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hyRAD RNA probes preparation and capture V.2

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Molecular Biogeography...

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Abstract

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Guidelines

You can change the number of samples used for the probes preparation by scaling the reaction.

For the capture, adjust the volumes accordingly to the numbers of libraries captured.

The final molarities in the capture reaction will be around a few pmol of probes and libraries, 50 pmol of each blocking oligo (around 10x the molarity of the DNA library), 2.3 ng of Cot-1 and 2.3 ng of salmon sperm DNA, in approximately 5.4x SSPE (0.8 M NaCl), 0.013 M EDTA, 1.05% SDS, 0.75x Denhardt's solution.

Literature:

Carpenter et al. (2013) Pulling the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. The American Journal of Human Genetics 93, 852-864.

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Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE 7(5): e37135. doi:10.1371/journal.pone.0037135

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Materials

MATERIALS

- MseI - 500 units **New England Biolabs Catalog #**R0525S
- PstI - 10,000 units **New England Biolabs Catalog #**R0140S
- T4 DNA Ligase - 20,000 units **New England Biolabs Catalog #**M0202S
- Nuclease-free Water
- Ethanol 100%
- Ethanol 70% [Note: freshly prepared]
- sodium dodecyl sulfate (SDS)
- Agencourt Ampure XP **Beckman Coulter Catalog #**A63880
- 1 M Tris-HCl pH 8.0
- HiScribe T7 High Yield RNA Synthesis Kit - 50 rxns **New England Biolabs Catalog #**E2040S
- ATP Solution (100 mM) **Thermo Fisher Scientific Catalog #**R0441
- 0.5 M EDTA pH 8.0
- Cot-1 DNA **Thermo Fisher Scientific Catalog #**15279011
- SSPE Buffer Concentrate (20x) **Life Technologies Catalog #**15591043
- Dynabeads MyOne Streptavidin C1 **Invitrogen - Thermo Fisher Catalog #**65001
- Denhardt's solution (50x) **Catalog #**750018
- UltraPure 20x SSPE Buffer **Life Technologies Catalog #**15591-043
- Salmon Sperm DNA Solution **Thermo Fisher Scientific Catalog #**15632011
- SUPERase-In RNase Inhibitor **Thermo Fisher Scientific Catalog #**AM2694
- TURBO DNase **Thermo Fisher Scientific Catalog #**AM2238
- Biotin 16 UTP **Thermo Fisher Scientific Catalog #**AM8452
- sodium acetate
- sodium chloride
- Kapa HiFi Hotstart Readymix **Kapa Biosystems Catalog #**KK2601
- Qubit RNA BR Assay Kit **Thermo Fisher Scientific Catalog #**Q10211

Before start

RAD P1 adapters, SbfI/PstI/NsiI-compatible (RAD-T7-P1)

RAD-P1.1_T7-PstI: ACACTCTTTCCCTACACGACGCTCTTCCGATCTAATACGACTCACTATAGTGCA
RAD-P1.2_T7-PstI: [5phos]CTATAGTGAGTCGTATTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

The above oligonucleotides are annealed to form adapter compatible with SbfI, PstI or NsiI restriction enzyme overhang (3'TGCA). The restiction site is not active anymore after adapter ligation.

When sequencing, the first 18 nt will be the T7 promoter sequence and the next 6 nt of the cut-site. Please keep in mind that the beginning of the reads will have zero diversity (the T7 promoter sequence + cut site). Pool it with other libraries of high diversity, spike-in with a lot of PhiX or consider using several adapters with different length of N-padding before the T7 sequence (not tested yet but should work):
RAD_P1.1_pad-T7-PstI: ACACTCTTTCCCTACACGACGCTCTTCCGATC[pad]TAATACGACTCACTATAGTGCA
RAD_P1.2_pad-T7-PstI: [5phos]CTATAGTGAGTCGTATTA[pad]GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

RAD P2 adapter, MseI-compatible (RAD-P2)

RAD_P2.1_MseI-bio: [5biotin]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
RAD_P2.2_MseI: [5phos]TAAGATCGGAAGAGCGAGAACAA

The above oligonucleotides are annealed to form adapter compatible with MseI restriction enzyme overhang (5'TA). Restriction site is present after ligation, so the adapter can be removed by MseI before probes transcription. One of the strands is 5'biotinylated, this allows separating strands with P2 adapter ligated using streptavidine beads and discarding fragments with P1 adapters on both ends.

PCR primers

PCR_F: AATGATACGGCGACCACCGAGAT
PCR_F_indexed: AATGATACGGCGACCACCGAGATCTACACxxxxxxxxACACTCTTTCCCTACACGACGC
PCR_R_indexed: CAAGCAGAAGACGGCATACGAGATxxxxxxxxGTGACTGGAGTTCAGACGTGTGC
PCR_R_post: CAAGCAGAAGACGGCATACGAGAT

Depending on the decision to use single or double indexing for your libraries and the probes use the combination of either unindexed forward and indexed reverse or both indexed primers. Use different indexed PCR primer for each sample. These indexed primers should be also different than the ones used for the preparation of genomic libraries to be captured.

For the post-capture PCR use combination of PCR_Fvd and PCR_Rev_post primers.

From 100 µM stock, prepare aliquots of each primer at 5 µM.

Blocking oligonucleotides

The sequences of DNA templates for blocking RNA transcription are listed below for single- and double-indexed libraries. You might need to change the poly-N fragment corresponding to the indices to match the lenth of the index you use.

For double-indexed libraries:

T7.prom: AGTACTAATACGACTCACTATAGG
BO.P5-multiplex:
AATGATACGGCGACCACCGAGATCTACACNNNNNNNNACACTCTTTCCCTACACGACGCTCTTCCGATCTCCTATAGTGAGTCGTATTAGTACT
BO.P7-multiplex:
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTGCCTATAGTGAGTCGTATTAGTACT

For single-indexed libraries:
T7.prom: AGTACTAATACGACTCACTATAGG
BO.P5
AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATTCCTATAGTGAGTCGTATTAGTACT
BO.P7-multiplex:
AGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNNNATCTCGTATGCCGTCTTCTGCTTGCCTATAGTGAGTCGTATTAGTACT

Prepare 200 µM stock. These oligonucleotides are transcribed into RNA blocking oligonucleotides that target one strand of the final libraries as explained in the protocol.

Other solutions to prepare:

- 10 mM Tris-HCl pH 7.5 or PCR-grade water
- EDTA 500 mM
- SDS 10%
- TEN buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 1M NaCl)
- Wash buffer 1 (1x SSC / 0.1% SDS)
- Wash buffer 2 (0.1x SSC / 0.1% SDS)
- 2x Bind and Wash buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl)

Use RNase-free water for all the solutions!

Preparation

1 Choose a few fresh samples coming from different populations (if possible, to capture the diversity of the targeted species). The DNA samples should be diluted to the same concentrations, ideally at 20-50 ng/µl. If you need to sequence the probes library, you should keep the samples separated for the next steps, until they are indexed in a PCR.

Restriction

2 Prepare master mix 1:

- 66 µL water
- 10 µL CutSmart buffer (10x)
- 2 µL MseI (10,000 U/ml)
- 2 µL PstI-HF (20,000 U/ml)

3 Mix 20 µl of DNA with 80 µl of master mix A1 (total volume = 100 µl).

4 Incubate 3 hours at 37°C, hold at 4°C.

5 Perform AMPure cleanup with the beads:sample ratio 2:1 according to the manufacturer's instructions. Resuspend in 20 µl of 10 mM Tris.

Adapter ligation

6 Prepare RAD-P1 adapters. You need to anneal:

1. RAD-P1.1_T7-PstI with RAD-P1.1_T7-Pst2 to obtain RAD-T7-P1 adapter (10 µM)
2. RAD_P2.1_MseI-bio with RAD_P2.2_MseI to obtain RAD-P2 adapter (10 µM)

	reagent	volume [µl]
	first oligonucleotide (100 µM)	10.0
	second oligonucleotide (100 µM stock)	10.0
	RNase-free water	70.0
	Annealing buffer (10x)	10.0

Heat the mix for 1 min at 95°C, cool down to 10°C with ramp 0.1°C/s.

The annealed adapters can be stored long-term at -20°C.

7 Prepare master mix 2:

- 0.2 µL water
- 2.8 µL Ligation buffer
- 2.0 µL T4 DNA ligase (400 U/µl)

8 Mix 19 µl of digested DNA with 2 µl of RAD-T7-P1 adapter (10 µM) and 2 µl of RAD-P2 adapter (10 µM). Briefly vortex and spin.

9 Add 5 µl of master mix 2 to the digested DNA with adapters (total volume = 28 µl). Briefly vortex and spin.

10 Incubate 3 hours at 16°C, hold at 4°C.

Note

There is no need to heat-kill the enzyme as you do not pool the samples until after the PCR step.

11 Perform AMPure cleanup with the beads:sample ratio 1.5:1 according to the manufacturer's instructions. Resuspend in 60 µl of 10 mM Tris.

Size selection

12 Perform Pippin Prep size selection using 30 ul of the sample according to the instructions, keeping another 30 µl in case the size selection fails. You obtain 40 µl of the size selected sample.

Note

Calculate the desired fragment sizes by doing in silico digestion. if you need to guess (i.e. no reference genome), aim for an insert of a mean lenght of 200 bp, a broader range for small genomes, narrower for large genomes. You need to add 90 nt of the technical sequences when calculating the size selection range.

13 Measure concentration and check the profile of the sample using Fragment Analyzer/Tapestation/Bioanalyzer.

Biotinylated fragment selection

14 Mix well Dynabeads C1, make sure that all the beads are resuspended.

15 Dispense 30 µl of beads in a PCR tube (for each capture).

16 Wash 1:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 1 ml of 2x Bind and Wash buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 2 M NaCl).

17 Wash 2:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 1 ml of 2x Bind and Wash buffer.

18 Wash 3:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 40 µl of 2x Bind and Wash buffer.

19 Combine 40 µl of the size-selected ligation product with 40 µl of the beads in 2x Bind and Wash buffer.

20 Incubate 15 min at room temperature with gentle agitation. In the meantime, prepare the 1x wash buffer (see next step).

21 For each sample, prepare 3 ml of 1x Bind and Wash buffer by mixing 1.5 ml of 2x Bind and Wash buffer and 1.5 ml of water.

22 Wash 1:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 1 ml of 1x Bind and Wash buffer.

23 Wash 2:
1. put on the magnetic rack, wait until the beads separate,

2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 1 ml of 1x Bind and Wash buffer.

24 Wash 3:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 1 ml of 1x Bind and Wash buffer.

25 Resuspend:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 30 µl of 10 mM Tris.

PCR amplification

26 Prepare indexed primer solutions (see Before starting). Use different combination of indexed primers for each starting sample.

27 Prepare master mix 3 for 3 PCR reactions:
- 7.0 µL water
- 12.5 µL KAPA HiFi HotStart ReadyMix
- 1.5 µL RAD primer forward (5 µM)
- 1.5 µL RAD primer reverse (5 µM)

28 Mix 2.5 µl of the size selected ligation product with 22.5 µl of master mix 3 (total volume = 25 ul). Prepare 3 PCR reactions for each sample like this.

> Note
>
> Only part of the size selected DNA is used in the PCR reaction. The rest can be used for PCR optimization (see next point) or for more amplification reactions if needed.

29 PCR program for KAPA HiFi HotStart ReadyMix:
3 min at 95°C
15 cycles* of:
- 20 s at 98°C
- 15 s at 60°C
- 30 s at 72°C

5 min at 72°C

hold at 4°C.

* optimize the number of PCR cycles. Overamplification leads to heteroduplex formation, visible on a gel as secondary peaks/bands. Product like this will sequence normally but is harder to quantify. Too many PCR cycles also increase chimera formation and PCR error rate.

30 Pool the triplicates of each sample and perform AMPure cleanup with the beads:sample ratio 1:1 according to the manufacturer's instructions. Elute in 20 µl of 10 mM Tris.

31 Quantify the library concentration using Qubit. Concentration around 10 ng/µl should be enough.

If not enough of probes is obtained, the rest of size-selected library can be amplified or the PCR product can be re-amplified: dilute the purified PCR product 10x and use in PCR reactions.

32 Pool the PCR products obtained from each sample together in equimolar ratios.

Keep a small aliquot for verifying the restriction reaction (step 24) and aliqot for sequencing.

Safety information

At this point, the aliquot of the RAD-seq library can be kept for the sequencing. Keep in mind that the beginning of the reads will have no diversity (the T7 promoter sequence). Pool it with other libraries of high diversity or consider using a few adapters with different length of N-padding before theT7 sequence.

Adapter removal

33 Prepare restriction digest reaction:

	reagent	volume [µl]
	water	3.35
	CutSmart buffer (10x)	1.5
	MseI (10,000 U/ml)	0.15
	PCR product	10.0

34 Incubate for 3 hours at 37°C.

35 Check the restriction by running gel or Fragment Analyzer/Tapestation/Bioanalyzer on the sample before and after the restriction. There should be a visible peak of cut adapters and the main peak should shift accordingly.

In vitro transcription of the libraries into RNA probes

36 In this step HiScribe T7 High Yield RNA Synthesis Kit is used. Usually 1 µl of the template yelds enough RNA. After the first reaction you can scale it up for the amount of probes needed.

Please make sure to read the HiScribeTM T7 Quick High Yield RNA Synthesis Kit Instruction Manual for instructions and troubleshooting.

37 Assemble the reaction:

	reagent	volume [µl]
	10X Reaction Buffer	1.5
	ATP (100 mM)	1.5
	GTP (100 mM)	1.5
	CTP (100 mM)	1.5
	UTP (100 mM)	1.0
	Biotin-16-UTP (10 mM)	5.0
	T7 RNA Polymerase Mix	1.5
	DNA template	1.0
	RNase-free water	5.5

38 Incubate at 37°C overnight (up to 16 h).

39 Add 1 µl TURBO DNase (4 U) and incubate at 37°C for 30 minutes.

40 Purify the RNA using RNEasy Mini kit (Qiagen) using standard protocol except adding 665 µl EtOH to the RNA + RTL mix before loading on the column and elute in 30 µl of RNase-free water.

41 Quantify using Qubit RNA kit. You can check the RNA size using Fragment analyzer/Tapestation or by TBE/Urea gel electrophoresis.

42 Dilute the RNA probes to around 100 ng/µl.

Add 1 µl of SUPERase-In for each 19 µl of the probes.
Store at −80°C if not used immediately.

Blocking RNA oligonucleotides synthesis

43 Anneal the oligonucleotides used as the template for T7 polymerase reaction. You need to anneal:

1. blocking RNA template for P5 adapter with T7 promoter,
2. blocking RNA template for P7 adapter with T7 promoter.

	reagent	volume [µl]
	one of the two blocking oligos template (200 µM)	12.5
	T7.prom oligo (200 µM)	12.5
	RNase-free water	20.0
	Annealing buffer (10x)	5.0

Heat the mix 1 min at 95°C, cool down to 10°C with ramp 0.1°C/s.

The annealed template can be stored long-term at -20°C.

44 Assemble the reaction for each annealed oligonucleotides pair:

	reagent	volume [µl]
	RNase-free water	10
	10X Reaction Buffer	1.5
	ATP (100 mM)	1.5
	GTP (100 mM)	1.5
	CTP (100 mM)	1.5
	UTP (100 mM)	1.5
	T7 RNA Polymerase Mix	1.5
	Annealed template oligonucleotides	1

45 Incubate at 37°C overnight (up to 16 h).

46 Add 2 µl TURBO DNase (4 U) and incubate at 37°C for 30 minutes.

47 Purify the RNA using RNEasy Mini kit (Qiagen) using standard protocol except adding 665 µl EtOH to the RNA + RTL mix before loading on the column and elute in 30 µl of RNase-free water.

48 Quantify again using Qubit RNA kit. You can check the RNA size using Fragment analyzer/Tapestation or by TBE/Urea gel electrophoresis.

49 Adjust the concentration of each blocking RNA to 200 µM. This corresponds to the following concentrations:

1. BO.P5: 3129 ng/µl
2. BO.P5-multiplex: 4192 ng/µl
3. BO.P7-multiplex: 4366 ng/µl

Add 1 µl of SUPERase-In for each 19 µl of the final probes volume

Store at −80°C if not used immediately.

Safety information

Perform more reactions and concentrate the RNA using RNEasy Mini kit if not enough blocking RNA is obtained.

50 Mix the same amount of both blocking RNA oligonucleotides (BO.P5 and BO.P7-multiplex for single-indexed libraries or BO.P5-multiplex and BO.P7-multiplex for double-indexed libraries). The final concentration of each will be 100 µM.

Hybridization reaction

51 Prepare the **hybridization mix in the PCR tubes**. Probes and blocking oligos are used in excess. So far, we tested the protocol using 500 ng of the genomic libraries and the same amount of the probes. For rare targets (aDNA for instance), you can increase library amount up to 2000 ng.

The below mixes are based on MyBaits protocol (http://www.mycroarray.com/pdf/MYbaits-manual-v3.pdf) and prepared in a bit larger quantities to account for evaporation and for easier handling.

Assemble the hybridization mix on ice:

	reagent	volume [µl]
	SSPE (20x)	9.0
	EDTA (500 mM)	0.5
	SDS (10%)	0.5
	Denhardt's solution (50x)	3.5
	SUPERase-In (20 U/µL)*	1
	hyRAD RNA probes (1000 ng)*	5.5

*keep on ice

Total volume = 20.0 µl, of which 18.0 µl is used in the hybridization.

52

Prepare the **blocking mix**:

	reagent	volume [µl]
	Human Cot-1 DNA (1 mg/ml)	2.5
	Salmon sperm DNA (1 mg/ml)	2.5
	Mix of blocking RNA oligos	0.5

Total volume = 5.5 µl, of which 5.0 µl si used in the hybridization.

Note

Do not use Human Cot-1 for DNA capture on plants; use 5 µl of Salmon sperm DNA instead. If available, substitute human Cot-1 DNA for the closest to your species of interest.

53 Put 7 µl of the prepared Illumina **library in the PCR tube** (500 ng), add 5 µl of the **blocking mix**. Mix by pippeting.

54 Program the termocycler for two steps (both steps with a heated lid):
1. 5 min at 95°C,
2. 16-24 h at hybridization temperature (see below)

Usual hybridization temperature is 65°C. For rare targets like aDNA, incubation can last for up to 40 hours at lower temprature of 60 or 55°C.

55 Incubate the **library + blocking mix** 5 min at 95°C (1st step).

56 When thermocycler temperature decrease to the hybridization temperature (2nd step), put the tubes with the **hybridization mix** in the thermocycler, incubate the library and hybridization mix for 5 more minutes at 65°C.

57 Still at the hybridization temperature, add 18 µl of the **hybridization mix** to the **library+blocking mix** and mix by pippeting. Total volume is now 30 µl. Do not take the mixture out from the PCR machine to vortex or centrifuge at this step.

58 Incubate at the hybridization temperature for 16-24 hours (up to 40 hours and lower temperature for rare targets like aDNA).

Preparation of Dynabeads

59 Mix well Dynabeads C1, make sure that all the beads are resuspended.

60 Dispense 30 µl of beads in a PCR tube (for each capture).

61 Wash 1:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 200 µl of TEN.

62 Wash 2:
1. put on the magnetic rack, wait until the beads separate,
2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 200 µl of TEN.

63 Wash 3:
1. put on the magnetic rack, wait until the beads separate,

2. remove and discard supernatant,
3. take the tube off the magnetic rack,
4. resuspend in 70 µl of TEN.

64 Store at room temperature until use.

Washes

65 Prepare the aliquots of the wash buffers and Dynabeads in TEN and prewarm them to the hybridization temperature in a thermocycler.

66 Keeping the hybridization reaction in a thermocycler at the hybridization temperature with the heated lid, add 70 ul of the Dynabeads in TEN to the 30 µl of the hybridization reaction and mix well by pipetting.

67 Incubate for 30 min at the hybridization temperature in a thermocycler.
00:30:00 incubation with Dynabeads

68 Wash 1:
1. put on the magnetic rack, wait until the beads separate,
2. remove supernatant,
3. resuspend beads in 180 µl of 1x SSC / 0.1% SDS prewarmed to the hybridization temperature,
4. mix well by vortexing, briefly centrifuge.

69 Incubate for 15 min at the hybridization temperature in a thermocycler.
00:15:00 incubation after the 1st wash

70 Wash 2:
1. put on the magnetic rack, wait until the beads separate,
2. remove supernatant,
3. resuspend beads in 180 µl of 0.1x SSC / 0.1% SDS prewarmed to the hybridization temperature,
4. mix well by vortexing, briefly centrifuge.

71 Incubate for 10 min at the hybridization temperature in a thermocycler.
00:10:00 incubation after the 2nd wash

72 Wash 3:
1. put on the magnetic rack, wait until the beads separate,
2. remove supernatant,
3. resuspend beads in 180 µl of 0.1x SSC / 0.1% SDS prewarmed to the hybridization temperature,

4. mix well by vortexing, briefly centrifuge.

73 Incubate for 10 min at the hybridization temperature in a thermocycler.
00:10:00 incubation after the 3rd wash

74 Wash 4:
1. put on the magnetic rack, wait until the beads separate,
2. remove supernatant,
3. resuspend beads in 180 µl of 0.1x SSC / 0.1% SDS prewarmed to the hybridization temperature,
4. mix well by vortexing, briefly centrifuge.

75 Incubate for 10 min at the hybridization temperature in a thermocycler.
00:10:00 incubation after the 4th wash

76 If you use Kapa HiFi Hot Start polymerase mix in the next steps you can keep the captured DNA on the beads and proceed to step 68.

Otherwise elute the DNA from the probes:
1. put on the magnetic rack, wait until the beads separate,
2. remove supernatant,
3. add 30 µl of 95°C 10 mM Tris,
4. mix well by pipetting.

77 Incubate for 10 min, 95°C in a thermocycler.
00:05:00 DNA elution incubation

78 Put on the magnet; remove and **retain the supernatant**.
This supernatant contains the hybridization-enriched products!
Discard the beads.

Post-capture library amplification

79 Prepare re-amplification master mix. The volumes are per one capture-enriched library:

	A	B
	reagent	volume [µl]
	water	7.5
	KAPA HiFi HotStart ReadyMix	25.0
	PCR_F primer (5 µM)	5.0
	PCR_R_post primer (5 µM)	5.0

80 Mix 42.5 µl of the master mix with 7.5 µl of the capture-enriched library.

81 PCR program for KAPA HiFi HotStart ReadyMix:
3 min at 95°C
15 cycles* of:
- 20 s at 98°C
- 15 s at 60°C
- 30 s at 72°C

5 min at 72°C
hold at 4°C.

* optimize the number of PCR cycles to obtain enough number of molecules for sequencing while using as few PCR cycles as possible.

82 Purify with AMPure using beads:sample ratio 1:1, elute in 30 µl of 10 mM Tris.

83 Verify the library profile and molarity using Fragment Analyzer/Tapestation/Bioanalyzer. Libraries can now be pooled in equimolar ratios.

Your libraries are now ready to be sent for sequencing, congratulations!