Endosymbiont Diagnostics

If not indicated otherwise, reagents are stored in Snoopy -20°C , top drawer, in box "Aphids 'Diagnostic PCR' reagents". Extra primer aliquots at 10 µM and 100 µM primer stocks are at -80°C in tower 105 in G 72.

1.1 Aphid Sampling

1) If aphids are used within a few days, freeze for >30 minutes at -20°C in a 1.5 ml Eppendorf tube
2) If aphids are to be stored long-term, add them to a clean Eppendorf tube containing 100% EtOH

1.2 DNA Extraction

An extraction control is to be done during each extraction: apply extraction protocol to an Eppendorf tube without aphid.

1.2.1 Salting out

Note: do not use this extraction protocol for quantitive assays that rely on ratios between species (e.g. qPCR of Spiro per Aphid) since bacteria are released from the aphid more easily than the aphid DNA when one uses pipet tips for crushing. If a ratio is calculated, use Pravin's protocols (using glass beads)

1) Switch on 55°C heat block
   Check that there is enough 100% and 70% EtOH in Snoopy -20, top drawer
2) If the aphid was stored in EtOH, let the ethanol evaporate by placing it on a tissue. Place aphid in a 1.5 mL Eppendorf tube.
3) Add 5 µl (or 7 µl if incubating for 1 h in step 5)) proteinase K (10mg/ml) to the 1.5 ml Eppendorf tube and use the tip to squish the aphid. Discard tip. Repeat for all aphids.
4) Add 300 µl TNES buffer (on shelf above bench) to each tube
5) Mix, spin down on bench, and incubate tubes for (1 -) 3 h at 55°C (or overnight at 37°C)
6) Add 85 µl 5M NaCl (on shelf abo bench), shake hard for 15 s by hand
7) On gel room centrifuge, spin tubes for 15-30 min at 21912 xG (14'000 rpm) to pellet proteins. When run is finished, start cooling centrifuge down to 4°C.
8) Remove supernatant (if not clear, spin longer) into a new labelled 1.5 Eppendorfe tube
9) Add ~400 µl (1 volume) ice-cold 100% EtOH. Invert tubes slowly five times and, optionally, store at -20°C for 1 h or overnight [Stopping point]
10) On gel room centrifuge, spin tubes for 10 minutes at 21912 xG (14'000 rpm) and 4°C
11) Discard supernatant and add ~500 µl ice-cold 70% EtOH
12) On gel room centrifuge, spin tubes for 10 minutes at 21912 xG (14'000 rpm) and 4°C
13) Discard all supernatant and air dry the pellet: place opened tube in heat block at 55°C, or overnight at RT covered with a tissue to avoid contamination
14) Re-suspend DNA in 100 µl 1xTE pH 7.5 (on shelf above bench)

15) If required, test DNA concentration in sample with Qubit (see protocol 'Amount and Quality of Extracted DNA')

16) Store DNA in Snoopy at -20°C, switch off heat block

1.3 PCR for symbiont presence[1]

The Excel Aphid_Diagnostic_PCR_Setup.xlsx helps with calculations, and lists appropriate primers and positive controls

To check whether PCR product was amplified with an agarose gel use GoTaq 2x Green Master mix as it doubles as a loading buffer. If you have more than 48 samples, it's worth using Qiaxcel instead of gels. In that case, use GoTaq 2x Colorless Mastermix, as green mix may damage capillaries, and increase amount of mastermix added to DNA sample from 9 µl to 10 µl. According to the manufacturer, Qiaxcel should have at least 10 ul sample to work with, and 11 µl total volume allows for some evaporation during PCR.

1.3.1 Controls

Master mix with primer but w/o template as PCR control
Extraction control
Negative and positive Control: DNA from symbiont-negative and symbiont-carrying aphids

1.3.2 Procedures

1) Book a PCR cycler
2) Decide whether to use agarose gels or Qiaxcel for analysis. For Qiaxcel, add 10 µl mastermix per 1 µl sample (instead of 9 µl) and use colorless mastermix (instead of green mastermix).
3) Retrieve and, at RT, thaw aliquots of
    a. the specific endosymbiont forward and reverse primer at 10 µM
    b. the GoTaq 2x Green Master mix (green cap, Promega M782A) [Agarose gel]
       OR
       GoTaq 2x Colorless Mastermix (Promega M714) [Qiaxcel]
    c. DNase/RNase free water
4) Positive control DNA can be taken from Corinne's or Marco's "Aphid DNA" box (Snoopy -20°C, top drawer)
5) Thaw DNA samples and positive control DNA at RT. Mix and spin down on bench.
   Working on ice is only necessary in case of large number of samples.
6) If working with strip tubes ('PCR strips, Vorburger lab' on shelf), label ends of strips and lids
7) Vortex and spin down all PCR reagents
8) Using filter tips, combine GoTaq Master mix (green or colorless), forward and reverse primer and water (if the amount allows: in the tube or well of the PCR control)

[1] Most primers are impossible to multiplex since product size is too similar.

9) Using filter tips, distribute 9 µl of the master mix (for agarose gels) or 10 µl mastermix (for Qiaxcel) to each tube or well, reuse tip
10) Add 1 µl of DNA to each tube or well (natural tips). If amount of DNA is limited, DNA can be diluted to 5-10 ng/µl. Otherwise, DNA is used as it results from the extraction (~30-40 ng/µl)
11) Vortex and spin down tubes; spin down plates in salad spinner
12) Place empty tubes at the four corners of the PCR cycler plate to support the silicon mat laid on top of the tubes. When using plates just use the grey re-useable PCR mat (bottom drawer).
13) Start program *CV- 01 diagnostics for symbionts*, store all reagents and samples at -20°C
14) To store, centrifuge 96-well plates at 4000 rpm for 20s on gel room centrifuge, exchange PCR mat for a cheap PCR foil (seal edges well), and store at -20°C, or 4°C if used the next day. Tubes can be stored directly.

1.4 TBE-Gel

All tips are discarded into the tip waste on the gel room bench
There is also a Promega 1 kb ladder available in the gel room fridge, which we do not use

For symbiont diagnosis, prepare a <u>2%</u> agarose gel

1) Apply masking tape to the open sides of the gel mold
2) Add comb(s)
3) Consult the chart posted near the microwave for appropriate agarose, TBE, and peqGreen amounts. With a spatula, add agarose (if scale does not work, check fuse on plug) to an Erlenmeyer flask. Measure out 1xTBE with a graduated cylinder (big plastic bottles on shelf) and pour into the Erlenmeyer flask. Swirl to mix.
4) Microwave slurry for 1 minute (put napkins underneath flask to catch spills) until all particles have dissolved. Protect hands with oven mittens.
5) Let agarose cool down until slightly warmer than body temperature (under flowing water)
6) Remove peqGreen from 4°C fridge and pipet appropriate amount into the agarose. Swirl well.
7) Slowly pour gel, remove bubbles with a pipette tip and let it set for ~20 minutes until opaque
8) Rinse glassware with dH_2O from the tap
9) Place gel in the running chamber (top at black electrode), cover with used TBE from flasks on bench and remove comb slowly.
10) Load lanes with 3-5 µl of PCR product or 3 µl of BenchTop 100bp DNA ladder (Promega, ready to load) from fridge in gel room
11) Let gel run at max 110 V and 500 mA for up to ~1 h in case of mini gels, ~1.5 h in case of midi gels – stop when the front of dye comes close to the end of the gel
12) Take pictures: switch on eBox, press 'WL', place gel in chamber (Enduro trays cannot be placed under UV), close chamber and press 'Live'. Switch to 'UV', adjust zoom and focus if necessary. 'Freeze', 'Print', 'Save'. Open CAPT software on computer, download picture and save on Q:\Abteilungsprojekte\eco\ResGroups\Vorburger\your_folder.

13) Discard the gel into the plastic bag provided on the bench and wipe down gel chamber
14) Fill out tick lists for Agarose, peqGreen and 100bp ladder
15) Label gel digitally (e.g. in PowerPoint) or enter information into the gel image excel (Hugo)