Procedure & Checklist - Greater Than 10 kb Template Preparation Using AMPure® PB Beads

Before You Begin

This procedure can be used to prepare greater than 10 kb libraries from 5 µg of sheared and concentrated DNA. If preparing larger amounts of DNA, scale all the reaction volumes proportionally (e.g., if the input amount of DNA is double the amount set forth in this procedure, then double all the reaction volumes listed in the tables).

If a Pippin Pulse™ size selection system is not available or if the SMRTbell® libraries are less than 500 ng, an alternative method using AMPure PB beads may be used to filter out most fragments in the 1 kb to 2 kb range. A more aggressive approach, using 0.40X AMPure PB beads (in the final step of the purification process) may also be used. Note that size selection using the AMPure PB beads does not completely remove all short SMRTbell templates. As a result, the average subread lengths may be shorter than a Pippin Pulse size selected library.

Insert Size Target	Insert Size Range	Sheared and Concentrated DNA Amount	Ligation	DNA Damage Repair
10 kb to 20 kb	> 10 kb	5 µg	Blunt	Required

Evaluate Genomic DNA Quality

We recommend using a Pulsed Field Electrophoresis system for evaluating gDNA quality. There are two commercially available systems capable of resolving HMW DNA fragments and smears.

- Bio-Rad® CHEF Mapper® XA Pulsed Field Electrophoresis system. The procedure is available here.
- Sage Science's Pippin Pulse Electrophoresis Power Supply. The procedure is available here.

Alternatively, Advanced Analytical Technologies, Inc. FEMTO *Pulse*™ is an automated pulsed-field capillary electrophoresis instrument for evaluating the integrity of genomic DNA with a run time of approximately 1.5 hours.

Fragment and Concentrate DNA

Use a Covaris® g-TUBE® device to shear your DNA sample. General recommendations for use of g-TUBEs can be found in the g- TUBE user manual available for download from the Covaris website (see http://covarisinc.com/products/g-tube/). Note that although Covaris provides recommendations for shearing gDNA to 20 kb, these parameters result in sheared gDNA with average insert sizes of less than 20 kb, and are **NOT** suitable for use in this protocol. To generate sheared gDNA with average insert sizes >20 kb, you **MUST** follow the specific instructions provided here:

1. Dilute DNA to 200-300 ng/µl in Elution Buffer (EB). The sample volume may range from 20-100 µl
2. Shear at 5500 rpm (2029 x g) for 2minutes in an Eppendorf® MiniSpin plus.
3. Check for any residual sample remaining in the upper chamber. If present, re-spin for another 2 minutes. Continue spinning until all the sample has passed through the orifice.
4. Invert and spin at 5500 rpm (2029 x g) until all the sample has passed through the orifice.
5. Recover your sample into a 1.5 mL LoBind microcentrifuge tube.

Depending upon the quality of your sample, approximately 20% sample loss is to be expected as a result of the shearing and concentration process. Therefore, be sure to have sufficient amounts of starting DNA in order to have at least 5 µg of sheared and concentrated DNA (140 ng/µL) for the subsequent repair steps.

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STEP	✓	Concentrate DNA	Notes
1		Add **0.45X** volume of AMPure® PB magnetic beads. _____ µL of sample X **0.45X** =_____µL of beads Note that the beads must be brought to room temperature before use and all AMPure PB bead purification steps should be performed at room temperature. Before using, mix the bead reagent well until the solution appears homogenous. Pipette the reagent slowly since the bead mixture is viscous and precise volumes are critical to the purification process.	
2		Mix bead/DNA solution thoroughly by tapping the tube gently. Do not pipet to mix.	
3		Quickly spin down the tube (for 1 second) to collect the beads.	
4		Allow the DNA to bind to beads by gentle end-over-end rotation for 15-20 minutes at room temperature. We recommend using a tube rotator.	
5		Spin down the tube (for 1 second) to collect beads.	
6		Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.	
7		With the tube still on the magnetic bead rack, slowly pipette off cleared supernatant and save in another tube. Avoid disturbing the bead pellet. If the DNA is not recovered at the end of this procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.	
8		Wash beads with freshly prepared 70% ethanol. Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. Let the tube sit for 30 seconds. – Do not disturb the bead pellet. – After 30 seconds, pipette and discard the 70% ethanol.	
9		Repeat step 8 above.	
10		Remove residual 70% ethanol. – Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. – Let beads separate fully. – Pipette off any remaining 70% ethanol.	

STEP	✓	Concentrate DNA	Notes
11		Remove the tube from the magnetic bead rack and allow beads to air-dry (with the tube caps open) for 30 to 60 seconds.	
12		Calculate appropriate volume of Elution Buffer. _____ ng X **0.5** / (____ng/µL) =_____µL of Elution Buffer needed The minimum DNA concentration required to proceed to the next step (End-Repair) is **140 ng/µL** with preferred mass of at least **5 µg**.	
13		Add the Pacific Biosciences® Elution Buffer volume (calculated in step 12 above) to your beads. Tap the tube with finger gently to mix, until beads are uniformly resuspended. Do not pipet to mix. – Elute the DNA by letting the mix stand at room temperature for 2 minutes. – Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the bead pellet, transfer supernatant to a new 1.5 mL Lo-Bind tube. – Discard the beads.	
14		Verify your DNA amount and concentration using a Qubit quantitation platform. – Measure the DNA concentration using a Qubit fluorometer. – Using 1 µL of the eluted sample, make a 1:10 dilution in EB. – Use 1 µL of this 1:10 dilution to measure the DNA concentration using a Qubit dsDNA HS Assay kit according to the manufacturer's recommendations. Yield up to this point should be **80%**. The remaining 9 µL of 1:10 diluted sample may be used for QC by pulsed-field gel electrophoresis or pulsed-field capillary electrophoresis.	
15		The sheared DNA can be stored for up to 24 hours at 4ºC or at -20ºC for longer duration.	
16		Actual recovery per µL and total available sample material: _____	

ExoVII Pre-Treatment of DNA

Use the following table to remove single-stranded ends from 5 μg of sheared gDNA at 140 ng/ μL. If starting with more than 5 μg of sheared gDNA, scale the reaction volumes proportionally (i.e., for a mass between 6-10 μg of DNA scale the total volume to 96 μL).

1. In a LoBind microcentrifuge tube, add the following reagents:

Reagent	Tube Cap Color	Stock Conc.	Volume	Final Conc.	✓	Notes
Sheared DNA (5 μg)			36.0 μL			
DNA Damage Repair Buffer		10 X	5.0 μL	1 X		
NAD+		100 X	0.5 μL	1 X		
ATP high		10 mM	5.0 μL	1 mM		
dNTP		10 mM	0.5 μL	0.1 mM		
ExoVII		10 U/μL	1.0 μL	0.2 U/μL		
Total Volume			48.0 μL			

2. Mix the reaction well by gently tapping the tube.
3. Spin down contents of tube with a quick spin in a microfuge.
4. Incubate at 37ºC for 15 minutes, then return the reaction to 4ºC.

Repair DNA Damage

Use the following table to prepare your reaction. For more than 5 µg input DNA, scale all reactions volumes proportionally.

Reagent	Tube Cap Color	Stock Conc.	Volume	Final Conc.	✓	Notes
DNA (ExoVII treated)			48.0 µL			
DNA Damage Repair Mix		25 X	2.0 µL	1X		
Total Volume			50.0 µL			

1. Mix the reaction well by gently tapping the tube.
2. Spin down contents of tube with a quick spin in a microfuge.
3. Incubate at 37ºC for 20-60 minutes, return the reaction to 4ºC for 1 to 5 minutes.

Repair Ends

Use the following table to prepare your reaction then purify the DNA. For more than 5 µg input DNA, scale all reactions volumes proportionally.

Reagent	Tube Cap Color	Stock Conc.	Volume	Final Conc.	✓	Notes
DNA (Damage Repaired)			50.0 µL			
End Repair Mix		20 X	2.5 µL	1X		
Total Volume			52.5 µL			

1. Mix the reaction well by gently tapping the tube.
2. Spin down contents of tube with a quick spin in a microfuge.
3. Incubate at 25ºC for 5-10 minutes, return the reaction to 4ºC. Proceed to the next step.

STEP	✓	Purify DNA	Notes
1		Add **0.45X** volume of AMPure PB beads to the End-Repair reaction.	
2		Mix the bead/DNA solution thoroughly by gently tapping the tube. Do not pipet to mix.	
3		Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.	
4		Allow the DNA to bind to beads by gentle rotation for 15-20 minutes at room temperature. We recommend using a tube rotator.	
5		Spin down the tube (for 1 second) to collect beads.	
6		Place the tube in a magnetic bead rack to collect the beads to the side of the tube.	
7		Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet. If the DNA is not recovered at the end of this procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.	
8		Wash beads with freshly prepared 70% ethanol. Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. – Do not disturb the bead pellet. – After 30 seconds, pipette and discard the 70% ethanol.	
9		Repeat step 8 above.	
10		Remove residual 70% ethanol. – Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. – Pipette off any remaining 70% ethanol.	
11		Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.	
12		For 5 µg of input sheared gDNA, elute in **23 µL** Elution buffer. If you started with more than 5 µg input sheared gDNA, scale volume of EB proportionally (i.e., for 6-10 µg of DNA, elute in 46 µL EB). Add the Pacific Biosciences® Elution Buffer volume to your beads. Tap the tube with finger gently to mix. Do not pipet to mix. – Elute the DNA by letting the mix stand at room temperature for 2 minutes. – Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the bead pellet, transfer supernatant to a new 1.5 ml Lo-Bind tube. – Discard the beads.	

13		Optional: Verify your DNA amount and concentration using a Qubit quantitation platform. – Measure the DNA concentration using a Qubit fluorometer. – Using 1 µL of the eluted sample, make a 1:10 dilution in EB. – Use 1 µL of this 1:10 dilution to measure the DNA concentration using a Qubit fluorometer and the dsDNA HS Assay kit according to the manufacturer's recommendations. – The remaining 9 µL of 1:10 diluted sample may be used for QC by pulsed-field gel electrophoresis or pulse-field capillary electrophoresis.	
14		The End-Repaired DNA can be stored overnight at 4ºC or at -20ºC for longer duration.	
15		Actual recovery per µL and total available sample material: _____	

Prepare Blunt Ligation Reaction

Use the following table to prepare your reaction, adding the components below in the order listed. Be sure to mix insert gDNA and adapter BEFORE adding ligase. For more than 5 µg input DNA, scale all reaction volumes proportionally.

Reagent	Tube Cap Color	Stock Conc.	Volume	Final Conc.	✓	Notes
DNA (End Repaired)			23.0 µL			
Blunt Adapter (20 µM)		20 µM	10.0* µL	5 µM		
Mix before proceeding						
Template Prep Buffer		10 X	4.0 µL	1X		
ATP low		1 mM	2.0 µL	0.05 mM		
Mix before proceeding						
Ligase		30 U/µL	1.0 µL	0.75 U/µL		
Total Volume			40.0 µL			

*Note that this increase in adapter during ligation minimizes the incidence of chimeras. Adapter dimers are then efficiently removed during size selection in the BluePippin System. This is not recommended for libraries which are not being size selected using the BluePippin System.

1. Mix the reaction well by gently tapping the tube.
2. Spin down contents of tube with a quick spin in a microfuge.
3. Incubate at 25ºC for 15 – 60 minutes. Ligation reactions can be left at 25 ºC overnight, if desired.
4. Incubate at 65ºC for 10 minutes to inactivate the ligase, then return the reaction to 4ºC.

ExoIII/VII Digestion to Remove Failed Ligation Products

Use the following table to prepare your reaction. For more than 5 µg input DNA, scale all reaction volumes proportionally.

Reagent	Tube Cap Color	Stock Conc.	✓	Volume
Ligated DNA				40.0 µL
Mix reaction well by pipetting				
ExoIII		100.0 U/µL		1.0 µL
ExoVII		10.0 U/µL		1.0 µL
Total Volume				42.0 µL

1. Mix the reaction well by gently tapping the tube.
2. Spin down contents of tube with a quick spin in a microfuge.
3. Incubate at 37ºC for 1 hour, then return the reaction to 4ºC.
4. You must immediately proceed with AMPure PB bead purification after this step.

Purify SMRTbell® Templates

There are 3 purification steps, 2 using 0.45X volume of AMPure PB beads and a final purification of 0.40X volumes of AMPure PB beads.

STEP	✓	Purify SMRTbell® Templates - First Purification	Notes
1		Add **0.45X** volume of AMPure® PB beads to the exonuclease-treated DNA.	
2		Mix bead/DNA solution thoroughly by tapping the tube gently. Do not pipet to mix.	
3		Quickly spin down the tube (for 1 second) to collect the beads.	
4		Allow the DNA to bind to beads by gentle end-over-end rotation for 15-20 minutes at room temperature. We recommend using a tube rotator.	
5		Spin down the tube (for 1 second) to collect beads.	
6		Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.	
7		With the tube still on the magnetic bead rack, slowly pipette off cleared supernatant and save in another tube. Avoid disturbing the bead pellet. If the DNA is not recovered at the end of this procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.	
8		Wash beads with freshly prepared 70% ethanol. Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. Let the tube sit for 30 seconds. – Do not disturb the bead pellet. – After 30 seconds, pipette and discard the 70% ethanol.	
9		Repeat step 8 above.	
10		Remove residual 70% ethanol. – Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. – Let beads separate fully. – Pipette off any remaining 70% ethanol.	
11		Remove the tube from the magnetic bead rack and allow beads to air-dry (with the tube caps open) for 30 to 60 seconds.	
12		For up to 10 µg input SMRTbell library, elute the DNA off the beads in **100 µL** of Elution buffer. If you have more than 10 µg library, scale volume of EB proportionally. Add the Pacific Biosciences® Elution Buffer volume to your beads. Tap the tube with finger gently to mix. Do not pipet to mix. – Elute the DNA by letting the mix stand at room temperature for 2 minutes. – Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the bead pellet, transfer supernatant to a new 1.5 ml Lo-Bind tube. – Discard the beads.	
13		The eluted DNA in **100 µL** Elution Buffer should be taken into the second **0.45X** AMPure PB bead purification step.	

STEP	✓	Purify SMRTbell® Templates - Second Purification	Notes
1		Add **0.45X** volume of AMPure PB beads to the eluted DNA from the first AMPure PB bead purification step above.	
2		Mix bead/DNA solution thoroughly by tapping the tube gently. Do not pipet to mix.	
3		Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.	
4		Allow the DNA to bind to beads by gentle rotation for 15-20 minutes at room temperature. We recommend using a tube rotator.	
5		Spin down the tube (for 1 second) to collect beads.	
6		Place the tube in a magnetic bead rack to collect the beads to the side of the tube.	
7		Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet. If the DNA is not recovered at the end of this procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.	
8		Wash beads with freshly prepared 70% ethanol. Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. – Do not disturb the bead pellet. – After 30 seconds, pipette and discard the 70% ethanol.	
9		Repeat step 8 above.	
10		Remove residual 70% ethanol. – Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. – Pipette off any remaining 70% ethanol.	
11		Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.	
12		For up to 10 µg input SMRTbell library, elute the DNA off the beads in **100 µL** of Elution buffer. If you have more than 10 µg library, scale volume of EB proportionally. Add the Pacific Biosciences® Elution Buffer volume to your beads. Tap the tube with finger gently to mix. Do not pipet to mix. – Elute the DNA by letting the mix stand at room temperature for 2 minutes. – Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the bead pellet, transfer supernatant to a new 1.5 ml Lo-Bind tube. – Discard the beads.	
13		The eluted DNA in **100 µL** Elution Buffer should be taken into the third **0.40X** AMPure PB bead purification step.	

STEP	✓	Purify SMRTbell® Templates - Third Purification	Notes
1		Add **0.40X** of AMPure PB beads to the eluted DNA. Note that for **0.40X** volumes, it is critical to accurately pipet the desired volume of AMPure PB bead solution; there is a steep drop-off in recovery for concentrations <**0.40X**.	
2		Mix the bead/DNA solution thoroughly by gently tapping the tube. Do not pipet to mix.	
3		Quickly spin down the tube (for 1 second) to collect the beads. Do not pellet beads.	
4		Allow the DNA to bind to beads by gentle rotation for 15-20 minutes at room temperature. We recommend using a tube rotator.	
5		Spin down the tube (for 1 second) to collect beads.	
6		Place the tube in a magnetic bead rack to collect the beads to the side of the tube.	
7		Slowly pipette off cleared supernatant and save (in another tube). Avoid disturbing the bead pellet. If the DNA is not recovered at the end of this procedure, you can add equal volumes of AMPure PB beads to the saved supernatant and repeat the AMPure PB bead purification steps to recover the DNA.	
8		Wash beads with freshly prepared 70% ethanol. Note that 70% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results. Also, 70% ethanol should be stored in a tightly capped polypropylene tube for no more than 3 days. – Do not remove the tube from the magnetic rack. – Use a sufficient volume of 70% ethanol to fill the tube (1.5 mL for 1.5 mL tube or 2 mL for 2 mL tube). Slowly dispense the 70% ethanol against the side of the tube opposite the beads. – Do not disturb the bead pellet. – After 30 seconds, pipette and discard the 70% ethanol.	
9		Repeat step 8 above.	
10		Remove residual 70% ethanol. – Remove tube from magnetic bead rack and spin to pellet beads. Both the beads and any residual 70% ethanol will be at the bottom of the tube. – Place the tube back on magnetic bead rack. – Pipette off any remaining 70% ethanol.	
11		Remove the tube from the magnetic bead rack and allow beads to air-dry (with tube caps open) for 30 to 60 seconds.	
12		For up to 5 µg SMRTbell library, elute in **10 µL** Elution buffer. For more than 5 µg of SMRTbell template, scale volume of EB proportionally (i.e., for up to 10 µg of input DNA, elute in 20 µL EB). Add the Pacific Biosciences® Elution Buffer volume to your beads. Tap the tube with finger gently to mix. Do not pipet to mix. – Elute the DNA by letting the mix stand at room temperature for 2 minutes. – Spin the tube down to pellet beads, then place the tube back on the magnetic bead rack. – Let beads separate fully. Then without disturbing the bead pellet, transfer supernatant to a new 1.5 ml Lo-Bind tube. – Discard the beads.	

13		Verify your DNA amount and concentration using a Qubit quantitation platform. – Using 1 µL of the purified sample, make a 1:10 dilution in EB. – Use 1 µL of this 1:10 dilution to measure the DNA concentration using a Qubit fluorometer and the dsDNA HS Assay kit according to the manufacturer's recommendations. The remaining 9 µL of 1:10 diluted sample may be used for QC by pulsed-field gel electrophoresis or pulse-field capillary electrophoresis. Note that typical DNA yield, at this point of the process (at the end of library preparation) is between approximately **5-20%** of the total starting DNA amount.	

Anneal and Bind SMRTbell® Templates

Before adding the primer to the SMRTbell template, pre-condition the primer by heating to 80ºC for 2 minutes, then place immediately on ice. (Note that if kept on ice during use, and stored at -20ºC, pre-conditioned primer may be used multiple times without re-heating.)

PacBio RS II System:
For the PacBio RS II System, follow the Binding Calculator. Anneal 20X sequencing primer at a template concentration of 0.833 nM and incubate at 20ºC for 30 minutes.

Bind 10X P6 polymerase at an annealed template concentration of 0.500 nM (according to the Binding Calculator). For polymerase binding, incubation at 30ºC for 30 minutes is sufficient.

For more information about using the Binding Calculator, see the Pacific Biosciences *Template Preparation and Sequencing Guide* and *QRC - Annealing and Binding Recommendations*.

Sequel System:
For Sequel Systems, follow the SMRT Link Sample Setup instructions. Anneal 10X sequencing primer at a template concentration of 0.833nM and incubate at 20ºC for 60 minutes.

Bind 10X Sequel polymerase at an annealed template concentration of 0.500 nM (per SMRT Link Sample Setup). For polymerase binding, incubation at 30ºC for 4 hours is required.

Prepare for Sequencing

PacBio RS II System:

For the PacBio RS II System, MagBead loading is required. Optimal loading of ~20 kb SMRTbell libraries using P6 polymerase can typically be achieved using an on-plate concentration of 10-30 pM. We recommend you perform an initial loading titration in this range to determine optimal loading for your sample.
For efficient binding to MagBeads, bound complexes (at 0.500 nM concentration) must be diluted in the appropriate ratio of MagBead Binding Buffer and MagBead Wash Buffer. Follow the Binding Calculator instructions to dilute your sample for MagBead binding.

Sequel System:

For the Sequel System, MagBead loading is required. Refer to SMRT Link Sample Setup for optimal loading recommendations.

Also, it is highly recommended to purify the complex using SMRTbell Clean Up Columns to remove excess primers and polymerase prior to sequencing. See the PacBio *Procedure & Checklist - Sample Purification Using SMRTbell Clean Up Columns v2 for MagBead Loading*.

Follow the SMRT Link Sample Setup instructions to anneal, bind and clean-up your samples.