Aug 13, 2025

High-throughput wasp larvae gut content metabarcoding

Forked from High-throughput individual insect metabarcoding for identification and interaction data

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyxzmpgx9/v1

Romuald Tcheutchoua[1], Jordan P Cuff[2], Seirian Sumner[1]

[1]University College London; [2]Newcastle University

Foraging Ecology Resea...

Jordan P Cuff
Newcastle University

DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyxzmpgx9/v1

Protocol Citation: Romuald Tcheutchoua, Jordan P Cuff, Seirian Sumner 2025. High-throughput wasp larvae gut content metabarcoding. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyxzmpgx9/v1

Protocol status: Working
We use this protocol and it's working

Created: March 25, 2025

Last Modified: August 13, 2025

Protocol Integer ID: 124971

Keywords: metabarcoding, biomonitoring, entomology, high-throughput sequencing, community ecology, field techniques, throughput wasp larvae gut content, dna from individual wasp larvae, individual wasp larvae, extracting dna, throughput method for molecular analysis, dna, molecular analysis

Abstract

This protocol is designed for extracting DNA from individual wasp larvae for metabarcoding to detect and identify their trophic interactions. The reagents and methods proposed offer a cost effective and high-throughput method for molecular analyses of individual wasp larvae using specialised lab equipment, with standard alternatives signposted throughout. Where specialist equipment is used, attempts are made to suggest low-cost alternatives.

Image Attribution

Created in BioRender. Cuff, J. (2025) https://BioRender.com/559f6hh

Materials

For field collection and initial storage:

- Small collection pots or tubes for storage of samples
- 100 % ethanol for preservation
- Chemgene/diluted bleach for sterilisation of equipment

For DNA extraction:

- Dissection tools (e.g., scalpel, scissors and Petri dishes)
- Hardened carbon steel ball bearings
- 2.2 mL deep well plates for initial lysis
- Deep-well and standard Kingfisher 96-well plates
- Plate seals for long-term storage

For DNA amplification and subsequent steps:

- Tagged PCR primers
- 2X hot-start Taq polymerase mastermix
- Molecular grade water
- 96-well PCR plates
- Mineral oil
- 1X SPRI beads
- Nanopore sequencing library prep kit
- Nanopore sequencing flow cell

Buffers and reagents:

- Sodium chloride
- Tris-HCl
- EDTA
- GITC
- Nuclease-free water
- SDS
- PEG
- Tris-HCl
- 100 % ethanol
- Papain

Equipment:

- -20 °C freezer
- Geno/Grinder 2010 or similar bead beater for homogenisation
- Thermocycler
- Magnetic stand (for plates and tubes)
- Centrifuge
- Microcentrifuge

- Vortex
- Pipettes (preferably including multichannel, ideally including 96-well)
- Ideally, Kingfisher Apex or similar
- Ideally, Qiagen Qiaxcel or similar
- Nanopore sequencer

Safety warnings

Check safety guidelines for individual reagents before commencing work. Some reagents will be toxic, corrosive or otherwise present health and safety risks. Appropriate personal protective equipment should be used at all times, not only for personal safety but also reduction of contamination risk.

Ethics statement

Check national and institutional policies for insect research. Follow best practice guidelines and stay up to date with the latest developments in insect welfare (e.g., through the Insect Welfare Research Society). Only kill as many invertebrates as is necessary, and always do so as humanely as possible.

Sampling of wasps and gut dissection

3d 0h 30m

1 Collect larvae directly from the comb of wasp nests by securing forceps on the mandibles of each larva and gently pulling them out individually. The larva should slide out of the cell smoothly, with little resistance. Place each larva into a microcentrifuge tube filled with [M] 100 % volume ethanol and store at -20 °C .

> Note
>
> Take care when handling wasp nests directly. Be sure to wear appropriate PPE and be vigilant for any allergic reactions or anaphylaxis if anyone is stung.
>
> Storing larvae in separate tubes is ideal, but not always practical. Given the dissection of the gut out of the body in STEP 3, surface contamination should at least be less pervasive as in other applications of dietary metabarcoding.

2 Add 100 µL TNES lysis buffer to each well of a 96-well plate. 5m

> Note
>
> For the TNES buffer, follow the recipe provided by BOMB-Bio ([M] 100 millimolar (mM) Tris-HCl, [M] 52 millimolar (mM) NaCl, [M] 10 millimolar (mM) EDTA, [M] 10 Mass / % volume SDS).

3 Remove individual larvae from tubes using sterilised forceps and transfer each into a sterilised petri dish. Under a stereomicroscope, make a careful longitudinal incision along the larval body using sterile scissors/scalpel and forceps, then gently extract the gut from the body cavity. Examine the extracted gut carefully and gently clear away any remaining non-gut tissue with ethanol and/or a scalpel to reduce the prevalence of wasp DNA downstream. Place the gut into a pre-designated well of a 96-well plate, as described in STEP 4. 3d

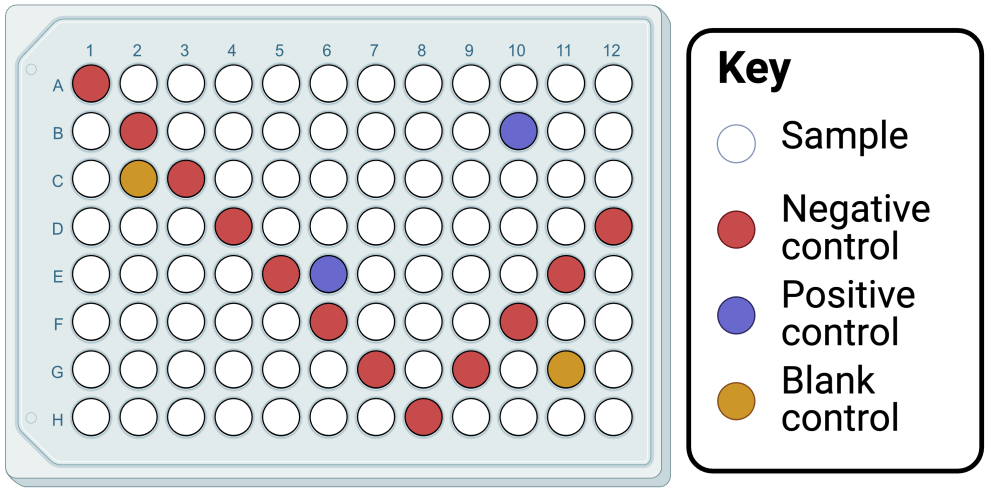
Note

Between each sample, thoroughly decontaminate dissection tools and any surfaces used (e.g., Petri dish) with Chemgene (or a similar disinfectant), rinsing with molecular biology-grade water and 100 % ethanol, and wiping dry with sterile tissue.

If pausing before proceeding to homogenisation and lysis, seal the 96-well plate and store it at -20 °C until ready.

Homogenisation and lysis

4 Transfer individual wasp larvae guts into 96-well plates (ideally deep-well, e.g., 2.2 mL, with fixable lids for grinding/washing). Consider the distribution of experimental controls ahead of subsequent steps to streamline downstream liquid handling. 15m



Our recommended PCR plate layout, which could be adopted here, during DNA extraction, for streamlining further into the protocol (i.e., to avoid having to redistribute samples across plates). Created in BioRender. Cuff, J. (2025) https://BioRender.com/559f6hh

Store samples at -20 °C until ready to process.

5 The DNA extraction protocol is largely adapted from the BOMB-Bio tissue nucleic acid extraction protocol. See their documentation for additional detail.

Citation

Oberacker P, Stepper P, Bond DM, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA, Jurkowski TP (2019)
. Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation.

https://doi.org/10.1371/journal.pbio.3000107 LINK

6 Add one 3 mm hardened carbon steel bead to each well. 5m

Note

Beads are usually shipped coated in manufacturing oil (especially the carbon steel beads). To remove this, place beads in a borosilicate glass beaker or Duran bottle with plastic pouring lip and lid removed then bake for at least 12 hours at 250 °C.

7 Grind the samples in a tissue grinder/homogeniser/lyser at 1750 rpm, Room temperature , 00:01:00 . 1m

8 Add 10 µL [M] 20 mg/mL papain to each well. 2m

9 Incubate overnight (~ 16:00:00) at 37 °C . 16h

DNA extraction 1h 5m

10 Centrifuge the plate at 2000 x g, Room temperature, 00:02:00 . 2m

11 Prepare Kingfisher Apex reagent plates as below.

Note

The Kingfisher Apex is ideal for automated high-throughput extraction of DNA, but will not always be available. Alternative equipment can achieve similar results, including just using magnetic racks. In that case, rather than transferring beads between plates, remove the supernatant and replace it with the next reagent, as described for normalisation below.

11.1 For the sample plate, to each well of a 96-well deep-well plate, add 60 µL of the lysate from STEP 11, 120 µL of 1.5X GITC buffer, 120 µL of [M] 1 mg/mL SeraMag Speed Beads in TE ([M] 10 millimolar (mM) Tris and [M] 1 millimolar (mM) EDTA) and 240 µL isopropanol. 5m

Note

For the 1.5X GITC buffer, follow the recipe provided by BOMB-Bio ([M] 6 Molarity (M) GITC, [M] 75 millimolar (mM) Tris-HCl, [M] 3 % volume sarkosyl, [M] 30 millimolar (mM) EDTA, [M] 0.15 % volume antifoam).

11.2 For the two ethanol plates, to each well of two 96-well deep-well plates, add 400 µL 80 % ethanol. 2m

11.3 For the isopropanol plate, to each well of a 96-well deep-well plate, add 400 µL isopropanol. 1m

11.4 For the elution plate, to each well of a 96-well standard plate, add 100 µL molecular biology grade water. 1m

12 Insert plates into the Kingfisher Apex and run a preset programme with the below steps. 30m

12.1 Pick up the 96 deep-well tip comb from a 96-well standard plate. 30s

12.2 Bind DNA to the beads by mixing at medium speed for 00:05:00 with a slow post-mix for 00:05:00 . 10m

12.3 Collect the beads in three 00:00:01 collections. 10s

12.4 Release the beads into the isopropanol plate and mix for 00:01:00 at medium speed, and collect the beads in three 00:00:01 collections. 1m 30s

12.5 Release the beads into one of the 80 % ethanol plates and mix for 00:01:00 at medium speed, and collect the beads in three 00:00:01 collections. 1m 30s

12.6 Release the beads into one of the 80 % ethanol plates and mix for 00:01:00 at medium speed, and collect the beads in three 00:00:01 collections. 1m 30s

12.7 Dry the beads above the well for 00:02:00 . 2m

12.8 Release the beads into the elution plate and mix for 00:02:00 at fast speed with a slow post-mix for 00:03:00 , and collect the beads in four 00:00:20 collections. 6m

12.9 Leave the tip comb in an empty 96-well standard plate. 30s

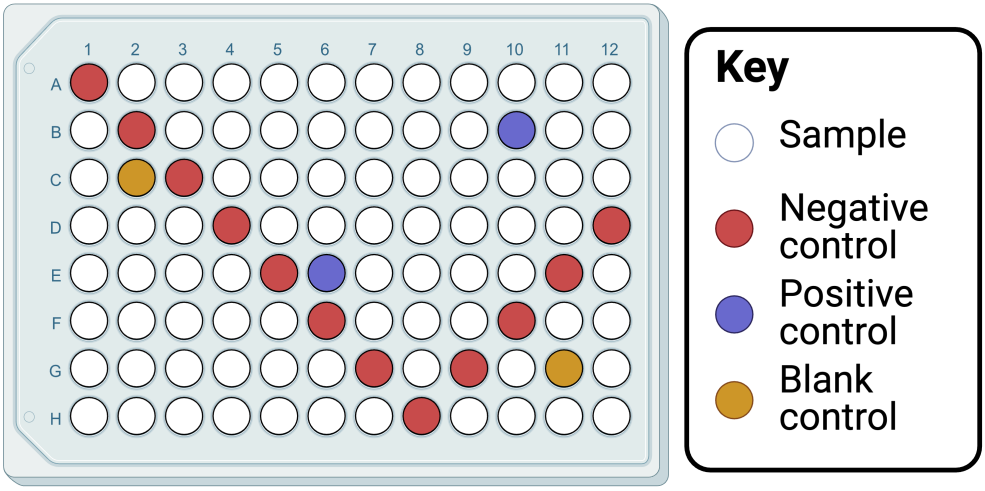
13 Store the eluted DNA at -20 °C until ready for subsequent steps.

PCR 3h 22m

14 Decide how samples will be distributed across plates (but don't distribute the DNA yet). Consider including a negative control in each row and column to detect any contaminants in each tagged forward and reverse primer. Among these wells, include any DNA extraction negative controls. Include positive controls (ideally mixed samples of species not found in the same study system), perhaps one adjacent to negative controls and the other adjacent only to samples (but both on separate rows and columns). Include blank controls (ideally wells into which no reagents or at least no primers are added), 10m

perhaps one adjacent to negative controls and the other adjacent only to samples (but both on separate rows and columns).

If using multiple PCR primer pairs, familiarise yourself with the annealing temperatures for each and prepare separate PCR plates for each. For optimal accuracy, consider running replicates of each reaction (e.g., triplicates).



Our recommended PCR plate layout, which could be adopted here for streamlining downstream. Created in BioRender. Cuff, J. (2025) https://BioRender.com/559f6hh

15 Prepare enough PCR mastermix for each sample. 2m

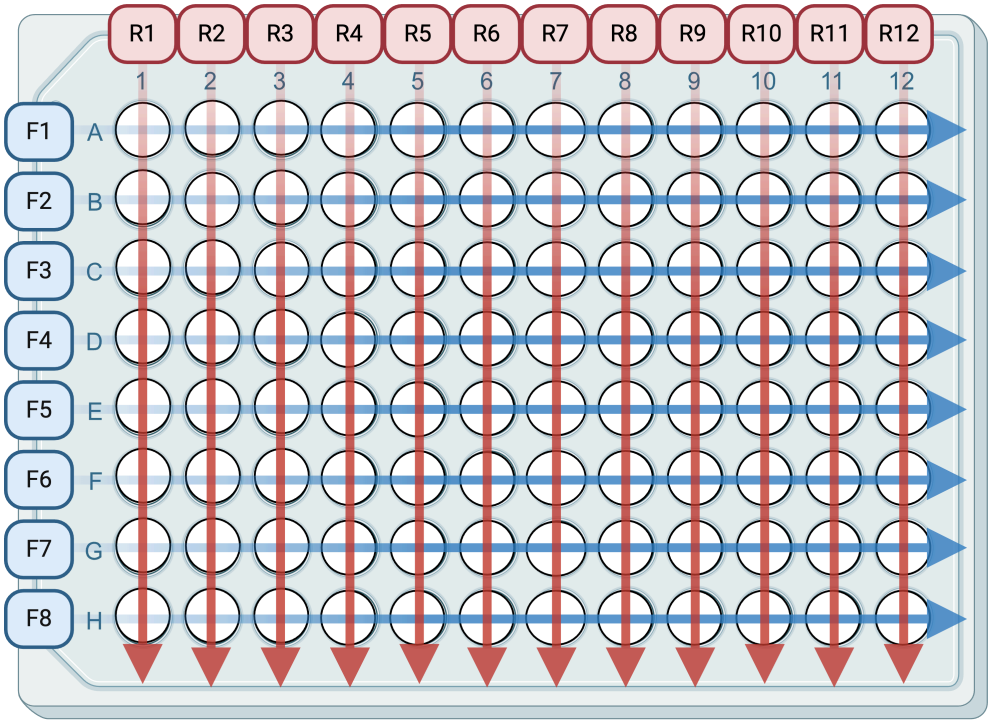
For a full plate, the below values will usually suffice (with some overage to account for pipetting error), but check your specific Taq polymerase mix for any differences:

	A	B
	Reagent	Volume (µL)
	Molecular grade water (DNase free)	1131.6
	2X hot-start PCR mastermix	1380

Note

These values are for 25 µL reaction volumes, which have been demonstrated to be effective for community metabarcoding. Consider running them in triplicate for more accurate results.

16 Using tagged primers, unique identifiers can be established for a full plate with 8 uniquely tagged forward primers and 12 uniquely tagged reverse primers. For ease, if able to use a 96-well pipette, consider creating a "primer plate" containing both PCR primers for each well at [M] 5 micromolar (µM) concentration; this is especially effective when using multiple plates. For 25 µL reaction volumes, this will subsequently involve adding 22.75 µL hot-start Taq polymerase and water master mix (described in the step above) to each well, followed by 1.25 µL of each primer mix to its corresponding well. It is possible to do this with a multichannel pipette. 15m



Distribution of tagged PCR primers across the 96-well plate. Created in BioRender. Cuff, J. (2025) https://BioRender.com/559f6hh

17 Add 1 µL DNA to each corresponding sample or positive control well, and 1 µL molecular grade water to each negative control other than extraction negative control(s). 10m

18 Distribute one drop of mineral oil into each well of the PCR plate(s) (~ 20 µL). 2m

Note

This can be achieved by taking a large volume of mineral oil into the pipette tip and then gently depressing the plunger so that a drop forms and falls from the tip into each well.

Mineral oil improves sealing of reactions by preventing evaporation and condensation. By reducing evaporation and thus loss of product, this also reduces potential cross-contamination.

19 Briefly centrifuge the plate to ensure that the oil is above the PCR mix and everything is at the bottom of each well without air bubbles. 1m

20 Load the PCR plate into a thermocycler. Ensure that the temperature regime matches the enzyme used (including any heat activation for hot-start Taq) and that the annealing temperature matches the PCR primers used. 2m

Note

Given differences between labs and samples, and inaccuracies in temperature calibration, considering running a temperature gradient PCR with known samples to check the specificity of your PCR primers.

21 Run your PCR programme. 2h

22 The samples should now be checked for successful amplification, contamination in negatives and any secondary banding. Gel electrophoresis will achieve this, but digital systems like the Qiagen Qiaxcel will do this and facilitate equalisation by generating amplicon-specific DNA concentations. 40m

Equalisation 13m

23

Note

Equalisation is more effective than normalisation, but requires amplicon concentration data. Both can be time and labour intensive though, so can be skipped if time restricted for large-scale projects, although at the expense of data recovery and evenness. This protocol presents equalisation, but see the protocol from which this is forked for a step-by-step description of normalisation.

If the PCRs were replicated (i.e., each sample run multiple times for each PCR primer pair used), these can be merged together into one plate at this point, or carried forward separately. Keeping the replicates separate increases the number of libraries to prepare and sequence later, but better facilitates identification of inconsistencies between samples that may arise from contamination or error. To merge triplicates, assuming use of 25 µL reaction volumes, pipette 22 µL from each well of two of the three plates into the corresponding well of the third. Briefly centrifuge the merged plate to move the oil to the top of the product again.

Note

To avoid pipetting oil from the oil-sealed PCR products, plunge the pipette to the first stop and fully insert the pipette tip into the bottom of the well, then release sharply. The PCR product will be taken up quickly, whereas the relatively viscous oil will be taken up slowly, thus being outcompeted by the PCR product.

24 Analyse the PCR products via digital electrophoresis (e.g., Qiagen Qiaxcel). This provides data on the concentration of specific amplicons (i.e., the target amplicon of the PCR primers used), which will be used in the next two steps.

Note

If digital electrophoresis (or an equivalent platform that facilitates quantification of concentration by band size) isn't available, you could proceed through the steps from STEP 31 in this section and quantify concentration using a fluorescence-based assay (e.g., Qubit dsDNA assay) and pool (as in STEPS 29-30) based on those values. It is important that primer dimers and other small fragments are removed (e.g., via magnetic bead-based purification) prior to these fluorescence-based assays as they can otherwise inflate and distort concentration values.

25 Based on the concentration of the target amplicon in each sample, calculate how much to pool from each sample in order to achieve equimolarity (i.e., equal representation of

samples). This can be achieved by dividing the maximum concentration within the plate by the concentration of each individual sample.

Note

The negatives, blanks and low concentration samples (for which the amount to pool exceeds the total volume available) should be pooled differently. This is summarised in STEP 26.

26 Pool the value calculated for each sample in the previous step as a volume in microlitres. Positive controls can be treated as samples. For negative controls, pool the average volume pooled across the samples (i.e., calculate the amount to be pooled from each sample to achieve equimolarity, calculate the mean of all of these values for each plate, and then use this value as the amount to pool from each negative control). For blanks, do not pool anything. If the volume to be pooled exceeds the total volume available, pool the total volume available. For samples with low or no concentration, the PCRs for these samples could be repeated, the samples could be excluded, or these can be pooled in the same manner as negative controls (although be wary in any interpretation of the data given increased potential for inaccuracy).

Note

If most of the volumes are similar and small, you can double (or otherwise multiply) the values.

If there is a large variation in the volumes to be pooled, the values can be halved to facilitate pooling more dilute samples proportionally.

27 To purify these pools ahead of library preparation, prepare a 1X solid phase reversible immobilisation (SPRI) bead solution and bring it to room temperature. The below steps detail how to make this solution, but it is also commercially available.

27.1 If using beads such as Sera-Mag Magnetic SpeedBeads (carboxylated, 1 µm, 3 EDAC/PA5), take 1 mL of well-mixed bead solution and wash the beads twice with TE+Tween buffer ([M] 10 millimolar (mM) Tris base, [M] 1 millimolar (mM) EDTA, [M] 0.05 % volume Tween 20, pH 8.0) by magnetising the beads, removing the supernatant, adding the TE+Tween, remagnetising the beads and removing the supernatant, and repeating the addition and removal of TE+Tween once more.

27.2 To the beads, add the following mix:

	A	B
	Reagent	Volume
	5 M NaCl	25 mL
	Molecular grade water	3.582 mL
	1 N HCl	0.168 mL
	1 M Tris base	0.5 mL
	0.1 M disodium EDTA	0.5 mL

27.3 Add 20 mL of [M] 50 % volume PEG to the tube to reach a 1X bead solution.

28 If ≥ 20 µL of PCR product is available for each pool, pipette 20 µL of 1X SPRI bead solution into each corresponding well of a 96-well plate or individual tubes. If less DNA is available, add a volume of SPRI bead solution equivalent to the full available volume of PCR product to the each corresponding well of the new 96-well plate or individual tubes.

Note

When working with magnetic bead solutions, ensure they are at room temperature and fully mixed, with no residue at the bottom of the container.

This is a good opportunity for size selection as well, especially if the PCR product contains any secondary bands. If using SPRI beads, adjust the volume added to select different fragment sizes. Refer to manufacturer details for more information on size selection.

Larger volumes can be used, so long as the corresponding volume of beads is used. For volumes greater than 100 µL, consider doubling the ethanol and water volumes in the remaining steps of this section.

29 Add 20 µL of PCR product (or whatever volume of beads was used in the last step) to each corresponding well of 1X bead solution, avoiding oil, and mix by vortexing (1500 rpm, Room temperature , 00:01:00).

> Note
>
> To avoid pipetting oil from the oil-sealed PCR products, plunge the pipette to the first stop and fully insert the pipette tip into the bottom of the well, then release sharply. The PCR product will be taken up quickly, whereas the relatively viscous oil will be taken up slowly, thus being outcompeted by the PCR product.

30 Incubate at Room temperature for 00:05:00 .

31 Place on a magnetic stand for 00:05:00 .

32 Remove all but 5 µL of the mixture from each well via pipette without disturbing the beads, which should be settled on the magnet.

> Note
>
> We recommend leaving 5 µL behind simply to avoid pipetting the beads themselves, but this can be avoided with care and experience.

33 Add 200 µL [M] 80 % volume ethanol to each well.

34 Remove the ethanol and add a further 200 µL [M] 80 % volume ethanol to each well.

35 Remove the ethanol as completely as possible by pipetting and allow the beads to air-dry until the aggregation of magnetic beads transitions from 'glossy' (shiny reflection of light) to 'matte' (dull dark brown mass), but not so long that it dries completely (i.e., begins to turn a rusty red and shows cracks).

36 Add 11 µL molecular grade water to each well, shake at 1500 rpm, Room temperature , 00:01:00 and incubate at Room temperature for 00:05:00 . 7m

37 Place on a magnetic stand for 00:05:00 . 5m

38 Remove 10 µL of the supernatant and place it into a new tube. This will be the library used in any subsequent library preparation. 1m

Library preparation and sequencing

39 Quantify the concentration of the library from the previous step (e.g., using Qubit dsDNA assay) and take this forward for library preparation. For Oxford Nanopore Technologies sequencing, follow the manufacturer protocols (or adaptations of them on here). For Illumina sequencing, consider protocols such as the one this is forked from if your primers include a Nextera overhang; otherwise, consider ligation-based library preparation methods. Check with your sequencing provider how many fmol they will need in how many µL. This will be based on the sequencer, sequencing cartridge and any QC processes they follow.

Protocol references

Citation

Oberacker P, Stepper P, Bond DM, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA, Jurkowski TP
(2019)
. Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation.

https://doi.org/10.1371/journal.pbio.3000107 LINK

Citations

Step 5

Oberacker P, Stepper P, Bond DM, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA, Jurkowski TP. Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation.
https://doi.org/10.1371/journal.pbio.3000107