

Protocol of construction of Blunt-End Illumina Libraries used in Twort et al. Museomics of a rare taxon: placing Whalleyanidae in the Lepidoptera Tree of Life

The original single index blunt-end Illumina library construction protocol was a modified version of Meyer and Kircher 2010 (Meyer oligos and NEBNext E6070L kit), provided by Nicolas Dussex (contact: nicolas.dussex@gmail.com) and subsequently modified by Victoria Twort (contact: vtwort@gmail.com)

This method can be used with existing DNA extracts, highly degraded DNA input, and low concentration samples. The protocol has successfully been used with samples varying in concentration from a few pg up to 1 µg of input DNA. Ideal input fragment size is < 250 bp, although less fragmented DNA can be sonicated prior to library preparation.

Two alternative magnetic bead size selection protocols are included. The data in the above mentioned manuscript was cleaned using the AMPURE beads, however subsequent sequencing runs have used the alternative speedbead protocol.

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Key Considerations:

1. Use low-binding Eppendorf tubes for all library preparation steps.
2. Avoid keeping finished libraries out of the fridge for too long, and avoid freeze thaw cycles.
3. IS1, IS2, IS3 should be made at a stock concentration of 500 μM .
4. Indexed primers stock should be 100 μM , then make 1:10 dilutions to working stock (10 $\mu\text{M}/\mu\text{L}$), this working solution cannot be kept.
5. Avoid vortexing solutions after adding enzymes, rather mix by pipetting
6. If you are only using single-end indexing the 2nd index primer should be IS4. However, its recommended to use a dual indexing approach.
7. I recommend aliquoting out the ATP into smaller volumes, to avoid freeze thaw cycles. Also, light damages the ATP as well, so make sure you cover it with foil when removing from freezer.
8. When setting up reactions, do so on a cooling block, but be sure the block does not freeze the reaction

DNA Extraction:

- DNA extractions were carried out with the Qiagen QIAamp DNA Micro Kit (Cat No. 56304).
- Abdomens were used, and were up to 8 mm in size, this protocol has also been used on small amounts of leg tissue.
- The 'Protocol: Isolation of Genomic DNA from Tissues' was used with some modifications:
 - Because we wanted the abdomens to be available for genitalia dissection crushing was not carried out. Rather the abdomens were simply incubated in the ATL buffer + proteinase K mix.
 - Incubations were carried out overnight.
 - After incubation samples were briefly centrifuged to collect abdomens at the bottom of the tubes, and supernatant transferred to new tube and the extraction process carried out as normal.
 - During DNA elution, the buffer was left on the column to incubate for 20 minutes prior to centrifugation.

The modified extraction protocol is detailed below:

1. Equilibrate Buffer AE to room temperature.
2. Set heating block or water bath to 56°C.
3. If buffer AL or ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
4. Ensure buffers AW1 and AW2 have had ethanol added to them, and mix by shaking.
5. Place sample in clean 1.5 mL Eppendorf, and add **180 µL** of buffer ATL, equilibrate to room temp.
6. Add 20 µl proteinase K and mix by pulse-vortexing for 15 s.
7. Incubate at 56°C overnight, incubations of 2 – 3 hours have also worked well.
8. Pulse spin tubes to collect abdomens at bottom, and transfer supernatant to a new Eppendorf tube (this will be what will be taken forward).
9. Add **200 µl** Buffer AL and mix by pulse-vortexing for 15s.
10. Add **200 µl** ethanol (96 – 100%) and mix by pulse-vortexing for 15 s.

11. Incubate at room temperature for 5 minutes.
12. Briefly centrifuge to remove drops from the lid.
13. Carefully transfer the entire lysate to the QIAamp MinElute column in a 2 ml collection tube, without wetting the rim. Close the lid and centrifuge at 6,000 xg (8,000 rpm) for 1 min.
14. Place column in a clean collection tube, discarding the collection tube containing the flow-through.
15. Carefully open the column and add **500 µl** Buffer AW1, without wetting the rim. Close lid, and centrifuge at 6,000 xg (8,000 rpm) for 1 min. Place column in a new collection tube, discard collection tube containing flow-through.
16. Add **500 µl** of Buffer AW2 to the column without wetting the rim, close lid and centrifuge at 6,000 xg (8,000 rpm) for 1 min. Place in a new collection tube, discarding collection tube containing flow-through.
17. Centrifuge at full speed (14,000 rpm / 20,000 xg) for 3 minutes to dry the membrane completely.
18. Place the column in a clean Eppendorf tube discarding tube containing the flow-through.
19. Carefully open the lid and apply **25 µl** Buffer AE to the center of the membrane, close the lid.
20. Incubate at room temperature for 20 minutes, before centrifuging at full speed (14,000 rpm / 20,000 xg) for 1 minute.
21. Open the lid and apply an additional **25 µl** of buffer AE to the center of the membrane, close the lid and incubate 20 minutes at room temperature.
22. Spin at full speed (14,000 rpm / 20,000 xg) for 1 minute.

DNA Quality Check and Sonication:

- The ideal size range for the protocol is fragment lengths < 300 bp in size, although longer fragments have also been used.
 - Due to the mixed nature of samples we process, we always check the fragmentation of our DNA before library prep.
1. Run 2 µl DNA on 0.8% w/v agarose gel stained with SYBR safe (Fisher Scientific: S33102), along with the 1kb+ DNA ladder (Fisher Scientific: 10787018) as the size standard.
 2. Based on the size of the DNA fragments, determine whether or not sonication is necessary and in case it is necessary for how long each sample should be sonicated.
 3. Follow the recommendations of the available sonicator to reach a final DNA range of 200–300 bp. Here we used the Bioruptor Sonicator so we proceeded as following:
 - a. At least 1 hour of precooling with ice is require, and max of 12 samples per run.
 - b. For very high MW DNA: ~ 45 minutes.
 - c. For mid-range: ~ 30 minutes.
 - d. Something in between mid-range and 200 – 300 bp: ~ 10 mins.
 4. In the sonication tubes, place the desired volume of DNA, half the extraction volume (~20 µl) was used to ensure we had DNA left over. Make up to a final volume of 100 µl with milliQ.
 5. Add precooled milliQ to the sonicator until the water fill line, ensure that there isn't too much ice left.
 6. Add tubes to the rotor, place in machine. Ensure sonicator is set to 30/90 (on/off), and M intensity.
 7. Turn on for desired time, ensure you place the warning signs on the door, and if staying you must wear ear protection.
 8. Once time is up, remove samples from sonicator. If doing additional rounds, place more ice in the container and wait to cool some more before continuing.
 9. Transfer the sample to clean low-bind eppendorf tube, and vacuum centrifuge until dry.
 10. Resuspend sample in **50 µl** milliQ, leave in fridge overnight or RT for a few hours to ensure resuspension.
 11. Rerun samples out on a 0.8% w/v gel to ensure desired fragmentation level has been reached.

Buffer and Reagent Preparation:

- Oligo Hybridisation Buffer is made once, and then aliquoted out.

1. Make Oligo Hybridisation Buffer (10X)

Reagent	Concentration	Volume
NaCl	500 mM	19.76 mL when stock is 0.5 M
Tris-Cl pH 8	10 mM	0.2 mL when stock is 1 M
EDTA pH 8	1 mM	0.04 mL when stock is 0.5 M

2. Make P5 hybridisation mix (200 µM) as follows:

Reagent	Final concentration	Volume (µL)
IS1_adapter_P5.F (500 µM)	200 µM	40
IS3_adapter_P5+P7.R (500 µM)	200 µM	40
Oligo Hybridization Buffer (10X)	1X	10
ddH2O		10

3. Make P7 hybridisation mix (200 µM) as follows:

Reagent	Final concentration	Volume (µL)
IS2_adapter_P7.F (500 µM)	200 µM	40
IS3_adapter_P5+P7.R (500 µM)	200 µM	40
Oligo Hybridization Buffer (10X)	1X	10
ddH2O		10

4. Incubate each reaction mix for 10 sec at 95°C, followed by a ramp from 95°C to 12°C at a rate of 0.1°C/sec (eg. -1°/10sec).
5. Combine the P7 and P5 reaction mixes, to get a ready-to-use 100 µM adapter stock mix. Aliquot into smaller volumes and store in the freezer. (Known as Oligo hybridization mix).

DNA Blunt-End Repair

1. Remove reagents from freezer, ensure ATP is covered with foil, and all enzymes are in the cold block.
2. Prepare the master mix as follows for the desired number of reactions:

Reagent	Final Concentration	x1 (µl)
Tango Buffer (10X)	1X	4
dNTPs (25 mM)	100 µM	0.16
ATP (100 mM)	1 mM	0.4
T4 PNK (10 U/µL)	0.5 U/µL	2
T4 DNA Polymerase	0.1 U/µL	0.8
MilliQ		12.64

3. Add **20 µl** master mix to clean labelled tubes.
4. Add **20 µl** Sonicated DNA to each reaction, mix by pipetting (total rxn volume: 40 µL).
5. Briefly spin tubes.
6. Incubate 15 min at 25°C, followed by 5 min at 12°C.
7. While incubating, take MinElute Columns from fridge AND turn on incubator to 37°C.
8. Place on ice or immediately proceed to the next step.

MinElute Reaction Clean-Up

1. Add **40 µL** reaction from above step to new labelled low-bind eppendorf tubes.
2. Apply **200 µL** PB buffer, mix briefly by pipetting.
3. Add to column (total volume of 240 µL).
4. Spin 13,000 rpm for 1 min.
5. Discard waste and change to new collection tube.
6. Add **700 µL** of PE Buffer to column.
7. Spin 13,000 rpm for 1 min.
8. Discard waste and change to new collection tube.
9. Add **700 µL** of PE Buffer to column.
10. Spin 13,000 rpm for 1 min.
11. Discard waste and change to new collection tube.
12. Spin 13,000 rpm for 1 min to dry column.
13. Change to new labelled low-bind eppendorf tube.
14. Leave column with open lid (in the fume hood) for 5 min to dry out column.
15. Add **22 µL** of EB buffer to column.
16. Incubate 5 min at 37°C.
17. Elute DNA by spinning down for 1 min at 13,000 rpm.

Adapter Ligation

1. Remove reagents from freezer. Check the buffer has no precipitate after thawing, if present warm to 37°C and vortex (only the buffer) until precipitate is dissolved.
2. Oligo hybridisation mix needs to be diluted 1:10 before adding to master mix. Note: This dilution needs to be made fresh each time.

3. Prepare master mix as follows for desired number of reactions:

Reagent	Final Concentration	X1 μ L
T4 DNA Ligase Buffer (10X)	1X	4
PEG-4000 (50%)	5%	4
Oligo hybridization mix dilution	10 pmol	1
T4 DNA Ligase (5U/ μ L)	0.125 U/ μ L	1
MilliQ		10

4. Add **20 μ l** master mix to new tubes (small tubes for thermocycler).
5. Add **20 μ l** of cleaned DNA from previous MinElute clean up step (total rxn volume: 40 μ l).
6. Incubate for 30 min at 22°C
7. While this is running, remove MinElute Columns from fridge, so they are room temperature for next step AND Turn on Incubator to 37°C.

MinElute Reaction Clean-Up 2

1. Add **40 µL** reaction from above step to new labelled low-bind eppendorf tubes.
2. Apply **200 µL** PB buffer, mix by pipetting.
3. Add to column.
4. Spin 13,000 rpm for 1 min.
5. Discard waste and change to new collection tube.
6. Add **700 µL** of PE Buffer to column.
7. Spin 13,000 rpm for 1 min.
8. Discard waste and change to new collection tube.
9. Add **700 µL** of PE Buffer to column.
10. Spin 13,000 rpm for 1 min.
11. Discard waste and change to new collection tube.
12. Spin 13,000 rpm for 1 min to dry column.
13. Change to new labelled low-bind eppendorf tube.
14. Leave column with open lid for 5 min to dry out column.
15. Add **22 µL** of EB buffer to column.
16. Incubate 5 min at 37°C.
17. Elute DNA by spinning down for 1 min at 13,000 rpm.

Adapter Fill-In

1. Remove reagents from freezer, ensure the thermopol reaction buffer has not precipitate before starting, if precipitates are present warm to 37°C and vortex.
2. Prepare the following master mix for the desired number of reactions:

Reagent	Final Concentration	X1 (µL)
Isothermal Amp buffer (10X)	1X	4
dNTPs (25 mM)	250 µM	0.4
Bst Polymerase, LF (8U/µL)	0.3 U/µL	1.5
MilliQ	---	14.1

3. Add **20 µL** master mix to new clean labelled tubes.
4. Add **20 µL** DNA from previous clean up step, mix briefly by pipetting, spin briefly (total reaction volume: 40 µL).
5. Incubate **20 min 37°C**, followed by **80°C for 20 min** (heat kill) (Saved as Fill-In).

Safe Stopping point – Libraries can be stored in the freezer overnight or longer until ready to do the next step.

Indexing PCR Test

1. Remove Index combinations from the freezer. Prepare 1:10 dilutions. These cannot be kept overnight. (indexes and combinations are given in Index_adapter_sequences.xls. All 60 unique dual index pairs are compatible with the Novaseq).
2. Prepare the following PCR master mix for the desired number of reactions, and 1 negative PCR.

Reagent	Final Concentration	X1 (μL)
AccuPrime reaction mix (10X)	1X	2.5
AccuPrime Pfx	---	0.5
milliQ		16.75

3. Add **20.5 μL** Mix master to each tube.
4. Add **0.75 μL** of diluted Index Primer 1 (10 μM).
5. Add **0.75 μL** of diluted Index Primer 2 (10 μM).
6. Add **3 μL** DNA from previous step (place the rest back in the freezer as you will need it again).
7. Spin tubes down.
8. Use following thermal cycler program, for my first test I always start at 15 cycles, this is in optimized for subsequent PCR reactions.

Step	Temperature (°C)	Time	
Initial Denaturation	95	2 min	
Denaturation	95	15 sec	15 cycles*
Annealing	60	30 sec	
Elongation	68	60 sec	
Hold	4	∞	

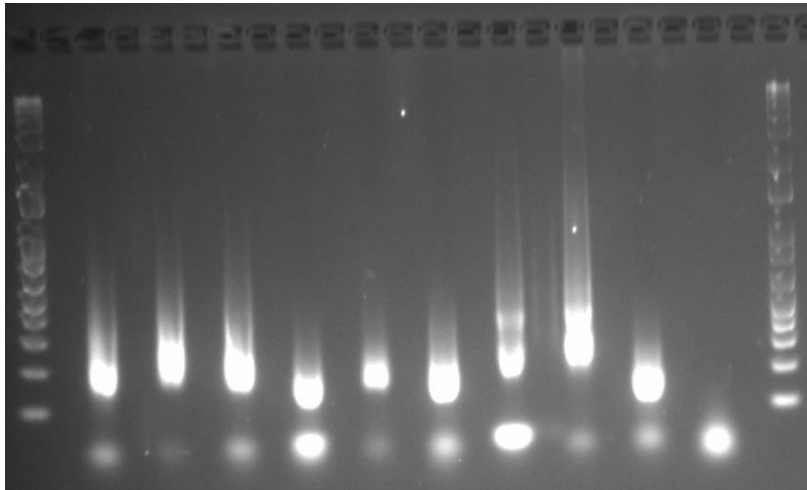
9. Make 1.2% w/v agarose gel, run 5 μl of PCR product on gel at 100V for 2 hours, ensure you also run 1kb+ ladder for sizing. It's important you can determine the size of the library and you can distinguish between primer and library bands for highly degraded samples.

10. Based on the brightness of the library determine how many cycles for the next round.

- a. If too bright, reduce to 12.
- b. If very weak/non-existent increase to 18.
- c. If looks ok leave at 15.

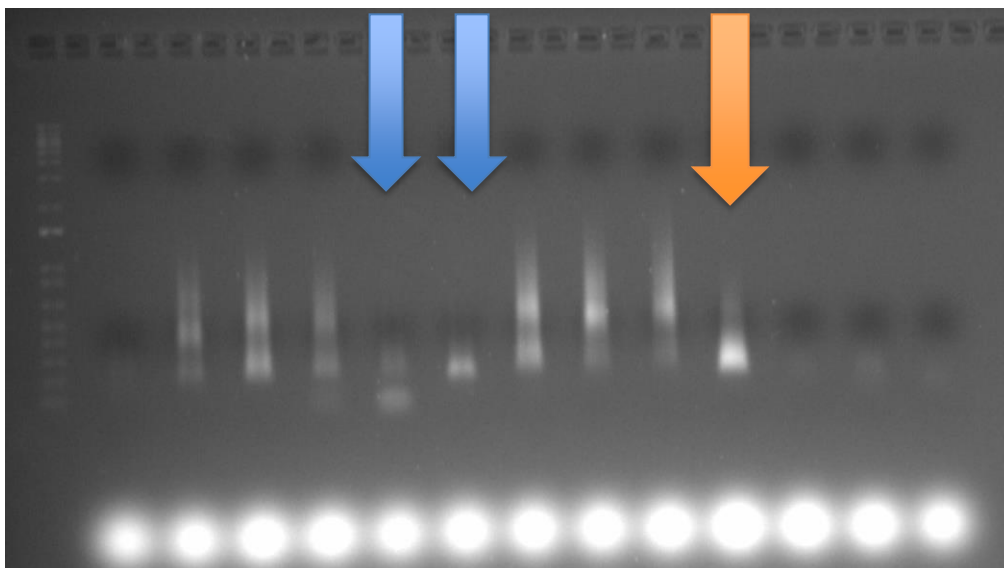
Example libraries are shown below:

In the samples below I would reduce the number of cycles:



The blue arrows indicate samples for which I would increase the number of cycles

The orange arrow indicates samples for which I would leave the number of cycles the same



Indexing PCR

1. If new index dilutions are needed, prepare first.
2. For each sample we are now preparing 5 independent PCR reactions with the same primer combinations used in the Index PCR test.
3. Prepare PCR master mix.

Reagent	Final Concentration	x1 (µL)
AccuPrime reaction mix (10X)	1X	2.5
AccuPrime Pfx	---	0.5
milliQ		16.75

4. Add **20.5 µL** Mix master.
5. Add **0.75 µL** of Index Primer 1 dilution (10 µM).
6. Add **0.75 µL** of Index Primer 2 dilution (10 µM).
7. Add **3 µL** DNA from the Adapter fill in step.
8. Use following thermal cycler program! ***Run the number of cycles you determined were optimum in the test!**

Step	Temperature (°C)	Time	
Initial Denaturation	95	2 min	
Denaturation	95	15 sec	
Annealing	60	30 sec	15 cycles *
Elongation	68	60 sec	
Hold	4	∞	

Size Selection and Purification Option 1 (Ampure beads)

IMPORTANT: Always use clean fresh 70% ethanol from clean and pure ethanol and thoroughly vortex before use

1. Remove AMPURE beads from fridge at least 30 mins before starting
2. Prepare TE-tween mix by taking 800 μL UV-treated TE buffer, and adding 40 μL 1% Tween. Cover eppendorf with foil (This is enough for ~22 samples, if making more increase volume).
3. Prepare fresh 70% Ethanol.
4. For each sample pull together all 6 PCR reactions (1 from the Index PCR test, and 5 from the Indexing PCR) in a clean low bind tube and mix.
5. Allocate **100 μL** of PCR product to another tube low bind tube (this will be what you carry forward).
6. Add **50 μL** Ampure beads to PCR product (0.5X volume), ensure the beads are well mixed before adding (this is critical).
7. Vortex, pulse spin and incubate at room temperature for 5 minutes.
8. Place tubes on magnetic rack for 1 minute (or until all beads have migrated to wall and supernatant is clear).
9. Carefully remove supernatant to new low binding tube without disturbing the pellet.
10. The pellet contains long fragments that are likely contaminants .
11. Add **270 μL** Ampure beads to supernatant (1.8X volume, again ensure bead mixture is mixed before adding, crucial step).
12. Vortex, pulse spin, and incubate at room temperature for 10 minutes.
13. Place tube on rack and incubate for 3 minutes or until beads have migrated to wall and supernatant is clear.
14. While tubes are on the magnetic rack, discard supernatant, keeping the pellet (beads are attached to the fragments you want).

15. While tubes are still on the rack, add **500 µL** 70%ETOH, leave for 1 min, then remove by pipetting.
16. Add **500 µL** 70 % ETOH, leave 1 min and remove by pipetting.
17. Add **500 µL** 70 % ETOH, leave 1 min and remove by pipetting.
18. Remove from rack, and very quick pulse spin to collect ethanol at bottom of tube.
19. Place back on magnetic rack, when beads have migrated to wall, open carefully to stop ethanol moving, and remove any excess with small pipette
20. Leaving the tubes open on the rack, air dry to remove all ethanol. This step is critical and any carryover ethanol will interfere with downstream sequencing etc. The pellet should look like mud when dry, if it is shiny wait until it is no longer. It is better to overdry your samples rather than underdry.
21. Add **36 µL** EB-tween buffer, remove from the rack and mix by pipetting.
22. Vortex for 20 sec.
23. Pulse spin and incubate at room temperature for 5 minutes.
24. Place on magnetic rack for 5 minutes or until the supernatant is clear.
25. Remove supernatant and transfer to clean labelled low bind tube, being careful not to disturb the beads. If you disturb the beads, pulse spin and place back on magnetic rack until supernatant is once again clear, and then remove. It is crucial you don't carry over the beads.
26. The supernatant in the new tube contains your final library. Store in the freezer until being sent off or pooling.

Size Selection and Purification Option 2 (SpeedBeads)

To use this method, you first need to create a workable solution of speedbeads (See Speedbead Preparation).

IMPORTANT: Always use clean fresh 80% ethanol from clean and pure ethanol and thoroughly vortex before use.

1. Remove Speedbeads beads from fridge at least 30 mins before starting
2. Prepare TE-tween mix by taking 800 μL UV-treated TE buffer, and adding 40 μL 1% Tween. Cover eppendorf with foil (This is enough for ~22 samples, if making more increase volume).
3. Prepare fresh 80% Ethanol.
4. For each sample pull together all 6 PCR reactions (1 from the Index PCR test, and 5 from the Indexing PCR) in a clean low bind tube and mix.
5. Allocate **100 μL** of PCR product to another tube low bind tube (this will be what you carry forward).
6. Add **50 μL** Speedbeads beads to PCR product (0.5X volume), ensure the beads are well mixed before adding (this is critical).
7. Vortex, pulse spin and incubate at room temperature for 5 minutes.
8. Place tubes on magnetic rack for 1 minute (or until all beads have migrated to wall and supernatant is clear).
9. Carefully remove supernatant to new low binding tube without disturbing the pellet.
10. The pellet contains long fragments that are likely contaminants.
11. Add **270 μL** Speedbeads beads to supernatant (1.8X volume, again ensure bead mixture is mixed before adding, crucial step).
12. Vortex, pulse spin, and incubate at room temperature for 10 minutes.
13. Place tube on rack and incubate for 3 minutes or until beads have migrated to wall and supernatant is clear.

14. While tubes are on the magnetic rack, discard supernatant, keeping the pellet (beads are attached to the fragments you want).
15. While tubes are still on the rack, add **500 µL** 80%ETOH, leave for 1 min, then remove by pipetting.
16. Add **500 µL** 80 % ETOH, leave 1 min and remove by pipetting.
17. Add **500 µL** 80 % ETOH, leave 1 min and remove by pipetting.
18. Remove from rack, and very quick pulse spin to collect ethanol at bottom of tube.
19. Place back on magnetic rack, when beads have migrated to wall, open carefully to stop ethanol moving, and remove any excess with small pipette.
20. Leaving the tubes open on the rack, air dry to remove all ethanol. This step is critical and any carryover ethanol will interfere with downstream sequencing etc. The pellet should look like mud when dry, if it is shiny wait until it is no longer. It is better to overdry your samples rather than underdry.
21. Add **36 µL** EB-tween buffer, remove from the rack and mix by pipetting.
22. vortex for 20 sec.
23. Pulse spin and incubate at room temperature for 5 minutes.
24. Place on magnetic rack for 5 minutes or until the supernatant is clear.
25. Remove supernatant and transfer to clean labelled low bind tube, being careful not to disturb the beads. If you disturb the beads, pulse spin and place back on magnetic rack until supernatant is once again clear, and then remove. It is crucial you do not carry over the beads.
26. The supernatant in the new tube contains your final library. Store in the freezer until being sent off or pooling.

Speedbead Preparation

We prepare speedbeads using the protocol by Faircloth & Glenn (17 May 2016) from

<https://baddna.uga.edu/protocols.html>

This work above was in turn derived from the following referenced protocol, Rohland N, Reich D. **Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture.** Genome Research 22: 939-946.

1. In a 50 mL sterile falcon tube combine **500 µL** 1 M Tris pH8 and **100 µL** 0.5 M EDTA.
2. Make up to 50 mL with dH₂O giving you a TE solution of 10 mM Tris-HCO, 1 mM EDTA.
3. Mix the Sera-mag SpeedBeads well and transfer **1 mL** to a 1.5 mL Eppendorf tube.
4. Place SpeedBeads on magnet rack until the supernatant is clear.
5. Remove supernatant with pipette being careful not to disturb the beads and discard.
6. Add **1 mL** TE solution prepared above to beads, then remove from magnet.
7. Mix beads solution, then return to magnet.
8. Remove supernatant with pipette.
9. Add **1 mL** TE to beads, remove from magnet, mix, return to magnet.
10. Remove supernatant with pipette.
11. Add **1 mL** TE to beads and remove from magnet.
12. Fully resuspend and set microtube in rack (i.e. not on magnet stand).
13. To a new sterile falcon tube add **9 g** PEG-8000.
14. To the PEG add **10 mL** 5 M NaCL to falcon tube.
15. Followed by **500 µL** 1 M Tris-HCL to falcon tube.
16. Followed by **100 µL** 0.5 M EDTA to falcon tube.

17. Fill falcon tube to ~ 49 mL using sterile dH₂O. You can do this by eye, just go slowly.
18. Mix falcon tube for about 3-5 minutes until PEG goes into solution (solution, upon sitting, should be clear).
19. Add **27.5 uL** Tween 20 to falcon tube and mix gently.
20. Mix the 1 mL SpeedBead + TE solution that has been sitting in your rack and transfer this to the 50 mL falcon tube
21. If the falcon tube is not at the 50 mL mark, fill up with dH₂O and gently mix 50 mL conical until brown.
22. Wrap in tinfoil (or place in dark container) and store at 4°C

Reagent Product Codes

Product	Product Code	Product Company
T4 DNA Polymerase (150 Units)	M0203S	NEB/Bionordika
T4 Polynucleotide Kinase (500 Units)	M0201S	NEB/Bionordika
T4 DNA Ligase	NEB M0202	NEB/Bionordika
Bst 3.0 Warmstart Polymerase (1.6 Units)	M0538S	NEB/Bionordika
Tango Buffer (10X)	11541505	Fisher Scientific
AccuPrime Pfx (200 rxn)	12344024	Fisher Scientific
Tris-Cl pH8 (100 ml)	10259194	Fisher Scientific
Agencourt AMPure XP (5 ml or 60 mL)	A63880	Beckman Coulter
MinElute PCR clean up kit (250 rxns)	28006	Qiagen
dNTPs	DNTP100-KT	Sigma-Aldrich
ATP	000000011140965001	Sigma-Aldrich
PEG-4000	1546569-1G	Sigma-Aldrich
Tween-20	P9416	Sigma-Aldrich
TE buffer	93283-100mL	Sigma-Aldrich
NaCl solution	71386-1L	Sigma-Aldrich
Lowbind eppendorf tubes	525-0130	VWR
1 kb plus ladder	10787018	Fisher Scientific
PEG-8000	1546605	Sigma-Aldrich
Sera-mag SpeedBeads	09-981-123	Fisher Scientific

Adapter Primer Sequences and Indexes

- IS4 is only needed if you are NOT dual indexing

Name	Sequence (a 5'-3'; * indicates a PTO bond)
IS1_adapter_p5.F	A*C*A*C*TCTTTCCCTACACGACGCTCTTCCG*A*T*C*T
IS3_adapter_p5+P7.R	A*G*A*T*CGGAA*G*A*G*C
IS2_adapter_P7.F	G*T*G*A*CTGGAGTTCAGACGTGTGCTCTTCCG*A*T*C*T
IS4_adapter_P5+P7.R	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTT

All 120 Index sequences and combinations are given in the file: Index_adapter_sequences.xls