Technique Notes

***Drosophila* stock transferring**.

Here we would like to cross-reference an interesting article by Adelaide Carpenter on page 147. Her paper, "Sturtevant Revisited", is both an historical note and a comment on a technique that is useful for transferring stocks under less than optimal media conditions.

An improved procedure in fly food preparation.

Zeng, C. Department of Biological Sciences, University of Wisconsin Milwaukee, Milwaukee, WI 53211.

The most frequently used procedure for preparing fly food includes mixing and cooking ingredients, dispensing the cooked mixture, allowing the condensation to evaporate, and properly storing the finished product. Typically, sheets of chess cloth are used to wrap trays of bottles or vials immediately after hot food is dispensed. This allows the condensation to escape while keeping free roaming flies out. In our experience, we have found that chess cloth is very susceptible to tearing after several uses. If the torn cloth is not noticed, airborne flies could gain entry into vials and bottles. To prevent such contamination, we have replaced chess cloth with regular sized pillow cases for this step. Pillow cases offer a good alternative because they are inexpensive and less likely to suffer such damage. Trays of vials or bottles of standard size can fit easily inside the pillow cases through the opening without any alterations. By simply folding the open side and tucking it underneath the tray, an adequate seal was formed to keep flies out. It usually took one day for all condensation to evaporate in our laboratory.

A new olfactory trap assay.

Sharma, Ruchira, Sonia Sen, and Gautam Dey. (Summer interns at the Department of Biological Sciences), Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai, India.

We have miniaturized and standardized a trap assay first described by Woodard *et al*. (1989), and modified in form by Park *et al*. (2002). The assay, originally named the "Hedgie trap assay", is used to quantify attraction of adult *Drosophila* flies to odorants. We have used wild type (CSBz) and an olfactory mutant, *smellblind* (*sbl*) (Rodrigues and Siddiqi, 1978; Lilly and Carlson, 1989; Lilly *et al*., 1994) to standardize this test.

Figure 1. Experimental setup as described in protocol. a: trap containing control (water). b: trap containing odorant (1:10 isoamyl acetate in water).

The assay was carried out in 500 ml beakers using modified 1 ml plastic pipette tips (Blue tips) as the traps. In contrast to the protocol described by Park *et al*. (2002), a control trap containing the diluent alone was also included in the same setup. The test was carried out using 1:10 dilution of isoamyl acetate (Sigma) in water as the odorant and was allowed to run for approximately 2 days. The experimental set-up is shown in Figure 1. Briefly, a thin layer of moist cotton was placed at the bottom of a 500 ml beaker. To construct the trap, the tip was cut off 1.5 cm from the end of the blue tip. The base was covered with parafilm and 500 µl of odorant solution was added to the pipette tip. A 200 µl plastic pipette tip (yellow tip) was used as a 'valve' to prevent flies from escaping from the trap once they had entered it. In order to construct this, we cut the tip cut 1 cm from the end, and inverted it into the trap (see Figure 1). An identical trap containing 500 microlitres of distilled water

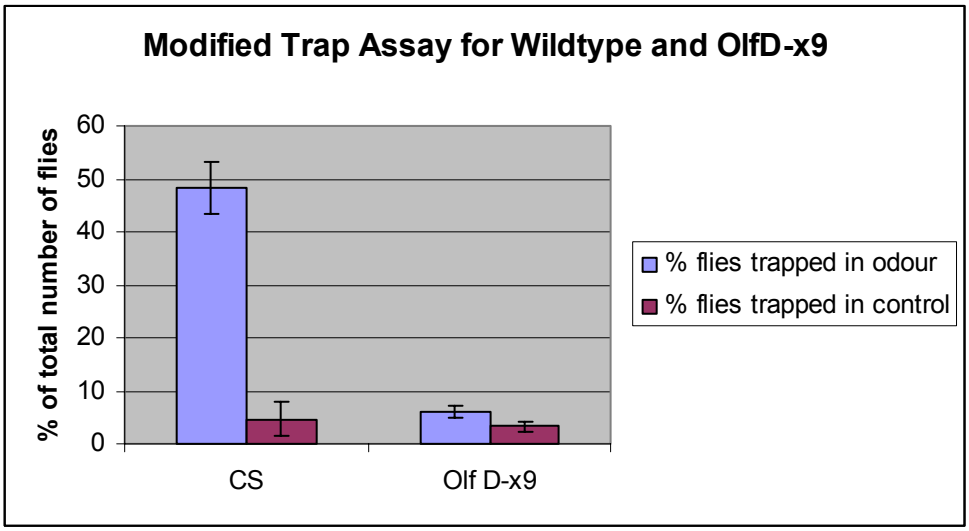


Figure 2. Responses of wild type and *sbl* $^{olfd\text{-}x9}$ flies to isoamylacetate. The trap shown in Figure 1 was used except that the 'yellow tip valves' were not used in this experiment.

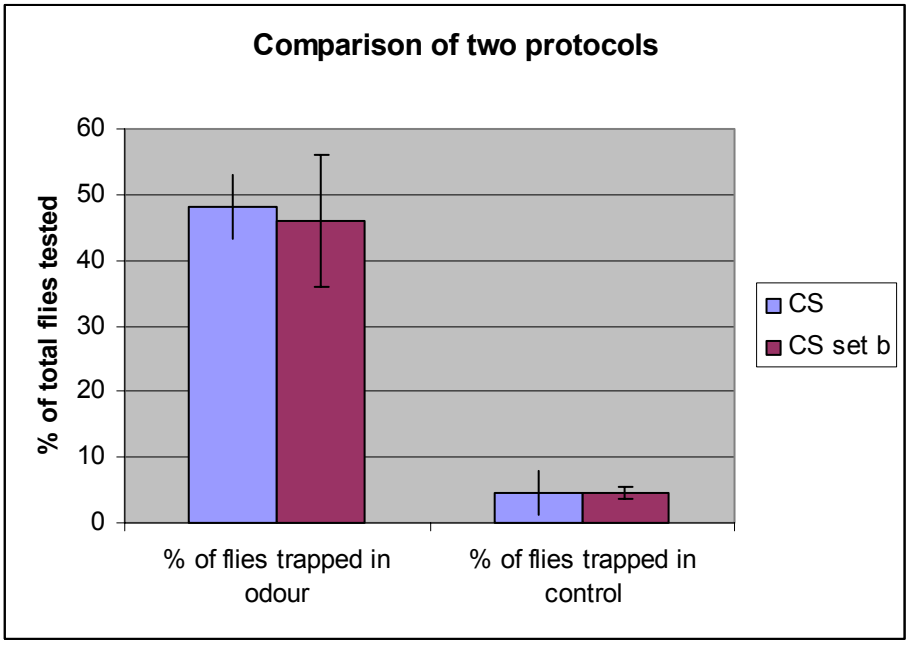


Figure 3. Responses of wild type flies when tested in a trap with (set b) and without the 'yellow tip valve'. The presence of the valve does not significantly alter the response of the flies.

was used as the control. The two traps were taped to diametrically opposite sides of the beaker. About a hundred two-to-three day old flies were introduced into the beaker and a layer of cling film was used to cover and seal the setup. Two 200 µl pipette tips were inserted into the cling film layer to provide air for the flies. The beakers were left undisturbed at 25°C for 2 days and the number of flies in both traps was counted to calculate the percentage response. The percentages of flies trapped within the odour trap and within the control trap were calculated (Figures 2 and 3). The mean and

standard deviation were calculated from ten independent experiments. In all experiments the number of flies recovered from the control trap after 2 days was very small. About 50% of the population of wild type flies could be trapped by 10^{-1} dilution of isoamylacetate. Under these conditions less than 7 % of *sbl* $^{olfd\text{-}x9}$ flies were captured. Ten sets were tested for each of the two lines.

We conclude that the modified trap assay can be used as an effective tool in identifying mutant strains with an olfactory attraction defect. *Sbl* $^{olfd\text{-}x9}$ show a significant decrease in attraction to the odorant solution. The advantage of using this assay lies in the fact that it is compact, convenient, and simple to set up and uses only basic materials. Furthermore, multiple experiments can be simultaneously set up, and can then be left unattended for the duration of the test. Thus, for example, it can be used as a preliminary test in large scale mutagenesis screens to quickly and efficiently identify olfactory mutants for further investigation.

References: Lilly, M., and J.R. Carlson 1990, Genetics 124(2): 293-302; Lilly, M., R. Kreber, B. Ganetzky, and J.R. Carlson 1994, Genetics 136: 1087-1096; Loughney, K., R. Kreber, and B. Ganetzky 1989, Cell 58(6): 1143-1154; Park, S.K., S.R. Shanbhag, A.E. Dubin, M. Bruyne, Q. Wang, P. Yu, N. Shimoni, S. D'Mello, J.R. Carlson, G.L. Harris, R.A. Steinbrecht, and C.W. Pikielny 2002, J. Neurobiol. 51(3): 248-260; Rodrigues, V., and O. Siddiqi 1978, Proc. Ind. Acad. Sci. 87B: 147-160.

A novel luciferase assay for the quantification of insulin signaling in *Drosophila*.

Slade, Jennifer D., Jamie M. Kramer, and Brian E. Staveley. Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland & Labrador, Canada, A1B 3X9; telephone (709) 737-4317; telefax (709) 737-3018; Corresponding author: Dr. Brian E. Staveley; e-mail address: bestave@mun.ca.

Introduction

Insulin signaling is implicated in the control of growth and cell survival. One component, the transcription factor FOXO, is activated under low levels of insulin signaling. The shuttling of FOXO between the nucleus and the cytoplasm is mediated by several mechanisms, including its phosphorylation by the akt kinase (van der Heide *et al.,* 2004). Downstream targets of FOXO include genes which influence apoptosis, DNA repair, cell cycle progression, metabolism and oxidative stress resistance (Barthel *et al.,* 2005). Thus, *FOXO* is an important mediator in the control of cellular growth and survival.

Studies in *Drosophila* demonstrate that the evolutionarily conserved insulin signaling pathway is involved in the control of body size and growth, and the reaction to nutritional stress (Kramer *et al.,* 2003; Jünger *et al.,* 2003; Puig *et al.,* 2003). Ectopic expression of *Drosophila FOXO* leads to inhibition of growth and generation of small adults. This occurs via a reduction in cell number and cell size (Kramer *et al.,* 2003). In addition, when there is an excess of *FOXO* activity in larvae, feeding behaviour is altered. Newly hatched *Drosophila* larvae require nutrients in order to increase their body mass via replication of cells in mitotic tissues. In contrast, when larvae are hatched into conditions of amino acid starvation, they live in a state of developmental arrest until nutrients become available (Beadle *et al.,* 1938). Ectopic expression of *FOXO* leads to a phenotype that resembles *Drosophila* deprived of nutrients (Kramer *et al.*, 2003). As *daf16*, the *Caenorhabditis elegans* homologue of *FOXO*, is required for the unique response to nutritional stress (dauer larva formation), it has been demonstrated that *Drosophila FOXO* also plays a key role in survival under

nutritional stress. Alterations to the insulin signaling pathway and availability of nutrients negatively regulate the activity of *FOXO* (Kramer, Slade and Staveley, submitted). We believe that it is important to understand the fine control mechanisms influencing *FOXO* activity in flies and thus have the basis to comprehend the subtly of the role of *FOXO* in human conditions such as diabetes and obesity.

Modern genetic analysis may entail the detection and quantification of gene expression. *In vivo,* this procedure requires the use of a reporter gene that, when introduced into the organism, yields a quantifiable phenotype (Wood, 1995). Elements that enhance transcription can be fused to the firefly *luciferase* gene, and then be introduced into the fruit fly. The following describes an assay for the detection of *luciferase* expression from a transgene that responds to alterations in *FOXO* activity.

The *FOXO* recognition element-*luciferase* (*FRE-luc*) transgene

The *FOXO* recognition element-*luciferase* (*FRE-luc*) transgene was created from the 8xFK1tkLuc plasmid construct, which had been demonstrated to respond extremely well to *FOXO* in cultured cell lines (Biggs *et al.*, 1999). The construct contains eight direct repeats of a *FOXO* recognition element (FRE), and the *luciferase* gene under the control of a herpes simplex virus thymidine kinase minimal promoter. The 8xFK1tkLuc construct was cloned into the *pP{CaSpeR-1}* transformation vector (Figure 1) and then injected into w^{1118} embryos by standard methods. Transgenic flies were selected based on eye colour.

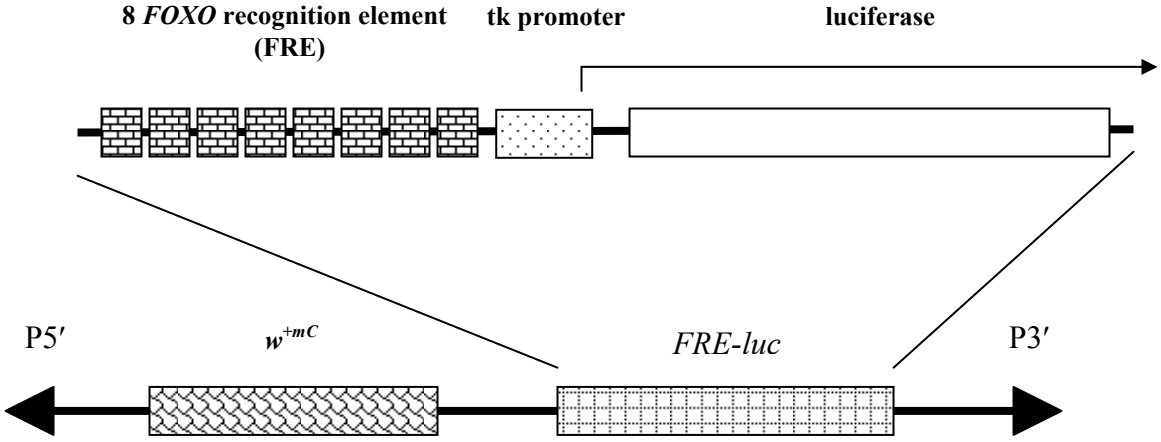


Figure 1. The *FRE-luc* transgene. Eight direct repeats of the *FOXO* recognition element are located upstream of a minimal herpes simplex thymidine kinase (tk) promoter fused to the *luciferase* gene. This has been inserted into the multiple cloning site of the *pP{CaSpeR-1}* transformation vector. Not drawn to scale.

Assay Procedure

Isolation of Drosophila lines containing the FOXO responsive luciferase transgene

Routinely adults are collected in triplicate groups of ten and then flash frozen at -70°C for a minimum of 5 minutes. Flies can be stored at -70°C until ready for protein extraction.

Protein extraction

Protein extraction is performed according to the Promega Luciferase Assay System manual (Promega Corporation, Madison, Wisconsin). Flies are placed in 200 µl of cell culture lysis buffer, and homogenized by grinding in a 1.5 ml microcentrifuge tube. Samples are then subjected to a freeze-thaw cycle three times, that includes flash freezing the samples in ethanol at -70°C, and thawing the samples in a 37°C water bath. The tubes are centrifuged at 10,000 rpm for 10 minutes to remove cellular debris. The supernatant is collected in a fresh tube. The procedure of homogenization, freezing and thawing, centrifugation and collection of supernatant is repeated on the pellet fraction, with the second supernatant pooled with the original.

Determination of protein concentration

Protein amounts are quantified using the *DC* Protein Assay kit from Bio-Rad (Bio-Rad Laboratories, Hercules, California). Protein concentrations of extracts were determined against a standard BSA curve and using manufacturer's instructions. Working reagent A, an alkaline copper tartrate solution, and reagent B, a dilute Folin reagent, are required for protein quantification. The proteins and copper react in the alkaline medium, and this leads to the reduction of Folin and colour production. 5 µl of each protein sample is added to wells of a clean, dry microtiter plate. 25 µl of reagent A and 200 µl of reagent B are then added to each protein sample. The plate is gently agitated to mix reagents and samples, and bubbles removed. After 15 minutes, the absorbance of each well is read at 750 nm by a spectrophotometer, such as Spectramax 190 microplate reader (Molecular Devices Corporation, Chicago, Illinois).

Quantification of luciferase expression

To quantify the luciferase activity present in each protein sample, 20 µl of sample plus 100 µl of Steady Glo reagent (Promega Luciferase Assay System) is added to wells in a dark 96-well plate. The plate is then placed into a luminometer, such as a Top Count NXT microplate scintillation and luminescence counter. Amounts of luciferase activity can then be quantified and compared to control samples.

Conclusions

This method represents a novel and accurate way of measuring the activity of endogenous and mutant *FOXO* genes. Indirectly, it can measure the activity of upstream components of the insulin pathway that regulate *FOXO*. For example, as *akt* negatively regulates *FOXO,* an increase in *akt* activity may lead to less *FOXO* activity. In addition, this method can be used to measure the effect of environmental factors, such as age and stress, on the activity of endogenous *FOXO*.

Acknowledgments: This work was funded by grants to BES from the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant program and The Banting Research Foundation. JDS was partially funded by a Memorial University of Newfoundland School of Graduate Studies Fellowship and a graduate student teaching assistantship from the Department of Biology of Memorial University. JMK was funded by NSERC Post-Graduate Scholarship (Doctoral) award. Many thanks are extended to Dr. W.H. Biggs III for the *luciferase* transgene, and Dr. H. Lipshitz for the w^{1118} line. Further thanks go to Justin N. Moores for his comments on the manuscript.

References: Barthel, A., D. Schmoll, and T.G. Unterman 2005, Trends Endocrinol. Metab. 16: 183-189; Beadle, G., E. Tatum, and C. Clancy 1938, Biol. Bull. 75: 447-462; Biggs, W.H., III,

J. Meisenhelder, T. Hunter, W.K. Cavenee, and K.C. Arden 1999, Proc. Natl. Acad. Sci. USA 96: 7241-7246; Jünger, M.A., F. Rintelen, H. Stocker, J.D. Wasserman, M. Végh, T. Radimerski, M.E. Greenberg, and E. Hafen 2003, J. Biol. 2: 20; Kramer, J.M., J.T. Davidge, J.M. Lockyer, and B.E. Staveley 2003, BMC Dev. Biol. 3: 5; Puig, O., M.T. Marr, M.L. Ruhf, and R. Tjian 2003, Genes Dev. 17: 2006–2020; van der Heide, L.P., M.F.M. Hoekman, and M.P. Smidt 2004, Biochem. J. 380: 297-309; Wood, K.V., 1995. Curr. Opin. Biotechnol. 6: 50-58.

Adapting a microcalorimeter to measure heat output of individual *Drosophila*.

Parisi, Michael[1], Philip D. Ross, and Brian Oliver. National Insititute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD. [1]LCDB, NIH, 50 South Drive, Bethesda MD 20892-8028; email: MichaelPa@intra.niddk.nih.gov.

Analyzing differences in metabolic rates and behavioral activity between different fly strains is essential to understanding key physiological processes in *Drosophila*. One of the rare drawbacks of *Drosophila* as a model organism is the difficulty inherent in studying the physiology of a small single insect. We were interested in investigating whether or not metabolic profiles differed between flies that had a functionally intact germline (GL+) and flies that have had their germlines ablated genetically (GL). One prediction suggests that a functional germline would prioritize metabolic resources for gamete development. Not having a germline might show metabolic differences, perhaps as an increase in muscular activity that would generate heat. Such metabolic trade-offs occur naturally in other insect systems. For example, nutrients, especially lipid stores dedicated to flight capability, are negatively associated with reproductive output in the sand cricket *Gryllus firmus* (Zera and Brink, 2000). Changes in activity rate can be easily measured using activity monitors such as those that detect motion across an infrared beam (Martin *et al*., 1999). However, changes in metabolic rate during periods of rest or activity are not measurable using these devices. Calorimetry studies have been performed on larger insects (4-50 mg) (Lovrien *et al*., 1981) and we wanted to adapt a system that would accommodate the smaller (~0.5-1.0 mg) *Drosophila*. We show in this study that metabolic rates and activity are measurable as heat output using a differential scanning microcalorimeter initially designed to measure thermal effects in solutions (Ross and Goldberg, 1974) (Figure 1).

We gauged germline-dependent metabolic differences by measuring heat generated by GL– male progeny of homozygous *tudor (tud^1/tud^1)* mutant females (Schupbach and Wieschaus, 1986) as compared to the GL+ male progeny of sibling *tud^1/CyO* control females. Single five-day-old adult flies were placed into a hollow stainless steel cell (2.6 cm × 2.0 cm × 0.5 cm) and inserted within a large insulated metal cylinder (33 cm ht by 32.5 cm diameter). The mass of the large metal cylinder facilitated temperature stability in the internal cell thus lessening environmental temperature fluctuations. At the center of the cylinder, a semiconductor thermoelectric module was positioned in contact with the cell. Heat output from the cell, detected by the thermopile, was read as voltage (V) by a Keithley model 181 nanovoltmeter (Keithley, Cleveland, OH) and recorded as a printed trace. The sensitivity of the thermopile was 5.36 Watts W/V as determined by electrical calibration by passing known voltage through a resistor mounted around the cell. Prior to performing an experiment, the cell was washed first in deionized water, then methanol and baked to remove all liquid. The empty cell was cooled, placed in the device and allowed to equilibrate for two to three

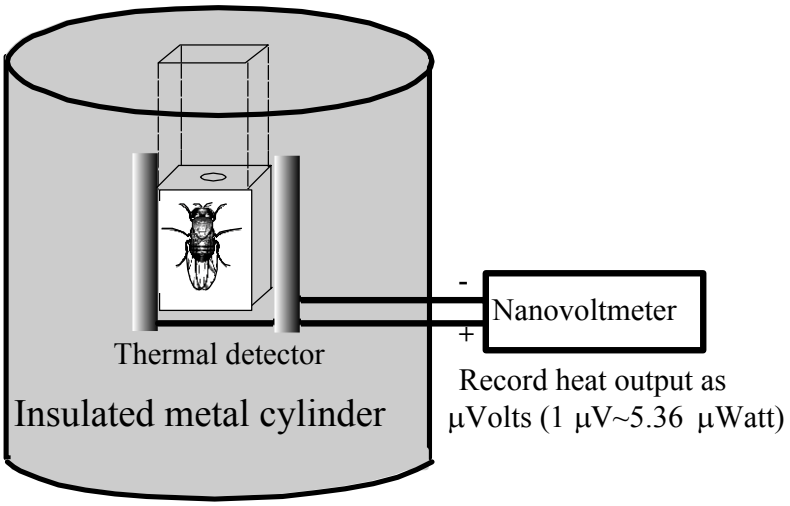


Figure 1. Diagram showing the microcalorimeter used to measure heat from a single fly.

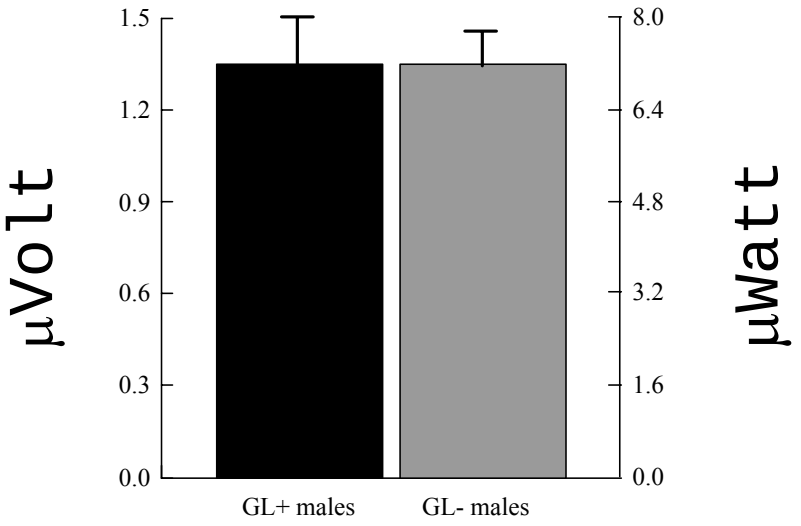


Figure 2. Graph shows average resting heat output from experimental runs performed on 9 (GL+) and 10 (GL−) flies.

hours to create a baseline signal for the instrument. A single fly was then placed in the cell for periods of 12 to 14 hours in most instances and up to two days in other experiments. The voltage differential from baseline was recorded and converted to power. Cyclic changes in heat output were observed indicating that both heat output at rest and during activity bouts were measured. In experiments where a fly died in the cell (due to dessication), the signal returned to the instrument baseline level. These data demonstrate that the microcalorimeter detects the heat output of single flies reliably.

Heat output at rest was calculated from signal above equilibration of the cell determined by averaging the initial and final resting voltage levels minus the pre-experiment instrument background.

An average of 1.35 ± 0.15 (2 sd) and 1.35 ± 0.11 (2 sd) µvolts were determined from GL+ (n = 9 flies) and GL– (n = 10 flies) males, respectively, (Figure 2) indicating that male *Drosophila* generate approximately 7.2 µWatts at rest. Activity spikes were consistently shown to go to ~ 2.0 µV. In one exceptional case, we saw very regular spikes in heat output at 20 minute intervals over an extended seven hour period (Figure 3). In this experiment, the resting voltage produced was 1.31 µV (7.02 µW) with activity bursts spiking to 2.15 µV (11.5 µW). Interestingly, heat output increases the most as activity commences, then falls slightly during the activity period before a rapid return to basal levels.

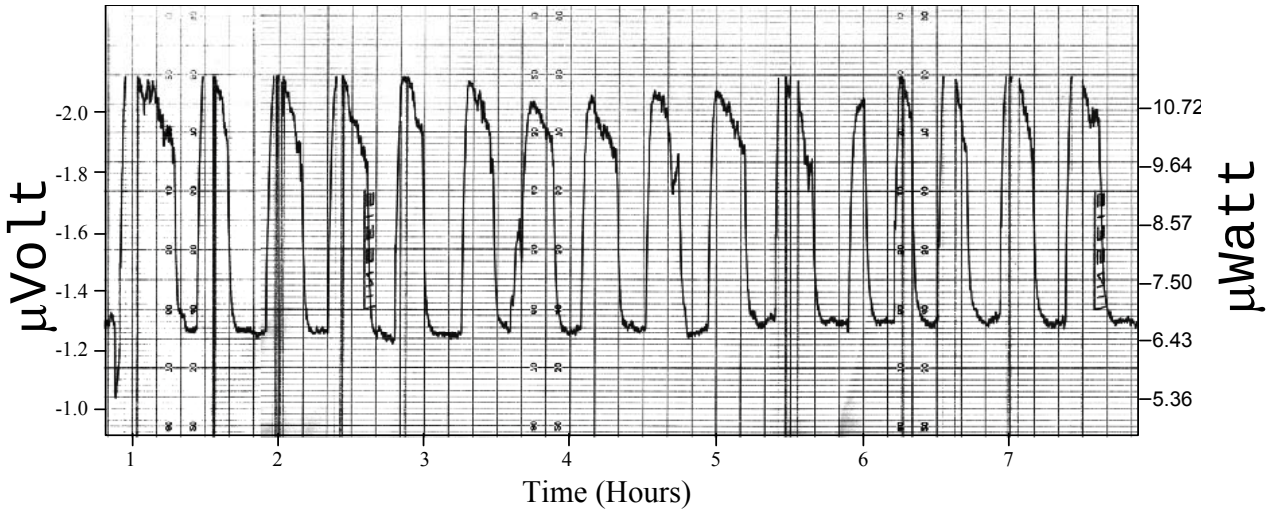


Figure 3. Trace of a GL+ male fly shows periodic activity bursts with a high degree of regularity. The spikes likely represent transient metabolism due to muscular exertion that then returns to the resting metabolic level.

We did not find significant differences in resting heat output between the two germline conditions tested suggesting that presence or absence of a germline, at least in males, does not significantly alter heat generation when the flies are in a resting state. This method shows that we can detect the heat generated from single flies that should be useful for studying mutant strains with altered metabolic profiles.

Acknowledgments: This research was supported in part by the Intramural Research Program of the NIH, NIDDK.

References: Lovrien, R.E., T.J. Kurtti, R. Tsang, and M. Brooks-Wallace 1981, J. Biochem. Biophys. Methods. 5(6): 307-318; Martin, J.-R., R. Ernst and M. Heisenberg 1999, J. Comp. Physiol. 184: 73-84; Ross, P.D., and R.N. Goldberg 1974, Thermochimica Acta. 10(2): 143-151; Schupbach, T., and E. Wieschaus 1986, Dev. Biol. 113: 443-448; Zera, A.J., and T. Brink 2000, J. Insect Physiol. 46 (8): 1207-1218.

pUAST-IR: A new *Drosophila* vector for easy-cloning of RNAi transgenes.

Lasbleiz, Christelle, Audrey Coiffic, Hervé Tricoire, and Véronique Monnier*.
Institut Jacques Monod, 2 place Jussieu, 75251 Paris, France; * Corresponding author: V. Monnier, Institut Jacques Monod, Université Paris 6-7, 2 place Jussieu, 75251 Paris, France; Tel: 33144278213; Fax: 33144273660; E-mail: monnier@ijm.jussieu.fr.

RNA interference (RNAi) has been demonstrated to be a powerful genetic tool to specifically inactivate genes of interest in a large variety of organisms. It relies on the ability of double-stranded RNA to cause sequence specific silencing of the corresponding gene (Fire *et al.,* 1998; Hunter, 1999). In *Drosophila*, methods have been developed to express double-stranded RNA (dsRNA) stably in transgenic flies, enabling studies of a spatially and temporally-controlled gene inactivation in the late stages of development and in adult flies (Lam and Thummel, 2000)**.** These methods usually involve transgenes exhibiting symmetric sequences of the targeted gene, under control of the upstream activating sequence (UAS) of the transcription factor GAL4 (Kennerdell and Carthew, 2000; Kirby *et*

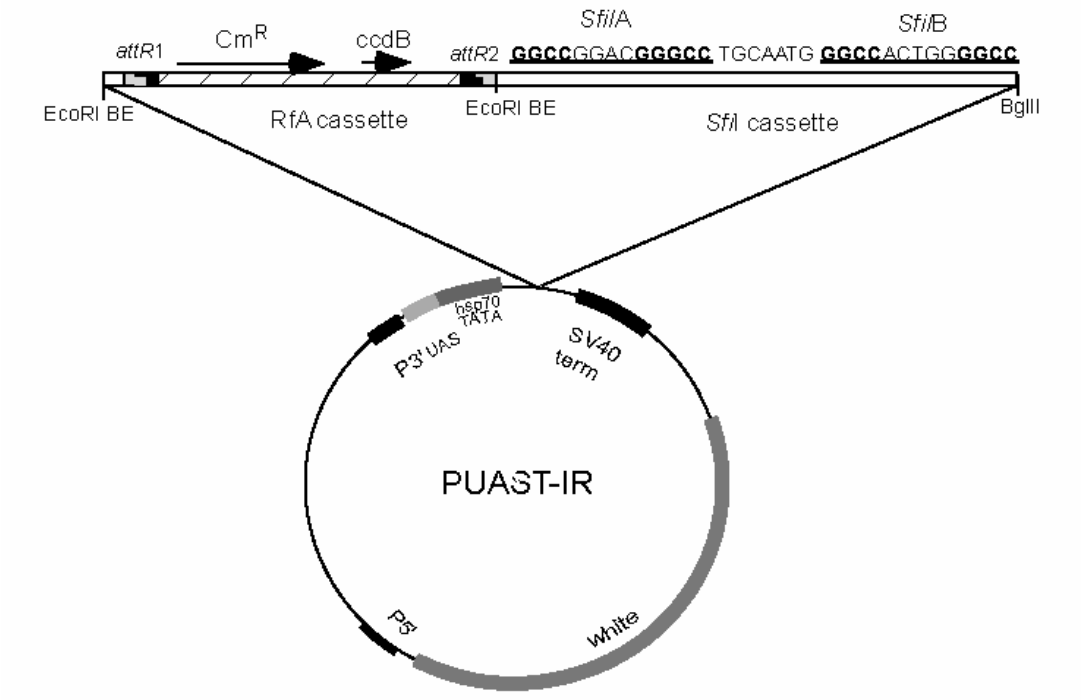


Figure 1. Map of pUAST-IR. pUAST-IR derives from pUAST transformation vector. We first introduced the *Sfi*I cassette (which contains *Sfi*IA and *Sfi*IB restriction sites) between *Eco*RI and *Bgl*II sites of pUAST polylinker. This vector was then digested by *Eco*RI, and the Klenow fragment was used to create blunt ends (*Eco*RI BE) to introduce the blunt-ended RfA cassette. To select and propagate this plasmid pUAST-IR, DB3.1^{TM} competent cells of Invitrogen (F$^-$ *gyr*A462 *end*A1 Δ(sr1-*rec*A) *mcr*B *mrr hsd*S20(r_B-, m_B-) *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Sm^R) *xyl*-5 λ- l*eu mtl*1) are used because they are resistant to the toxic effects of CcdB, and selection should be made on chloramphenicol (30 μg/ml) and ampicillin (100 μg/ml)-containing media.

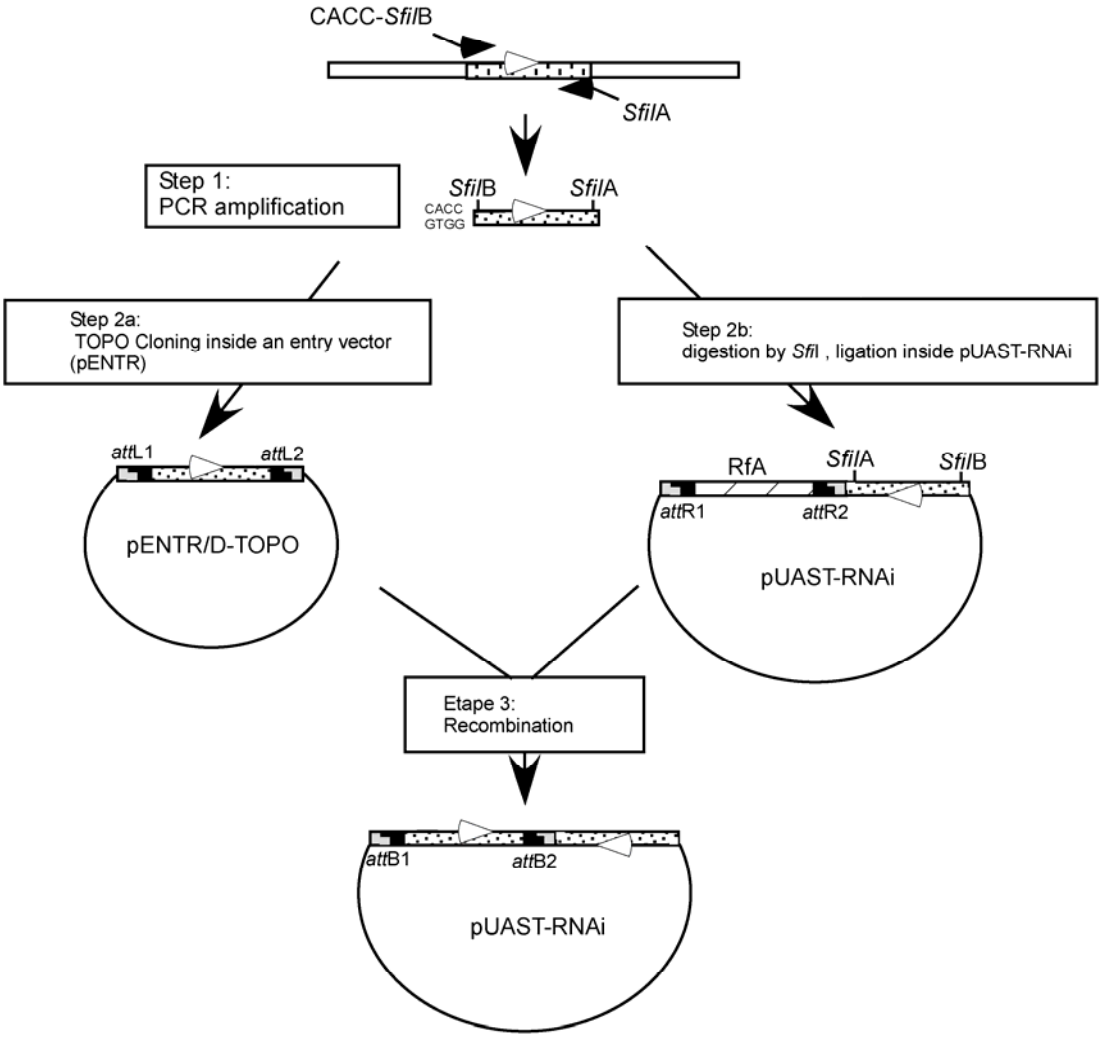


Figure 2. Strategy for generation of RNAi constructs with pUAST-IR. Step 1: amplification of the sequence of interest by PCR, with a polymerase producing blunt-end products. One primer should contain ATCC upstream of the *Sfi*IB site at its 5' end, and the over the *Sfi*IA site. Step 2: Cloning of the PCR product into an entry vector (pENTR/D TOPO, kanamycin resistance) by TOPO cloning (Step 2a) and in parallel, after *Sfi*I digestion (at 50°C) into pUAST-IR (Step 2b). Step 3: Recombination with the LR clonase mix (Invitrogen) as described by the supplier. Transformation of the recombination product should be made in the SURE® bacterial strain of Stratagene (*e14– (McrA-) Δ(mcrCB-hsdSMR-mrr)171 endA1 supE44 thi-1 gyrA96 relA1 lac recB recJ sbcC umuC::Tn5* (Kan^r) *uvrC* [F' *proAB lacI^{q}ZΔM15 Tn10* (Tet^r)]) to maximize the stability of the inverted repeats. Only plasmids with successful recombination are able to amplify in these *ccdB*-sensitive cells, on ampicillin-containing media (100 μg/ml).

al., 2002; Roignant *et al.*, 2003). In presence of GAL4, dsRNA is expressed as extended hairpin-loop RNA, leading to biological effects similar to RNAi-mediated interference by injected dsRNA. However, such methods involves the cloning of inverted-repeat (IR) sequences in a *Drosophila* transformation plasmid vector, and IRs cloning is subjected to variable efficiency in bacterial strains.

Here, we describe an efficient time-saving method for the cloning of IR transgenes under control of UAS, using a new pUAST-IR vector. IR transgenes can easily be introduced in this vector, derived from pUAST (Brand and Perrimon, 1993), by two sequential insertions of the same PCR product. The first relies on classical cloning using adequate restriction sites and the second step uses the Gateway Recombination System developed by Invitrogen (Figure 1). pUAST-IR contains the RfA Cassette of Invitrogen, that we have inserted downstream of the hsp70 TATA box and transcriptional start. This cassette contains *att*R1 and *att*R2 recombination sites, flanking the ccdB gene, and the chloramphenicol resistance gene. This modified vector can be used as a destination vector as defined in the Gateway System. Downstream the RfA cassette, we have also inserted a S*fi*I cassette that contains two adjacent restriction sites, called *Sfi*IA and *Sfi*IB. These sites are both recognized by *Sfi*I enzyme, but lead to non cohesive ends after digestion, preventing plamid recircularisation.

To construct a transgene using pUAST-IR, a DNA fragment corresponding to the gene of interest is amplified by PCR with two primers containing respectively *Sfi*IA and *Sfi*IB sites at their 5' ends (Figure 2, Step 1). The primer sharing the *Sfi*IB site also contains the four bases CACC at its 5' end to enable directional TOPO cloning of the PCR product in pENTR TOPO vector, between *attL* recombination sites (Step 2a). In parallel, the same PCR product is introduced in pUAST-IR by directional cloning in *Sfi*I sites (Step 2b).

An LR recombination reaction is then performed between this pENTR clone, and the pUAST-IR containing the sequence introduced in the *Sfi*I cassette (Step 3). The recombination occurs between attL and attR sites, and leads to a pUAST-IR vector containing the sequence of interest in inverted orientations. This last step, corresponding in our hands to the problemmatic step of IR constructions by ligation of enzymatic digested fragments, appears to be much more efficient using LR recombination. Indeed, we have already made five transgenic RNAi constructs for genes under study in our laboratory and 50% to 100% of clones were positive after LR recombination.

We introduced four of these RNAi constructs in *Drosophila* by classical transgenic methods. Expression of IR transgenes, under control of an ubiquitously expressed GAL4, lead to the expected phenotypes and to a decrease of transcript levels (data not shown).

Thus, this new vector appears to provide high efficiency and time saving to obtain RNAi constructs for subsequent *Drosophila* transgenesis, as all cloning steps can be performed in one week. The principle of our method could be easily extended to other types of vectors.

References: Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, and C.C. Mello 1998, Nature 391: 806-811; Hunter, C.P., 1999, Curr. Biol. 9: R440-442; Kennerdell, J.R., and R.W. Carthew 2000, Nat. Biotechnol. 18: 896-898; Kirby, K., J. Hu, A.J. Hilliker, and J.P. Phillips 2002, Proc. Natl. Acad. Sci. USA 99: 16162-16167; Lam, G., and C.S. Thummel 2000, Curr. Biol. 10: 957-963; Roignant, J.Y., C. Carre, B. Mugat, D. Szymczak, J.A. Lepesant, and C. Antoniewski 2003, Rna 9: 299-308.

An updated chemically-defined medium for *Drosophila melanogaster*.

Blatch, S.A., and J.F. Harrison. School of Life Sciences, Arizona State University, Tempe, AZ 85287-4601 USA. Correspondence: sydella.blatch@asu.edu.

For many nutritional or biochemical studies, it is often necessary to raise insects on completely chemically-defined (holidic) diets so exact nutrient content is known and can be

controlled. Holidic diets typically used for *Drosophila melanogaster* are Sang's Medium C (Sang, 1956) or are based on this. However, holidic diets are unable to produce *D. melanogaster* as fit as those reared on diets that include yeast (Rapport *et al.*, 1984). In an attempt to increase fitness, many modifications have been made to existing holidic media (Bruins *et al*., 1991; Geer and Vovis, 1965; Rapport *et al*., 1984). In this contribution, we have combined most of the alterations of prior studies and added several new modifications in order to provide an accessible, updated holidic diet for *D. melanogaster*.

Table 1. Composition of Holidic Medium.

Nutrient	Grams/liter water
Cholesterol	0.2901
Agar	29.0135
Casein (Vitamin free)	53.1915
Sucrose	9.6712
Choline	0.0580
Yeast RNA	3.8685
Thiamin HCl	0.0116
Riboflavin	0.0097
Nicotinic acid	0.0464
Calcium pantothenate	0.0193
Pyridoxine	0.0058
Biotin	0.0006
Folic Acid	0.0029
L-Carnitine	0.0097
L-Ascorbic acid	0.5377
L-Tryptophan	2.4178
To Make Stock Salt Solutions	**Grams**
$NaHCO_3$	13.3333
KH_2PO_4	9.4667
K_2HPO_4	49.7333
$MgSO_4$, $7H_2O$	8.2667
$FePO_4$	0.1333
$CaCl_2$	0.2667
$MnSO_4$	0.1720
Na_2HPO_4	25.2000
$CuSO_4$	0.0667
$Zn(CH_3COO)_2$	0.2667
Dissolve each salt in 200 mL distilled water. 14.5 mL of each stock solution is added to the medium	
Total Amount Distilled Water	1 liter

Media Composition and Preparation

We modified Sang's Medium C by adding L-tryptophan (Sang, 1956). Quantity of sucrose and some of the salts was based on the media of Geer and Vovis (1965). Addition of carnitine followed Geer *et al.* (1971), ascorbate followed Bruins *et al.* (1991), and choline and other salts followed Rapport *et al.* (1984). The remaining changes (the amounts of water, thiamin, nicotinic acid, pantothenic acid, biotin, and pyridoxine) were established in our lab (Table 1).

Agar, casein, sucrose, choline, and yeast RNA are ground in a blender for approximately 2 minutes. A second mixture is prepared by stirring the cholesterol into warm 20% ethanol (500 mL 20% ethanol per gram of cholesterol). After the cholesterol is dissolved, the mixture is autoclaved (20 lbs., 120°C) for ten minutes, and then further mixed in a blender for one minute. Excluding folic acid, each of the vitamins, carnitine, and tryptophan are weighed on a microbalance and placed into a large beaker. 665 mL distilled water is then added and stirred until all the vitamins have dissolved. The folic acid is dissolved separately in 20% ethanol (25.5 mL 20% ethanol per mg of folic acid) and then added to the rest of the vitamins. The mineral salts are prepared as stock solutions and added to the medium.

All the components (dry ingredients, cholesterol suspension, vitamins in the water, and salt solutions are combined, stirred thoroughly, and autoclaved for 20 minutes (20 lbs., 120°C). The total amount of water in the media is one liter. Once cooled to 50°C, Tegosept (methylparaben) or other antimicrobials can be added.

Masses of Flies Reared on Holidic and Standard Yeast Media

The yeast medium we used for comparison contained 116.11 g D-glucose, 9.44 g agar, 49.49 g cornmeal gluten, and 29.02 g ground yeast for every 0.9 L distilled water. All fruit flies were Oregon R stain and reared in incubators kept at 25°C. Male and female mass was taken from adults

developed from eggs laid in either the updated holidic or yeast-based medium. All parent flies used to lay the eggs had been reared on the yeast media.

As in prior studies, flies reared on holidic diets were smaller than those reared on yeast-based diets. However, the decrease in mass appeared smaller for this holidic diet than reported for Rapport *et al*.'s holidic diet (which was much more similar to Sang's original diet, at least for females) (Table 2). Percent survival from egg to adulthood (averaged for offspring of 16 to 19 females) was also less in this holidic diet (43.2%) than the yeast-based diet (71.3%).

Table 2. The effect of diet on fruit fly masses (mean + standard deviation in mg).

:	Male mass	Female mass
Current Study		
Holidic	0.63 + 0.069 (n = 62)	0.94 + 0.162 (n = 67)
Yeast-Based	0.82 + 0.056 (n = 69)	1.36 + 0.15 (n = 62)
% lower mass in holdic diet	77%	69%
From Rapport *et al*. (1984):		
Holidic	0.51 + 0.03	0.62 + 0.05
Yeast-Based	0.67 + 0.04	1.13 + 0.02
% lower mass in holidic diet	76%	55%

Although several modifications have been made to Sang's holidic medium for *D. melanogaster* in this and other studies, a considerable decrease in health is still observed on holidic-reared flies compared to those on yeast-based diets. Thus, even in this updated diet, there are likely multiple unknown nutrients of importance to the fruit fly. This highlights a need for further research in micronutrient requirements and metabolism in the fruit fly.

References: Bruins, B.G., W. Scharloo, and G.E.W. Thorig 1991, Insect Biochem. 21: 535-539; Geer, B.W., and G.F. Vovis 1965, J. Exp. Zool. 158: 223-236; Geer, B.W., W.W. Dolph, J.A. Maguire, and R.J. Dates 1971, J. Exp. Zool. 176: 445-460; Rapport, E.W., D. Stanleysamuelson, and R.H. Dadd 1984, Arch. Insect Biochem. Physiol. 1: 243-250.

A cautionary note on the use of SSAP (sequence-specific amplified polymorphism) to study transposons in *Drosophila*.

Vázquez, J.F., J. Albornoz, and A. Domínguez. Departamento de Biología Funcional, Area de Genética. Universidad de Oviedo, 33071 Oviedo, Spain. sanjurjo@uniovi.es.

Abstract

The technique of sequence-specific amplified polymorphism (SSAP) was proposed as an alternative to classical Southern analysis to study population genetics of transposable elements. SSAP was applied to analyse mutations of the retrotransposon *297* of *Drosophila melanogaster* on 82 mutation accumulation lines. The method was remarkably repeatable, but amplification was non-specific. Only 5 out of 11 bands extracted from the gel, reamplified, and sequenced were related to the retrotransposon. Lack of specificity of the technique precludes its use to analyze copy numbers and frequencies of polymorphic variants.

Introduction

SSAP (sequence-specific amplification polymorphism) was presented as an approach for obtaining polymorphic molecular markers in barley (Waugh *et al*., 1997; Ellis *et al*., 1998). The same

strategy was presented with the name of "transposon display" as a method that allows simultaneous detection of individual transposable elements of the *dTph 1* transposon family, in high copy number lines of *Petunia hybrida* (Van den Broeck *et al*., 1998). SSAP combines the AFLP technique (Vos *et al*., 1995) with a nested PCR to specifically amplify and display fragments containing a transposon sequence at one end and a flanking host restriction site at the other. The method was proposed as an alternative to classical Southern analysis to study population genetics of transposable elements, in particular for high copy number families where the resolution of Southern techniques is often insufficient to resolve individual elements (De Keukeleire *et al*., 2001). In addition SSAP presents the advantage that the PCR product can be extracted from the polyacrylamide gel, reamplified, cloned, sequenced and used as a probe in further analysis.

SSAP has been used mainly in plants but has been only rarely used in *Drosophila* (Perdue and Nuzhdin, 2000; Yang and Nuzhdin, 2003; Guimond *et al*., 2003). Here we present an attempt at using SSAP to study the nature of structural mutations involving retrotransposon *297* in a set of *Drosophila melanogaster* mutation-accumulation lines (Domínguez and Albornoz, 1996, 1999; Albornoz and Domínguez, 1999). Our results show that the rather permissive conditions of PCR amplifications used in SSAP can often lead to the amplification of sequences not related to the transposon.

Materials and Methods

The analysis was performed on *D. melanogaster* mutation accumulation lines derived from a starter population made isogenic for all chromosomes. The lines (named lines Oviedo) were maintained with high inbreeding, thus natural selection against middle deleterious mutations would be ineffective. Eighty-two lines representing 14,460 generations of mutation accumulation were used in this study.

To perform SSAP *Taq*I was used as cutter enzyme and a specific primer homologous to the LTR of *297* was designed, 5'-AGATAAACGAGTCTGCGCAGCTGG-3'. The fragments detected after electrophoresis are expected to represent both internal sequences of the transposon from the right LTR to the *Taq*I target and fragments that flank the transposon from the left LTR (Figure 1).

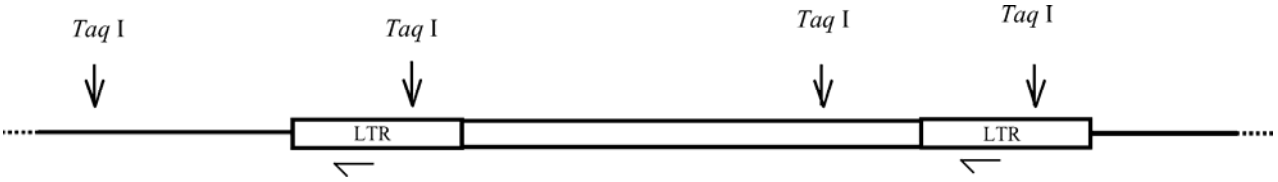


Figure 1. Diagram of *297* element (not to scale) showing the sites of *Taq*I and the locations of the specific primer.

SSAP analysis was performed following Waugh *et al*. (1997) with modifications. Genomic DNA (1 µg) was restricted with 10 U *Taq*I overnight at 65ºC in 1 × RL buffer (10 mM Tris-Acetate pH 7.5, 10 mM Mg Acetate, 10 mM K Acetate, 5 mM DTT, 50 ng/µl BSA) (Vos *et al*., 1995). Each DNA sample was ligated with Taq adapters (5'-ATGAGTCCTGAA-3' and 5'-CGTTCAGGACTCAT-3'; from Ellis *et al*., 1998) by adding 5 µl of ligation mix (3 U T4 ligase (Promega), 50 pmol *Taq*I adapters, 2 mM ATP, 1 × RL buffer) and incubating at 37ºC during 3 h. About 30 ng of the ligation product was preamplified with the primer homologous to the adapter 5'-ATGAGTCCTGAACG-3' in the following reaction conditions: 50 pmol of *Taq*I primer, 1 U Taq DNA polymerase (Promega), 100 µM dNTPs, 2.5 mM $MgCl_2$, and 1 × Taq buffer in a final 20 µl

volume. The program for PCR reaction starts with 95ºC for 1 min, followed by 30 cycles of 94ºC (1 min), 60ºC (1 min), and 72ºC (1 min), and it ends with 72ºC for 7 min. Specific amplifications were made with the primer homologous to the adapter and the above *297* LTR primer fluorescently labeled with 6-carboxyfluorescein (6-FAM) at the 5' end to permit subsequent analysis. Amplification conditions were as follows: two µl of a 3 times diluted preamplified material, 10 pmol of labeled *297* specific primer, 10 pmol of *Taq*I primer, and 1 U Taq DNA polymerase in a PCR buffer with the following components and concentrations: 100 µM dNTPs, 2.5 mM $MgCl_2$, and 1 × Taq buffer in a final 20 µl volume. The thermal program for selective PCR was 95ºC for 1 min, 13 cycles of denaturing at 94ºC (1 min), and annealing at temperature starting at 65ºC and descending with 0.7ºC every cycle (1 min), followed by 22 cycles of 94ºC (1 min) and 56ºC (1 min), and terminated with one cycle at 72ºC (10 min). Fragments were electrophoresed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) at 15 kV for 30 min at 60ºC.

To sequence specific fragments, PCR products were analyzed on 5% denaturing polyacrylamide gels and visualized by silver staining (Promega). The gel was moistened with distillated H_2O and the band was sliced out of the gel, distillated H_2O was added up to 50 µl. Following overnight incubation at room temperature, the gel material was pelleted by centrifugation, and 5 µl of the supernatant was used as template in standard PCR amplification. Reamplified material was cloned in a pMOS*Blue* vector (pMOS*Blue* Blunt Ended Cloning kit, Amersham Biosciences). Clones were sequenced with a BigDye v3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). SSAP sequences were compared to sequences deposited in Genbank using nucleotide to nucleotide BLAST search (BLASTN, www. ncbi.nlm.nih.gov/BLAST/).

Table 1. Characteristics of the fragments amplified.

Fragment	Size bp	Amplified region	Map	Accession	5`Primer	5`Target homology	3`Primer	3`Target homology
297-SSAP1	70	gene CG8991, CDS	48C	NM_136859	*Taq*I	enzyme target	297 specific	8 nt
297-SSAP2	216	LTR plus intergenic region	13B	AC018488	297 specific	conserved LTR	297 specific	7 nt
297-SSAP3	229	LTR plus intron of gene prosap	50D	AC007852	297 specific	conserved LTR	297 specific	4 nt
297-SSAP4	241	not defined pericentromeric repeat region	40F	AE002603	297 specific	-	297 specific	-
297-SSAP5	270	LTR plus intergenic region	83F	AC008001	297 specific	conserved LTR	297 specific	3 nt
297-SSAP6	296	gene RE56869, CDS	43E	AY071516	297 specific	6 nt	297 specific	6 nt
297-SSAP7	319	gene CG9632, CDS	89A	NM_142247	297 specific	8 nt	297 specific	15 nt
297-SSAP8	350	gene CG8272, CDS	44F	NM_136569	297 specific	9 nt	297 specific	9 nt
297-SSAP9	381	gene CG3572. CDS	42E	AE003790	297 specific	8 nt	297 specific	8 nt
297-SSAP10	406	repeat region with homology to element F	80E	AE002665	297 specific	-	297 specific	-

Results

Eighty-two lines were analysed and their electrophoregrams compared. The common profile showed 33 fragments between 40 and 400 bp. Only one line showed a consistent distinctive profile with a new fragment of approximately 70 bp representing a putative *297* mutation (Figure 2). The fragments amplified from this line were electrophoresed on polyacrylamide gels and the new band was cut off the gel, reamplified, and sequenced. The new fragment was found to correspond to a bacterial tRNA Ala gene. One of its ends is homologous to the last seven 3` nucleotides of the *297* LTR specific primer and the other corresponds to the target of the restriction enzyme.

After this finding ten additional bands of varying sizes were sequenced to verify whether amplification occurred as expected and if fragments corresponded primarily to transposon sequences. Nine out of 10 analysed fragments did not amplify from the two hypothetical primers, but the same oligonucleotide primed both sides. In addition, only five fragments were related to element *297* (Table 1). Three of these sequences were amplified from the left LTR to the DNA flanking the

retrotransposon at the insertion site. The other two sequences corresponded to pericentromeric repeated regions that may well include degenerate *297* elements. Overall, non-specific priming (at one or both sides) occurred in 12 cases, and in every instance the target sequence had a limited homology, between 3 and 15 nucleotides, with the *297* primer at the 3`end.

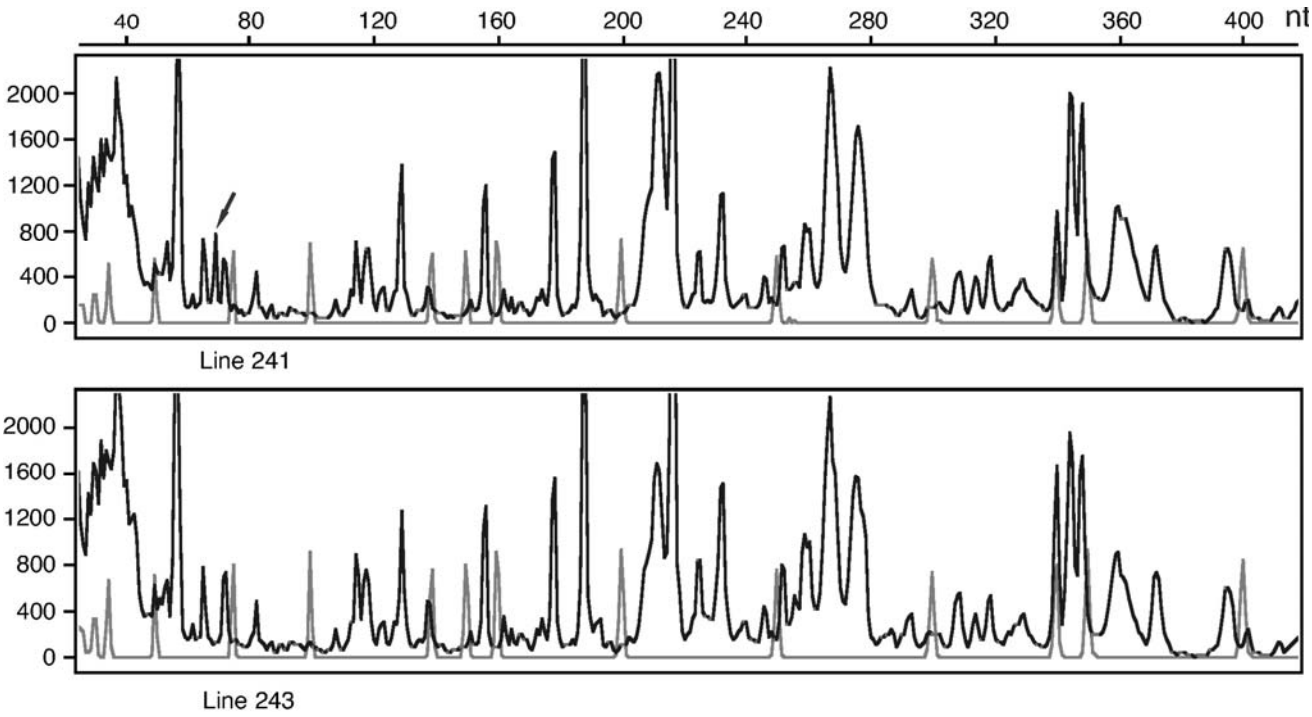


Figure 2. SSAP of *297* element by capillary electrophoresis. The above profile, corresponding to line 241, shows an extra fragment (marked with an arrowhead). In grey, size standard.

Discussion

SSAP not only displayed fragments homologous to the element *297* but also other non-related fragments. This result can be attributed to the rather permissive conditions of the specific PCR inherent to the technique. Many cycles take place at low annealing temperature, and then homology of a few bases at the 3´end of the primer is enough to allow amplification. It is amazing that none of the 11 sequenced fragments were as expected, that is with a string of 24 specific nucleotides at one end and the target for the restriction enzyme at the other.

SSAP represent a good method to obtain polymorphic repeatable markers as initially proposed (Waugh *et al*., 1997; Ellis *et al*., 1998). Despite the varied origin of fragments, the profile obtained was remarkably repeatable, contrary to other multiple polymorphic markers as Randomly Amplified Polymorphic Fragments (RAPDs, Pérez *et al*., 1998). Only 2 out of the 82 lines analysed showed a distinctive profile, each with an extra band. In one case the result was not repeated in a second replicate while in the other the extra band was consistently obtained in two successive replicates. The band resulted to be homologous to a bacterial tRNA and probably originated from a bacterial contamination of the line.

Only three out of the eleven fragments sequenced showed a clear homology to element *297* and contained the expected end of the left LTR at side of the specific primer. The flanking sequences

are at map positions 13B, 50D and 83F from the BLAST search of *Drosophila melanogaster* genome. These sites had been previously identified by *in situ* hybridization to polytene chromosomes (Domínguez and Albornoz, 1999). Two other bands had homology to centromeric repeated regions and thus could be presumed to share homology to degenerate copies of *297* in heterochromatin. The other six fragments are not related to the retrotransposon. It follows from these observations that bands obtained by SSAP can not be readily assumed to be related to the transposon, and hence the method can hardly be used to make inferences about the number of copies of different polymorphic variants within a family of transposons. Hence conclusions that master copy of *copia* is not the responsible for the transposition of this element (Perdue and Nuzhdin, 2000) or that *Doc* copies with promoters are not more selected against than *Doc*s lacking promoters (Yang and Nuzhdin, 2003) must be taken with caution.

SSAP has been described as a strategy that allows the isolation of transposon tagged sequences without cloning (Van der Broeck *et al*., 1998). We provide here an example of this utility. The tree identified elements are now located in the genome, and individual copies can be subsequently amplified and sequenced. The tree elements are within AT repeats illustrating the preference of *297* for inserting in AT rich regions that was previously reported (Inouye *et al*., 1986).

Acknowledgments: We thank Andy Flawell for sending us a detailed protocol of the SSAP method.

References: Albornoz, J., and A. Dominguez 1999, Heredity 83: 663-670; De Keukeleire, P., T. Maes, M. Sauer, J. Zethof, M.Van Montagu, and T. Gerats 2001, Mol. Genet. Genomics 265: 72-81; Domínguez, A., and J. Albornoz 1999, Genetica 105: 239-248; Domínguez, A., and J. Albornoz. 1996, Mol. Gen. Genet. 251: 130 - 138; Ellis, T.H.N., S.J. Poyser, M.R. Knox, A.V. Vershinin, and M.J. Ambrose 1998, Mol. Gen. Genet. 260: 9-19; Guimond, N., D.K. Bideshi, A.C. Pinkerton, P.W. Atkinson, and D.A. O'Brochta 2003, Mol. Genet. Genomics 268: 779-790; Inouye, S., S. Yuki, and K. Saigo 1986, Eur. J. Biochem. 154: 417-425; Perdue, S., and S.V. Nuzhdin 2000, Mol. Biol. Evol. 17: 984-986; Pérez, T., J. Albornoz, and A. Domínguez 1998, Mol. Ecol. 7: 1347-1357; Van den Broeck, D., T. Maes, M. Sauer, J. Zethof, P. De Keukeleire, M. D´Hauw, M. Van Montagu, and T. Gerats 1998, Plant J. 13: 121-129; Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau 1995, Nucleic Acids Res. 23: 4407-4414; Waugh, R., K. McLean, A.J. Flavell, S.R. Pearce, A. Kumar, B.B. Thomas, and W. Powell 1997, Mol. Gen. Genet. 253: 687-694; Yang, H.P., and S.V. Nuzhdin 2003, Mol. Biol. Evol. 20: 800-804.

Cleaning up Southern pictures.

Junakovic, Nikolaj,* and Wolfgang J. Miller. *Istituto di Biologia e Patologia Molecolari C.N.R., Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza", 00185 Roma, Italy; Email: nikolaj.junakovic@uniroma1.it; tel : +39-0649912786; fax: +39-0649912500; °Laboratories of Genome Dynamics, Center of Anatomy and Cell Biology, Medical University of Vienna, Waehringerstr. 10, A-1090 Vienna, Austria; Email: wolfgang.miller@meduniwien.ac.at.

While preventing the background signal is a key goal of all variants of the Southern blotting technique, no protocol has been reported so far for removing the unwelcome signal after it has shown up. Here we describe a simple remedy that removes or reduces at least two types of unwanted ^{32}P signal and does not impair subsequent use of the filter.

Abstract

Soon after its publication the Southern blotting technique became a fundamental tool of molecular biology and has remained so for the following 30 years up to now (Southern, 1975). In spite of such vast collective experience, occasional background interfering with the expected banding pattern is still an unpredictable, unwelcome outcome that may be particularly frustrating if the DNA being analyzed is unique or it cost money, time and labour to prepare. On the other hand, it is difficult to study an irreproducible phenomenon that can be due to a wide range of parameters, including carelessness. Here we show that the background caused by a probe labeled with ^{32}P can be removed by rubbing the membrane with towel paper.

Protocol

At the end of the routine washing, lay the membrane still soaked in the washing liquid, on a slab made of glass or plexiglass and covered with cling film. Position one side of the membrane close and parallel to one side of the slab. Immobilize the membrane by pinching it along one end with the aid of a plexiglass rod kept in place with the thumb; the rod should be as long as the membrane (or longer) and robust enough (*e.g.* square section, sides of 8 mm) to exert homogeneous pressure. Rub the membrane with a dry piece of towel paper as if a drop of honey were to be removed. To monitor removal of the unspecific signal, check the membrane and towel paper by a Geiger counter. Repeat on the other side of the membrane. Wash an additional 10 min prior to exposure. If heavy signal has been removed, repeat once.

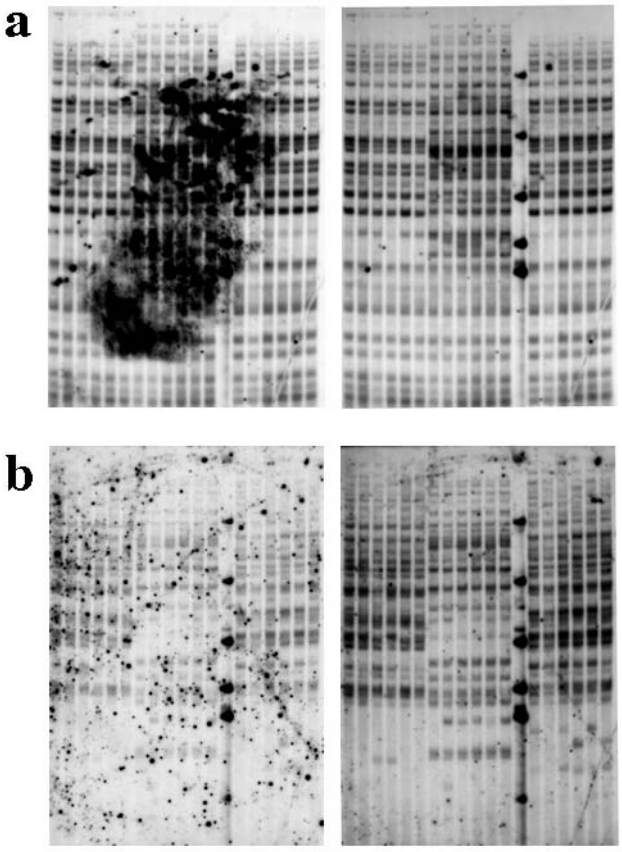


Figure 1. A and B show the pictures revealed on the same membrane (Hybond +, Amersham) with probes homologous to different transposon families of *Drosophila* as described in (Junakovic, 2004). The pictures to the left illustrate the two types of background observed; to the right the same pictures after cleaning, as described. **A:** The heavy background could not be removed by washing in 2 × SSC, 0.1% SDS at 65 °C for 6 hrs, with several changes; both pictures have been exposed for 24 hrs. **B:** Because probing with the Geiger counter was not informative at the end of washing, the membrane has been exposed for four hrs, cleaned, and re-exposed for about 30 hrs. The ratio between the wanted and unwanted signals has clearly improved. The same filter has been successfully probed and stripped an additional nine times (not shown).

Conclusion

This protocol helps with more than one type of background (Figure 1) suggesting that it may prove useful in additional cases. The DNA and membrane appear to be unaffected in subsequent cycles of hybridization and stripping. This shows that the protocol described can be used as a safe preemptive step whenever unspecific signal is suspected to persist after the traditional washing.

References: Junakovic, N., 2004, Southern blot analysis of individual Drosophila flies. Methods Mol. Biol., Humana Press Publ. 260: 41-57; Southern, E.M., 1975, J. Mol. Biol. 98: 503-517.

A new method to analyze habitat preference and mate choice.

Hurtado, J., E. Soto, and M. Cortese. Laboratorio de Evolución, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria Pab. II. (C1428EHA) Buenos Aires, Argentina; Phone: +54-011-4576-3384. Author for correspondence: juanhurtado@ege.fcen.uba.ar.

Habitat preference and mate choice are key components in models of sympatric speciation. Thus, the close relationship between these complex traits makes their joint study necessary. Such studies not only require an experiment for habitat preference and another for mate choice, but also the combined analysis of both. In order to facilitate such analysis and to allow the simultaneous analysis of both traits, we designed a device that allows the choice between two different habitats and to test for mate choice with the same sample of flies. These two habitats may differ in their resources, for example different odors quests.

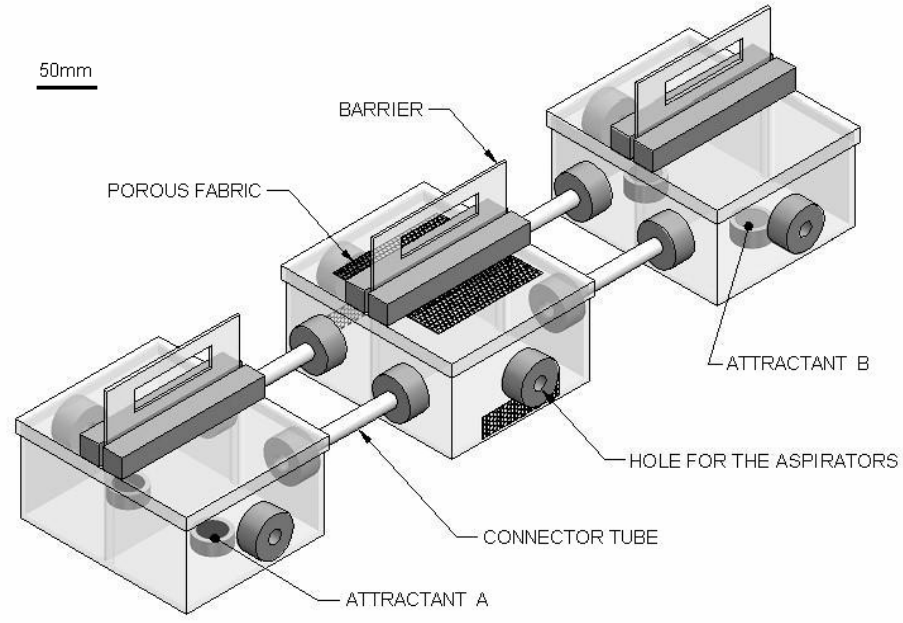


Figure 1. Three-dimensional view of the device. In this model the lateral boxes have dishes containing different attractants for the flies, the barriers are closed and the aspirators are not shown.

The device consists of three adjacent boxes, each one made of transparent acrylic and divided into two equal size compartments by a mobile barrier. One tube connects each compartment of the lateral boxes with its adjacent compartment of the central box. The latter, in turn, presents pieces of a porous fabric, which allows air circulation defining two different odorous zones (Figure 1). Lateral boxes have holes to which insect aspirators can be attached for fly collecting purposes (Figure 2).

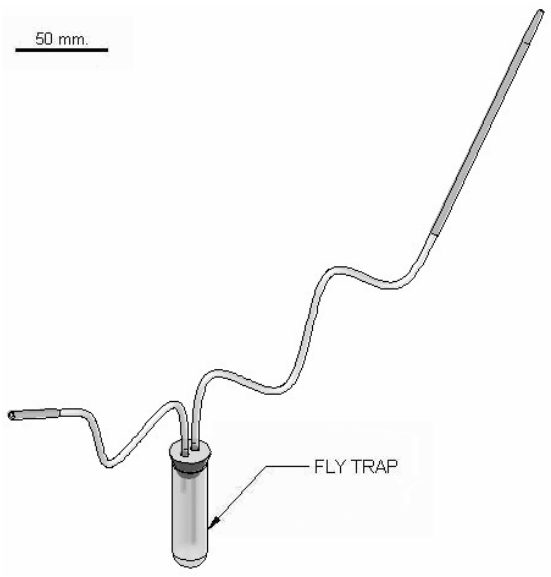


Figure 2. Three-dimensional view of the insect aspirator. The tube allows trapping each mating couple.

A sample of flies is introduced in the central box, males in one of the compartment and females in the other, and allowed to choose between two environments by following volatile compounds of different attractants situated in each one of the lateral boxes. This choice simulates habitat preference. Once the flies made their choice, the barriers, dividing the compartments of the lateral boxes, are opened, and then it is possible to test mate choice within each environment and between different strains by aspirating mating couples and subsequent individual identification. As a variant, it can be used a dark and opaque central box in order to increase the dispersal rate and reduce returning rate to the central box.

We also recommend this method for jointly testing habitat preference and mate choice or any interaction between different strains. In addition, the device may be used as a new alternative in artificial selection experiments. For example, it may be useful for testing the correlation between habitat preference and mate choice by comparing the efficiency of artificial selection either for habitat preference or mate choice with artificial selection for both traits at the same time.

Acknowledgments: The authors wish to thank Ignacio Soto and Esteban Hasson for criticisms on earlier versions of this report. This work was supported by grants of ANPCyT (PICT 11084) and UBA (X299) awarded to Esteban Hasson.

Selecting a nutritional media for multigenerational growth of *Drosophila melanogaster* in space.

Sanchez, M.E., M. Shenasa, E. Blazevic, A. Maldonado, A. Kakavand, and S. Bhattacharya. NASA Ames Research Center, Moffett Field, CA 94035.

Introduction

Future long-term missions to the Moon and to Mars will have physiological effects on humans that need to be investigated. In order to optimize the performance of humans in these new environments, models can be used to predict and to assess the effects of altered gravity, radiation, and dust on living organisms. *Drosophila melanogaster*, along with other model specimens, has provided valuable information for the physiological effects of environmental stimuli. In order to use *Drosophila* to conduct multigenerational experiments in space, it is critical to ensure that the food media used to support these cultures can be stored and preserved for long periods of time with no refrigeration requirements.

Laboratories across the world use a variety of recipes to make fly media. Fly food is often made on a weekly basis to support experiments in a laboratory. In space, since it is not feasible to make fresh media on orbit on a regular basis, the media will most likely be made several weeks prior

to launch and stored at around 25ºC until it is needed to sustain a new generation of flies. Furthermore, the media will need to withstand this long-term storage while maintaining its nutritional value and palatability to the flies.

Our laboratory tested four media recipes, a Semidefined medium developed by Backhaus *et al*. (1984), a variation of the Semidefined, and two other recipes that were modified from the standard dextrose medium developed by Brent and Oster (1974). In this study, these four media recipes were prepared and assayed for storage durations of up to three months. All media recipes were able to support fly growth after three months of storage at room temperature. The media were prepared and packed under sterile conditions. In addition, proton beams at a range of 25-33 kgrays were used to further irradiate a subset of our samples, to test whether the additional sterilization helped store food for long durations of time.

Materials and Methods

Media Preparation and Storage

In this test, four media recipes were made, stored, and vacuum packed in Mylar™ bags type PAKVF 3.5M Silver (Impak Corporation, 2004). To vacuum seal the food vials in the Mylar™ bags, we used a PAC vacuum impulse sealer model number PV-G36 (Packaging AIDS Corporation. San Rafael, CA). The following media recipes were assayed: **A**. Semidefined (1% Agar, 8% Brewer's Yeast, 2% yeast extract, 2% peptone, 3% sucrose, 6% glucose, 0.05% magnesium sulfate, 0.05% calcium chloride, 0.6% propionic acid, 1% of 10% p-Hydroxy-benzoic acid methyl ester in 95% ethanol); **B.** Semi-defined with cornmeal (same as semidefined with the addition of 60 g of cornmeal); **C.** EMCS Fab Feast 1:1 (6.47% dextrose 6.47% molasses, 0.93% agar, 6.12% cornmeal, 3.24% yeast, and 2.0% Tegosept); **D.** EMCS Fab Feast 3:1 (Same as EMCS Fab Feast 1:1, with the exception of 9.7% dextrose and 3.23% molasses). All food media were prepared using sterile water and poured under a laminar flow hood.

Sterilization with Proton Beams

A subset of the food samples were exposed to proton beams at a range of 25-33 kgrays as a means of providing additional sterilization to the food medium. This was done through the Nutek Corporation (30958 San Antonio St., Hayward, CA 94544).

Food Test

All food media were tested in standard laboratory polystyrene vials covered with Buzz Plugs (Fisher Scientific AS-520 and AS-275, respectively) with a starting population of 20 virgin females and 10 males. After three days, the parental populations were removed from the vials. The F_1 progenies were allowed to develop for 12 days. At the end of this period, all adult flies were counted. The same test was repeated each month thereafter on food that had been stored and packed. All food was stored and tested under ambient conditions of 25ºC. For each time point, both proton irradiated and unirradiated food were used. Wild type Oregon-R flies were used in all experiments.

Results

All four media recipes were able to support fly growth (Figures 1A and 1B). The unirradiated media performed better than the proton irradiated media, by supporting larger fly populations. Furthermore, no bacterial or fungal contamination was found in any of the food media, irradiated or unirradiated. The decrease in performance of the proton-sterilized food is unclear, but may have resulted from the degradation of constituents in the media as a result of proton irradiation. Our data

indicate, therefore, that additional sterilization by proton irradiation (25-33 kgrays) is not required nor recommended for fly food media that has been prepared as described in the Methods and Materials section.

It was also found that the addition of cornmeal into the Semidefined recipe increased the progeny sizes by about 40%. Both EMCS Fab Feast medium recipes yield nearly the same population sizes in the non-proton sterilized media, but not in the proton-sterilized ones. For purposes of supporting our experiments, which consisted of three-day egg lays, we found that the standard Semidefined medium performed well. The cultures were not overcrowded and yet population sizes were large and allowed optimal multigenerational studies. However, during space missions in which crew schedules are limited, the Fab Feast medium can be used for longer egg lays or the Semidefined with cornmeal medium for shorter ones.

Another important observation from these tests was that the food did not dry out or separate from the sides of the vials, even at the end of the storage period, as food stored in laboratories usually do over time. Therefore this packaged and stored food maintained its integrity and usability over the entire period of storage.

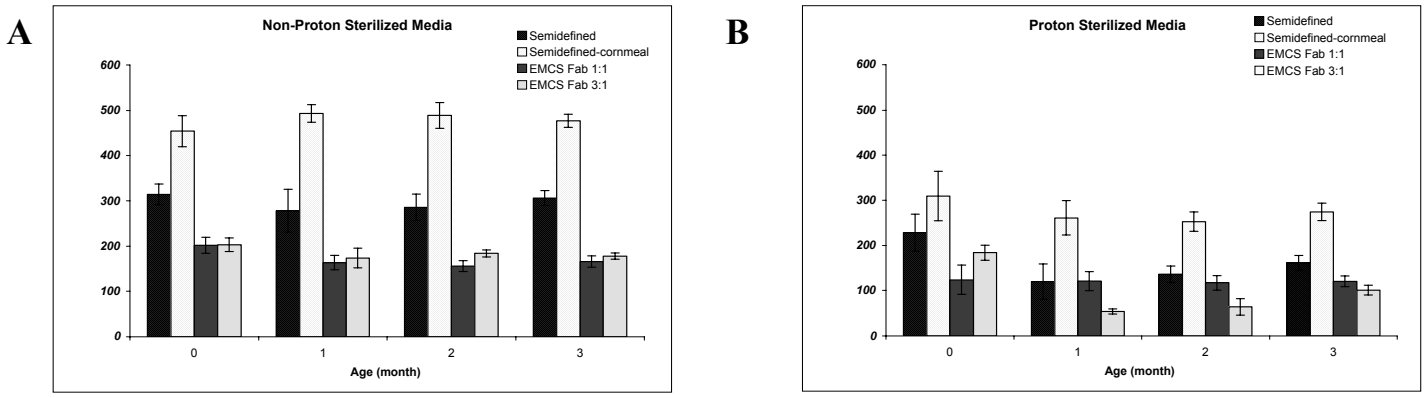


Figure 1. Figures A and B show the results from the unirradiated and proton irradiated media respectively. In general, the unirradiated media performed better than the proton irradiated media for fly cultures. Data shown here represent four independent studies. All studies were conducted at room temperature (25ºC).

Discussion

We have identified a method of packaging four different media recipes that are able to sustain fly growth after three months of storage. This will be ideal for long-term experiments in space but also in standard laboratories where making fresh food regularly is impractical for any reason.

In the past, there have been a variety of attempts to prolong the shelf life of fly food. Most fly labs maintain their fly media at 4°C for 1-4 weeks. The Bloomington Stock Center (2004) uses a material called Press'n Seal®. They regularly cover the food trays with this material and store it at 4°C. By using this methodology, they have been able to maintain the integrity of the food for about a month. There are additional drawbacks to using this method due to non-uniformity of heights between vials. These irregularities between vials result in wrinkles in the Press'n Seal® material creating small gaps that allow wandering flies to enter the food and contaminate the medium.

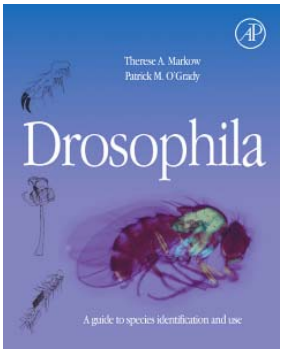
In a previous effort to prolong the shelf life of the standard Semidefined recipe, the EMCS Fab Feast 1:1, and the standard dextrose recipe, each medium recipe was packaged in FoodSaver®

Bags (Bags are commercially available from many of our local stores such as Bed Bath & Beyond; http://www.bedbathandbeyond.com/) and vacuum sealed in our laboratory. The food was stored for 16 months at room temperature. Semidefined and the EMCS Fab Feast 1:1 media were able to support fly growth after 16 months of storage. No flies grew in the 16-month old standard dextrose recipe (data not shown). In order to generate data from the above results, further experiments are currently in progress. A lower dosage of proton beam exposure on the food will be used for further assessments. These data will be presented in a subsequent publication. These data are promising, and while this paper shows the feasibility of storage for up to three months, in reality the semidefined and Fab Feast medium can be stored and used for well over a year.

Storing fly food can be useful or even critical for fly laboratories. This methodology has proven to be functional for a minimum of three months, and we have preliminary evidence to show that some of the media remain usable for over a year. This has great practical as well as commercial implications, as it can allow laboratories to reduce the cost and overhead of producing fresh fly food on a regular basis.

References: Backhaus, B., E. Sulkowski, and F.W. Schlote 1984, Dros. Inf. Serv. 60: 210-212; Brent, M.M., and I.I. Oster 1974, Dros. Inf. Serv. 51: 155-157; Impak Corporation. Mylar® Bags. Sorbent Systems. January 18, 2004. (http://www.sorbentsystems.com); Bloomington *Drosophila* Stock Center. *Drosophila* Media Recipes and Methods. Indiana University. January 4, 2006. (http://fly.bio.indiana.edu/).

New Books



Markow, Therese, and Patrick O'Grady. 2005. Drosophila: *A Guide to Species Identification and Use*. ISBN: 0-12-473052-3. Academic Press, NY. 500 pages, $89.95.

This book is an excellent source of keys and illustrations for identifying Drosophila species. Life histories of many species are also presented along with distribution maps, media recipes, and collection tips. For more information, see http://books.elsevier.com/us/lifescience/us/subindex.asp?maintarget=&isbn=0124730523&country=United+States&srccode=&ref=,%20&subcode=&head=&pdf=&basiccode=&txtSearch=&SearchField=&operator=&order=&community=lifescience.

Held, Lewis I., Jr. 2002. *Imaginal Discs: The Genetic and Cellular Logic of Pattern Formation*. Cambridge University Press, Cambridge, UK. 460 pp.

This excellent resource on the genetics of pattern formation in *Drosophila* is now available in paperback. The ISBN for the paperback edition is: 0-521-01835-8. For a review, see Dros. Inf. Serv. 85: 107 (2002). All figures and legends are also now posted at the Interactive Fly website.