Pollen metabarcoding

Tomasz Suchan

Abstract

Citation: Tomasz Suchan Pollen metabarcoding. **protocols.io**
dx.doi.org/10.17504/protocols.io.rpmd5k6
Published: 16 Jul 2018

Guidelines

Perform reactions in small batches until you are confident that there is no cross-contamination among the samples. Including isolation blanks and PCR blanks is crucial for the quality control.

Before start

Prepare 5 µM primer solutions:

ITS2 primers used in the 1st PCR:
ITS2-4R GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNTCCTCCGCTTATTGATATGC
ITS2-S2F ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNATGCGATACTTGGTGTGAAT

Indexing primers used in the 2nd PCR (xxxxxxxx - index):
AATGATACGGCGACCACCGAGATCTACACxxxxxxxxACACTCTTTCCCTACACGACGC
CAAGCAGAAGACGGCATACGAGATxxxxxxxxGTGACTGGAGTTCAGACGTGTGC

Materials

Q5 Hot Start High-Fidelity DNA Polymerase - 100 units M0493S by New England Biolabs

Water, nuclease free by Contributed by users

dNTP mix (25 mM of each) by Contributed by users

Phire Plant Direct PCR Kit F130WH by Thermo Fisher Scientific

Protocol

Pollen extraction

Step 1.

Vortex the butterfly in 50 uL of water with 0.1% SDS.

SAFETY INFORMATION

Add blank sample at this step (= "isolation blank")

Pollen extraction

Step 2.

Evaporate water in speedvac.

Pollen extraction

Step 3.

Add 5 uL of the Phire Plant Direct sample buffer.

Pollen extraction

Step 4.

Spin max speed for 2 min.

1st PCR

Step 5.

Prepare the mix:

AMOUNT

14 µl Additional info: molecular grade water

AMOUNT

25 µl Additional info: Phire Plant Direct PCR mix

AMOUNT

5 µl Additional info: ITS2-S2F primer

AMOUNT

5 µl Additional info: ITS2-4R primer

1st PCR

Step 6.

Add 1 uL of the sample to the mix. Use water for the blanks.

SAFETY INFORMATION

Add another blank sample at this step (= "PCR blank")

1st PCR

Step 7.

Run the PCR program: initial denaturation at 98°C for 5 min; 20 cycles of denaturation at 98°C for 40 s, annealing at 49°C for 40 s and elongation at 72°C for 40 s; followed by a final extension step at 72°C for 5 min.

Purification

Step 8.

Perform AMPure purification with ratio 1x. Elute in 10 µl.

PROTOCOL

. AMPure purification protocol

CONTACT: Tomasz Suchan

Step 8.1.

Remove beads from the fridge, equilibrate to the room temperature, mix thoroughly but do not vortex.

Step 8.2.

Add desired ratio of AMPure beads to the purified sample and mix well by pipetting.

Step 8.3.

Incubate 5 minutes.

Step 8.4.

Place on the magnetic rack.

Step 8.5.

Let it stand for 5 minutes on the rack, aspirate and discard supernatant.

Step 8.6.

Add 200 µl of freshly prepared 70% EtOH, incubate 30 seconds, aspirate and discard EtOH.

Step 8.7.

Repeat the wash: add 200 µl of freshly prepared 70% EtOH, incubate 30 seconds, aspirate and discard EtOH.

Step 8.8.
Wait until the pellet dries out completely. DO NOT remove the plate/tubes from the magnetic rack, as the dried AMPure might pop out from the tubes!

Step 8.9.
Add desired volume of Tris 10 mM or water, wetting the dried AMPure pellets (add 1 µl to the final volume to avoid pipetting out the beads)

Step 8.10.
Remove from the magnetic rack.

Step 8.11.
Resuspend by pipetting or vortexing.

Step 8.12.
Incubate 10 minutes, incubating in 37°C can improve DNA yield.

Step 8.13.
Place on the magnetic rack.

Step 8.14.
Let it stand for 5 minutes, pippete out and save supernatant. The eluted DNA is in the supernatant, do not discard it!

2nd PCR

Step 9.
8volume: 7.0 336.0Mix 1 uL of the template with 7 uL of the mix.Add 2 uL of each 5 uM primer (forward and reverse).

AMOUNT

4.82 µl Additional info:
molecular grade water

AMOUNT

2 µl Additional info: Q5
reaction buffer

AMOUNT

0.08 µl Additional info:
dNTPs (25 mM each)

AMOUNT

0.1 µl Additional info: Q5
Hot Start polymerase

SAFETY INFORMATION

Use different pair of indexed primers for each sample

2nd PCR

Step 10.

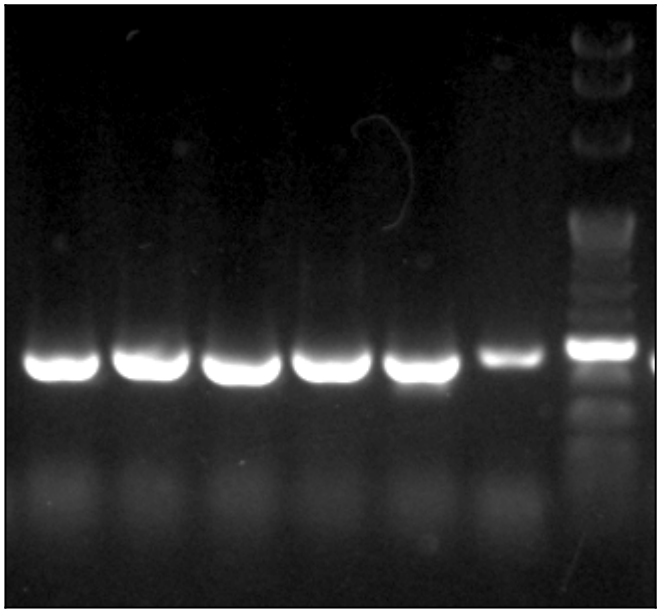
Run the PCR program: 30 s denaturation at 98°C; 12 cycles of denaturation at 98 °C for 10 s, combined annealing and extension at 72°C for 30 s (shuttle PCR); the final extension at 72°C for 5 min.

2nd PCR

Step 11.

Check profiles on a gel.

EXPECTED RESULTS



Pooling

Step 12.

Pool all the samples together.

Pooling

Step 13.

lPerform AMPure purification with ratio 1x. Elute in 100 µl.

PROTOCOL

. AMPure purification protocol

CONTACT: Tomasz Suchan

Step 13.1.
Remove beads from the fridge, equilibrate to the room temperature, mix thoroughly but do not vortex.

Step 13.2.
Add desired ratio of AMPure beads to the purified sample and mix well by pipetting.

Step 13.3.
Incubate 5 minutes.

Step 13.4.
Place on the magnetic rack.

Step 13.5.
Let it stand for 5 minutes on the rack, aspirate and discard supernatant.

Step 13.6.
Add 200 µl of freshly prepared 70% EtOH, incubate 30 seconds, aspirate and discard EtOH.

Step 13.7.
Repeat the wash: add 200 µl of freshly prepared 70% EtOH, incubate 30 seconds, aspirate and discard EtOH.

Step 13.8.
Wait until the pellet dries out completely. DO NOT remove the plate/tubes from the magnetic rack, as the dried AMPure might pop out from the tubes!

Step 13.9.
Add desired volume of Tris 10 mM or water, wetting the dried AMPure pellets (add 1 µl to the final volume to avoid pipetting out the beads)

Step 13.10.
Remove from the magnetic rack.

Step 13.11.
Resuspend by pipetting or vortexing.

Step 13.12.
Incubate 10 minutes, incubating in 37°C can improve DNA yield.

Step 13.13.
Place on the magnetic rack.

Step 13.14.
Let it stand for 5 minutes, pippete out and save supernatant. The eluted DNA is in the supernatant, do not discard it!

Pooling

Step 14.
Check the concentration using Qubit and the profile using Tapestation/Fragment Analyzer. Calculate molarity from that and proceed to the sequencing. Add 15% PhiX to the sequencing run.