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## Amplification of DNA from 30-year-old aceto-orcein stained salivary gland squash slides.

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Salivary gland squash slides have been used since the early days of genetics to visualize the fruit fly's polytene chromosomes. These slides represent an immense archive of genetic material for *Drosophila* and other genera. Aceto-orcein is commonly used to stain salivary gland preparation, as well as other cytological specimens, especially when trying to visualize chromosomal material. However, aceto-orcein stained tissue proves recalcitrant to yielding DNA for amplification. I describe here a simple procedural modification of a basic DNA extraction protocol that yields PCR-amplifiable DNA from aceto-orcein stained salivary glands.

The slides in question were made over 30 years ago from *Drosophila melanogaster* strains with presumptive tandem duplications of the maroon-like (*mal*) locus. These were apparently the result of spontaneous non-homologous unequal crossing over (Johnson and Smith, 1976; Johnson, 1977). These slides were prepared using a standard protocol involving 1) coating one slide with a gelatin subbing solution, 2) squashing salivary glands in 45% acetic acid between two slides using a bench vice, 3) freezing the slides on dry ice then popping them apart, 4) staining with aceto-orcein, 5) dehydration in alcohol, serial toluene treatment, and mounting a coverslip with CoverBond (Harelco). Various protocols to recover DNA were attempted repeatedly, including using the Pinpoint Slide DNA Isolation System™ (Zymo Research), all yielding no detectable DNA after PCR.

The key that made DNA isolation possible was a brief acidification step, presumably to remove the DNA-bound aceto-orcein. The successful protocol below is a modification of the Wizard® Genomic DNA Purification Kit procedure (Promega). The use of toluene here is to remove CoverBond which is toluene soluble.

### Protocol

1) Carefully scrape salivary gland tissue off the slide with a new razor blade then soak the tissue in toluene for 5 minutes.
2) Add an excess of 95% ethanol and mix gently.
3) Centrifuge briefly to pellet the tissue flake then discard the supernatant.
4) Wash three more times with 95% ethanol (repeat steps 2 and 3).
5) Remove remaining ethanol in a vacuum centrifuge.
6) Dissolve the flake (as much as possible) in 600 µL Wizard Nuclei Lysis Solution.
7) Incubate at 65ºC for 30 minutes.

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8A21    1     TAGATGTGAAAACGAATTTAATGTTCTGTAGTTAAAAAACATTTTATAAATGTTTTAAAT  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    TAGATGTGAAAACGAATTTAATGTTCTGTAGTTAAAAAACATTTTATAAATGTTTTAAAT

8A21    61    AATAAGATTGTAGTTTCCAAAAATTATTGTCATACAATTGGCCAATTAGCCATTAGCAAC  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    AATAAGATTGTAGTTTCCAAAAATTATTGTCATACAATTGGCCAATTAGCCATTAGCAAC

8A21    121   CATTTTCCTAACACCAATTGTTTGCCAACACTCAGAACATATGTTTGAATAGCCATGCAA  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    CATTTTCCTAACACCAATTGTTTGCCAACACTCAGAACATATGTTTGAATAGCCATGCAA

8A21    181   AGTGCACAACGACTAGTTAATACCGTACAATTTGAGTTTAAAATTCTATGCAATTCAGAG  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    AGTGCACAACGACTAGTTAATACCGTACAATTTGAGTTTAAAATTCTATGCAATTCAGAG

8A21    241   TTAATAATTGGAAAAGATTTGGTAAAATGCTACATCTATAATATTGGTGAGCAGTGTGAG  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    TTAATAATTGGAAAAGATTTGGTAAAATGCTACATCTATAATATTGGTGAGCAGTGTGAG

8A21    301   AATATGATATATATATTACAATAAGCAAACATAATGCACAAGAAGCTTAATAAATTGAGC  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    AATATGATATATATATTACAATAAGCAAACATAATGCACAAGAAGCTTAATAAATTGAGC

8A21    361   TTCGTAAAATGTGTAGGGTGGAGTAAGTTCAAAGAGAAGTCAAAGCAAATGCTCAACTGA  420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    TTCGTAAAATGTGTAGGGTGGAGTAAGTTCAAAGAGAAGTCAAAGCAAATGCTCAACTGA

8A21    421   ACGTGATAGTTGATGTATACTGTCCGTGGCTTTTTCCAGCGAAGAAGAGTATGGCGCAAC  480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    ACGTGATAGTTGATGTATACTGTCCGTGGCTTTTTCCAGCGAAGAAGAGTATGGCGCAAC

8A21    481   AAATGCAAGCCGGTCGCAAATAAATAAAGCCCGCTTTGCAGCCAACACGAATAGTTAGTT  540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    AAATGCAAGCCGGTCGCAAATAAATAAAGCCCGCTTTGCAGCCAACACGAATAGTTAGTT

8A21    541   CCCAACATGACATCATATCGGCCGGAGTTCTCCGCATCGGAGCAGTCGCAAATAGACGCG  600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    CCCAACATGACATCATATCGGCCGGAGTTCTCCGCATCGGAGCAGTCGCAAATAGACGCG

8A21    601   GAATTCTCTAGATTGGCCAGTAAGTGGAGAACCATGGTGAAATACCCAGCGCAAACAGAT  660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AE014298.4    GAATTCTCTAGATTGGCCAGTAAGTGGAGAACCATGGTGAAATACCCAGCGCAAACAGAT

8A21    661   AACGAACCTAACCATCCGTCTATCGAACAGGCAACA  696
              |||||||||||| ||||||||||||||||||||||
AE014298.4    AACGAACCTAACTATCCGTCTATCGAACAGGCAACA
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Figure 1. Alignment the entire fragment amplified from a slide (8A21) and the *Drosophila melanogaster* chromosome X *mal* region DNA (AE014298.4).

8) Add 10 µL of Wizard RNase and 10 µL of Proteinase K (20 mg/mL) and incubate at 37ºC for 20 minutes.
9) Incubate at 95ºC for 5 minutes to denature Proteinase K.
10) Add 200 µL of Wizard Protein Precipitation Solution and vortex for 20 seconds.
11) Chill on ice for 5 minutes.
12) Centrifuge for 4 minutes at 13,000-16,000 ×g.

13) Transfer supernatant to a clean 1.5 mL microfuge tube containing 600 µL of isopropanol.
14) Gently mix by inversion.
15) Centrifuge for 1 minute at 13,000-16,000 ×g.
16) Carefully remove isopropanol with a pipette and discard.
17) Add 600 µL of 70% ethanol and gently mix by inversion.
18) Centrifuge for 1 minute at 13,000-16,000 ×g.
19) Carefully remove ethanol with a pipette and discard.
**20) Add 100 µL 45% acetic acid and mix gently; let stand for 2 minutes.**
**21) Add 300 µL Wizard Rehydration Solution and 1/10 volume 7.5 M ammonium acetate and mix gently.**
**22) Add 2 volumes 95% ethanol and mix gently.**
**23) Incubate at 70ºC for 5 minutes.**
**24) Centrifuge for 1 minute at 13,000-16,000 ×g.**
**25) Carefully remove supernatant with a pipette and discard.**
**26) Add 1000 µL ethanol, mix gently, and centrifuge for 1 minute at 13,000-16,000 ×g.**
**27) Carefully remove supernatant with a pipette and discard.**
28) Invert tube on a paper towel and air-dry the pellet for 10–15 minutes.
29) Add 40 µL of Wizard DNA Rehydration Solution and incubate at 65°C for 1 hour.
30) Store DNA at 2–8°C.

PCR was performed on these extracts using GoTaq® (Promega) and primers that amplify an approximately 744 nt segment at the beginning of the *mal* mRNA (primers: 5'CAGCTGTATGTGTAGGCTATCGTC3' and 5'CCGCATGATCCAGGTAAACACTCT3'). Longer extension times were used (94ºC for 1 minute, 55ºC for 1 minute, 72ºC for 3 minutes for 33 cycles) and hot start PCR was performed using AmpliWax® PCR Gem wax beads (Applied Biosystems). A typical reaction used 10.5 µL of DNA in the lower layer and 12.5 µL of GoTaq and 1 µL of each primer in the upper layer. DNA amplified from two slides using these primers was indistinguishable on agarose gels from similarly amplified DNA from wild-type flies. PCR products were cloned using the TOPO® TA method (Invitrogen) and sequenced (Macrogen, Korea). As shown in Figure 1, this amplified DNA is from the *mal* locus of *Drosophila melanogaster*. All clones from the two slides gave similar results.

It is my hope that this report will make such archived *Drosophila* DNA more accessible.

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**An organizational strategy for deficiency mapping: A computational approach**.

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## Introduction

Deficiency mapping provides *Drosophila* geneticists with the unique ability to physically map mutations by studying a single generation of animals. By crossing a lethal mutation in an unknown