

Small scale genomic DNA preparation

Solutions

Solution A: 0.1M Tris HCl pH9.0
 0.1M EDTA
 1% v/v SDS
 1% DEPC (add just before use)

8M KAc
Isopropanol
70% v/v EtOH
TE

Procedure

- Collect flies in an eppendorf tube (fill with CO₂, put on ice).
- Complete Solution A by adding DEPC.
- Add Solution A: 100µl to 1-5 flies
 200µl to 6-10 flies
 400µl to 50 flies
 (= 1 volume).
- Homogenize the flies.
- Incubate 30' at 70°C (secure covers, or tubes will pop open).
- Add 14µl of KAc per 100µl of Solution A.
- Incubate 30' on ice.
- Spin 15' at RT.
- Move SN to a new tube.
- Phenolize twice with 1 volume of phenol/chloroform (1:1).
- Add 1/2 volume of isopropanol.
- Spin 5' at RT.
- Remove SN.
- Wash pellet with 0.5ml of 70% EtOH.
- Dry pellet 10' under vacuum.
- Redissolve in 100µl of TE.

Reference: N. Walter (1991). Site-selected P element mutagenesis Methods booklet. Hafen-Lab, Zurich.