

Drosophila DNA extraction

(Gloor, G and Engels, W 1992 Single fly DNA prep for PCR D.I.S 71: 148-179)

Reagents

Squishing buffer (100 ml)	Final concentrations
1 ml 1M TrisHCl pH8.2	10 mM TrisHCl pH8.2
0.5 ml 0.2M EDTA	1 mM EDTA
0.5 ml 5M NaCl	25 mM NaCl
98 ml H ₂ O	

20 mg/ml proteinase K

Protocol

For 10 samples 1ml buffer = 990 μ l squishing buffer & 10 μ l proteinase K (i.e. final concentration 200 mg/ml).

1. Put fly in eppendorf in freezer for 5 min
2. 50 μ l per fly, squash with pipette tip before expelling solution
3. Leave at 37-55°C >1h
4. Boil for 2 min
5. Freeze until used

Gel electrophoresis

Gel sizes

Size	0.5X TBE	Eth Br
Small	50 ml	2.5 μ l
Medium	150 ml	7.5 μ l
Large	300 ml	15 μ l

Pre/post-staining

If gel run for a long time (Medium, Large) add 50 μ l EtBr per lt of buffer directly into gel tank. Will need to label the tank as containing EtBr and dispose of the buffer accordingly. Also need to keep the buffer protected from light.

The only reason to stain after running is in restriction enzyme assays, where the better picture obtained is worth the effort of staining-destaining.

Gel resolving power

Strength	Fragment size
0.50%	1-30 Kb
0.70%	0.8-12 Kb
1%	0.5-10 Kb
1.50%	0.3-3 Kb

- Ethidium Bromide working concentration: 0.5 μ g/ml
- Ideal Voltage is: 1-10 V/cm

PCR troubleshooting

Weak products

- Decrease annealing temp
- Use more primer
- Use more DNA
- Use more taq
- Increase cycle number

Longer unspecific products

- Increase annealing temp
- Decrease annealing temp
- Decrease extension temp to 62-68°C
- Decrease extension time
- Decrease MgCl₂ concentration
- Decrease primers
- Decrease DNA

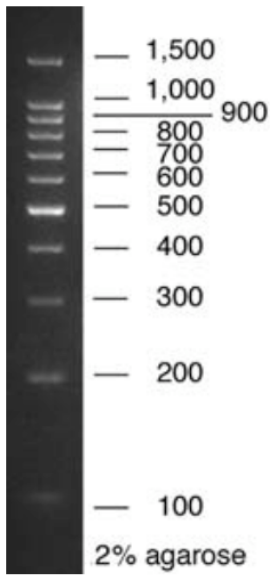
Shorter unspecific products

- Increase annealing temperature
- Increase annealing time
- Increase extension time
- Increase extension temp to 74-78°C
- Increase MgCl₂ concentration
- Decrease primer
- Decrease DNA
- DNA degradation?

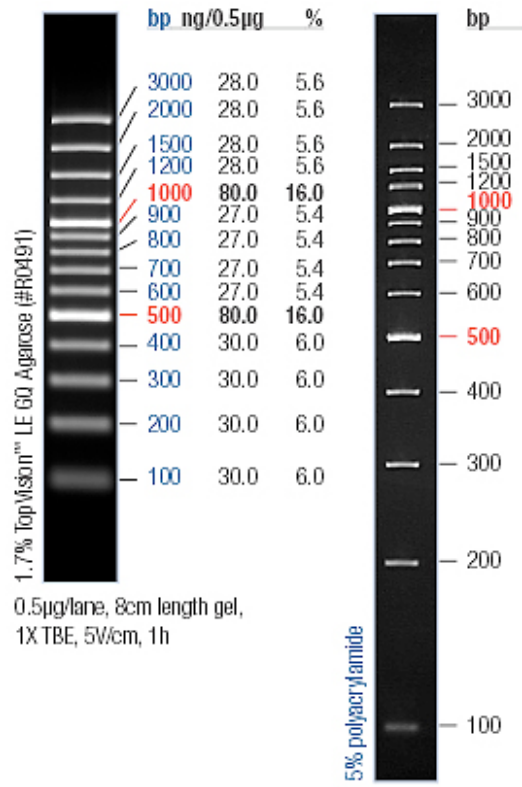
Reaction used to work but not anymore

- Change dNTPs
- Increase primer
- Increase DNA
- Decrease annealing temp by 6-10°C
- The less MgCl₂ the better (stringency/specificity).

Promega 100 bp ladder



Generuler Plus 100 bp



0.5µg/lane, 8cm length gel,
1X TBE, 5V/cm, 1h

