Aphid molecular ecology – useful protocols and recipes

1. Protocols

1.1. DNA extraction

1.1.1. "Salting out" extraction of DNA from single aphids (Sunnucks & Hales, 1996)

1) Put the aphid in a 1.5 ml Eppendorf tube. If the aphid was stored in ethanol, place the aphid on a tissue for a few minutes to let the ethanol evaporate.

2) Add 5 µl of proteinase K (10 mg/ml) and squish the aphid with a pestle or melted pasteur pipette. Wash pestle in a large volume of distilled water and dry after every use.

3) Add 300 µl TNES buffer by letting it run down the pestle to wash all of the squised aphid into the tube.

4) Mix and incubate tube at 55 °C for 1 – 3 h (or at 37 °C over night).

5) Add 85 µl of 5M NaCl and shake hard for 15 s (proteins become precipitated).

6) Microfuge at full speed for 5 – 10 min (longer is better, but people are impatient) – proteins become pelleted.

7) Carefully take off the supernatant (which should be clear) and put it in a new labelled tube. Add 1 volume (~400 µl) of cold 100% ethanol (store bottle in freezer). Invert tubes slowly a couple of times (DNA is precipitated).

8) Microfuge at full speed for at least 5 min (DNA is pelleted).

9) Carefully remove all the ethanol (sometimes needs a second spin).

10) Rinse pellet in ~500 µl 70% ethanol and microfuge at full speed again for 5 min.

11) Air-dry the pellet (open tube in hotblock at 37 – 55 °C or at room temperature over night), cover tubes with a tissue to avoid contamination.

12) Re-suspend the DNA in the required amount of 1× TE buffer. For PCR with dye-labelled primers, low concentrations work better, so add 150 – 200 µl, for other applications, higher concentrations are better (add 30 – 50 µl). Can always be diluted further.

13) Store frozen.

1.1.2. Chelex extraction of DNA from single aphid eggs

1) Put 30 µl of 5% Chelex solution into an Eppendorf tube (Make sure the Chelex is well suspended, best have a small stirrer in the bottom of the bottle and have the solution stirring for dispension).

2) Transfer the egg to the Eppendorf tube with a yellow tip and crush it on the side of the tube with the tip (if the egg was stored in ethanol, put the egg on a kleenex first and let the ethanol evaporate for a couple of minutes).
   Leave at room temperature for 5 – 10 minutes.

3) Incubate for 15 min at 65 °C in block heater (or water bath).

4) Vortex briefly.

5) Boil for 6 min (block heater set to 100 °C). Either fix lids with clips or weigh them down to prevent popping).

6) Vortex briefly.

7) Microfuge at full speed to spin down the Chelex.

8) Transfer the supernatant into a new labelled tube, taking care to leave the Chelex beads behind (setting pipette at around 20 µl works all right).

9) Store in freezer. For microsatellite PCR, use 1 µl in a 10 µl reaction.

1.1.3. Chelex extraction of DNA from single aphids

Same protocol, but start with larger volume of 5% Chelex solution, ~50 µl for DNA at high concentration, ~200 µl for DNA at low concentration (for PCR with dye-labelled primers and visualization on automated sequencers). Squish aphid well.

The resulting DNA concentrations for different volumes of Chelex solution have not been worked out. It would be worth the effort to do so.

2. Solutions & Reagents

Good idea to autoclave solutions if they are going to sit on the bench for a while. Detergents and volatiles cannot be autoclaved. Add those after. If available, Milli-Q water can of course be used instead of ddH_2O. Mark the level of the solution on the bottle prior to autoclaving. If it has dropped substantially, top up with autoclaved ddH_2O.

BSA (bovine serum albumin, PCR additive)

Usually comes dry.
Dissolve in ddH_2O or, if applicable, the buffer recommended by the manufacturer at the required concentration (typically 10 mg/ml, for long storage maybe higher).
Store frozen.

5% Chelex solution

Autoclave a Schott bottle and a small magnetic stirrer. Put stirrer in bottle.
Measure out the right amount of Chelex resin (5 g for 100 ml, 10 g for 200 ml).
Add ddH_2O, shake and autoclave the solution.

dNTPs

Depends on how they are purchased.
If stocks are bought at 100 mM each, pipette together at equal volumes to produce aliquots, e.g. 20 µl each into an Eppendorf tube, resulting in 80 µl at 25 mM each to be stored frozen. Dilute 1:20 for use at desired concentration of 1.25 mM (e.g. 80 µl + 1520 µl ddH_2O gives an Eppendorf full ready to use).

0.5 M EDTA pH 8.0

for 500 ml:

Dissolve 93.5 g EDTA in 400 ml ddH_2O
Adjust to pH 8.0 with NaOH (~10 g pellets)
Adjust to 500 ml
Autoclave

5 M NaCl

for 200 ml:
Dissolve 58.44 g NaCl in 150 ml ddH_2O
Adjust to 200 ml
Autoclave

TBE electrophoresis buffer

For 1 l of 10× TBE:

108 g Tris base
55 g boric acid
9.3 g EDTA solid/or 40 ml 0.5 M EDTA dissolve all in ~0.8 l of ddH_2O

make up to 1 l with ddH_2O. No need to autoclave.

Use at 1× concentration.

TE buffer pH 7.5

10 mM Tris.HCl (pH 7.5)
1 mM EDTA (pH 8.0)

for 400 ml of 10 × TE:

40 ml 1 M Tris.HCl (pH 7.5)
8 ml 0.5 M EDTA (pH 8.0)
352 ml ddH_20
Autoclave

TNES buffer

50 mM Tris.HCl (pH 7.5)
400 mM NaCl
20 mM EDTA
0.5 % SDS

for 200 ml:

10 ml 1 M Tris.HCl (pH 7.5)
16 ml 5 M NaCl
8 ml 0.5 M EDTA
161 ml ddH_2O

Autoclave, then add 5 ml of 20% SDS. Check pH 7.5.

1 M Tris.HCl pH 7.5

for 500 ml:

Dissolve 60.6 g of Tris base in 400 ml of ddH_2O
Adjust the pH with concentrated HCl to 7.5 (~60-70 ml)
Allow the solution to cool before fine-adjusting pH (pH of Tris solutions is T-dependent)
Adjust to 500 ml
Autoclave

3. PCR

3.1. General pipetting scheme for msat PCR with dye-labelled primers

<u>for 10 µl reactions:</u>

H_2O (PCR quality)	4.0 µl
10 × Taq buffer (usually supplied with Taq polymerase)	1.0 µl
$MgCl_2$ 25 mM (usually supplied with Taq polymerase)	0.8 µl
dNTPs (at 1.25 mM each)	1.6 µl
BSA (10 mg/ml)	0.5 µl
Forward primer (dye-labelled, 1 µM)	0.5 µl (often much less is enough)
Reverse primer (unlabelled, 1 µM)	0.5 µl (often much less is enough)
Taq polymerase (5 U/µl)	0.1 µl
DNA	1.0 µl

Notes:

Final concentrations in reaction: 2 mM $MgCl_2$, 200 µM of each dNTP, 5 µg BSA, 0.05 µM of each primer.
There's nothing magical about this. $MgCl_2$ concentration can be modified if required, BSA can even be omitted if it doesn't improve PCR. General recommendation is to use it (never seems to hurt, sometimes does a lot of good...).

Primer concentrations are as recommended by Li-Cor. For most loci, this turned out to be far too much. Often 0.1 – 0.2 µl are sufficient.

For multiplexing just add primers for more than one locus, the relative concentrations will have to be worked out by trial and error. Adjust the volume of H_2O accordingly to keep the reaction at 10 µl.

It does not have to be the forward primer that is labelled, can also be the reverse.

3.2. PCR protocols (Sloane *et al.*, 2001)

PMS2 ("general-purpose" touch-down, works well with M37, M40, M63, M86, M107, myz2 & myz9)

Cycle	Step	Temperature (°C)	Duration	
1	1	94	2 min	Initial denaturation
2	1	94	15 s	
	2	55	30 s	
	3	72	45 s	
3	1	94	15 s	
	2	53	30 s	
	3	72	45 s	
4	1	94	15 s	
	2	51	30 s	
	3	72	45 s	
5	1	94	15 s	
	2	49	30 s	
	3	72	45 s	
6	1	94	15 s	⎫
	2	47	30 s	⎬ 30 ×
	3	72	45 s	⎭
7	1	72	2 min	Final extension
8	1	Lowest possible	24 h	"fridge", e.g. when run over night

PMS1 (touch-down with higher annealing temperatures, try when more specificity is required, possibly at the expense of lower yield)

Cycle	Step	Temperature (°C)	Duration	
1	1	94	2 min	Initial denaturation
2	1	94	15 s	
	2	62	30 s	
	3	72	45 s	
3	1	94	15 s	
	2	61	30 s	
	3	72	45 s	
4	1	94	15 s	
	2	59	30 s	
	3	72	45 s	
5	1	94	15 s	
	2	57	30 s	
	3	72	45 s	
6	1	94	15 s	⎫
	2	55	30 s	⎬ 30 ×
	3	72	45 s	⎭
7	1	72	2 min	Final extension
8	1	Lowest possible	24 h	"fridge", e.g. when run over night

5. References

Sloane MA, Sunnucks P, Wilson ACC, Hales DF (2001) Microsatellite isolation, linkage group identification, and determination of recombination frequency in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Genetical Research*, **77**, 251-260.

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510-524.

Christoph Vorburger, 24/8/04