# Equipment and materials needed for sequencing



Quick reference card

This Quick reference card lists examples of third-party laboratory equipment and other materials that are recommended for running specific types of SMRT sequencing applications on PacBio® Sequel® II, Sequel IIe and Revio systems. Note that although vendor part numbers are listed herein, they may not always be up to date. Verify and obtain the latest part numbers and relevant documentation from the specific vendor or manufacturer's website. For more detailed guidance regarding specific equipment recommendations, refer to the appropriate PacBio **Procedure & checklist**.

#### **SMRT®** sequencing application categories

Main application	Workflow	Procedure & checklist						
Whole genome	Large genome & small (microbial) genome WGS	Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0 [ 102-166-600 ]						
sequencing (WGS)	Ultra-low DNA input WGS	Preparing HiFi SMRTbell libraries from ultra-low DNA input [ 101-987-800]						
Viral sequencing	HiFiViral SARS-CoV-2	PacBio HiFiViral high-throughput multiplexing for full-viral genome sequencing of SARS-CoV-2 using SMRTbell prep kit 3.0 [ 102-396-100 ]						
(VIRAL)	AAV	Preparing multiplexed AAV SMRTbell libraries using SMRTbell prep kit 3.0 [ 102-126-400 ]						
RNA sequencing	Iso-Seq method	Preparing Iso-seq libraries using SMRTbell prep kit 3.0 [ 102-396-000 ]						
(RNA)	MAS-Seq single-cell method	Preparing MAS-Seq libraries using MAS-Seq for 10x 3' concatenation kit [ 102-678-600 ]						
Metagenomics	Full-length 16s	Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 [ 102-359-000 ]						
(META)	Shotgun metagenomics	Preparing whole genome and metagenome libraries using SMRTbell prep kit 3.0 [ 102-166-600 ]						
Targeted sequencing	Amplican acquencies	Preparing multiplexed amplicon libraries using SMRTbell prep kit 3.0 [ 102-359-000 ]						
(TARG)	Amplicon sequencing	Preparing multiplexed amplicon libraries using PacBio barcoded M13 primers & SMRTbell prep kit 3.0 [ 101-921-300 ]						

#### Laboratory equipment and material recommendations guide

+	Compatible with a specific application
-	Not recommended for a specific application
Highly recommended	Where multiple compatible equipment options may be available for a particular category, specific products that are highly recommended to enable generation of optimal SMRT sequencing data quality are also highlighted*



Note:

Use of other compatible equipment options that are not specifically designated as "Highly Recommended" may require adjustment of input genomic DNA amounts (depending on the sample genome size and target coverage requirements) for HiFi whole genome sequencing projects to accommodate the DNA sizing QC steps. For more detailed guidance regarding specific equipment recommendations, refer to the appropriate PacBio **Procedure & checklist**.



#### A. DNA sizing analysis

PacBio highly recommends using the Femto Pulse system (Agilent Technologies) for DNA sizing QC analysis of genomic DNA and SMRTbell libraries in HiFi whole genome sequencing (WGS) sample preparation workflows. Use of other compatible equipment options that are not specifically designated as "Highly Recommended" may lead to lower HiFi data yields and/or lower HiFi data quality, or may require adjustment of input genomic DNA amounts (depending on the sample genome size and target coverage requirements) for HiFi WGS projects to accommodate the DNA sizing QC steps. For more detailed guidance regarding specific equipment recommendations, refer to the appropriate PacBio **Procedure & checklist**.



Femto Pulse system for DNA sizing analysis up to ~165 kb

		Femto Pulse System											
Highly recommended	Agilent technologies	<ul> <li>Rapid analysis time (&lt;1.5 hours)</li> <li>Requires &lt;1 ng of sample</li> <li>Suitable for analysis of SMRTbell libraries and genomic DNA up to ~165 kb</li> </ul>	M5330AA	+	+	+	+	+	+	+	+	+	
		Genomic DNA 165 kb Kit for Femto Pulse											
	Agilent technologies	THE	FP-1002- 0275	+	+	+	+	+	+	+	+	+	

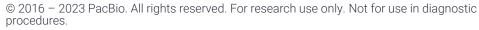


# DNA sizing analysis (cont.)

			WGS	VIRAL	RNA	META	TARG
Vendor	Product	PN	Large & small genome WGS Ultra-low DNA input WGS	HiFiViral SARS-CoV-2 AAV	Iso-Seq Method MAS-Seq single-cell Method	Full-Length 16S Shotgun Meta-G	Amplicon Seq.

Bioanalyzer 2100 system for DNA sizing analysis up to ~7 - 15 kb

Diodilalyzer 210	oo system for DNA sizing analys	no up to '	1010									
Agilent technologies	• Suitable for analysis of SMRTbell libraries up to ~15 kb	G2939BA	-	-	+	+	+	+	+	-	+	
Agilent technologies	DNA 7500 Kit for Bioanalyzer 2100	5067-1506	-	-	+	-	-	-	+	-	+	
Agilent technologies	DNA 12000 Kit for Bioanalyzer 2100	5067-1508	-	-	+	+	+	+	+	-	+	
Agilent technologies	High Sensitivity Analysis Kit for Bioanalyzer 2100	5067-4626			+	+	+	+	+		+	

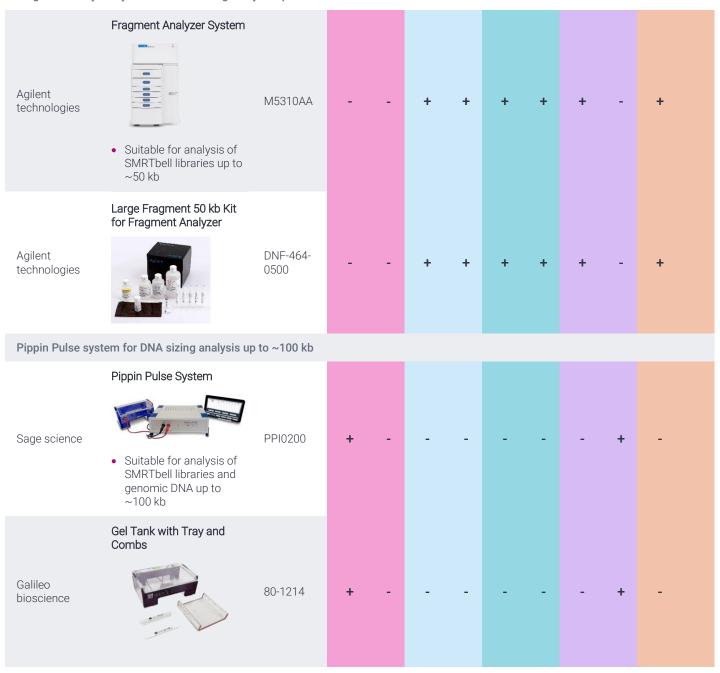




#### DNA sizing analysis (cont.)

	WGS	VIRAL	RNA	META	TARG
Vendor Product PN	Large & small genome WGS Ultra-low DNA input WGS	HiFiViral SARS-CoV-2 AAV	Iso-Seq Method MAS-Seq single-cell Method	Full-Length 16S Shotgun Meta-G	Amplicon Seq.

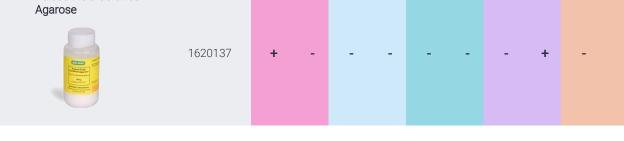
Fragment Analyzer system for DNA sizing analysis up to ~50 kb



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											Š	
Sage science	10X KBB Buffer  Sage Gestrophorest All  sage KKFF	KBB1001	+		-	•	-	-	-	+	-	
Lonza	SeaKem® Gold Agarose	50150	+		-	-	-	-		+		
CHEF Mapper X	A system for DNA sizing analys	sis up to ~10 M	lb									
Bio-Rad	Pulsed Field Gel Electrophoresis System: CHEF Mapper® XA  • Suitable for analysis of SMRTbell libraries and genomic DNA up to ~10 Mb	170-3670	+	-	-		-	-	-	+	-	
Bio-Rad	10X TBE Electrophoresis Buffer	1610770	+	-	-	-	-	-	-	+	-	





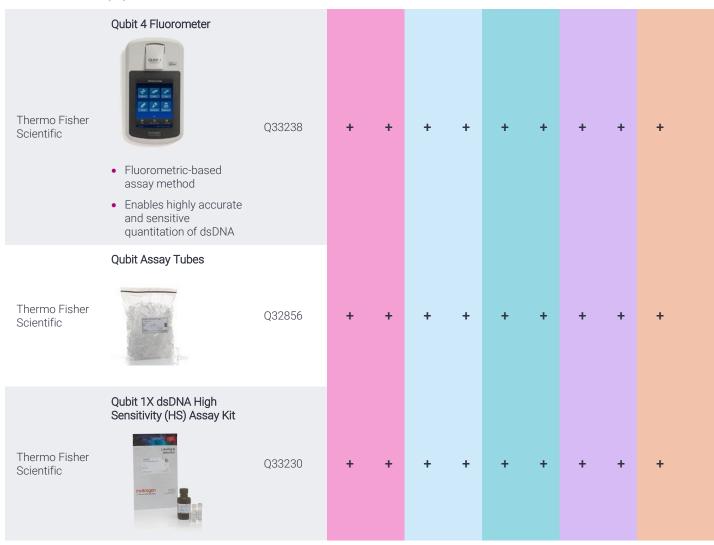
Bio-Rad

Pulsed Field Certified

#### B. DNA quantification analysis

			V	WGS		RAL	RNA		A ME		TA	RG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.	

#### Fluorometric assay systems





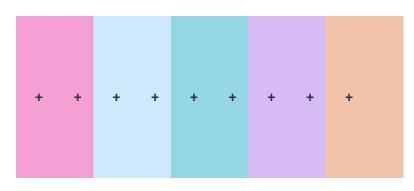
#### C. DNA purity analysis



Spectrophotometric assay systems

NanoDrop™ 2000/2000c Spectrophotometer

Thermo Fisher Scientific ND-2000



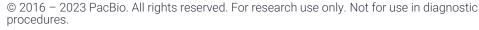
#### D. DNA shearing

PacBio highly recommends using the Megaruptor 3 system (Diagenode) for DNA shearing in HiFi whole genome sequencing (WGS) sample preparation workflows. Use of other compatible equipment options that are not specifically designated as "Highly Recommended" may lead to lower HiFi data yields and/or lower HiFi data quality, or may require adjustment of input genomic DNA amounts (depending on the sample genome size and target coverage requirements) for HiFi WG projects to accommodate the DNA sizing QC steps. When a Megaruptor 3 System is unavailable, the Covaris g-TUBE presents an alternative shearing method that does not require instrumentation beyond a standard microcentrifuge – see Technical note: Covaris g-TUBE DNA shearing for SMRTbell prep kit 3.0 (102-326-501) for more information. For more detailed guidance regarding specific equipment recommendations, refer to the appropriate PacBio Procedure & checklist.

			٧	WGS		RAL	RNA		META		TA	RG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	lso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.	

Megaruptor 3 system for shearing DNA to 5 - 100 kb fragment sizes

		system for shearing black to 5	3									
pap	Diagenode	Megaruptor 3 System  Includes 8-sample cassette	B06010003	+	+	-	-	-	-	-	+	-
Highly recommended	Diagenode	Megaruptor 3 cassette for 12 samples	B06010020	+	+	-	-	-	-	-	+	-
Hig	Diagenode	Megaruptor 3 Shearing Kit	E07010003	+	+	-	-	-	-	-	+	-
	Diagenode	Megaruptor 3 DNAFluid+ Kit	E07020001	+	-	-	-	-	-	-	+	-

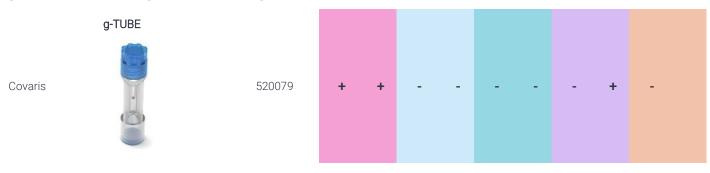




# DNA shearing (cont.)



g-TUBE devices for shearing DNA to 6 - 20 kb fragment sizes



#### E. DNA size selection

In Procedure & checklist – Preparing whole genome and metagenome sequencing libraries using SMRTbell prep kit 3.0 (102-166-600), AMPure PB bead size selection is the recommended default method to remove short DNA fragments and enrich for the long fragments that are most valuable for downstream HiFi data analysis. Although size-selection beads have many advantages, automated DNA size selection systems that utilize gel cassettes offer more flexibility in defining a size cutoff. This section lists three automated DNA size selection tools that may optionally be employed for performing size selection on SMRTbell libraries for HiFi WGS applications. Note that use of these tools requires higher input DNA amounts ( $\geq 1.5 \, \mu \text{g/SMRT}$  Cell 8M or  $\geq 3 \, \mu \text{g/Revio}$  SMRT Cell). For more information, refer to Technical Note: Alternative Size Selection Methods for SMRTbell Prep Kit 3.0 (102-326-503), which provides detailed guidance for size selection of WGS libraries using automated DNA size selection tools.

	WGS	VIRAL	RNA	META	TARG
Vendor Product PN	Large & small genome WGS Ultra-low DNA input WGS	HiFiViral SARS-CoV-2 AAV	Iso-Seq Method MAS-Seq single-cell Method	Full-Length 16S Shotgun Meta-G	Amplicon Seq.

SageELF system for DNA size selection 100 bp - 40 kb





#### DNA size selection (cont.)



PippinHT system for DNA size selection 100 bp - 50 kb

	PippinHT System											
	ALVO A											
Sage science	<ul> <li>Separates DNA samples by size, and collects all fragments above a size threshold set by the user</li> </ul>	HTP0001	+	+	-	-	-	-	-	-	-	
	Up to 10 samples (plus a Size Marker) can be fractionated on a single precast agarose cassette, and up to two cassettes (20 samples) may be processed at one time											
	0.75% Agarose Cassette for PippinHT (with Marker 75E)											
Sage science		HPE7510	+	+	-	-	-	-	-	-	-	

# DNA size selection (cont.)

	WGS	VIRAL	RNA	META	TARG
Vendor Product PN	Large & small genome WGS Ultra-low DNA input WGS	HiFiViral SARS-CoV-2 AAV	Iso-Seq Method MAS-Seq single-cell Method	Full-Length 16S Shotgun Meta-G	Amplicon Seq.

PippinHT system for DNA size selection 100 bp - 50 kb

	BluePippin System							
Sage science	<ul> <li>Separates DNA samples by size, and collects all fragments above a size threshold set by the user</li> <li>Up to four samples (plus a Size Marker) can be fractionated on a single precast agarose cassette, and only one cassette may be processed at one time</li> </ul>	BLU0001	+	+	-	-	 	-
Sage science	0.75% Agarose Cassette for BluePippin (with Marker S1)	BLF7510	+	+	-	-	 	

# F. General laboratory equipment

			V	VGS	VI	RAL	R	NA	М	ETA	TARG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	ААV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.
Benchtop mi	crocentrifuges										
Eppendorf	See recommended models below  MiniSpin® Plus  Centrifuge 5425	022620100	+	+	+	+	+	+	+	+	+
Mini microce	entrifuges										
VWR	Mini Microcentrifuge	10153-842	+	+	+	+	+	+	+	+	+
Plate centrifu	uges										

EP022628168



Eppendorf

Plate Centrifuge

			W	IGS	VIRAL		RNA		META		TARG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.
Vortex mixe	ers										
VWR	Digital Vortex Mixer (or equivalent)	10153-842	+	+	+	+	+	+	+	+	+
VWR	Microtube and microplate holder kit with insert retainer for vortex mixer	Q33230	+	+	+	+	+	+	+	+	+
Thermal mix	xers										
Eppendorf	Provides stable incubation temperatures for low-throughput SMRTbell library construction reactions using 1.5 mL or 2.0 mL tubes	2231000667	+	+	+	+	+	+	+	+	+





#### Thermal cyclers

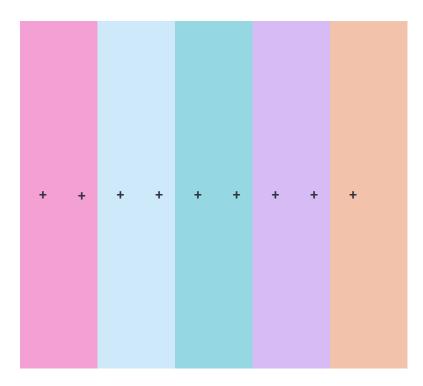
SimpliAmp Thermal Cycler (or equivalent)



Thermo Fisher Scientific

A24811

- Provides stable incubation temperatures for highthroughput SMRTbell library construction reactions using microplates or 0.2 mL PCR tubes
- Controllable heated lid preferred
- 96-well capacity recommended for highthroughput environments



	WGS	VIRAL	RNA	META	TARG
Vendor Product PN	Large & small genome WGS Ultra-low DNA input WGS	HiFiViral SARS-CoV-2 AAV	Iso-Seq Method MAS-Seq single-cell Method	Full-Length 16S Shotgun Meta-G	Amplicon Seq.

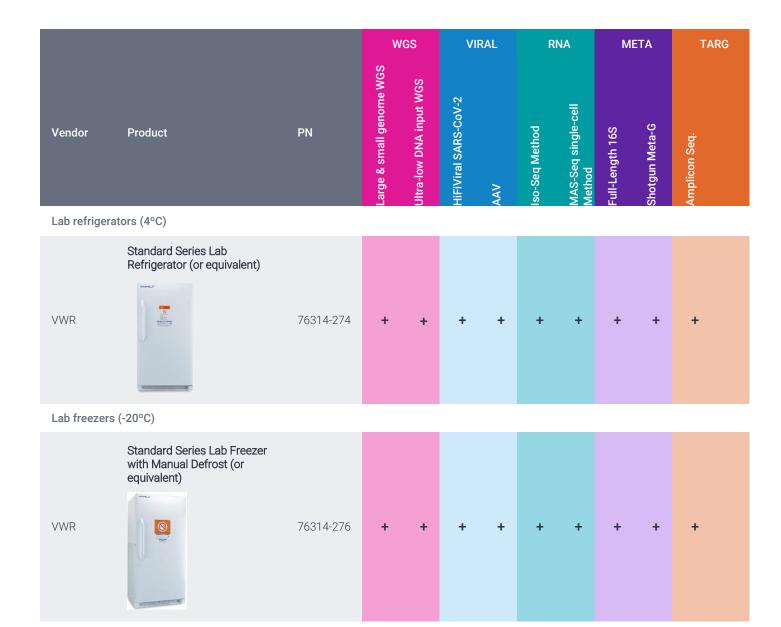
Heat sealer (Required for Sequel IIe system sequencing workflows ONLY. A heat sealer in NOT required for Revio system workflows.)





			V	/GS	VI	RAL	R	NA	М	ETA	TARG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.
Ice makers											
VWR	Flake Ice Maker with Bin (or equivalent)	89166-534	+	+	+	+	+	+	+	+	+
Magnetic se	parator racks										
Thermo Fisher Scientific	DynaMag™-2 Magnet Separation Stand for 1.5-2 mL Microcentrifuge Tubes (or equivalent)	12321D	+	+	+	+	+	+	+	+	+
V & P Scientific, Inc.	Low Profile Magnetic Separation Stand for 0.2 mL, 8- Strip PCR Tubes (or equivalent)	VP772F4-1	+	+	+	+	+	+	+	+	+







#### G. General laboratory reagents and consumables

			V	/GS	VI	RAL	R	NA	М	ETA	TARG
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.
Tubes*											
USA Scientific	0.2 mL TempAssure PCR 8-tube strips (or equivalent)	1402-4708	+	+	+	+	+	+	+	+	+
Eppendorf	Eppendorf DNA LoBind Tube 0.5 mL, PCR clean	022431005	+	+	+	+	+	+	+	+	+
Eppendorf	Eppendorf DNA LoBind Tube 1.5 mL, PCR clean	022431021	+	+	+	+	+	+	+	+	+
Eppendorf	Eppendorf DNA LoBind Tube 2.0 mL, PCR clean	022431048	+	+	+	+	+	+	+	+	+
VWR	Falcon™ Conical Centrifuge Tubes, PP (Polypropylene), 15 mL / 50 mL	21008-936 21008-940	+	+	+	+	+	+	+	+	+

<sup>\*</sup> Corning® Axygen® consumables are not compatible with SMRT sequencing and should not be used in the preparation of PCR amplicons or SMRTbell® templates.

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# General laboratory reagents and consumables (cont.)

			W	IGS	VII	RAL	R	NA	М	ETA	TARG	
Vendor	Product	PN	Large & small genome WGS	Ultra-low DNA input WGS	HiFiViral SARS-CoV-2	AAV	Iso-Seq Method	MAS-Seq single-cell Method	Full-Length 16S	Shotgun Meta-G	Amplicon Seq.	
Ethyl alcohol	, pure											
Sigma- Aldrich	Ethyl Alcohol, Pure, 200 Proof, Molecular Biology or ACS Reagent Grade CH <sub>3</sub> CH <sub>2</sub> OH	459844	+	+	+	+	+	+	+	+	+	
Molecular bi	ology grade water											
Teknova	Nuclease-Free Water, for Molecular Biology (or equivalent) H20	W3330	+	+	+	+	+	+	+	+	+	
Sample and	buffer plate accessories for Agiler	nt Fragment An	alyzer a	nd Fem	to Pulse	system	าร					
Eppendorf	twin.tec® PCR Plate 96, semi-skirted, 250 µL, PCR clean, colorless plates  • Recommended sample plate for Agilent Fragment Analyzer & Femto Pulse systems	951020303	+	+	+	+	+	+	+	+	+	
Thermo Fisher Scientific	Fisherbrand™ 96-Well DeepWell™ Polypropylene Microplates, 1 mL  • Recommended buffer plate for Agilent Fragment Analyzer & Femto Pulse systems	12-566-120	+	+	+	+	+	+	+	+	+	

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Revision history (description)	Version	Date
Removed No-Amp SMRT sequencing application category and added clarifying note that a heat sealer tool is not required for Revio system sequencing workflows. Added Megaruptor 3 cassette for 12 samples and Megaruptor 3 DNAFluid+ Kit to list of recommended DNA shearing materials.	08	May 2023

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