Human Affinities v1

Population Genomics Hybridization Capture for Targeted NGS

User Manual
Version 1.1 December 2022

For myBaits Expert Human Affinities - Complete
myBaits Expert Human Affinities - Prime Plus
myBaits Expert Human Affinities - Ancestral Plus

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Human Affinities v1 Kit Protocol

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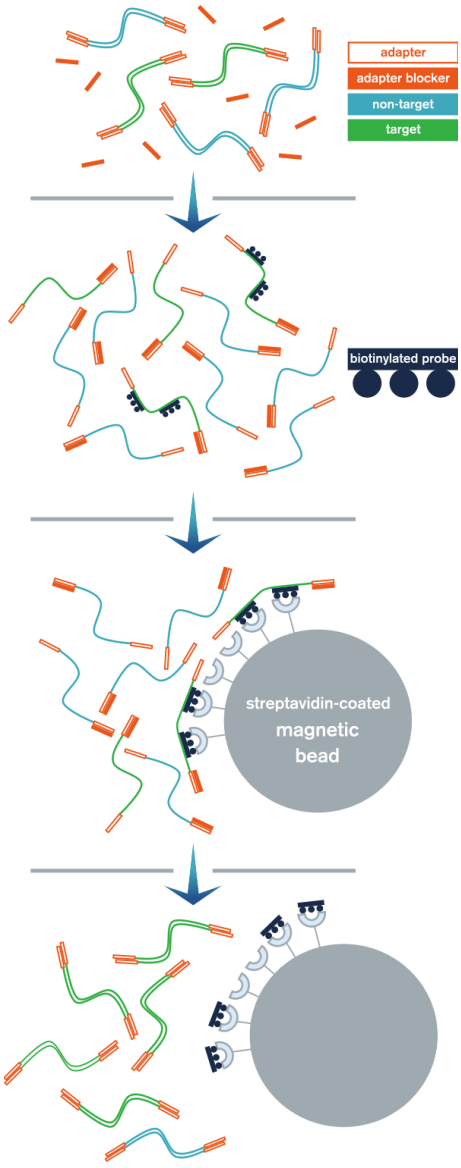
INTRODUCTION

The myBaits® Expert Human Affinities kit is an in-solution next-generation sequencing (NGS) library target enrichment system for modern and ancient DNA sequencing libraries. The kit uses biotinylated oligonucleotide probes to capture over 1.6M sites in the human genome that contain known single-nucleotide sites polymorphic in living and ancient populations. Probes and other reagents for NGS target enrichment are supplied with the kit. After enrichment, libraries can be sequenced on NGS platforms such as those supplied by Illumina® or Thermo Fisher Scientific®.

Procedure overview

1. Amplified sequencing library, adapter blockers, and other reagents are combined.
2. Libraries are denatured, allowing blockers to hybridize to adapters. Probes are then introduced and hybridized to targets for several hours.
3. Probe-target hybrids are bound to streptavidin-coated magnetic beads and pulled out of suspension with a magnet. Most non-target DNA is washed away.
4. The remaining enriched library is amplified, enriched again, and either taken directly to sequencing or further treated.



Daicel Arbor Biosciences products compatible with this manual

This manual is compatible with Daicel Arbor Biosciences catalog numbers beginning with 351, 352 and 353 (for example Cat# 351016: “myBaits Expert Human Affinities Kit - Complete, 16 rxn”).

This procedure is only compatible with the myBaits kits listed above. For standard myBaits ancient DNA procedures, see the myBaits High Sensitivity protocol in myBaits Manual version 5.0.

Kit components and stability

	Reagent	Cap color	Volume (16 rxn)	Volume (48 rxn)
Box A ***Store at 4˚C***	Beads	Clear	1070 µL	2 × 1600 µL
	BB 2X	-	12 mL	36 mL
	WB1	-	5.5 mL	16.5 mL
	WB2	-	17 mL	51 mL
	WB3	-	5.5 mL	16.5 mL
Box B ***Store at -20˚C***	Hyb D	Yellow	125 µL	375 µL
	Probes	White	2 × 56 µL	6 × 56 µL
	Block O	Blue	90 µL	270 µL
	Block C	Dark Green	90 µL	270 µL
	Block X	Orange	18 µL	2 × 27 µL
	Buffer E	Light Green	1070 µL	2 × 1600 µL

At the recommended storage temperatures, myBaits kit components have a shelf life of one year.

Probes are supplied as 1 tube per 8 reactions (1 reaction = two rounds of enrichment).

REQUIREMENTS AND RECOMMENDATIONS

Input library

TYPE Use myBaits with PCR-amplified and amplifiable NGS libraries, including Illumina TruSeq®-style, Illumina Nextera® Flex-style, Ion Torrent®, or other libraries with universal adapter priming sites. Dual-indexed libraries are strongly recommended to reduce the hazard of mis-indexing induced by PCR jumping events. 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.

VOLUME Each myBaits Expert Human Affinities target enrichment reaction has space for 10 µL total NGS library volume. Many libraries will require concentration to 10 µL with vacuum centrifugation or other means to maximize input mass. Perform this in advance of your enrichment experiments, resuspending libraries in nuclease-free buffer or water.

MASS & POOLING A wide range of total library mass can be successfully enriched with this kit (nanograms to micrograms). Optimal input amounts will vary between library qualities and endogenous constituents and may require trials to identify. See below for recommendations for different sample types:

Degraded and contaminated DNA libraries: Pooling libraries from ancient, degraded or heavily contaminated DNA prior to enrichment is not recommended. Instead, enrich individual libraries using up to 1 µg of library per reaction. If pooling is required, pool as few libraries as possible. Make certain that libraries are balanced by endogenous content to ensure each sample contributes the same amount of target content. For example, if two libraries are to be pooled, and library A has 2% endogenous template and library B has 1% endogenous template, include twice the mass of library B as library A in the enrichment pool.

Non-degraded, non-contaminated DNA libraries: Pool up to 4 libraries per enrichment reaction, 250 ng each.

Blocking oligos

Block X is compatible with most Illumina library configurations, including single- and dual-index TruSeq-style, Nextera-style, and several custom styles containing P5 and P7 priming sites. For different adapter configurations, we recommend Custom IDT® xGen® Blocking Oligos. At a concentration of 1 µg/µL, custom adapter-blocking oligos can be used in lieu of myBaits Block X.

Sequencing depth

Following enrichment, percent reads on-target (and depending on sequencing depth, percent duplicates) can vary dramatically between libraries, especially for ancient DNA. As a starting point, consider sequencing 20 million read-pairs per sample.

Data usage

The Human Affinities kit can be used to generate data for a wide range of population genetics analyses. However, because this kit exhibits allelic read coverage bias distinct from biases in other assays (like shotgun sequencing and other targeted sequencing kits), we recommend that data generated with the Human Affinities kit be used alone for whatever analyses you may perform. Daicel Arbor Biosciences is currently in the process of generating a list of sites that are not expected to show allelic coverage bias, and thus can be used in conjunction with the unbiased portion of data from other assays. If you would like to be notified when this list becomes available, please contact techsupport@arbor.daicel.com with a request to be added to our Human Affinities product notification mailing list.

Equipment required

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 0.2 mL strips	1 (e.g., Permagen Cat# S500)
* Magnetic particle collector for microcentrifuge tubes	1 (e.g., ThermoFisher Cat# 12321D)
Vortex mixer and mini-centrifuge for tubes and strips	
Rotator capable of holding 0.2 mL strips	
Water bath or incubation oven at 55°C	

* *Recommended for preparing a multi-reaction batch of Beads; see Tip in section A2.3, page 11.*
† *Ensure that the thermal cycler and strips allow no more than 4 µL of 30 µL volume evaporation overnight at 70°C*

Reagents required

Reagent	Notes
Nuclease-free (“NF”) water	900 µL per reaction
2X Hi-RPM Buffer	**Agilent Part# 5188-5242: one order supplies enough for 71 dual-round enrichment reactions** ***- or -*** **Agilent Part# 5190-0403: one order supplies enough for 625 dual-round enrichment reactions**
PCR primers to amplify sequencing libraries after capture, e.g.:	
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	1 per reaction (e.g. Roche Cat# 07958927001)
PCR purification system, e.g., silica columns or SPRI beads	1 cleanup per reaction

PROCEDURE

PART 1: Hybridization setup

Libraries are mixed with various blocking nucleic acids, denatured, and then combined with other hybridization reagents (including Probes). These hybridization reactions incubate overnight to allow probes to encounter and hybridize with target library molecules.

A1.1 Prepare materials

Reagents	Equipment
2X Hi-RPM Buffer (from Agilent Part# 5188-5242)	Nuclease-free microcentrifuge tubes (×2)
Hyb D from Box B	0.2 mL strips with attached lids (×1 per 8 reactions)
Block C, O, and X from Box B	Pipettors and tips; multichannel for 20 µL recommended
Probes from Box B	Vortex mixer and mini-centrifuge for above tube types
Sequencing libraries for enrichment in 10 µL	Thermal cycler(s); 2 blocks recommended for >23 reactions
NF Water	

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	70°C	5m
3	70°C	∞

A1.2 Hybridization Mix setup

1. Once the hybridization reagents have thawed, vortex to homogenize and then briefly centrifuge.
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 1st enrichment round	µL / Reaction 2nd enrichment round
2X Hi-RPM Buffer	19.7	19.7
Hyb D	3.5	3.5
NF Water	0.0	2.2
Probes	4.4	2.2
TOTAL	**27.6**	**27.6**

3. For each capture reaction, aliquot 25.5 µL of Hybridization Mix to a 0.2 mL well/tube.

   These reaction aliquots of Hybridization Mix are now referred to as "HYBs".
   Proceed immediately to step A1.3.

A1.3 Blockers Mix setup

1. Assemble the Blockers Mix in an appropriate size tube and mix by pipetting. **The following volumes are already adjusted for pipetting error:**

Component	µL / Reaction
Block C	2.5
Block O	2.5
Block X	0.5
TOTAL	**5.5**

2. For each capture reaction, aliquot 5 µL of Blockers Mix to a 0.2 mL well/tube.
3. Add 10 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".
   Proceed immediately to step A1.4.

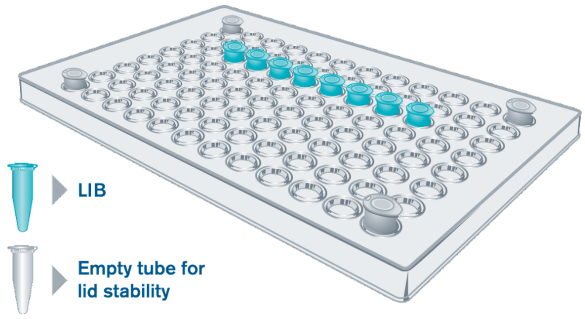
A1.4 Reaction assembly

Double-check the thermal program:

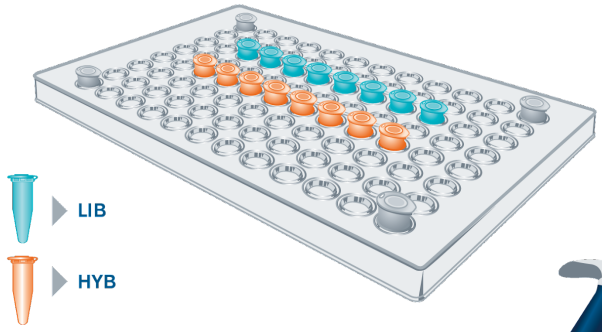
Set lid temperature 5 to 10°C above each step temperature to minimize evaporation

Step	Temperature	Time
1	95°C	5m
2	70°C	5m
3	70°C	∞

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

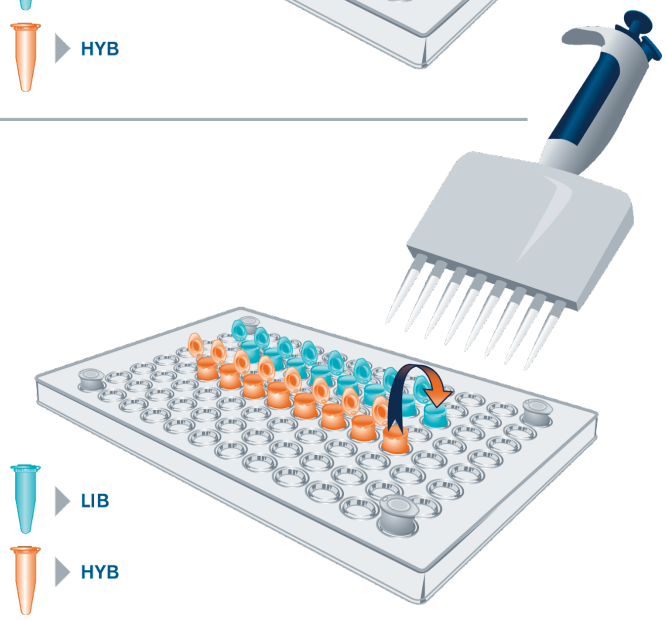


2. Once the cycler reaches 70°C during step 2, pause the program, put the **HYBs** in the thermal cycler, close the lid, and resume the program.



3. After step 2 of the program is complete, leaving all tubes in the thermal cycler, **pipette 24 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 2: Bind and Wash ("Cleanup")

After overnight incubation, probe-target hybrids are bound to streptavidin-coated magnetic beads, and then most non-target DNA is removed with several rounds of washing with three different solutions.

A2.1 Prepare materials

Start at least 90 minutes before intended hybridization stop time

Reagents
BB 2X (Box A) *Bring to room temperature*
WB1, WB2, and WB3 (Box A) *Bring to room temperature*
Beads (Box A)
Nuclease-free (NF) Water, 340 µL per enrichment reaction

Equipment
Water bath or incubation oven set to 55°C
Receptacles for 15 or 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
Rotator for 0.2 mL strips
Nuclease-free 0.2 mL PCR strips with individually-attached lid, 1 vessel per enrichment reaction
Thermal cycler set to 55°C
Pipettors and tips for 20 – 200 µL; **multichannel pipettor strongly recommended**

A2.2 Prepare BB 1X and WB2

This step dilutes BB 2X to working concentration (1X) and warms BB 1X and WB2 before use.

1. Allow BB 2X to come to room temperature and vortex thoroughly.
2. In a 15 mL (for up to 22 enrichment reactions) or 50 mL (for up to 74 enrichment reactions) tube, for each capture reaction combine 370 µL BB 2X and 370 µL NF water. (16 reactions: 5.92 mL each reagent; 48 reactions: 17.76 mL each reagent). Vortex thoroughly and label "BB 1X".
   BB 1X can be stored at 4°C for 1 year.
3. Heat the WB2 to 55°C in the water bath or oven for at least 30 minutes before use.

A2.3 Prepare Beads *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 BB 1X 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 BB 1X. If proceeding to washing in 0.2 mL format, transfer the aliquots to PCR strips.

TIP: *Prepare Beads in 8 (or fewer) reaction batches (240 µL) in a microcentrifuge tube and matched MPC. 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 BB 1X, then aliquot 70 µL suspension to individual tubes.*

A2.4 Bind Beads and probe-library hybrids

1. Incubate the prepared bead aliquots at 55°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 10 minutes at 55°C. Agitate at the 2.5, 5, and 7.5 minute marks by flicking or inverting the tubes to keep the beads suspended, followed by briefly centrifuging.

A2.5 Washes

1. Briefly spin down the tubes to collect condensate. Pellet the beads in the MPC until the solution is clear. Remove and discard the supernatant.
2. Remove the tubes from the MPC and add 150 µL WB1 and resuspend by pipetting. Rotate for 15 minutes at room temperature.
3. Briefly spin down the tubes and pellet the beads in the MPC. Remove and discard the supernatant.
4. Add 120 uL warmed WB2 and resuspend by pipetting. Incubate in the thermal cycler at 55°C for 10 minutes. Agitate at the 2.5, 5, and 7.5 minute marks to resuspend the beads.
5. Briefly spin down the tubes and pellet the beads in the MPC. Remove and discard the supernatant.
6. Repeat steps 4 and 5 three times for a total of four WB2 washes.
7. Remove the beads from the MPC and add 150 µL WB3 and resuspend by pipetting. Incubate at room temperature for 5 minutes, agitating the tube regularly to maintain a suspension.
8. Briefly spin down the tubes and pellet the beads in the MPC. Remove and discard the supernatant. **Remove as much liquid as possible without touching the bead pellet - briefly spinning down the tubes before a final liquid removal may help maximize removal.**

Proceed immediately to Part 3.

PART 3: Library Resuspension and Amplification

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

A3.1 Prepare materials

Reagents	Equipment
Buffer E (Box B)	Tubes appropriate for PCR master mix assembly
PCR primers for amplifying libraries (e.g., P5 and P7)	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

A3.2 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 KAPA HiFi HotStart or NEB Ultra II Q5 polymerase systems for amplification***	**WORKFLOW B:** ***When not using the polymerase systems for amplification listed 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 transfer the supernatant containing enriched libraries to new tubes.

Resuspended, bead-bound libraries can be stored at -20°C for several months if desired.

A3.3 Library amplification

This is an example post-capture amplification using KAPA HiFi HotStart ReadyMix and Illumina libraries.

If this is the first time performing step A3.3, 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 Conc.	µL / Reaction	Sequence
NF Water	-	5	-
2X KAPA HiFi HotStart Ready Mix	1 X	25	-
Forward library primer (at 10 µM)	500 nM	2.5	e.g. P5: AATGATACGGCGACCACCGA
Reverse library primer (at 10 µM)	500 nM	2.5	e.g. P7: CAAGCAGAAGACGGCATACGA
Enriched library (on- or off-bead)	-	15	-
	TOTAL	**50**	**-**

A3.3 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	×20, 10 or 8 cycles*
3	60°C	30 seconds	
4	72°C	45 seconds	
5	72°C	5 minutes	
6	8°C	∞	

Library type	*Cycles after Round 1	*Cycles after Round 2
Ancient	20	10
Modern	10	8

3. After amplification with your preferred PCR cleanup system (e.g., silica columns, SPRI beads, etc).

   - **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.

   - For SPRI cleanups, be sure to use an appropriate ratio for your library molecule lengths.

A3.4 Perform a second round of enrichment

If this is the end of the first time through step A3.3 (one of two):

1. Combine both purified amplification reactions generated above and concentrate to 10 µL.
2. Repeat steps A1.1 through A3.3 using this once-enriched template as input.

If this is the end of the second time through step A3.3 (two of two), the enriched libraries are now ready for quantification, quality-assessment, and sequencing. See Page 5 for discussion of sequencing depth.

APPENDIX

AA1: Troubleshooting

During hybridization, my thermal cycler dropped below 70°C

You can expect a lower on-target read proportion and target read complexity for these libraries than if the temperature had 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

Not all combinations of starting percent endogenous or input amounts will result in sufficient product to visualize with electrophoresis. Sequencing library total quantitative PCR should be used to determine final concentration of the enriched libraries. Insert length distributions can be determined by running one of the products of a qPCR on gel or similar, so long as amplification did not cycle in the plateau stage.

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 cycling without template extension (i.e., past PCR plateau). 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.

Sequence less deeply, use more DNA per library preparation and/or more library per capture reaction, and avoid diluting probes before capture. In a tube, the proportion of molecules generated through PCR is a single value, but the percent of duplicate reads in sequencing data (often referred to as "clonality" or "duplication rate") increases as you sequence a library more deeply. 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 to resolve your targets, you sequenced more deeply than necessary. 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).

AA2: myBaits Procedure Quick Guide - Human Affinities v1

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

Hybridization Mix	
Component	**µL / Reaction**
2X Hi-RPM Buffer	19.7
Hyb D	3.5
H2O (round 1 / 2)	0.0 / 2.2
Probes (round 1 / 2)	4.4 / 2.2
TOTAL	**27.6**

Blockers Mix	
Component	**µL / Reaction**
Block C	2.5
Block O	2.5
Block X	0.5
TOTAL	**5.5**

2. For each reaction, aliquot **25.5 µL** of Hybridization Mix to their own tubes – now **"HYBs"**.
3. For each reaction, aliquot **5 µL** of Blockers Mix and then add **10 µL** of each library – now **"LIBs"**.
4. Incubate the LIBs in the thermal cycler for 5 minutes @ 95°C and then drop to 70°C. **Be sure to use a heated lid.**
5. Put the HYBs in the thermal cycler and warm to 70°C for 5 minutes.
6. Transfer **24 µL** of each HYB to each LIB, mix by pipetting, and incubate for 16-24 hours.
7. **1.5 hours before step 9**, for each reaction, prepare BB 1X by combining 370 µL BB 2X and 370 µL nuclease-free molecular biology-grade water in a 15 or 50 mL tube; i.e., 5.92 mL BB 2X and 5.92 mL H2O for 16 cleanups; 17.76 mL BB 2X and 17.76 H2O for 48 cleanups. Vortex thoroughly.
8. Warm WB2 to 55°C for at least 30 minutes in a water bath.
9. Prepare 30 µL of beads per reaction by washing three times in 200 µL BB 1X. Resuspend washed bead aliquots in 70 µL Binding Buffer and incubate suspensions at 55°C for at least 2 minutes before step 10.
10. Combine the warmed beads with the hybridization reactions and incubate for 10 minutes at 55°C, agitating every 2.5 minutes to keep beads suspended. Pellet the beads and remove the supernatant.
11. Wash the beads once with 150 µL WB1, rotating for 15 minutes. Pellet the beads and remove the supernatant.
12. Wash the beads four times with 120 µL warmed WB2, each time incubating 10 minutes at 55°C with agitation every 2.5 minutes to maintain suspension, pelleting in the MPC and removing the supernatant.
13. Wash the beads once with 150 µL room temperature WB3, incubating 5 minutes with periodic agitation. Pellet the beads and remove the supernatant, removing as much of the liquid as possible.
14. 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 for 20 cycles if this is the first round of enrichment; do one amplification for 10 cycles if this is the second and last round of enrichment.
15. 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.**
16. If this is the end of your first round of enrichment: combine both post-capture amplifications and concentrate to 10 uL, then repeat steps 1-12. Otherwise, the enriched libraries are ready for QC and sequencing.