Modified paired end rapid library preparation protocol for 454 GS Junior 8 kb library preparation using Covaris g-tubes and BluePippin electrophoresis

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Method Article

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Abstract

This protocol describes an alternative approach to performing Roche's Paired End Rapid Library Preparation Method for 8 kb span libraries. This method uses the Corvaris g-tube for DNA fragmentation, eliminating the need for a HydroShear apparatus, and a Sage Science BluePippin electrophoresis platform to size select the 8 kb fragments, eliminating the need for agarose gel electrophoresis and sample recovery using a Whatman Elutrap device. These two modifications allow the protocol to be completed in significantly less time \(eliminate an overnight step) and require significantly less capital investment in laboratory equipment. This modified library preparation protocol has yielded paired end libraries meeting all the quality control benchmarks the original protocol stipulates and has been combined with sequencing on a GS Junior instrument to generate excellent sequencing results. The equipment substitutions presented allow paired end library preparation to be practical for smaller research groups.

Introduction

In large-scale sequencing centers it is commonplace to have a range of DNA sequencing platforms \(e.g. 454, Illumina, SOLiD, and PacBio) and also specialized devices for shearing of DNA molecules common in the preparation of paired-end libraries. With the introduction of laboratory-scale devices like the GS Jr and MiSeq there is a shift occurring where DNA sequencing technology is becoming directly accessible within small labs. The ability to perform whole genome sequencing and assembly within a lab is now reasonable to consider for Bacteria using the GS Jr. A crucial type of data for scaffolding of genome assemblies is paired-end data. By generating information from sequencing reads linked across some known or estimated spanning distance, bioinformatic techniques are capable of ordering and orienting contigs produced from shotgun sequencing. In terms of the GS Jr platform the system is inherently capable of sequencing paired-end libraries but there is a challenge for laboratory-scale use as the conventional protocol for library production includes shearing of DNA molecules using a Hydroshear. As a specialized device the Hydroshear represents a significant investment for a laboratory-scale setting and may present a barrier to the use of paired-end sequencing in lab settings. This protocol describes an alternative approach to performing Roche's Paired End Rapid Library Preparation Method for 8 kb span libraries. This method uses the Corvaris g-tube for DNA fragmentation, eliminating the need for a HydroShear apparatus, and a Sage Science BluePippin electrophoresis platform to size select the 8 kb fragments, eliminating the need for agarose gel electrophoresis and sample recovery using a Whatman Elutrap device. These two modifications allow the protocol to be completed in significantly less time \(by eliminating an overnight step) and simplify sheared fragment purification. This modified library preparation protocol yielded paired-end libraries meeting all the quality control benchmarks of the original protocol and has been combined with shotgun sequencing on a GS Junior to generate excellent assemblies for a range of Bacteria. The equipment substitutions and associated savings in time provide a practical protocol for laboratory-scale paired-end sequencing on the GS Jr.

Reagents

Reagents unique to modified protocol: Covaris g-tube \(sold as package of 10) - Covaris #: 520079 Sage Science pre-cast gels - Sage Science #: BLF7510 Sage Science control DNA - Sage Science #: CON7504 Regents in common with original Roche paired end rapid library preparation method: GS FLX Titanium Library Paired End Adaptors \(Shared with GS FLX System) - Roche #: 5463343001 GS Rapid Library Preparation Kit - Roche #: 5608228001 AMPure XP 5 ml kit - Beckman coulter \(Agencourt) #: A63880 GenFind DNA Isolation Tube kit - Beckman coulter \(Agencourt) #: A41499 High Sensitivity DNA kit \(for BioAnalyzer) - Agilent #: 5067-4626 Plasmid-Safe ATP-Dependent DNase \(10,000 U/ml) - Epicentre biotechnologies #: E3101K Amicon Ultra-0.5 ml Centrifugal filters - Fisher Scientific #: UFC501096 Molecular Biology Grade water - Fisher Scientific #: BP2819-100 Dynabeads M-270 Streptavidin - Life Technologies #: 65305 Qubit BR dsDNA assay kit - Life Technologies #: Q32850 Qubit ssDNA Assay Kit - Life Technologies #: Q10212 Cre recombinase - New England Biolabs #: M0298L Exonuclease I \(20,000 U/ml) - New England Biolabs #: M0293L Bst DNA Polymerase, Large fragment \(8,000 U/ml) - New England Biolabs #: M0275L MinElute PCR Purification Kit \(50 columns) - Qiagen #: 28004 FastStart Taq DNA polymerase - Roche #: 12032902001 1,4-Dithiothreitol \(ultrapure) - Roche #: 3117006001 Bovine Serum Albumin \(20 mg/ml) - Roche #: 10711454001 ATP, lithium salt, pH 7 \(100 mM) - Roche #: 11140965001 PCR Nucleotide mix \(10 mM each) - Roche #: 11581295001 T4 DNA Polymerase \(1,000 U/ml) - Roche #: 11004794001 Polynucleotide Kinase \(PNK) \(10,000 U/ml) - Roche #: 10633542001 5 M NaCl - made in-house 10x TE buffer - made in-house 100% ethanol - supplied locally General lab consumables \(microfuge tubes, PCR tubes, plugged pipette tips, etc.)

Equipment

Equipment unique to modified protocol: Eppendorf 5415R or 5424 or MiniSpin plus microcentrifuge Sage Science BluePippin electrophoresis platform Equipment in common with original Roche paired end rapid library preparation method: Waterbath LabQuake rotator Magnetic particle concentrator \(MPC) Thermocycler Qubit fluorometer \(Life Technologies) Nitrogen gas tank General lab equipment \(pipettors)

Procedure

Base protocol is the Paired End Rapid Library Preparation Method Manual, 20 kb and 8 kb Span, GS Junior Titanium Series, March 2012. See attached "Supplementary Manual":http://www.nature.com/protocolexchange/system/uploads/3223/original/GSJuniorPairedEndLibraryPrepMethodManual-20kb-8kbSpan_March2012.pdf?1409053168. Modifications by Hill lab \(Saskatoon) group: Start with 15 µg of sample genomic DNA in 150 µL Tris-HCl pH 8.0 \(genomic DNA preparation of 100 ng/µL) - same as original protocol Section 3.1 - DNA Fragmentation \(HydroShear) - completely disregard this section. Instead, use Covaris g-tubes. • Save a few µL of original genomic DNA to visualize on an agarose gel later. • Add the 150 µL genomic DNA prep to a g-tube. Follow instructions with tubes - Spin in an Eppendorf MiniSpin plus microcentrifuge at 8,600 rpm for 1 min, flip tube over, repeat spin, remove sample from tube. • Keep 2 µL of the sheared sample to run on an agarose gel later. Section 3.2 - Fragment End Repair • For 25°C steps throughout protocol, set waterbath to maintain this temperature. Room temperature does not always work. • Disregard step 3 \(don't need this agarose gel). • Step 4m - use the arm on the Labquake to keep the tube from flipping completely over when doing this gentle mixing \(just rock the tube back and forth). Section 3.3 - Circularization Adaptor Ligation • Step 1 - change the 2x rapid ligase buffer for 10x rapid ligase buffer \(New England Biolabs product) and only add 10 µL \(reduces mixture to 100 µL from 190 µL). • Stop at the end of Step 4 and disregard Steps 5-11. Instead, take the entire sample and load onto a BluePippin cassette. Save a few µL of the sample pre-loading to run on an agarose gel later. • To load the sample, bring the sample volume to 120 µL with Tris-HCl pH 8.0 and add 40 µL BluePippin loading dye. Follow cassette instructions to prepare Blue Pippin cassette and load 4 lanes of a cassette by adding 40 µL of sample into each lane. Load the 5th lane with BluePippin marker. • Run the BluePippin with the range selection from 5,000-11,000 bp. • When finished, remove the size-selected DNA from the elution well into a microfuge tube and rise the elution chamber with 40 µL 0.1% Tween 20 solution \(supplied with BluePippin), adding this to the tube with the sample. • Concentrate and wash the size-selected sample by loading all the BluePippin eluted DNA into an Amicon filter column \(0.5 mL, 10 K) and spinning for 10 min at 14,000 x g. Discard flow-thru and add 250 µL Tris-HCl to the column. Spin for 15 min at 14,000 x g. Invert column in a clean collection tube and spin for 2 min at 1,000 x g. • Save a few µL of concentrated sample to run on an agarose gel later. • Run an agarose gel of the original genomic DNA \(unsheared), g-tube sheared DNA, sample before BluePipping and sample after BluePippin. • Continue to next section Section 3.5 - Fill-In Reaction • At Step 3, quantify with Life Technologies's Qubit BR dsDNA quantification kit. You need to achieve at least 6 ng/µL to continue. Section 3.6 - Circularization • No changes to protocol Section 3.7 - Nebulizer Assembly \(3.7.1) and DNA Nebulization and Collection/Purification of the Fragmented DNA \(3.7.2) • No changes to protocol Section 3.8 - Fragment End Repair • No changes to protocol Section 3.9 - Immobilization Bead Preparation • For Step 1 - prepare 2x Library Binding Buffer by mixing 5 mL Molecular Biology Grade Water, 4 mL of 5 M NaCl and 1 mL of 10x TE. Section 3.10 - Adaptor Ligation • For Step 4 - incubate at 25°C for 1 hour in a thermocycler. Section 3.11 - Library Immobilization • No changes to protocol Section 3.12 - Library Amplification • Prepare the PCR mixture as indicated for the sample and a second mixture to run without sample as a negative control. Save a few µL of the sample PCR after amplification and the negative PCR sample to run on the BioAnalyzer chip at the end. Section 3.13 - Sizing Mix Preparation • No changes to protocol Section 3.14 - Final Library Size Selection • No changes to protocol Section 3.15 - Library Quality Assessment • Step 1 - use BioAnalyzer chip and run the PCR sample and negative control from Section 3.12, as well as the final sample. Also, quantify final sample using Life Technologies's Qubit ssDNA quantification kit.

Timing

Sections 3.1 to 3.5 can be performed in a day \(including the BluePippin run). A good spot to pause overnight is either right after the BluePippin run \(end of Section 3.4) or after the fill-in reaction and quantification \(end of Section 3.5). Sections 3.5 or 3.6 to the end of Section 3.11 is a good second day of work. Finishing Section 3.12 to 3.15 on the third morning allows for plenty of time to continue on with sequencing emPCR later in that day.

Troubleshooting

Things that we have noted: • The AMPure size selection at the end \(Section 3.13 and 3.14) can be variable, depending on the AMPure lot and day you do it. • We have considered running the BluePippin cassette selecting for the tighter ~500 bp range \ (instead of the larger range in the protocol) to standardize the paired end fragment size and make this selection more precise. However, if the genomic DNA shearing wasn't precisely around the 8 kb mark, keeping the larger size window gives more DNA to work with and appears to work just fine.

Anticipated Results

Figure 1 shows typical results of shearing with the Covaris g-tubes and the purification of appropriately sized fragments using the Blue Pippin. Table 1 shows sequencing metrics generated on the GS Junior apparatus for three bacteria.

References

Paired End Rapid Library Preparation Method Manual, 20 kb and 8 kb Span, GS Junior Titanium Series, March 2012

Figures

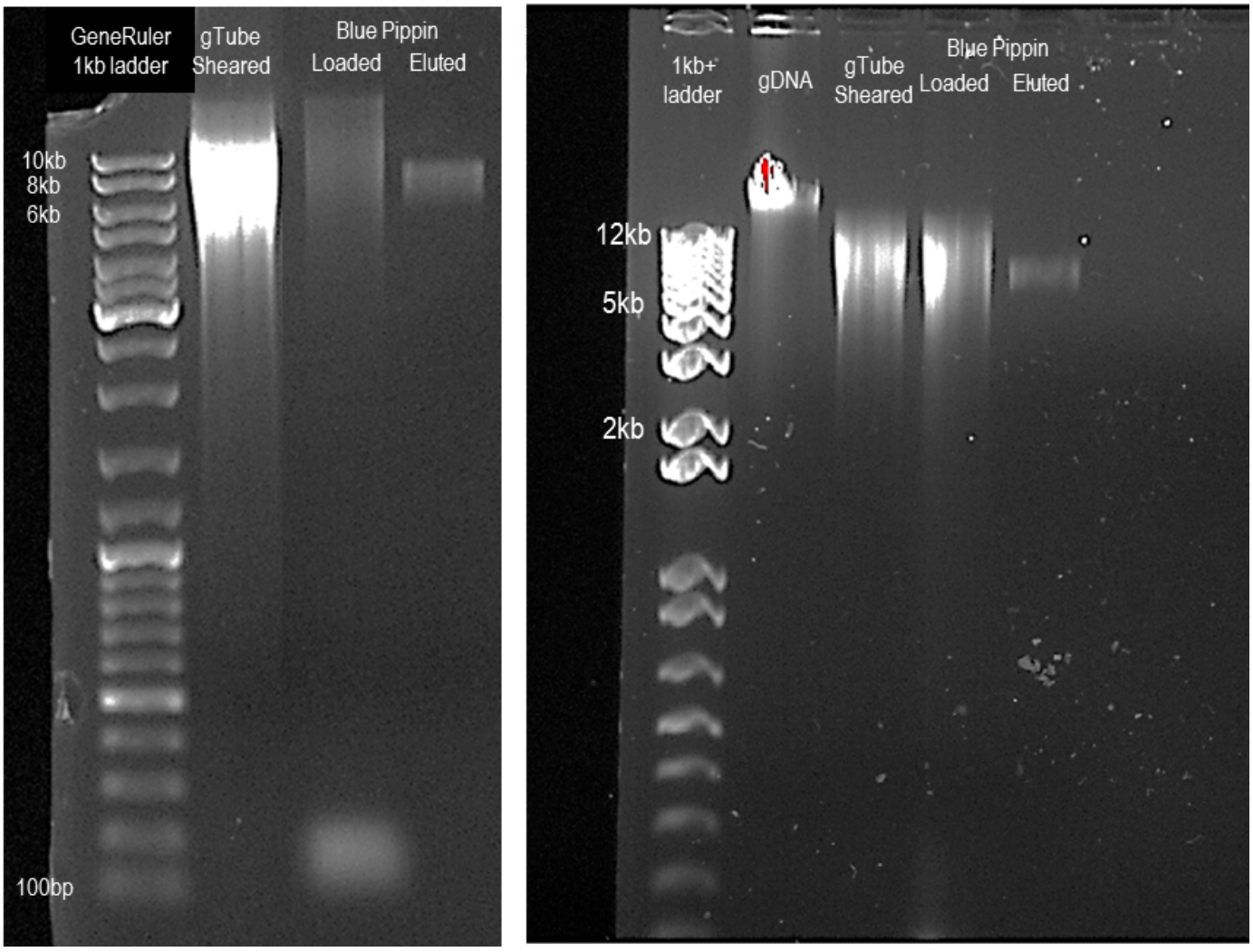


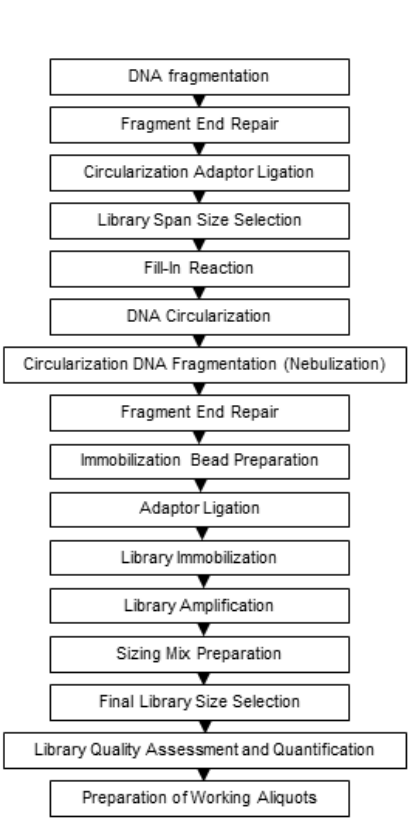
Figure 1

Gel electrophoresis of sheared DNA Agarose gel of DNA before and after shearing and before and after BluePippin.

	BRG100			KL180			Pagg4		
	Shotgun	Paired End	Combined	Shotgun	Paired End	Combined	Shotgun	Paired End	Combined
Reads	290,085	203,771	493,856	300,116	137,252	437,368	151,364	80,667	232,031
Paired End Reads		112,190	112,190		70,713	70,713		12,560	12,560
% Aligned	99.38	99.21	99.64	82.1	92.97	91.32	98.67	41.66	98.91
Coverage	21x	12x	33x	2.3x	4x	2.4x	13x	3x	16x
Estimated Genome Size	6.4MB	6.9MB	6.6MB	67.3MB	13.4MB	78MB	5.3MB	3.2MB	4.9MB
Scaffolds		6	4		32	6		1	12
Largest Scaffold		6.195MB	6.145MB		1.014MB	3.082MB		0.003MB	2.198MB
Large Contigs	46	231	43	344	1121	380	62	282	50
Contigs	60	254	59	753	1478	605	75	3545	61
N50 Contigs	323,897	58,094	344,991	28,233	3,023	45,775	201,512	626	271,189
Pair Distance		7,373±1843	7,373±1843		5,980±1495	5,980±1495		5,937±1484	5,937±1484

Figure 2

Table 1. Sequencing metrics Sequencing metrics using shotgun and paired-end protocols on the GS Junior (Titanium chemistry). Results are shown for three genomes: Pseudomonas sp. BRG100, Brachyspira sp. KL180, and Pantoea agglomerans isolate 4.



	Standard protocol	Modified protocol
DNA fragmentation	HydroShear	Eppendorf microcentrifuge
Circularization Adaptor Ligation	Agarose gel electrophoresis system	Blue Pippin electrophoresis system
Library Span Size Selection	Whatman Elutrap device	

Step	Equipment	Capital cost	Consumable cost	Procedure time
DNA fragmentation	HydroShear	$26000	Negligible (microfuge tube)	30 min
	Eppendorf microcentrifuge	$1650	Corvaris g-tube; $28 per sample	5 min
Circularization Adaptor Ligation	Agarose gel electrophoresis system	$500	$10 per gel	15-17 hrs
Library Span Size Selection	Whatman Elutrap device	$1700	BT2 membrane; $3 per sample	2.5 hrs
	Blue Pippin electrophoresis system	$20000	Gel cassette; $45 per sample	4 hrs

Figure 3

flowchart flowchart of modified paired-end protocol

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement0.pdf