary contig and haplotigs PacBio assembly.

Conclusions

- New library workflow enables high-quality *de novo* genome assemblies from as little as 50 ng starting genomic DNA.
- Protocol requires partially fragmented, HMW DNA with few fragments less than 20 kb.
- No shearing of genomic DNA or size-selection of library.
- Enables sequencing single individuals with no need for inbreeding or pooling multiple samples.
- Scalable protocol can be applied to 1 Gb genomes.
- For more information, please see *An. coluzzii* preprint: https://www.biorxiv.org/content/early/2018/12/19/499954

References

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4. https://www.vectorbase.org/organisms/anopheles-gambiae/pest/agamp4 [cited 7 Aug 2018]

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