myBaits® Microbial Collection

Targeted sequencing workflow (library prep + hybridization capture) for panels within the Microbial Collection

User Manual
Version 1.0 May 2026

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© Daicel Arbor Biosciences
5840 Interface Drive, Suite 101
Ann Arbor, MI 48103 (USA)
+1 (734) 998-0751
info@arbor.daicel.com

INTRODUCTION

The myBaits® Microbial Collection provides reagents and baits (probes) for targeted next-generation sequencing (NGS) of specific microbes and pathogens from complex samples, via in-solution hybridization capture. Bait panel options are available for targeted genome sequencing for a wide range of specific microbes including bacteria, viruses, or parasites. The current list of available Microbial Collection panels can be found at arborbiosci.com/mybaits-microbial-collection.

For compatible pathogens with DNA genomes where Illumina® sequencing is desired, Daicel Arbor Biosciences offers reagents for the complete workflow including library preparation. Alternatively, users can optionally make libraries using library preparation reagents from any other vendor prior to myBaits enrichment (if so, the Library Preparation sections of this manual can be skipped). Please see additional details below and in subsequent sections regarding choosing the correct workflow for your desired experimental goals, or reach out at techsupport@arbor.daicel.com for consultation.

Library Preparation Kit for myBaits

The Library Preparation Kit for myBaits converts double-stranded DNA to sequencing-ready libraries for the Illumina short-read sequencing platform, prior to enrichment with the myBaits hybridization capture reagents. It provides an excellent conversion rate and minimal sequence composition bias.

Input:	1-500 ng dsDNA in water or Tris-based buffer (≤10 mM Tris and ≤0.1mM EDTA)
Shearing:	Enzymatic
Adapter ligation:	A/T-overhang-based
Post-ligation PCR:	Yes
Intended use:	Preparation of NGS libraries from dsDNA, prior to myBaits hybridization capture

Included with the library preparation reagents are all enzymes necessary for fragmentation, end-repair and adenylation, adapter ligation (with user-supplied adapters), library amplification (with user-supplied indexing primers or kit-supplied P5/P7 primers) both pre- and post-myBaits capture, and SPRI beads for library purification both pre- and post-myBaits capture.

myBaits Hybridization Capture Kit (Reagents + Baits)

myBaits is an in-solution NGS library target enrichment system, compatible with Illumina, Element Biosciences®, and essentially any linear and amplifiable sequencing library. Daicel Arbor Biosciences uses our versatile nucleic acid synthesis technology to produce biotinylated RNA “baits” or “probes” that are complementary to your desired microbial genome sequence targets, which are used with myBaits hybridization capture reagents to “enrich” libraries for those targets of interest prior to next-generation sequencing. After enrichment with myBaits and subsequent universal amplification, libraries may then be sequenced on the aforementioned platforms directly, or further prepared for PacBio® or Oxford Nanopore Technologies® sequencing.

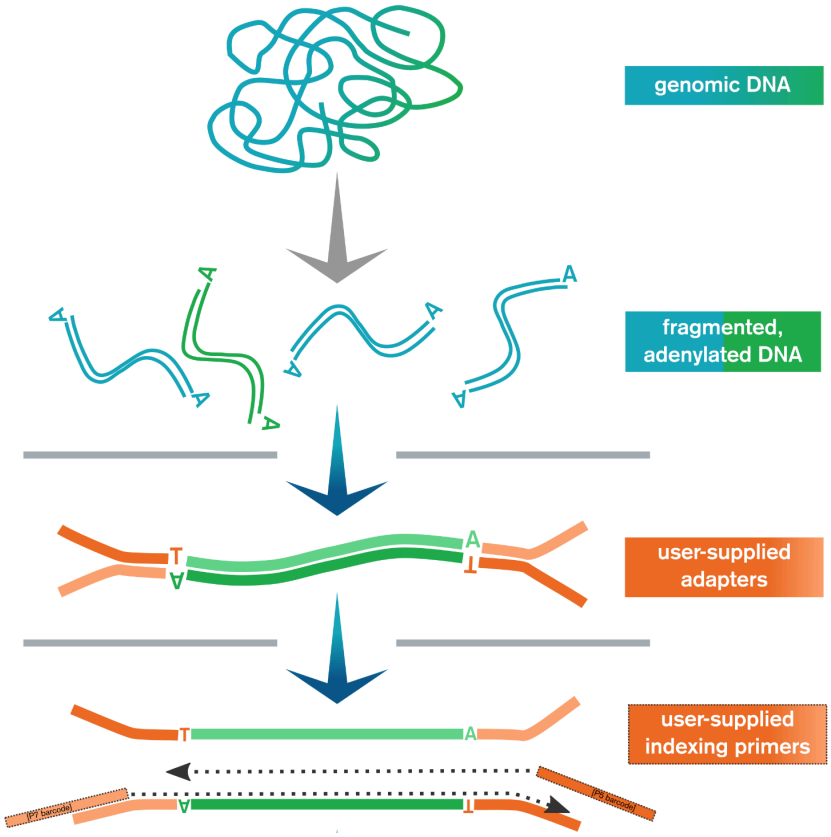
Workflow Overview

Library preparation with the **Library Preparation Kit for myBaits**

1. Genomic or other double-stranded DNA (dsDNA) is enzymatically fragmented, end-polished, and adenylated.

2. User-supplied adapters are ligated to the end-repaired fragments.

3. Ligation products are purified with SPRI beads and then amplified for a limited number of cycles using user-supplied or kit-supplied primers. Libraries are SPRI-purified again and assayed for quantity and morphology.



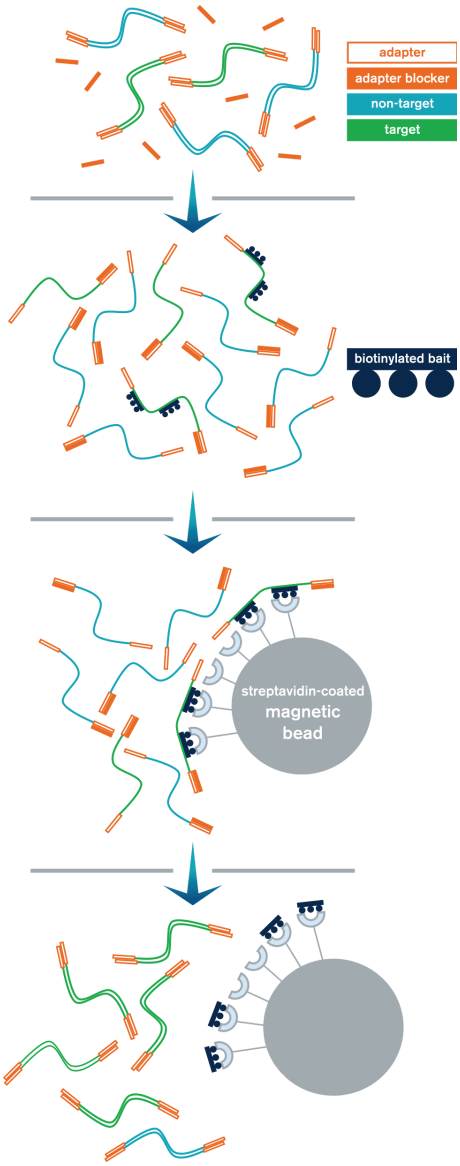
Hybridization capture with **myBaits kit reagents and baits**

4. Amplified barcoded sequencing libraries (typically, 4 per capture reaction), adapter blockers, and other hybridization reagents are combined.

5. Libraries are denatured, allowing blockers to hybridize to adapters. **Baits** (biotinylated molecules that are sequence-specific for the desired target microbial genome(s)) are then introduced and hybridized to targets for several hours.

6. Bait-target hybrids are bound to streptavidin-coated magnetic beads and pulled out of suspension with a magnet.

7. Most non-target DNA is washed away. The remaining library is then amplified and either taken directly to sequencing or further treated.



Products compatible with this manual

This manual is compatible with current generation myBaits Microbial Collection reagents and baits.

Type	Name	Cat#(s)
Library prep† reagents only	Library preparation for myBaits kit, 32 samples	910032
	Library preparation for myBaits kit, 128 samples	910128
Hyb reagents only	Hyb reagents, 32 samples (8 rxn)	3P0008.V5
	Hyb reagents, 64 samples (16 rxn)	3P0016.V5
	Hyb reagents, 128 samples (32 rxn)	3P0032.V5
	Hyb reagents, 256 samples (64 rxn)	3P0064.V5
	Hyb reagents, 768 samples (192 rxn)	3P0192.V5
Library prep† plus hyb reagents bundle	Library prep + hyb reagents, 32 samples	9BUNDLE-032.V5 = 1x 910032, 1x 3P0008.V5
	Library prep + hyb reagents, 64 samples	9BUNDLE-064.V5= 2x 910032, 1x 3P0016.V5
	Library prep + hyb reagents, 128 samples	9BUNDLE-128.V5 = 1x 910128, 1x 3P0032.V5
	Library prep + hyb reagents, 256 samples	9BUNDLE-256.V5 = 2x 910128, 1x 3P0064.V5
	Library prep + hyb reagents, 768 samples	9BUNDLE-768.V5 = 6x 910128, 1x 3P0192.V5
Baits only*	** Cat# will vary depending on the specific targeted microbe(s). FOR EXAMPLE:*	
	Baits, M. tuberculosis, 32 samples	*3P-M-008_Mtube*
	Baits, M. tuberculosis, 64 samples	*3P-M-016_Mtube*
	Baits, M. tuberculosis, 128 samples	*3P-M-032_Mtube*
	Baits, M. tuberculosis, 256 samples	*3P-M-064_Mtube*
	Baits, M. tuberculosis, 768 samples	*3P-M-192_Mtube*

Please visit arborbiosci.com/mybaits-microbial-collection for a full list of available myBaits Microbial Collection bait panels.

To complete this entire workflow, you will need reagents for **library preparation** († DNA genomes only; optional), reagents for **hybridization capture**, as well as **baits** (probes) for the specific microbial target genome(s) that you wish to sequence, for at least as many samples as you wish to process. For example, for *M. tuberculosis* genome sequencing for 96 samples, you could purchase 128 samples worth of library preparation and/or hybridization capture reagents (e.g. 1x Cat# 910128 + 1x Cat# 3P0032.V5; or 1x Cat# 9BUNDLE-128.V5) as well as *M. tuberculosis* whole genome capture baits (Cat# 3P-M-032_Mtube).

Manuals for other myBaits products can be accessed at arborbiosci.com/mybaits-manual. Please email techsupport@arbor.daicel.com if you need help identifying the correct reagents or protocol needed to process your samples.

Kit components and stability

Reagents for library preparation

Storage Conditions	Reagent	Tubes and volumes			
		32 sample kit		128 sample kit	
		Vessels	Volume each	Vessels	Volume each
Box A *Store at -20˚C*	Frag/AT Buffer	1	150 µL	1	590 µL
	Frag/AT Enzyme Mix	1	220 µL	1	890 µL
	Ligation Mix	1	740 µL	2	1480 µL
	Amplification Mix	1	1150 µL	3	1550 µL
	P5/P7 Primer Mix	1	230 µL	1	920 µL
Box B *Store at 4˚C*	SPRI Beads	1	7.5 mL	4	7.5 mL
	Buffer P	1	5.0 mL	2	5.0 mL

Library preparation kit reagents are shipped with cold packs. Store at indicated temperatures immediately upon arrival.

Reagents and baits for hybridization capture

Box & Storage	Reagent	Cap color	Volume per kit				
			32 samples	64 samples	128 samples	256 samples	768 samples
Box #1 *Store at 4˚C*	**Hyb N**	Red	400 µL	400 µL	1000 µL	1000 µL ×2	1000 µL ×4
	Hyb S	Blue	1500 µL	1500 µL	1500 µL	1500 µL ×2	1500 µL ×4
	Beads	Clear	550 µL	550 µL	1600 µL	1600 µL ×2	1600 µL ×4
	Binding Buffer	Clear	12 mL	12 mL	36 mL	36 mL ×2	36 mL ×4
	Wash Buffer	Clear	20 mL	20 mL	60 mL	60 mL ×2	60 mL ×4
Box #2 *Store at -20˚C*	**Hyb D**	Yellow	140 µL	140 µL	400 µL	400 µL ×2	400 µL ×4
	Hyb R	Purple	50 µL	50 µL	150 µL	150 µL ×2	150 µL ×4
	Block C	Green	50 µL	50 µL	130 µL	130 µL ×2	130 µL ×4
	Block O	Blue	90 µL	90 µL	270 µL	270 µL ×2	270 µL ×4
	Block X	Orange	5 µL	9 µL	9 µL ×2	9 µL ×4	27 µL ×4
	Buffer E	Clear	550 µL	550 µL	1600 µL	1600 µL ×2	1600 µL ×4

Box & Storage	Reagent	Cap color	Volume per kit
Box #3 *Store at -80˚C*	**Baits**	Clear	50 µL per 32 samples (= 8 capture reactions)

To minimize freeze-thaw cycles, it is strongly recommended that sub-aliquots of Baits are made in reaction sizes appropriate for your experiment plans.

Both hyb capture reagents and baits are shipped at ambient temperature and are stable during transit. If stored at indicated temperatures upon arrival, myBaits hyb capture reagents and baits expire one year from the date they were received.

Equipment required

For library preparation

Item	Notes
Ice and/or cold block	For reaction setup and benchtop reagent storage during setup
Thermal cycler	
0.2 mL tubes or strips	Compatible with thermal cycler
Plate seals or strip lids	Compatible with selected 0.2 mL tubes or strips
Magnetic particle collector	Compatible with selected 0.2 mL tubes or strips
Assorted nuclease-free tubes	For master mix preparation
Vortexer	
Mini centrifuge	
Digital microfluidic electrophoresis device (dsDNA)	Including consumables. For example, Bioanalyzer® (Agilent)
DNA quantification fluorometer (dsDNA)	Including consumables. For example, Qubit™ (Thermo Fisher)

For hybridization capture

Item	Notes
50 mL tubes	1 per 44 reactions
Microcentrifuge tubes (1.5, 1.7, or 1.8 mL)	2 per 8 reactions
0.2 mL PCR strips with attached lids	2 per 8 reactions (e.g., VWR® Cat# 93001-118)
Pipettors and tips for 0.5 - 500 µL	**Multichannel for 20 and 500 µL recommended**
† Thermal cycler with heated lid compatible with 0.2 mL strips	1 or 2
Magnetic particle collector for microcentrifuge tubes	1 (e.g., Thermo Fisher Cat# 12321D)
Magnetic particle collector for 0.2 mL strips	1 (e.g., Permagen Labware® Cat# S500)
Vortex mixer and mini-centrifuge for tubes and strips	
Water bath or incubation oven at 65°C	
Heat block for microcentrifuge tubes at 60°C	

† *Ensure that the thermal cycler and strips allow no more than 4 µL of 30 µL volume evaporation overnight at 65°C*

Reagents required

For library preparation

Reagent	Notes
T-overhang-containing double-stranded adapters	These can be either short ("stubby") adapters OR full-length adapters that contain sample-specific barcodes. If using stubby adapters, indexing primers that add universal P5 and P7 priming sites are also required. See **Appendix A** for further information about adapters and primers.
Molecular grade water	
200 proof ethanol	

For hybridization capture

Reagent	Notes
Nuclease-free ("NF") water	900 µL per reaction
PCR primers to amplify sequencing libraries after capture, e.g.:	If using "Library Preparation Kit for myBaits", use 'P5/P7 Primer Mix'
Illumina P5: AATGATACGGCGACCACCGA	2.5 µL @ 10 µM per reaction
Illumina P7: CAAGCAGAAGACGGCATACGA	2.5 µL @ 10 µM per reaction
PCR reagents for post-capture amplification	If using "Library Preparation Kit for myBaits", use 'Amplification Mix' If not using "Library Preparation Kit for myBaits", obtain reagents for 1 amplification per capture reaction (e.g. Roche® Cat# 07958927001)
PCR purification system, e.g., silica columns or SPRI beads	1 cleanup per reaction

SPECIAL NOTES

High Sensitivity capture protocol. The High Sensitivity protocol includes two rounds of enrichment. This may require that you purchase additional myBaits Hybridization Capture Reagents (without Baits). For example, if you plan to process 32 samples using the High Sensitivity protocol, you will need only 32 samples worth of Baits, but Reagents for at least 64 samples. There are 5 sizes of Microbial Collection reagents to choose from (from 32 to 768 samples; see page 5), but standard myBaits V5 reagents are also compatible (16 or 48 reaction sizes).

Capturing <4 libraries per capture reaction. The Microbial Collection hybridization capture workflow is designed for pooling 4 NGS libraries together in each myBaits capture reaction (see page 17 for more information). If you choose to pool <4 libraries per capture, you will need to purchase additional hyb cap reagents and baits to accommodate your increased capture reagent usage.

Input requirements and recommendations for library preparation

Adapters and barcodes

Dual-indexed libraries are strongly recommended to reduce the hazard of mis-indexing induced by PCR jumping events. The recommended, and most common, final library configuration prior to myBaits enrichment has Illumina TruSeq-style or Nextera-style adapters with dual 6-12 bp barcodes or indexes. This library format, as well as single 6-12 bp indexing, is directly compatible with the standard adapter blocking reagent provided with the myBaits hybridization capture reagents (Block X), but this component can be replaced with a custom blocking oligo if necessary (see page 17 for additional information).

If using the **Library Preparation Kit for myBaits from Daicel Arbor**:

The Library Preparation Kit for myBaits is designed for preparing libraries from samples with 1 to 500 ng of double-stranded DNA in water or Tris-based buffer (≤10 mM Tris and ≤0.1mM EDTA). RNA transcripts or RNA genomes must first be converted to double-stranded cDNA prior to use in the Library Preparation Kit for myBaits. Heavily degraded or predominantly single-stranded DNA samples are not suitable for use with the Library Preparation Kit for myBaits.

This workflow is compatible with either full-length barcoded adapters paired with universal P5/P7 primers for initial amplification OR 'stubby' adapters paired with indexing primers for initial amplification; please see page 17, the "Adapters and barcodes" section above, as well as Appendix A for more information.

Please proceed to the next page for the protocol for the Library Preparation Kit for myBaits.

If using a **library preparation kit from other vendor:**

It is critical to select a library preparation kit that is appropriate for and compatible with your target nucleic acid molecules (DNA and/or RNA; quality; quantity). myBaits hybridization capture is compatible with most library preparation methods on the market that produce PCR-amplified and amplifiable NGS libraries, including Illumina TruSeq®-style, Illumina Nextera® Flex-style, or other libraries with universal adapter priming sites. However, it is NOT recommended to use myBaits with PCR-free libraries; additionally, myBaits are incompatible with libraries made using original Nextera or Nextera XT library preparation kits, or any library type containing biotin. For long insert library preparation, we recommend the procedure described in Witek et al. 2016 (doi: 10.1038/protex.2016.027).

Once your libraries are made, please proceed to page 17 for information on how to prepare them for myBaits capture.

Library Preparation Kit for myBaits

For most targeted sequencing applications

CONTENTS

PROCEDURE

Library Preparation Kit for myBaits

IMPORTANT! The following protocol is for preparing libraries using the **Library Preparation Kit for myBaits** purchased from Daicel Arbor Biosciences. If you are preparing your NGS libraries with a kit from another vendor, please proceed to the requirements of input libraries for hybridization capture starting on page 17.

PART P1: Fragmentation and end-repair

P1.1 Prepare materials

Reagents		Equipment
1-500 ng dsDNA in 40 µL water or buffer containing ≤10 mM Tris and ≤0.1mM EDTA		Ice in vessel that can hold reagents and reactions
		0.2 mL strips or PCR plate, one well per prep reaction
Frag/AT Buffer	**KEEP ON ICE**	Lids or film for above compatible with thermal cycler
Frag/AT Enzyme Mix	**KEEP ON ICE**	Thermal cycler

P1.2 Program and start the thermal cycler

Final insert length is driven by the time and temperature of the fragmentation reaction. Program and start the thermal cycler according to your fragment length goals, 50 µL volume setting.

Step	Temperature	Time
Lid	105°C	∞
1	4°C	∞
2	30°C	4 or 15 m*
3	65°C	30 m
4	4°C	∞

** See Part P4, section 2 for examples of final amplified libraries using different fragmentation settings. When starting with intact high molecular-weight dsDNA, use 4 minutes for final 500-700 bp average insert lengths, and 15 minutes for final 300-400 bp average insert lengths. For low molecular weight or degraded DNA, optimization will be required, but start with 4 minutes. Samples can vary in their final lengths and sample sets may require experimentation to achieve the desired length distribution. For further assistance, review Appendix B, and contact techsupport@arbor.daicel.com for additional technical support.*

P1.3 Set up the fragmentation and end-repair reaction

WORK ON ICE

1. Invert the Frag/AT Buffer and Frag/AT Enzyme Mix 10 times to mix. Briefly centrifuge to collect the material at the bottom of each tube.
2. Build the Frag/AT mastermix. **The following volumes are already adjusted for pipetting error:**

Component	µL / Reaction
Frag/AT Buffer	4.4
Frag/AT Enzyme Mix	6.6
TOTAL	**11**

3. Vortex gently for 4 seconds and briefly centrifuge to collect.
4. Add 10 µL Frag/AT mastermix to each 40 µL DNA sample.
5. Mix by pipetting 10 times using 25 µL volume, or by briefly vortexing. Ensure mixing is consistent between reactions. Centrifuge briefly to collect fluid.
6. Place the reactions in the 4°C thermal cycler and proceed to the next step of the program.

PART P2: Adapter ligation

P2.1 Prepare materials

Reagents	Equipment
Adapters at 15 or 3 µM*	Thermal cycler
Ligation Mix **KEEP ON ICE**	Magnetic Particle Collector (MPC)
SPRI Beads **Bring to room temp**	Microcentrifuge
200 proof ethanol (~250 µL per reaction)	Vortexer
Molecular grade water (~90 µL per reaction)	

** For 11-500 ng dsDNA inputs, use 15 µM adapters; for 1-10 ng input, use 3 µM adapters. See Appendix A for additional information on the adapters required for ligation.*

1. Bring the SPRI Beads to room temperature before use.
2. Prepare 80% ethanol solution for washes, ~300 µL per reaction (e.g. 248 µL 200 proof ethanol + 62 µL molecular-grade water, or similar).

P2.2 Program the thermal cycler

Step	Temperature	Time
Lid	OFF	∞
1	20°C	15 m

P2.3 Set up the ligation reaction

1. Invert the Ligation Mix 10 times to mix.
2. Remove the fragmentation and end-repair reaction tubes from the thermal cycler.
3. Add 5 µL of adapter to each tube.
4. Add 20 µL of Ligation Mix to each tube.
5. Mix by pipetting 10 times using 40 µL volume.
6. Place the reactions in the thermal cycler and start the program, 75 µL volume setting.
7. Prepare a volume of 80% ethanol equal to 300 µL × the number of ligation reactions.
8. Once the incubation ends, remove reactions from the thermal cycler and proceed to 2.4.

P2.4 Clean up the ligation reaction

1. Once equilibrated to room temperature, vortex the SPRI Beads to thoroughly resuspend.
2. Add **60 µL** SPRI Beads to each reaction.
3. Mix by pipetting 10 times, or by briefly vortexing. Briefly spin down to collect fluid.
4. Incubate the bead+ligation reaction mixture for 5 minutes at room temperature.
5. Pellet the beads in the MPC for 5 minutes.
6. Remove and discard the supernatant. Leave the reactions in the MPC.
7. Add 150 µL 80% ethanol. Incubate at RT for 30 seconds and remove and discard the ethanol.
8. Repeat step 7 above an additional time for two total washes.
9. Briefly spin down the tubes and return to the MPC.
10. Using a smaller pipette tip than used for the washes, remove any remaining visible ethanol from the bottom of the tube. Remove from the MPC.
11. Let the open vessels dry for 3-5 minutes. Once cracks form in the pellet, they are sufficiently dry.
12. Add 22 µL water and pipette up and down several times to thoroughly resuspend the pellet.

    At this stage, ligation products can be stored for 1 week at -20°C.

PART P3: Library amplification

P3.1 Prepare materials

Reagents		Equipment
Ligation reactions from 2.4		Thermal cycler
Primers at 20 µM*		Magnetic Particle Collector (MPC)
Amplification Mix	**KEEP ON ICE**	Microcentrifuge
SPRI Beads	**Bring to room temp**	Vortexer
200 proof ethanol (250 µL per reaction)		
Molecular grade water (65 µL per reaction)		
Buffer P		

** If full-length adapters were used, use kit-supplied P5/P7 Primer Mix. If short adapters were used, use indexing primers at 20 µM. See Appendix A for information on primers for library amplification.*

1. Bring the SPRI Beads to room temperature before use.
2. Prepare 80% ethanol solution for washes, ~310 µL per reaction (e.g. 248 µL 200 proof ethanol + 62 µL molecular-grade water, or similar).

P3.2 Program the thermal cycler

Step	Temperature	Time	
1	98°C	45 s	
2	98°C	15 s	×4-10 cycles*
3	60°C	30 s	
4	72°C	45 s	
5	72°C	60 s	
6	12°C	∞	

gDNA input (ng)	cycles for ~1 µg
** 400-500:*	*4*
200-400:	*5*
50-200:	*6*
< 50:	*10-15*

P3.3 Set up the amplification reaction

1. (Thaw, if applicable, and) pellet the ligation reactions from step 2.4. Transfer 20 µL of the supernatant to a 0.2 mL reaction well of a strip or plate compatible with your thermal cycler.
2. To each reaction, if full-length adapters with barcodes were used in the ligation reaction, add 5 µL of P5/P7 Primer Mix to each well. If short adapters without barcodes were used in the ligation reaction, add 5 µL of each indexing primer pair (at 20 µM) to each well.
3. Add 25 µL Amplification Mix to each well, and pipette several times to mix. Briefly spin down the tubes.
4. Place the reactions in the thermal cycler and begin the program, 50 µL volume setting.

P3.4 Clean up the library amplification reactions

1. Once equilibrated to room temperature, vortex the SPRI Beads to thoroughly resuspend.
2. If starting with high molecular-weight DNA, Add **50 µL** (1× ratio) SPRI Beads to each reaction. If starting with degraded DNA, add **75 µL** (1.5× ratio) SPRI Beads to each reaction.
3. Mix by pipetting 10 times, or by briefly vortexing. Briefly spin down to collect fluid.
4. Incubate the beads+ligation reaction mixture for 5 minutes at room temperature.
5. Pellet the beads in the MPC for 5 minutes.
6. Remove and discard the supernatant. Leave the reactions in the MPC.
7. Add 150 µL 80% ethanol. Incubate at RT for 30 seconds and remove and discard the ethanol.
8. Repeat step 7 an additional time for two total washes.
9. Briefly spin down the tubes and return to the MPC.
10. Using a smaller pipette than used for the washes, remove any remaining visible ethanol from the bottom of the tube. Remove from the MPC.
11. Let the open vessels dry for 3-5 minutes. Once cracks form in the pellet, they are sufficiently dry.
12. Add 30 µL Buffer P and pipette up and down several times to thoroughly resuspend the pellet.
13. Briefly centrifuge the tubes to collect fluid.
14. Pellet on the MPC for 1 minute; remove and save the supernatant.
15. The amplified libraries are now ready for quantification and visualization.

    Amplified libraries can be stored for up to 12 months at -20°C

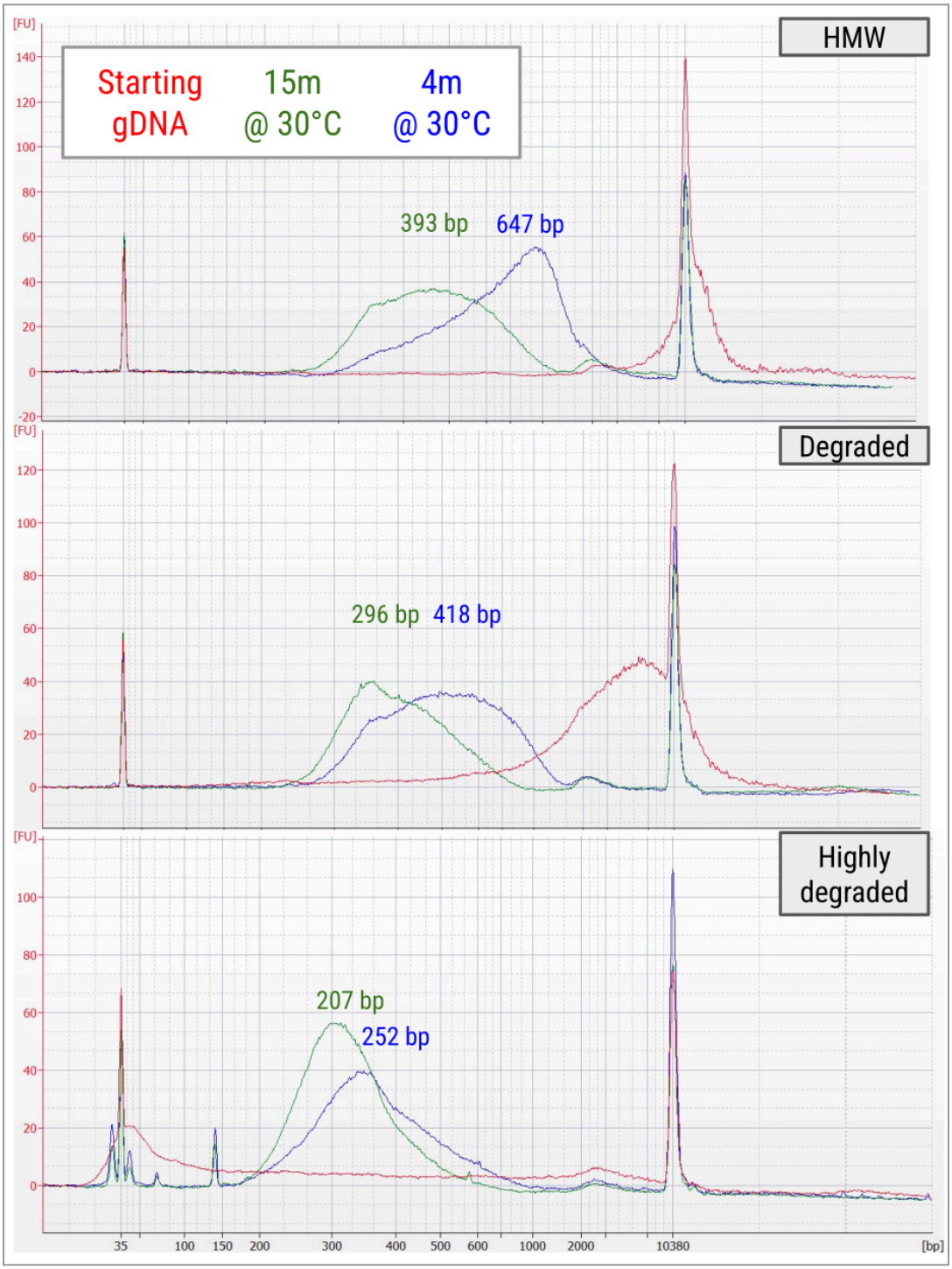
PART P4: Library quantification and visualization

1. Use 2 µL of each library in a fluorescent dsDNA quantification assay, e.g. Qubit High Sensitivity dsDNA Assay.

   If the number of cycles used followed the recommendations in the Library Amplification thermal program, you can expect between 500 ng and 1.5 µg total mass per library. **If your post-amplification yields are not at least triple the input mass of dsDNA** used for input, it is likely the library preparation was suboptimal, and conditions should be reviewed (see Appendix B).

2. Use 1-2 ng of each library in a capillary electrophoresis platform, e.g. Agilent Bioanalyzer.

   Note that after generation of Illumina TruSeq-style dual 8 bp barcode format libraries, a total of 136 bp of synthetic DNA is added to the starting sample DNA fragments. Libraries should be largely free of adapter dimer (peak at ~120-140 bp), though starting with low amounts of DNA or degraded DNA can result in retention of unused adapters or primers (17-60 nt) or adapter dimer.



Data Figure 1. Example library morphologies following amplification, as visualized on Agilent Bioanalyzer platform. Three different starting genomic DNA samples (top, high-molecular weight [HMW]; middle, degraded; bottom, highly degraded) are shown in red, with post-amplification traces for libraries made from that sample shown in green (using 15 minute fragmentation) and blue (using 4 minute fragmentation). Average insert lengths (total length minus 136 bp adapter) are shown above the primary peak of each library.

Input requirements of libraries for myBaits hybridization capture

Quantifying and pooling libraries prior to capture

TYPE myBaits hybridization capture is compatible with most library preparation methods on the market that produce PCR-amplified and amplifiable NGS libraries, including Illumina TruSeq-style, Illumina Nextera Flex-style, or other libraries with universal adapter priming sites. However, it is NOT recommended to use myBaits with PCR-free libraries; additionally, myBaits are incompatible with libraries made using original Nextera or Nextera XT library preparation kits, or any library type containing biotin. For long insert library preparation, we recommend the procedure described in Witek et al. 2016 (doi: 10.1038/protex.2016.027). Dual-indexed libraries are strongly recommended to reduce the hazard of mis-indexing induced by PCR jumping events.

If you have prepared libraries with the Library Preparation Kit for myBaits (including use of user-supplied adapters and/or indexes following the guidelines in Appendix A) OR an equivalent protocol, then proceed to curate your libraries into suitable pools for myBaits capture following the guidelines below.

ADAPTERS The standard adapter blocking reagent provided with each myBaits hybridization capture kit **(Block X)** is compatible with Illumina TruSeq-style or Nextera-style libraries with single 6-12 bp or dual 6-12 bp indexing. For libraries with different adapter configurations, we recommend ordering Custom IDT® xGen® Blocking Oligos customized for your NGS library adapter sequences. At a concentration of 1 µg/µL, any custom adapter-blocking oligos can be used in lieu of myBaits Block X.

MASS & POOLING The myBaits Microbial Collection workflow is designed for pooling **4* libraries per capture reaction**. We recommend an **input mass† of 500 ng per library** for short-insert libraries (Standard or High-Sensitivity protocols) or 250 ng per library for long-insert libraries (Long-Insert protocol), for a total of 1-2 µg of pooled libraries per capture reaction. We strongly recommended using library qPCR to measure mass, rather than e.g. dye assay or electrophoresis.

* Special note about pooling: *Optimal* pooling parameters (both in terms of number of libraries and total mass per library) will vary between library types, bait sets, and samples, and will require trials to identify. However, many configurations should work well. To minimize variation in performance among pooled samples, we recommend to pool libraries based on target abundance (e.g. based on microbial-specific qPCR assay), as well as other factors such as anticipated bait-genome sequence identity (i.e., taxon), DNA quality, starting DNA quantity, and library insert length. Whenever possible, prioritize balancing the libraries based on target content for best chance of similar performance between co-enriched samples. For example, if two libraries are to be pooled, and library A has 20% endogenous template and library B has 10% endogenous template, include twice the mass of library B as library A in the enrichment pool. If information about target content is not available, then pool equal amounts of each library. If you decide to pool <4 libraries per capture reaction, then additional reagents and baits will be needed for the workflow.

† Special note about mass**:** A wide range of total library mass amounts can be successfully enriched with myBaits (nanograms to micrograms). For best results we do not recommend going below a total input of 100 ng of pooled libraries, especially for samples with high background nucleic acid content from host

and/or other microbiome source. In some cases, increasing library input, up to ~12 µg per capture reaction, is beneficial, especially for extremely rare or low-abundance targets.

VOLUME Each myBaits target enrichment reaction has space for 7 µL total NGS library volume. Many pools of libraries will require concentration to 7 µL with vacuum centrifugation or other means, prior to myBaits capture. Complete this in advance of your myBaits experiments. Libraries should be suspended in nuclease-free buffer or water.

Generating non-enriched data metrics

To generate non-enriched ("shotgun" or "whole genome sequencing" or "WGS") data and/or metrics for your libraries, do not proceed to myBaits hybridization capture, and instead proceed directly to sequencing (for libraries prepared with the Library Preparation Kit for myBaits, this is after part P4 (page 15). Non-enriched libraries will need to be sequenced on a separate run from their myBaits-enriched counterparts, as they will have the same barcodes.

IMPORTANT
READ BEFORE PROCEEDING

Choose your capture protocol

This manual includes three separate myBaits protocols. Please select from the list below depending on the nature of your sequencing libraries. If you are unsure which protocol to follow, email techsupport@arbor.daicel.com for consultation with one of our NGS scientists.

Note that these are suggested starting points. Identification of optimal parameters for enrichment with a specific bait set and library will require testing and evaluation. These protocols have worked well across many bait set and library combinations.

Standard Begins on Page 20

For most targeted sequencing applications

Recommended when enriching NGS libraries that:

- derive from high-quality genomic DNA, with target inserts of 80-1,000 bp in length,
- contain a mean target sequence GC content of >25%,
- are free of substantial (>50%) contamination from non-target organisms, and
- do not have extensive base substitutions (from e.g. deamination or bisulfite conversion)

High Sensitivity Begins on Page 28

For high background contamination, ancient DNA, high bait-target divergence, OR short target sequences

Recommended when enriching NGS libraries that have ANY of the following characteristics:

- derive from DNA samples heavily contaminated with exogenous DNA (e.g. very rare pathogen targets in host DNA background, ancient DNA, environmental DNA),
- contain mostly target sequences shorter than 80 bp,
- contain a mean target sequence GC content of <25%, or
- contain targets that are on average ≥25% divergent from the bait sequences

Long Insert Begins on Page 36

For target enrichment of NGS libraries containing inserts 1-10 kilobase pairs in length

Recommended when enriching NGS libraries that:

- are dominated by inserts longer than 1,000 bp, and
- can be amplified with universal adapter primers prior to platform-specific library prep (e.g. PacBio or Nanopore library prep)

Standard Protocol

For most targeted sequencing applications

CONTENTS

PROCEDURE

myBaits Standard Protocol

PART S1: Hybridization setup

Sequencing libraries are mixed with various blocking nucleic acids, denatured, and then combined with other hybridization reagents (including baits). These hybridization reactions incubate for several hours to allow baits to encounter and hybridize with target library molecules.

S1.1 Choose a hybridization temperature (T_H)

65°C

When bait-target sequence divergence is expected to be less than 10%

62°C

When bait-target sequence divergence is expected to be 10 to 15%

60°C

When bait-target sequence divergence is expected to be 15 to 25%

S1.2 Prepare materials

Reagents
Hyb N and Hyb S from Box 1
Hyb D and Hyb R from Box 2
Block C, Block O, and Block X from Box 2
Baits from Box 3 **KEEP ON ICE**
Libraries or library pools in 7 µL per reaction

Equipment
Nuclease-free microcentrifuge tubes (×2)
0.2 mL strips with attached lids (×1 per 8 reactions)
Pipettors and tips; multichannel for 20 µL recommended
Vortex mixer and mini-centrifuge for above tube types
Heat block set to 60°C
Thermal cycler(s); 2 blocks recommended for 24 or more reactions

Program the thermal cycler:

Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Temperature	Time
1	95°C	5m
2	T_H	5m
3	T_H	∞

S1.3 Hybridization Mix setup

1. Once the Hyb reagents have thawed, vortex to homogenize and then briefly centrifuge.

   Heat Hyb N and Hyb S to 60°C and vortex to dissolve any precipitate present after thawing

2. Assemble the Hybridization Mix in a microcentrifuge (MC) tube, briefly vortex and briefly centrifuge the contents to collect. **The following volumes are already adjusted for pipetting error:**

Component	µL / Reaction
Hyb N	9.25
Hyb D	3.5
Hyb S*	0.5
Hyb R	1.25
Baits	5.5
TOTAL	**20**

**Cloudiness caused by Hyb S addition will clear after step 3*

3. Incubate the Hybridization Mix at 60°C for 10 minutes in the heat block. Vortex occasionally to collect the condensate. Remove from the heat block and let sit 5 minutes at room temperature.

4. For each capture reaction, aliquot 18.5 µL of Hybridization Mix to a 0.2 mL well/tube.

These reaction aliquots of Hybridization Mix are now referred to as "HYBs"

S1.4 Blockers Mix setup

1. Assemble the Blockers Mix **specific for your target taxon/taxa** in an appropriately-sized tube and mix by pipetting. **The following volumes are already adjusted for pipetting error:**

MOST TAXA	
Component	**µL / Reaction**
Block O	2.5
Block C	2.5
Block X	0.5
NF Water	-
TOTAL	**5.5**

PLANTS	
Component	**µL / Reaction**
Block O	5.0
Block C	-
Block X	0.5
NF Water	-
TOTAL	**5.5**

SALMONIDS	
Component	**µL / Reaction**
Block O	-
Block C	2.5
Block X	0.5
NF Water	2.5
TOTAL	**5.5**

2. For each capture reaction, aliquot 5 µL of Blockers Mix to a 0.2 mL well/tube.

3. Add 7 µL of individual or pooled libraries to each Blockers Mix aliquot and mix by pipetting.

These libraries mixed with Blockers Mix aliquots are now referred to as "LIBs"

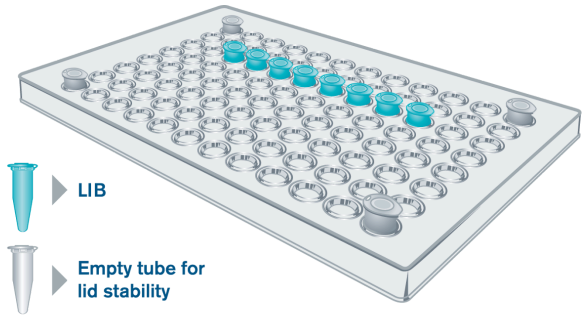
S1.5 Reaction assembly

Double-check the thermal program:

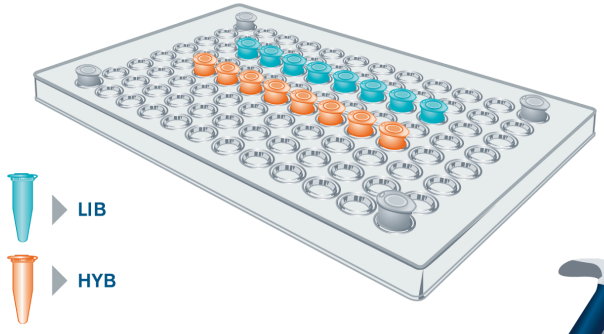
Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Temperature	Time
1	95°C	5m
2	T_H	5m
3	T_H	∞

1. Put the **LIBs** in the thermal cycler, close the lid, and start the thermal program.

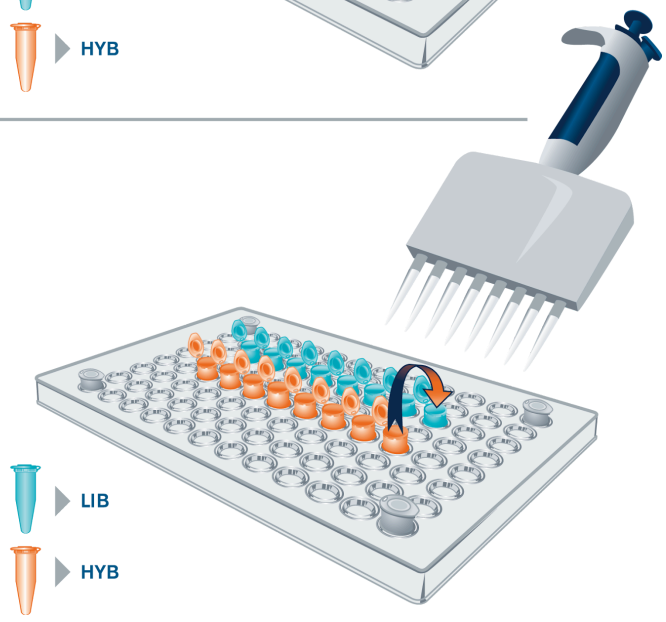


2. Once the cycler reaches the hybridization temperature during step 2, pause the program, put the **HYBs** in the thermal cycler, close the lid, and resume the program.



3. After step 2 of the program is complete, leaving all tubes in the thermal cycler, **pipette 18 µL of each HYB to each LIB.** Use a multichannel pipettor for easier execution. Gently homogenize by pipetting up and down 5 times.

4. Dispose of the HYB tubes. Briefly spin down the LIBs, return to the thermal cycler, close the lid, and allow the reactions to incubate overnight (16 to 24 hours).



PART S2: Bind and Wash ("Cleanup")

Bait-target hybrids are bound to streptavidin-coated magnetic beads, and then most non-target DNA is removed with several rounds of washing with a warm buffer. This is usually performed the day following completion of Part 1.

S2.1 Choose a wash temperature (T_W - typically identical to T_H)

65°C

When bait-target sequence divergence is expected to be 10% or less

62°C

When bait-target sequence divergence is expected to be 10 to 15%

60°C

When bait-target sequence divergence is expected to be 15 to 25%

S2.2 Prepare materials

Start at least 90 minutes before intended hybridization stop time

Reagents
Hyb S (Box 1) *
Binding Buffer (Box 1) *
Wash Buffer (Box 1) *
Beads (Box 1)
Nuclease-free (NF) Water (up to 900 µL per reaction)

** Allow these reagents to come to room temperature before use; warm to 60°C and vortex to dissolve precipitate if necessary*

Equipment
Water bath or incubation oven set to the T_W (e.g., 65°C)
Receptacles for 50 mL tubes, 0.2 mL strips and microcentrifuge tubes compatible with above incubation device
Vortex mixer and mini-centrifuge for 0.2 mL strips and MC tubes
Magnetic particle collector(s) (MPC) for above strips and/or tubes

When using only a microcentrifuge (MC) tube-compatible MPC	When using a 0.2 mL tube-compatible MPC
Nuclease-free 50 mL tube, 1 per 44 cleanups	Nuclease-free 50 mL tube, 1 per 68 cleanups
Nuclease-free MC tubes, 1 per reaction	Nuclease-free 0.2 mL PCR strips with individually-attached lids, 1 vessel per reaction
Heat block set to the T_W	Thermal cycler set to T_W
Pipettors and tips for 20 – 500 µL	Pipettors and tips for 20 – 200 µL; **multichannel pipettor strongly recommended**

S2.3 Wash Buffer X preparation

This step generates enough Wash Buffer X for 44 reactions in microcentrifuge ("MC") tube cleanup format, and 68 reactions in 0.2 mL cleanup format; scale up or down if needed.

1. Thaw and thoroughly homogenize Wash Buffer and Hyb S prior to aliquoting in order to dissolve any visible precipitate; warm slightly if necessary.
2. Combine 400 µL Hyb S, 39.6 mL NF water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly, label "Wash Buffer X." *Wash Buffer X can be stored at 4°C for 1 month*
3. Heat the Wash Buffer X to the T_W in the water bath or oven for at least 30 minutes before use.

S2.4 Bead preparation

 *Prepare beads immediately prior to use*

1. For each capture reaction, aliquot 30 µL beads to a microcentrifuge tube.
2. Pellet the beads in the MPC until the suspension is clear (1-2 minutes).
   Leaving the tubes on the magnet, remove and discard the supernatant.
3. Add 200 µL Binding Buffer to each bead aliquot. Vortex to resuspend the beads and centrifuge briefly. Place tube in the MPC and pellet beads; remove and discard the supernatant.
4. Repeat Step 3 above twice for a total of three washes.
5. Resuspend each washed bead aliquot in 70 µL Binding Buffer. If proceeding to washing in 0.2 mL format, transfer the aliquots to PCR strips.

TIP: *Beads can be prepared in 8 (or fewer) reaction batches (240 µL) in a microcentrifuge tube. Multiply all volumes by the number of reactions in the batch; i.e., for 8 reactions-worth, wash with 1.6 mL and resuspend in 560 µL Binding Buffer, then aliquot 70 µL suspension to individual tubes.*

S2.5 Binding beads and hybrids

1. Heat the bead aliquots to the T_W (e.g., 65°C) for at least 2 minutes.
2. Transfer each capture reaction to the heated bead aliquots. Mix by pipetting.
3. Incubate the libraries + beads on the heat block or thermal cycler for 5 minutes. Agitate at the 2.5 minute mark by flicking or inverting to keep the beads suspended, followed by briefly centrifuging.

S2.6 Bead washing

1. Pellet the beads with the MPC until the solution is clear. Remove and discard the supernatant.
   TIP: *This supernatant can be optionally saved as a backup for re-capture with the same or different baits.*
2. Add 375 µL (MC tube format) or 180 µL (0.2 mL format) warmed Wash Buffer X to the beads, remove from the MPC, place on heat block for 15 seconds, and briefly vortex or mix by pipetting. Briefly centrifuge the mixture.
3. Incubate for 5 minutes at the T_W in the heat block or thermal cycler. Agitate at the 2.5 minute mark via gentle vortexing and then briefly centrifuge.
4. Repeat steps 1 through 3 two times for MC tube format (three washes total), or three times for 0.2 mL format (four washes total). **After the last wash and pelleting, remove as much liquid as possible without touching the bead pellet.**

PART S3: Library Resuspension and Amplification

Bead-bound enriched library is resuspended in Buffer E and amplified.

S3.1 Prepare materials

Reagents
Buffer E (Box 2)
PCR primers for amplifying libraries, e.g. P5/P7 Primer Mix
PCR reagents for post-capture amplification
PCR purification system, e.g. silica columns or SPRI beads
Nuclease-free (NF) Water

Equipment
Tubes appropriate for PCR master mix assembly
Tubes or strips for 50 µL PCR amplification
Pipettors and tips capable of 5 – 100 µL volumes
Vortex mixer and mini-centrifuge for above tube types
Thermal cycler

S3.2 Enriched library recovery

1. Add 30 µL Buffer E to the washed beads and thoroughly resuspend by pipetting.
   Then, depending on your library amplification system, choose workflow A or B:

WORKFLOW A: *When using Arbor's 'Amplification Mix', KAPA® HiFi HotStart, or NEB Ultra II Q5 polymerase systems for amplification*	**WORKFLOW B:** *When <u>not</u> using the polymerase systems for amplification in workflow A*
2A. Proceed directly to section S3.3 using this bead resuspension as the template in amplification	2B. Incubate the suspension at 95°C for 5 minutes 3B. Immediately pellet the beads in the MPC and collect the supernatant containing the enriched libraries

S3.3 Library amplification

This is an example post-capture amplification using the Amplification Mix in the Library Preparation Kit for myBaits and Illumina libraries. If using user-supplied primers, the final concentration of each primer should be 500 nM.

1. Assemble the following PCR master mix:

Component	Final Concentration	µL / Reaction
NF Water	-	5
Amplification Mix	1 ×	25
P5/P7 Primer Mix	0.1 ×	5
Enriched Library (on-bead)	-	15 *
	TOTAL	**50**

**Remaining bead-bound library can be stored at -20°C for several months.*

S3.3 Library amplification (continued)

2. Cycle the reactions with the following thermal program:

Step	Temperature	Time	
1	98°C	2 minutes	
2	98°C	20 seconds	×8 to 14 cycles*
3	60°C	30 seconds	
4	72°C	45 seconds	
5	72°C	5 minutes	
6	8°C	∞	

**Minimize cycles where possible. Cycles required to meet molarity requirements of sequencing platform may exceed 14.*

3. After amplification:
   - **If beads were included in the amplification reaction and you intend to use silica columns for purification**, pellet the beads first and purify only the supernatant.
   - Otherwise, purify the reaction using your preferred PCR cleanup (e.g., silica columns or SPRI beads).

The enriched libraries are now ready for quantification, quality-assessment, and sequencing.

High Sensitivity Protocol

For high background contamination, ancient DNA, high bait-target divergence, and/or short insert fragments

CONTENTS

PROCEDURE

myBaits High Sensitivity Protocol

IMPORTANT! This High Sensitivity protocol includes two rounds of enrichment. This may require that you purchase additional myBaits Reagents (without Baits). For example, if you plan to process 32 samples using the High Sensitivity protocol, you will need only 32 samples worth of Baits, but Reagents for at least 64 samples. There are 5 sizes of Microbial Collection reagents to choose from (from 32 to 768 samples; see page 5), but standard myBaits V5 reagents are also compatible (16 or 48 reaction sizes).

PART H1: Hybridization setup

Sequencing libraries are mixed with various blocking nucleic acids, denatured, and then combined with other hybridization reagents (including baits). These hybridization reactions incubate for several hours to allow baits to encounter and hybridize with target library molecules.

H1.1 Choose a hybridization temperature (T_H)

63°C

For DNA libraries with low GC content or with expected bait-target divergence of less than 15%

60°C

For libraries with expected bait-target sequence divergence of 15 to 25%

55°C

For libraries with insert length distributions mostly shorter than the myBaits probe length, or expected bait-target sequence divergence higher than 25%

H1.2 Prepare materials

Reagents
Nuclease-free Water (“H2O”)
Hyb N and Hyb S from Box 1
Hyb D and Hyb R from Box 2
Block C, Block O, and Block X from Box 2
Baits from Box 3 **KEEP ON ICE**
Libraries or library pools in 7 µL per reaction

Equipment
Nuclease-free microcentrifuge tubes (×2)
0.2 mL strips with attached lids (×1 per 8 reactions)
Pipettors and tips; multichannel for 20 µL recommended
Vortex mixer and mini-centrifuge for above tube types
Heat block set to 60°C
Thermal cycler(s); 2 blocks recommended for 24 or more reactions

Program the thermal cycler:

Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Temperature	Time
1	95°C	5m
2	T_H	5m
3	T_H	∞

H1.3 Hybridization Mix setup

1. Once the Hyb reagents have thawed, vortex them to homogenize and then briefly centrifuge.

Heat Hyb N and Hyb S to 60°C and vortex to dissolve any precipitate present after thawing

2. Assemble the Hybridization Mix in a microcentrifuge (MC) tube, briefly vortex and briefly centrifuge to collect the sample. **The following volumes are already adjusted for pipetting error:**

Component	µL / Reaction	
Hyb N	9.25	
Hyb D	3.5	
Hyb S*	0.5	**Cloudiness caused by Hyb S addition will clear after step 3*
Hyb R	1.25	
H2O (round 1 / round 2)	1.1 / 4.4	*First enrichment round: 1.1 µL Second round: 4.4 µL*
Baits (round 1 / round 2)	4.4 / 1.1	*First enrichment round: 4.4 µL Second round: 1.1 µL*
TOTAL	**20**	

3. Incubate the Hybridization Mix at 60°C for 10 minutes in the heat block. Vortex occasionally to collect condensate. Remove from the heat block and let sit 5 minutes before proceeding.

4. For each capture reaction, aliquot 18.5 µL of Hybridization Mix to a 0.2 mL well/tube.

These reaction aliquots of Hybridization Mix are now referred to as "HYBs"

H1.4 Blockers Mix setup

1. Assemble the Blockers Mix **specific for your target taxon/taxa** in an appropriately-sized tube and mix by pipetting. **The following volumes are already adjusted for pipetting error:**

MOST TAXA	
Component	**µL / Reaction**
Block O	2.5
Block C	2.5
Block X	0.5
NF Water	-
TOTAL	**5.5**

PLANTS	
Component	**µL / Reaction**
Block O	5.0
Block C	-
Block X	0.5
NF Water	-
TOTAL	**5.5**

SALMONIDS	
Component	**µL / Reaction**
Block O	-
Block C	2.5
Block X	0.5
NF Water	2.5
TOTAL	**5.5**

2. For each capture reaction, aliquot 5 µL of Blockers Mix to a 0.2 mL well/tube.

3. Add 7 µL of individual or pooled libraries to each Blockers Mix aliquot and mix by pipetting.

These libraries mixed with Blockers Mix aliquots are now referred to as "LIBs"

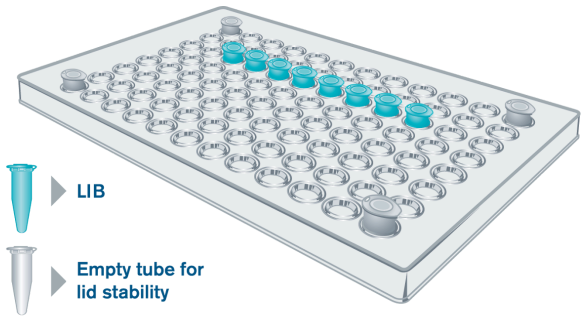
H1.5 Reaction assembly

Double-check the thermal program:

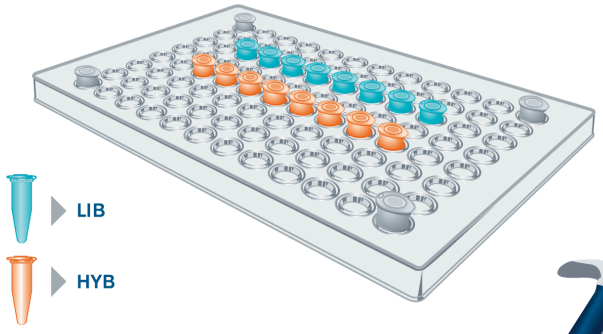
Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Temperature	Time
1	95°C	5m
2	T_H	5m
3	T_H	∞

1. Put the **LIBs** in the thermal cycler, close the lid, and start the thermal program.

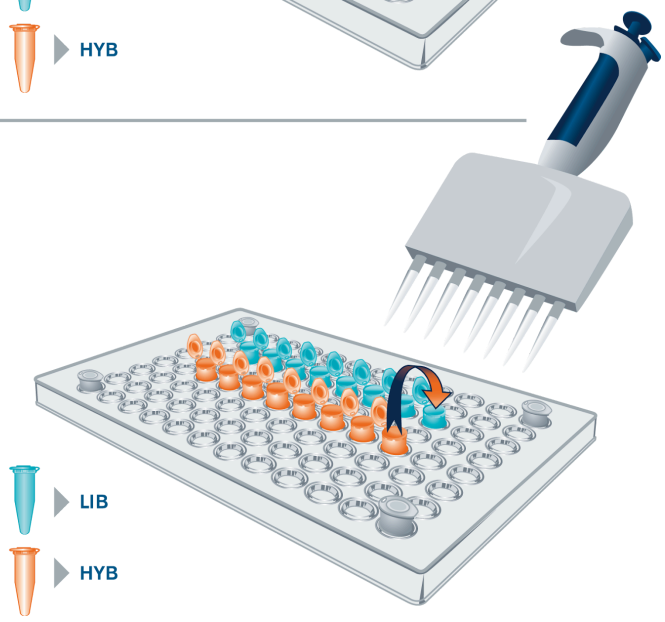


2. Once the cycler reaches the hybridization temperature during step 2, pause the program, put the **HYBs** in the thermal cycler, close the lid, and resume the program.



3. After step 2 of the program is complete, leaving all tubes in the thermal cycler, **pipette 18 µL of each HYB to each LIB.** Use a multichannel pipettor for easier execution. Gently homogenize by pipetting up and down 5 times.

4. Dispose of the HYB tubes. Briefly spin down the LIBs, return to the thermal cycler, close the lid, and allow the reactions to incubate overnight (16 to 24 hours).



PART H2: Bind and Wash ("Cleanup")

Bait-target hybrids are bound to streptavidin-coated magnetic beads, and then most non-target DNA is removed with several rounds of washing with a warm buffer. This is usually performed the day following completion of Part 1.

H2.1 Choose a wash temperature (T_W - typically identical to T_H)

63°C

When bait-target sequence divergence is expected to be 15% or less

60°C

When bait-target sequence divergence is expected to be 15 to 25%

55°C

When bait-target sequence divergence is expected to be higher than 25%

H2.2 Prepare materials

Start at least 90 minutes before intended hybridization stop time

Reagents
Hyb S (Box 1) *
Binding Buffer (Box 1) *
Wash Buffer (Box 1) *
Beads (Box 1)
Nuclease-free (NF) Water (up to 900 µL per reaction)

** Allow these reagents to come to room temperature before use; warm to 60°C and vortex to dissolve precipitate if necessary*

Equipment
Water bath or incubation oven set to the T_W (e.g., 65°C)
Receptacles for 50 mL tubes, 0.2 mL strips and microcentrifuge tubes compatible with above incubation device
Vortex mixer and mini-centrifuge for 0.2 mL strips and MC tubes
Magnetic particle collector(s) (MPC) for above strips and/or tubes

When using only a microcentrifuge (MC) tube-compatible MPC	When using a 0.2 mL tube-compatible MPC
Nuclease-free 50 mL tube, 1 per 44 cleanups	Nuclease-free 50 mL tube, 1 per 68 cleanups
Nuclease-free MC tubes, 1 per reaction	Nuclease-free 0.2 mL PCR strips with individually-attached lids, 1 vessel per reaction
Heat block set to the T_W	Thermal cycler set to T_W
Pipettors and tips for 20 – 500 µL	Pipettors and tips for 20 – 200 µL; **multichannel pipettor strongly recommended**

H2.3 Wash Buffer X preparation

This step generates enough Wash Buffer X for 44 reactions in microcentrifuge ("MC") tube cleanup format, and 68 reactions in 0.2 mL cleanup format; scale up or down if needed.

1. Thaw and thoroughly homogenize Wash Buffer and Hyb S prior to aliquoting in order to dissolve any visible precipitate; warm slightly if necessary.
2. Combine 400 µL Hyb S, 39.6 mL NF water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly, label "Wash Buffer X." *Wash Buffer X can be stored at 4°C for 1 month*
3. Heat the Wash Buffer X to the T_W in the water bath or oven for at least 30 minutes before use.

H2.4 Bead preparation

 *Prepare beads immediately prior to use*

1. For each capture reaction, aliquot 30 µL beads to a microcentrifuge tube.
2. Pellet the beads in the MPC until the suspension is clear (1-2 minutes).
   Leaving the tubes on the magnet, remove and discard the supernatant.
3. Add 200 µL Binding Buffer to each bead aliquot. Vortex to resuspend the beads and centrifuge briefly. Place tube in the MPC and pellet beads; remove and discard the supernatant.
4. Repeat Step 3 above twice for a total of three washes.
5. Resuspend each washed bead aliquot in 70 µL Binding Buffer. If proceeding to washing in 0.2 mL format, transfer the aliquots to PCR strips.

TIP: *Beads can be prepared in 8 (or fewer) reaction batches (240 µL) in a microcentrifuge tube. Multiply all volumes by the number of reactions in the batch; i.e., for 8 reactions-worth, wash with 1.6 mL and resuspend in 560 µL Binding Buffer, then aliquot 70 µL suspension to individual tubes.*

H2.5 Binding beads and hybrids

1. Heat the bead aliquots to the T_W (e.g., 63°C) for at least 2 minutes.
2. Transfer each capture reaction to the heated bead aliquots. Mix by pipetting.
3. Incubate the libraries + beads on the heat block or thermal cycler for 5 minutes. Agitate at the 2.5 minute mark by flicking or inverting to keep the beads suspended, followed by briefly centrifuging.

H2.6 Bead washing

1. Pellet the beads with the MPC until the solution is clear. Remove and discard the supernatant.
   TIP: *This supernatant can be optionally saved as a backup for re-capture with the same or different baits.*
2. Add 375 µL (MC tube format) or 180 µL (0.2 mL format) warmed Wash Buffer X to the beads, remove from the MPC, place on heat block for 15 seconds, and briefly vortex or mix by pipetting. Briefly centrifuge to collect.
3. Incubate for 5 minutes at the T_W in the heat block or thermal cycler. Agitate at the 2.5 minute mark via gentle vortexing and briefly centrifuge.
4. Repeat steps 1 through 3 two times for MC tube format (three washes total), or three times for 0.2 mL format (four washes total). **After the last wash and pelleting, remove as much fluid as possible without touching the bead pellet.**

PART H3: Library Resuspension and Amplification

Bead-bound enriched library is resuspended in Buffer E and amplified.

H3.1 Prepare materials

Reagents
Buffer E (Box 2)
PCR primers for amplifying libraries, e.g. P5/P7 Primer Mix
PCR reagents for post-capture amplification
PCR purification system, e.g., silica columns or SPRI beads
Nuclease-free (NF) Water

Equipment
Tubes appropriate for PCR master mix assembly
Tubes or strips for 50 µL PCR amplification
Pipettors and tips capable of 5 – 100 µL volumes
Vortex mixer and mini-centrifuge for above tube types
Thermal cycler

H3.2 Enriched library recovery

1. Add 30 µL Buffer E to the washed beads and thoroughly resuspend by pipetting.
   Then, depending on your library amplification system, choose workflow A or B:

WORKFLOW A: ***When using Arbor's 'Amplification Mix', KAPA HiFi HotStart, or NEB Ultra II Q5 polymerase systems for amplification***	**WORKFLOW B:** ***When <u>not</u> using the polymerase systems for amplification in workflow A***
2A. Proceed directly to section H3.3 using this bead resuspension as template in amplification	2B. Incubate the suspension at 95°C for 5 minutes 3B. Immediately pellet the beads in the MPC and collect the supernatant containing the enriched libraries

H3.3 Library amplification

This is an example post-capture amplification using the Amplification Mix in the Library Preparation Kit for myBaits and Illumina libraries. If using user-supplied primers, the final concentration of each primer should be 500 nM.

If this is the first time performing step H3.3, generate two of the following reactions per enrichment reaction (each with 15 µL enriched library as template). Otherwise, generate only one:

1. Assemble the following PCR master mix:

Component	Final Concentration	µL / Reaction
NF Water	-	5
Amplification Mix	1 ×	25
P5/P7 Primer Mix	0.1 ×	5
Enriched Library (on- or off-bead)	-	15 *
	TOTAL	**50**

**Remaining bead-bound library can be stored at -20°C for several months.*

H3.3 Library amplification (continued)

2. Cycle the reactions with the following thermal program:

Step	Temperature	Time		
1	98°C	2 minutes		
2	98°C	20 seconds	×14 or 8 cycles*	*First round of enrichment: 14 Second round of enrichment: 8*
3	60°C	30 seconds		
4	72°C	45 seconds		
5	72°C	5 minutes		
6	8°C	∞		

3. After amplification:
   - **If beads were included in the amplification reaction and you intend to use silica columns for purification**, pellet the beads first and purify only the supernatant.
   - Otherwise, purify the reaction using your preferred PCR cleanup (e.g., silica columns or SPRI beads).

H3.4 Perform a second round of enrichment

If this is the end of the first time through step H3.3 (one of two):

1. Combine both purified amplification reactions generated above and concentrate to 7 µL.
2. Repeat steps H1.1 through H3.3 using this once-enriched template as input.

If this is the end of your second time through step H3.3 (two of two), the enriched libraries are now ready for quantification, quality-assessment, and sequencing.

Long Insert Protocol

For target enrichment of NGS libraries containing inserts 1-10 kilobase pairs in length

CONTENTS

PROCEDURE

myBaits Long-Insert Protocol

IMPORTANT! Special note for handling long-insert libraries: Use gentle repeated pipetting rather than vortexing to homogenize solutions that contain both long-insert library and any type of beads (whether capture Beads or purification SPRI beads). This helps reduce potential mechanical shearing.

PART L1: Hybridization setup

Sequencing libraries are mixed with various blocking nucleic acids, denatured, and then combined with other hybridization reagents (including baits). These hybridization reactions incubate for several hours to allow baits to encounter and hybridize with target library molecules.

L1.1 Choose a hybridization temperature (T_H)

65°C

When bait-target sequence divergence is expected to be less than 10%

62°C

When bait-target sequence divergence is expected to be 10 to 15%

60°C

When bait-target sequence divergence is expected to be 15 to 25%

L1.2 Prepare materials

Reagents
Hyb N and Hyb S from Box 1
Hyb D and Hyb R from Box 2
Block C, Block O, and Block X from Box 2
Baits from Box 3 **KEEP ON ICE**
Libraries or library pools in 7 µL per reaction

Equipment
Nuclease-free microcentrifuge tubes (×2)
0.2 mL strips with attached lids (×1 per 8 reactions)
Pipettors and tips; multichannel for 20 µL recommended
Vortex mixer and mini-centrifuge for above tube types
Heat block set to 60°C
Thermal cycler(s); 2 blocks recommended for 24 or more reactions

Program the thermal cycler:

Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Parameters
1	60°C, 10 minutes
2	95°C, 10 minutes
3	Reduce 0.1°C per second to T_H
4	Hold at T_H

L1.3 Hybridization Reaction Setup

1. Once all reagents have thawed, vortex them to homogenize and then briefly centrifuge.

   Heat Hyb N and Hyb S to 60°C and vortex to dissolve any precipitate present after thawing

2. Assemble the Capture Mix in a microcentrifuge (MC) tube combining the reagents in the order indicated. **The following volumes are already adjusted for pipetting error:**

MOST TAXA	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S*	0.5
Hyb R	1.25
Block O	2.5
Block C	2.5
Block X	0.5
NF Water	-
Baits	5.5
TOTAL	**25.5**

PLANTS	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S*	0.5
Hyb R	1.25
Block O	5.0
Block C	-
Block X	0.5
NF Water	-
Baits	5.5
TOTAL	**25.5**

SALMONIDS	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S*	0.5
Hyb R	1.25
Block O	-
Block C	2.5
Block X	0.5
NF Water	2.5
Baits	5.5
TOTAL	**25.5**

**Cloudiness caused by Hyb S addition will clear after step 3*

   Briefly vortex and centrifuge to collect.

3. For each capture reaction, aliquot **23 µL** of Capture Mix to a 0.2 mL well/tube.
4. Add 7 µL of individual or pooled libraries to each Capture Mix aliquot and mix by gently pipetting.
5. Place the reactions in the thermal cycler and run the thermal program, incubating overnight.

Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Parameters
1	60°C, 10 minutes
2	95°C, 10 minutes
3	Reduce 0.1°C per second to T_H
4	Hold at T_H

PART L2: Bind and Wash ("Cleanup")

Bait-target hybrids are bound to streptavidin-coated magnetic beads, and then most non-target DNA is removed with several rounds of washing with a warm buffer. This is usually performed the day following completion of Part 1.

For long insert libraries, mix reactions using gentle pipetting or inversion rather than vortexing in order to minimize shearing effects

L2.1 Choose a wash temperature (T_W - typically identical to T_H)

65°C

When bait-target sequence divergence is expected to be 10% or less

62°C

When bait-target sequence divergence is expected to be 10 to 15%

60°C

When bait-target sequence divergence is expected to be 15 to 25%

L2.2 Prepare materials

Start at least 90 minutes before intended hybridization stop time

Reagents
Hyb S (Box 1) *
Binding Buffer (Box 1) *
Wash Buffer (Box 1) *
Beads (Box 1)
Nuclease-free (NF) Water (up to 900 µL per reaction)

** Allow these reagents to come to room temperature before use; warm to 60°C and vortex to dissolve precipitate if necessary*

Equipment
Water bath or incubation oven set to the T_W (e.g., 65°C)
Receptacles for 50 mL tubes, 0.2 mL strips and microcentrifuge tubes compatible with above incubation device
Vortex mixer and mini-centrifuge for 0.2 mL strips and MC tubes
Magnetic particle collector(s) (MPC) for above strips and/or tubes

When using only a microcentrifuge (MC) tube-compatible MPC	When using a 0.2 mL tube-compatible MPC
Nuclease-free 50 mL tube, 1 per 44 cleanups	Nuclease-free 50 mL tube, 1 per 68 cleanups
Nuclease-free MC tubes, 1 per reaction	Nuclease-free 0.2 mL PCR strips with individually-attached lids, 1 vessel per reaction
Heat block set to the T_W	Thermal cycler set to T_W
Pipettors and tips for 20 – 500 µL	Pipettors and tips for 20 – 200 µL; **multichannel pipettor strongly recommended**

L2.3 Wash Buffer X preparation

This step generates enough Wash Buffer X for 44 reactions in microcentrifuge ("MC") tube cleanup format, and 68 reactions in 0.2 mL cleanup format; scale up or down if needed.

1. Thaw and thoroughly homogenize Wash Buffer and Hyb S prior to aliquoting in order to dissolve any visible precipitate; warm slightly if necessary.
2. Combine 400 µL Hyb S, 39.6 mL NF water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly, label "Wash Buffer X." *Wash Buffer X can be stored at 4°C for 1 month*
3. Heat the Wash Buffer X to the T_W in the water bath or oven for at least 30 minutes before use.

L2.4 Bead preparation

 *Prepare beads immediately prior to use*

1. For each capture reaction, aliquot 30 µL beads to a microcentrifuge tube.
2. Pellet the beads in the MPC until the suspension is clear (1-2 minutes).
   Leaving the tubes on the magnet, remove and discard the supernatant.
3. Add 200 µL Binding Buffer to each bead aliquot. Vortex to resuspend the beads and centrifuge briefly. Place tube in the MPC and pellet beads; remove and discard the supernatant.
4. Repeat Step 3 above twice for a total of three washes.
5. Resuspend each washed bead aliquot in 70 µL Binding Buffer. If proceeding to washing in 0.2 mL format, transfer the aliquots to PCR strips.

TIP: *Beads can be prepared in 8 (or fewer) reaction batches (240 µL) in a microcentrifuge tube. Multiply all volumes by the number of reactions in the batch; i.e., for 8 reactions-worth, wash with 1.6 mL and resuspend in 560 µL Binding Buffer, then aliquot 70 µL suspension to individual tubes.*

L2.5 Binding beads and hybrids

1. Heat the bead aliquots to the T_W (e.g., 65°C) for at least 2 minutes.
2. Transfer each capture reaction to the heated bead aliquots. Mix by pipetting.
3. Incubate the libraries + beads on the heat block or thermal cycler for 5 minutes. Agitate at the 2.5 minute mark by flicking or inverting to keep the beads suspended, followed by briefly centrifuging.

L2.6 Bead washing

1. Pellet the beads with the MPC until the solution is clear. Remove and discard the supernatant.
2. Add 375 µL (MC tube format) or 180 µL (0.2 mL format) warmed Wash Buffer X to the beads, remove from the MPC, place on heat block for 15 seconds, and mix by pipetting. Briefly centrifuge to collect.
3. Incubate for 5 minutes at the T_W in the heat block or thermal cycler. Agitate at the 2.5 minute mark by gentle pipetting. Briefly centrifuge to collect the mixture.
4. Repeat steps 1 through 3 two times for MC tube format (three washes total), or three times for 0.2 mL format (four washes total). **After the last wash and pelleting, remove as much liquid as possible without touching the bead pellet.**

PART L3: Library Resuspension and Amplification

Bead-bound enriched library is resuspended in Buffer E and amplified.

L3.1 Prepare materials

Reagents	Equipment
Buffer E (Box 2)	Tubes appropriate for PCR master mix assembly
PCR primers for amplifying libraries, e.g. P5/P7 Primer Mix	Tubes or strips for 50 µL PCR amplification
PCR reagents for post-capture amplification	Pipettors and tips capable of 5 – 100 µL volumes
PCR purification system, e.g., silica columns or SPRI beads	Vortex mixer and mini-centrifuge for above tube types
Nuclease-free (NF) water	Thermal cycler

S3.2 Enriched library recovery

1. Add 30 µL Buffer E to the washed beads and thoroughly resuspend by pipetting.
   Then, depending on your library amplification system, choose workflow A or B:

WORKFLOW A: ***When using Arbor's 'Amplification Mix', KAPA HiFi HotStart, or NEB Ultra II Q5 polymerase systems for amplification***	**WORKFLOW B:** ***When <u>not</u> using the polymerase systems for amplification in workflow A***
2A. Proceed directly to section L3.3 using this bead resuspension as template in amplification	2B. Incubate the suspension at 95°C for 5 minutes 3B. Immediately pellet the beads in the MPC and collect the supernatant containing the enriched libraries

L3.3 Library amplification

This is an example post-capture amplification using the Amplification Mix in the Library Preparation Kit for myBaits and Illumina libraries. If using user-supplied primers, the final concentration of each primer should be 500 nM.

1. Assemble the following PCR master mix:

Component	Final Concentration	µL / Reaction
NF Water	-	10
Amplification Mix	1 ×	25
P5/P7 Primer Mix	0.1 ×	5
Enriched Library (on- or off-bead)	-	10 *
	TOTAL	**50**

**Remaining bead-bound library can be stored at -20°C for several months.*

L3.3 Library amplification (continued)

2. Cycle the reactions with the following thermal program:

Step	Temperature	Time		
1	98°C	3 minutes		
2	95°C	30 seconds	×25 cycles*	**Minimize cycles where possible. Cycles required to meet molarity requirements of sequencing platform may exceed 25.*
3	62°C	20 seconds		
4	68°C	10 minutes		
6	8°C	∞		

3. After amplification:
   - **If beads were included in the amplification reaction and you intend to use silica columns for purification**, pellet the beads first and purify only the supernatant.
   - Otherwise, purify the reaction using your preferred PCR cleanup (e.g., silica columns or SPRI beads).

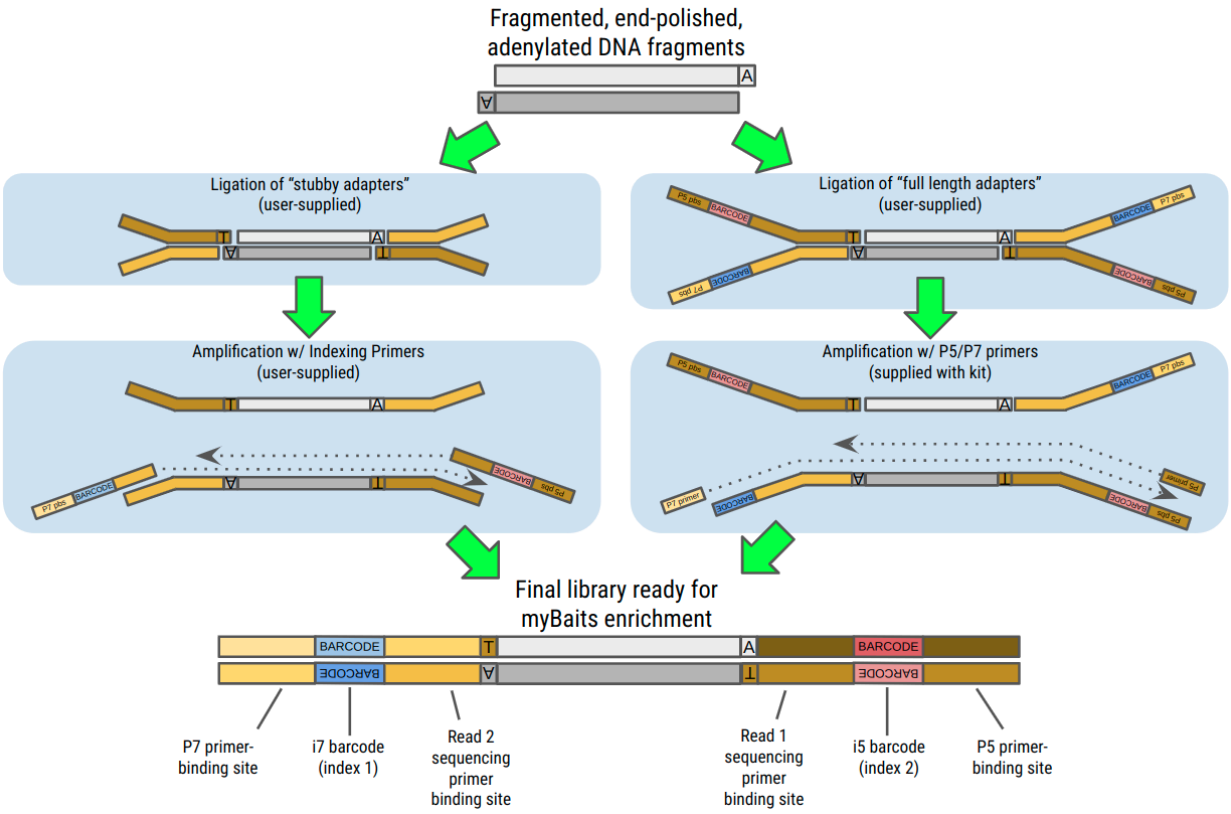
   The enriched libraries are now ready for quantification, quality-assessment, additional size-selection (if required), and then platform-specific library preparation and sequencing.

   If insufficient total mass was acquired from a single amplification reaction for e.g. PacBio or Oxford Nanopore library preparation, perform additional amplifications using the remaining non-amplified enriched library.

APPENDICES

APPENDIX A. Adapters and primers for library preparation

The end-repair step of the **Library Preparation Kit for myBaits** produces 3'-A-tailed dsDNA ends ready for "sticky" ligation to a 5'-T-tailed dsDNA adapter. In Illumina contexts, the adapter is typically either a short configuration that lacks the flowcell binding sites ("P5" and "P7") and sample-specific indexes/barcodes, or a full-length adapter that contains P5, P7, and barcodes. When coupled with short adapters, libraries must be amplified prior to capture with primers that add the P5/P7 sites and barcodes, while with barcoded full-length adapters libraries can be amplified with the universal P5/P7 Primer Mix supplied with the kit. After myBaits capture, both types can be amplified with the P5/P7 Primer Mix.



What sequencing platform can I use? We recommend consulting the sequencing vendor and/or service provider to determine if the libraries made using the Library Preparation Kit for myBaits and your user-supplied adapters and/or indexes will be compatible with your intended platform. For example, Element Biosciences short-read sequencers can be compatible with myBaits-enriched sequencing libraries made with Illumina-style adapters; however, other platforms such as PacBio or Oxford Nanopore Technologies may require extra steps after myBaits enrichment to render those library molecules compatible with their sequencing instruments. Please contact us at techsupport@arbor.daicel.com with any questions.

APPENDIX B. Troubleshooting library preparation

My libraries are longer/shorter than desired (if using the Library Preparation Kit for myBaits reagents)

Input quality, input purity, fragmentation time, and post-amplification SPRI ratio all interact to determine library insert length. These can be adjusted to tune the desired insert lengths, which for standard myBaits hybridization capture are typically in the 300-500 bp insert length range.

Input quality: The starting length distribution of the dsDNA will often impact the final length distribution (see Data Figure 1).

Input purity: EDTA and carryover from DNA extraction can impact enzymatic activity. Ensure your dsDNA is thoroughly purified and in nuclease-free water or a Tris-based buffer containing ≤0.1 mM EDTA, and is free of viscosity or color.

Fragmentation time: This can be tuned to match your target lengths. We recommend increasing/decreasing in 20% increments to identify the most suitable fragmentation time for your sample set.

SPRI ratio: After amplification, the ratio of SPRI beads will impact the lower length-cutoff, and thus the mean insert length. A 1× SPRI ratio, or 50 µL SPRI beads, during post-amplification cleanup will typically produce lower insert cutoffs like Data Figure 1, "HMW" and "degraded" traces, while a 1.5× SPRI ratio, or 75 µL SPRI beads, will produce lower cutoffs like Data Figure 1, "highly degraded" panel.

My library yields are lower than desired (if using the Library Preparation for myBaits reagents)

Yields can vary significantly, but several outcomes can result in a library of sufficient complexity for downstream use. If your yields are less than triple your starting dsDNA input, consider tuning parameters to improve the recovery rate and thereby final library complexity. Input quality, input quantity, amplification cycles, and post-amplification SPRI ratio all interact to determine library yield. Generally speaking, tripling your input will typically ~double the final yield, while you can expect a doubling of yield with every ~2 cycles of indexing amplification. Experimentation will sometimes be required to determine the appropriate pairing of input mass and amplification cycles to achieve your desired yield.

The following are example average yields and insert lengths for libraries prepared from a commercially-sourced HMW human genomic DNA.

	4 min fragmentation		15 min fragmentation	
input (ng)	avg yield (ng)	avg insert length (nt)	avg yield (ng)	avg insert length (nt)
10	112	479	338	292
50	500	502	1194	301
100	1290	444	1730	312
200	1425	465	2726	314

Data Table 1: Example average library yields and insert lengths (final library length minus 136bp) after 4 and 15 minutes of fragmentation time, followed full-length adapter ligation and then 6 amplification cycles, for a range of mass inputs of HMW gDNA.

I have visible “adapter dimer” in my libraries (if using the Library Preparation for myBaits reagents)

Adapter dimer, which is often a visible sharp peak around 120-140 bp depending on final adapter configuration, is often dramatically reduced or eliminated altogether via myBaits capture. However if it persists, you can:

- Re-purify the genomic DNA input and elute in Tris-Tween buffer or Buffer E (from myBaits capture kit).
- Use a lower adapter concentration; standard recommendation is 15 µM for 10-500 ng input, 3 µM for 1-10ng, but consider 0.5 µM if adapter dimer remains a problem.
- If full-length adapters were used, try short adapters with indexing amplification instead.
- Reduce post-amplification (either before or after capture) cleanup SPRI ratios from e.g. 1.5 to 1.2×.
- Use an electrophoresis selection system to physically separate the dimers from the rest of the library.

APPENDIX C: Troubleshooting myBaits hybridization capture

During hybridization, my thermal cycler dropped below the hybridization temperature.

You can expect a lower on-target read proportion and target read complexity for these libraries than if the temperature remained where intended, but not outright enrichment failure. Shallow preliminary sequencing will determine whether targets are likely to be retrieved at sufficient coverage within budget.

My enriched and amplified library is not visible on electrophoresis gel or similar.

Successful captures frequently yield a total mass of just a few nanograms even after re-amplification, which can be difficult to visualize with electrophoresis. This is most common when capturing especially small targets (<100 bp), or targets that are present at low frequency in the starting library (like those in degraded/ancient/environmental DNA), or if there is under-reamplification of the library post-capture. Often a few more cycles of library amplification will render the captured product sufficiently high in concentration to view with electrophoresis. Alternatively, determine with library qPCR whether the library is of sufficient mass for sequencing. If cycling is halted before reaching PCR plateau, the qPCR product can be visualized with electrophoresis to determine length distribution. Consult with your sequencing provider for library concentration and volume requirements.

My enriched and amplified library appears significantly longer than my original library, or has two peaks.

This may happen if the libraries are over-amplified and have formed 'daisy-chains' or 'bubbles' by experiencing cycles of denature-renature without template extension. These can be reverted to their original appearance in electrophoresis by applying three PCR cycles using regular library amplification.

I observe a high ratio of PCR duplicates in my enriched library sequence data.

Percent duplicates in sequencing data (i.e. "clonality" or "duplication rate") increases as you sequence deeper, and therefore it can only be fairly compared between experiments when the sequencing depth is normalized before analysis. Evaluate whether you have simply over-sequenced the libraries by plotting raw sequencing reads obtained on the X axis, and unique reads observed on the Y axis. If this **complexity curve** has plateaued, but you achieved sufficient unique reads, you sequenced more deeply than necessary. If it has not flattened, or you need to increase the total potential unique read yield of the library, **use more DNA per library preparation and/or more library per capture reaction. Avoid diluting baits before capture.** When working with heavily contaminated or damaged DNA target molecules, consider reducing temperatures used in all steps to improve capture sensitivity. Reducing PCR cycles when possible may also improve target coverage uniformity and complexity for a given sequencing depth, in some cases having an indirect effect on duplication rate. For more information about library complexity for any NGS application, we recommend Daley & Smith 2013 (doi: 10.1038/nmeth.2375).

APPENDIX D: myBaits Procedure Quick Guide - Standard Protocol

1. For each reaction, build the following Mixes; pipetting error is built in:

Hybridization Mix	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S	0.5
Hyb R	1.25
Baits	5.5
TOTAL	**20**

Blockers Mix	
Component	**µL / Reaction**
Block X	0.5
Block C	2.5 †
Block O	2.5*
NF Water	0 †
TOTAL	5.5

† Plants: 0; Salmonids: 2.5
*Plants: 5.0; Salmonids: 0

2. After pre-warming the Hybridization mix for 10 minutes @ 60°C, for each reaction, aliquot **18.5 µL** of Hybridization Mix to their own tubes – now **"HYBs"**.
3. For each reaction, aliquot **5 µL** of Blockers Mix and then add **7 µL** of each library – now **"LIBs"**.
4. Incubate the LIBs in the thermal cycler for 5 minutes @ 95°C and then drop to the hybridization temperature (e.g., 65°C). **Be sure to use a heated lid.**
5. Put the HYBs in the thermal cycler and warm to the hybridization temperature for 5 minutes.
6. Transfer **18 µL** of each HYB to each LIB, mix by pipetting, and incubate for 16-24 hours.
7. **1.5 hours before step 9**, prepare Wash Buffer X by combining 400 µL Hyb S, 39.6 mL nuclease-free molecular biology-grade water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly and warm to the hybridization temperature for at least 45 minutes.
8. Prepare 30 µL of beads per reaction by washing three times in 200 µL Binding Buffer. Resuspend the washed bead aliquots in 70 µL Binding Buffer and warm the suspensions to the hybridization temperature for at least 2 minutes.
9. Combine the warmed beads with the hybridization reactions and incubate for 5 minutes at the hybridization temperature, agitating at 2.5 minutes to keep beads suspended.
10. Pellet the beads and remove the supernatant. If using microcentrifuge tubes for cleanup, wash the beads three times with 375 µL warmed Wash Buffer X, incubating 5 minutes at the hybridization temperature. Wash four times with 180 µL washes if using a 96-well magnetic particle concentrator and 0.2 mL strips/tubes.
11. Resuspend the beads in 30 µL Buffer E and then use 15 µL of this in a 50 µL amplification reaction with KAPA HiFi or NEB Ultra II Q5 polymerase systems. If not using these polymerase systems, instead elute the library from the beads by incubating the suspension for 5 minutes at 95°C, immediately pellet the beads, and then use 15 µL of the supernatant in a 50 µL amplification reaction.
12. Purify the amplification reactions using silica columns or SPRI beads. **If using silica columns and beads were included in the amplification reaction, pellet the beads first and purify only the supernatant.**

APPENDIX E: myBaits Procedure Quick Guide - High Sensitivity Protocol

1. For each reaction, build the following Mixes; pipetting error is built in:

Hybridization Mix	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S	0.5
Hyb R	1.25
Baits (round 1 / 2)	4.4 / 1.1
H2O (round 1 / 2)	1.1 / 4.4
TOTAL	**20**

Blockers Mix	
Component	**µL / Reaction**
Block X	0.5
Block C	2.5 †
Block O	2.5*
NF Water	0 †
TOTAL	5.5

† Plants: 0; Salmonids: 2.5
*Plants: 5.0; Salmonids: 0

2. After pre-warming the Hybridization mix for 10 minutes @ 60°C, for each reaction, aliquot **18.5 µL** of Hybridization Mix to their own tubes – now **"HYBs"**.
3. For each reaction, aliquot **5 µL** of Blockers Mix and then add **7 µL** of each library – now **"LIBs"**.
4. Incubate the LIBs in the thermal cycler for 5 minutes @ 95°C and then drop to the hybridization temperature (e.g., 63°C). **Be sure to use a heated lid.**
5. Put the HYBs in the thermal cycler and warm to the hybridization temperature for 5 minutes.
6. Transfer **18 µL** of each HYB to each LIB, mix by pipetting, and incubate for 16-24 hours.
7. **1.5 hours before step 9**, prepare Wash Buffer X by combining 400 µL Hyb S, 39.6 mL nuclease-free molecular biology-grade water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly and warm to the hybridization temperature for at least 45 minutes.
8. Prepare 30 µL of beads per reaction by washing three times in 200 µL Binding Buffer. Resuspend washed bead aliquots in 70 µL Binding Buffer and warm the suspensions to the hybridization temperature for at least 2 minutes.
9. Combine the warmed beads with the hybridization reactions and incubate for 5 minutes at the hybridization temperature, agitating at 2.5 minutes to keep beads suspended.
10. Pellet the beads and remove the supernatant. If using microcentrifuge tubes for cleanup, wash the beads three times with 375 µL warmed Wash Buffer X, incubating 5 minutes at the hybridization temperature. Wash four times with 180 µL washes if using a 96-well magnetic particle concentrator and 0.2 mL strips/tubes.
11. Resuspend the beads in 30 µL Buffer E and then use 15 µL of this in a 50 µL amplification reaction with KAPA HiFi or NEB Ultra II Q5 polymerase systems. If not using these polymerase systems, instead elute the library from the beads by incubating the suspension for 5 minutes at 95°C, immediately pellet the beads, and then use 15 µL of the supernatant in a 50 µL amplification reaction. Do two amplifications if this is the first round of enrichment; do one if this is the second and last round of enrichment.
12. Purify the amplification reactions using silica columns or SPRI beads. **If using silica columns and beads were included in the amplification reaction, pellet the beads first and purify only the supernatant.**
13. If this is the end of your first round of enrichment: combine both post-capture amplifications and concentrate to 7 uL, then repeat steps 1-12. Otherwise, the enriched libraries are ready for QC and sequencing.

APPENDIX F: myBaits Procedure Quick Guide - Long Insert Protocol

1. For each reaction, build in the following Capture Mix appropriate to your target taxon; pipetting error is built in:

MOST TAXA	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S	0.5
Hyb R	1.25
Block O	2.5
Block C	2.5
Block X	0.5
NF Water	-
Baits	5.5
TOTAL	**25.5**

PLANTS	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S	0.5
Hyb R	1.25
Block O	5.0
Block C	-
Block X	0.5
NF Water	-
Baits	5.5
TOTAL	**25.5**

SALMONIDS	
Component	**µL / Reaction**
Hyb N	9.25
Hyb D	3.5
Hyb S	0.5
Hyb R	1.25
Block O	-
Block C	2.5
Block X	0.5
NF Water	2.5
Baits	5.5
TOTAL	**25.5**

2. For each enrichment reaction, aliquot **23 µL** of Capture Mix to their own tubes.
3. To each Capture Mix aliquot, add **7 µL** of each library or library pool.
4. Incubate the reactions in the thermal cycler for 10 minutes @ 60°C, then 10 minutes @ 95°C, and then drop to the hybridization temperature (e.g., 65°C) at a rate of 0.1°C per second. **Be sure to use a heated lid.** Incubate at the hybridization temperature for 16-24 hours.
5. **1.5 hours before step 9**, prepare Wash Buffer X by combining 400 µL Hyb S, 39.6 mL nuclease-free molecular biology-grade water and 10 mL Wash Buffer in a 50 mL tube. Vortex thoroughly and warm to the hybridization temperature for at least 45 minutes.
6. Prepare 30 µL of beads per reaction by washing three times in 200 µL Binding Buffer. Resuspend the washed bead aliquots in 70 µL Binding Buffer and warm the suspensions to the hybridization temperature for at least 2 minutes.
7. Combine the warmed beads with the hybridization reactions and incubate for 5 minutes at the hybridization temperature, agitating at 2.5 minutes to keep beads suspended.
8. Pellet the beads and remove the supernatant. If using microcentrifuge tubes for cleanup, wash the beads three times with 375 µL warmed Wash Buffer X, incubating 5 minutes at the hybridization temperature. Wash four times with 180 µL washes if using a 96-well magnetic particle concentrator and 0.2 mL strips/tubes.
9. Resuspend the beads in 30 µL Buffer E and then use 10 µL of this in a 50 µL library amplification reaction with KAPA HiFi or NEB Ultra II Q5 polymerase systems. If not using these polymerase systems, instead elute the library from the beads by incubating the suspension for 5 minutes at 95°C, immediately pellet the beads, and then use 10 µL of the supernatant in a 50 µL amplification reaction.
10. Purify the amplification reactions using silica columns or SPRI beads. **If using silica columns and beads were included in the amplification reaction, pellet the beads first and purify only the supernatant.** The enriched libraries are now ready for quantification, quality-assessment, and sequencing.