Drosophila genomic DNA extraction

Reagents:

Phenol-Cloroform: 1:1
KAc 8M
Isopropanol
EtOH 100% and 70%
TE Buffer pH 8.0
Grinding buffer: pH 8.0
- 0.1M NaCl
- 0.1M Tris (pH 8.0)
- 0.05M EDTA
- 0.5% SDS
- 0.2M sucrose

Procedure:

1. Freeze 50-500 flies on dry ice.
2. Add 0.4-2.0ml of grinding buffer, and homogenize the flies in a ground glass dounce.
3. Heat sample to 65°C and incubate for 30 min.
4. Add 8M KAc to final concentration of 1M.
5. Incubate on ice for 30 min.
6. Spin at 13.000 rpm at 4°C for 10-30 min.
7. Move supernatant using 1ml tip gently to a new tube without any pellet. If necessary, repeat this step.
8. Add 2-2.5 volumes of 100% ethanol. Incubate at RT for 2 min or -20°C O/N.
9. Spin at 13.000 rpm at 4°C for 10 min. Discard the supernatant and dry pellet under vacuum for 2 min.
10. Resuspend pellet in TE buffer at about 1ul/fly.
11. Add 5ul of RNase A+T and incubate 30 min at 37°C.
12. Add 5ul of proteinase K, and incubate 30 min at 37°C.
13. Add 1:1 of Phenol-Chloroform to the sample and shake thoroughly (or vortex gently). Spin at 13.000 rpm at RT for 5 min.
14. Move supernatant to a new tube. Add equal volume of Chloroform, shake thoroughly (or vortex gently), and spin at 13.000 rpm at RT for 5 min.
15. Move supernatant to a new tube. Add 2-2.5 volumes of ethanol, Na-acetate to final concentration of 0.15M and 1ul of GlycoBlue and shake. Put on dry-ice 15min or -20°C O/N. Spin at 13.000 rpm at RT for 5 min.
16. Suck off supernatant (don't lose pellet!). Wash the pellet with 1 ml of 70% EtOH twice.
17. Spin at 13,000 rpm at RT for 5 min.
18. Dry the pellet 2 min under vacuum.
19. Resuspend the pellet in 100µl of TE buffer.