Laser Pressure Catapulting and downstream DNA examination of single cells

MATERIAL

To reduce the chance of contamination of RNA and DNA we use pipetters and workstations separately for RNA and DNA.

Buffers, Enzymes and Solutions

- *dNTP solution containing all four dNTPs (2mM each)*

- *Thermo-Start DNA polymerase* for all PCRs. Buffer and $MgCl_2$ are derived hereof. *Thermo-Start DNA Polymerase* (250 units, 5units/µl; ABgene, Hamburg, Germany). Catalog number # AB-0908

- *Proteinase K solution* 20mg/ml (Qiagen GmbH, Hilden, Germany) Catalog number # 19131

For DNA-prep from paraffine sections we usually use a Proteinase K containing buffer.

PK buffer	0,5M EDTA pH8,0	20µl
	1M Tris pH8,0	200µl
	Tween 20	50µl
	Proteinase K 20mg/ml	100µl
	ddH2O	9,63ml

METHODS

Preparation of DNA from microdissected cell samples

1) Microdissection and Laser Pressure Catapulting

(a) take an autoclaved PALM cap
(b) pipette 2-3 µl of PK-Buffer, PCR oil, PCR buffer or RNase and DNase free water in the middle of the cap
(c) put the cap into the cap holder

(d) perform laser microdissection and laser pressure catapulting of wanted cells or cell areas
(e) remove the cap from the cap holder and put it onto a 0,5 ml microfuge tube
(f) centrifuge the sample at full speed for 1-2 minutes; discare the cap
(g) incubate at 55°C about 12 hours (may be shortened). Heat the samples immediately after digestion for 10 minutes at 99°C to inhibit Proteinase K activity

(h) At best perform digestion in a thermal cycler with a heating lid. If not going on immediately store the samples in the fridge at 4°C.

*Start the first PCR whilst using the **entire** amount of solution after digestion.*

PCR protocol for sex determination (Amelogenin); nested PCR

1st PCR

The product size of the DNA fragment is 330 bp for the X chromosome and 340 bp for the Y chromosome.
The 5´primer sequence is located in the exon/intron boundary of exon 1 and the 3´primer is located in intron 1.

2mM dNTP-Mix	2,0 µl
10 x Buffer	2,0 µl
25mM $MgCl_2$	1,6 µl
50pM/µl 5´outer primer	0,5 µl
50pM/µl 3´outer primer	0,5 µl
5units/µl Thermo Start Taq	0,1 µl
DNA	10 µl
H_2O double destilled	3,3 µl
total volume	20,0 µl

Cycle number	Denaturation	Annealing	Extension
1 cycle	15 min at 94°C		
35 cycles	30 sec at 94°C	30 sec at 54°C	40 sec at 72°C
1 cycle			10 min at 72°C

cool down to 4°C

2nd PCR

The product size of the DNA fragment is 104bp for the X chromosome and 112bp for the Y chromosome.

2mM dNTP-Mix	2,0 µl
10 x Buffer	2,0 µl
25mM $MgCl_2$	1,6 µl
50pM/µl 5´inner primer	0,5 µl
50pM/µl 3´inner primer	0,5 µl
5units/µl Thermo Start Taq	0,1 µl
1st PCR reaction product	5,0 µl
H_2O double destilled	8,3 µl
total volume	20,0 µl

Cycle number	Denaturation	Annealing	Extension
1 cycle	15 min at 94°C		
30 cycles	30 sec at 94°C	30 sec at 62°C	40 sec at 72°C
1 cycle			10 min at 72°C

cool down to 4°C