UNIVERSITY OF CAMBRIDGE
Department of Genetics

Fly Facility Services - August 2013

Services for Cambridge University and external users:

1. **Embryo injection service**
   a. *P*-element / piggyBactransformation
   b. *phiC31*integrase-mediated transgenesis

2. **Fly pushing** – crosses, screens, stock construction etc.

Services for Cambridge University users only:

3. **Fly Stock Keeping**

4. **Fly media** – tubes, bottles, plates

Service details and pricing:

1. Embryo injection service

Please direct all embryo injectionenquiries to flyinject@gen.cam.ac.uk(this address reaches Sang Chan,Simon Collier and Glynnis Johnson).

Please bring/sendDNA samples for injection- along with a completed **Request for Drosophila embryo injection service form**(seeend of this document)–direct to Sang Chan or (if Sang is not available) to Simon Collier in the Fly Facility office (Room 115a, Department of Genetics).

DNA quality and quantity for embryo injection - The plasmid DNA for embryo injection needs to be of a Qiagen midi prep quality, or equivalent, because the survival rate directly depends on the purity of the plasmid. The ratio of OD260/280 should be 1.80-1.90. The concentration of the plasmid for injection should be 1µg/µl (0.4µg/µl for *phiC31* injections) dissolved in dd (distilled,deionized) sterile water (not T.E.), and a minimum of 10µl is required. We provide the P-element helper plasmid (as required), and the injection buffer.

a. *P*-element / piggyBactransformation

	Description	Price
Plan 1	Inject 200 embryos - provide surviving larvae	**£130**
Plan 2	Inject 200 embryos – provide unbalanced G2 transformants	**£180**
Plan 3	Inject 200 embryos – provide balanced lines	**£250**

b. *phiC31*integrase-mediated transgenesis

A list of *phiC31* stocks currently available in the Fly Facility is here.

	Description	Price
Plan 4	Inject 200 *phiC31; attP* embryos - provide surviving larvae	**£130**
Plan 5	Inject 200 *phiC31; attP* embryos – provide balanced lines (integrase removed)	**£210**

Note: For **Plans 2, 3**, and **5**, we will inject a further 200 embryos (at no extra cost) if notransformants are obtained. **Plans 1** and **4** are injection only – we do not routinely re-inject if no transformants are obtained.

Note: We can usually provide other embryo injections services on request (price negotiable):

- Transformation of other (non-*melanogaster*) Drosophila species
- Wolbachiainfection
- TALEN mRNA injection

2. Fly pushing

We can discuss individual needs for fly pushing,and give advice on screen design and stock construction (no charge!). Please contact Simon Collier (65124) or email flyadmin@gen.cam.ac.uk (this address reaches Simon Collier and Glynnis Johnson). Costs for fly pushing services are arrived at by negotiation between the Fly Facility and the Group Leader/Grant Holder involved.

3. Fly Stock Keeping - (Billing Cycle here)

We provide a stock keeping service with the option to keep 1 or 2 tubes of each stock (the 1 tube option is for less 'valuable' stocks e.g. those that are easily obtainable from other sources). Stocks are available to users at any time during the working week.**Note:** we will also mail your stocks to anywhere in the world, free of charge, on your request.

Stocks are kept at 18°C (unless otherwise requested) and are transferred on a 5-week cycle. There is a stock keeping fee of **£30/tray/month** – trays hold 70 stocks (2 tube option) or 140 stocks (1 tube option) – users are charged according to the number of whole, and part, trays that their stocks occupy.

Note: The Fly Facility itself maintains a small stock collection, which is available to Cambridge users. However, the Fly Facility is not a Stock Centre and there is a discretionary fee of £20/stock (plus postage) for users outside of Cambridge.

Stock keeping cost estimates for grant applications

- Our stock keeping fee is **£30/tray/month,** so a tray of stocks costs **£360** annually
- Stocks at 18°C are transferred on a 5-week cycle i.e. 10 transfers/year. Therefore, a tray of stocks uses 10 trays of media annually at a cost of £30/tray = **£300**.
- Therefore, the annual cost of keep a tray of stocks is **£360 +£300 = £660**.

Annual charge for 18°C stock keeping (2 tubes of each stock):
Cost = Number of stocks/70 x £660

Annual charge for 18°C stock keeping (1 tube of each stock):
Cost = Number of stocks/140 x £660

4. Fly media - (Billing Cycle here)

Media can be ordered here (Raven password required):
http://www.gen.cam.ac.uk/department/flylab/flymedia/
Note: you will need to be registered as a media user – please contact Simon Collier s.collier@gen.cam.ac.uk to do this)

Media ordered before midday will normally be available the following weekday

Media is sold by the tray or part tray; one tray holds 150 tubes (large glass, small glass or plastic), 20 bottles or 7 Kilner jars. Tubes can be ordered unplugged.
Cost per tray (plugged): £30
Cost per tray (unplugged): £24

Note: To order KIlner jars, select 'bottles' on the media website and specify 'Kilner' in the comment column.

Any number of plates (50mm or 90mm) of media can also be ordered. On the media website, plates are ordered by 'quantity' not by 'tray'. Prices are:
50mm - £0.15 each
90mm - £0.30 each
Chip Plates (for Cage Room collections)**- £0.30 each**
Note: To order Chip Plates, select '90mm plates' on the media website and specify 'Chip Plates' in the comments column.

Media types available:

- Cornmeal
- Yeast Glucose
- Propionic
- Opuntia / banana / malt
- Apple Agar
- Grape Agar

- we can normally accommodate requests for alternate media where a detailed recipe is supplied.

Appendix I:Billingcycle (media and stock keeping)

We anticipate that future charges for media and stock keeping will be levied quarterly to cover the preceding 3 months.However, we can normally accommodateoccasional requests for alternate billing dates to match grant cycles.
Please note: Fly embryo injection and fly pushing services are billed upon completion of service, or as negotiated.

Billing date	Period
End January	Nov-Jan
End April	Feb-April
End July	May-July
End October	Aug-Oct

AppendixII:*phiC31* stocks currently available in the Fly Facility

(Please note that we will inject into any *phiC31* line providing the user can supply a healthy stock for embryo collection).

- **Stock 13-18: *nos-int; attp2***

Genotype: *y w P{y[+t7.7]=nos-phiC31\int.NLS}X #12;; P{y[+t7.7]=CaryP}attP2*
attP site: 3rd chromosome (68A4), marked with *yellow*$^+$
phiC31integrase: X chromosome, *nanos* promoter, marked with *yellow*$^+$

- **Stock 13-20**: ***vas-int; attp40***

Genotype: *y w M(eGFP, vas-int, dmRFP)ZH-2A; P{CaryP}attp40*
attP site: 2nd chromosome (25C6), marked with *yellow*$^+$
phiC31integrase: X chromosome (2A3), *vasa* promoter, marked with GFP and RFP

- **Stock 13-14: *vas-int; attp-86Fb***

Genotype: *y w M(eGFP, vas-int, dmRFP)ZH-2A;; M(attP)ZH-86Fb*
attP site: 3rd chromosome (86F8) – unmarked
phiC31integrase: X chromosome, *vasa* promoter, marked with GFP and RFP
Note: Bloomington stock 24749 with attP2 RFP marker removed (cre-ed out)

- **Stock 12-100:*vas-int; attp-51D***

Genotype: *y w M(eGFP, vas-int, dmRFP) ZH-2A; M(attP)ZH-51D*
attP site: 2nd chromosome (51D) – unmarked
phiC31integrase: X chromosome, *vasa* promoter, marked with GFP and RFP
Note: Bloomington stock 24483 with attP2 RFP marker removed (cre-ed out) – may be problems with enhancer trap effects at 51D

- **Stock 13-106: *vas-int; attp-3B VK00033***

Genotype: *y w M(eGFP, vas-int, dmRFP)ZH-2A;; PBac{y[+]-attP-3B}VK00033*
attP site: 3rd chromosome, marked with *yellow*$^{+}$
phiC31integrase: X chromosome (2A3), *vasa* promoter, marked with GFP and RFP

- **Stock 13-22: *VK00005***

Genotype: *y w P{y[+t7.7]=nos-phiC31\int.NLS}X #12; PBac{y+-attP-9A}VK00005*
attP site: 3rd chromosome (75A10), marked with *yellow*$^{+}$
phiC31integrase: X chromosome, *nanos* promoter, marked with *yellow*$^{+}$

- **Stock 13-23: *VK00027***

Genotype: *y w P{y[+t7.7]=nos-phiC31\int.NLS}X #12; PBac{y+-attP-9A}VK00027*
attP site: 3rd chromosome (89E11), marked with *yellow*$^{+}$
phiC31integrase: X chromosome, *nanos* promoter, marked with *yellow*$^{+}$

- **Stock 13-24: *VK00040***

Genotype: *y w P{y[+t7.7]=nos-phiC31\int.NLS}X #12; PBac{y+-attP-9A}VK00040*
attP site: 3rd chromosome (87B10), marked with *yellow*$^{+}$
phiC31integrase: X chromosome, *nanos* promoter, marked with *yellow*$^{+}$

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Request for Drosophila embryo injection service

User: _____

User email: _____

Group Leader: _____

Department: _____

Grant Code _____

External groups (outside of University of Cambridge Department of Genetics) must provide a purchase order and VAT exemption certificate

DNA sample name	Injection stock (embryos)	Service required (1-5)