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Using the Q system in *Drosophila*

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Abstract

In *Drosophila,* the GAL4/UAS/GAL80 repressible binary expression system is widely used to manipulate or mark tissues of interest. However, complex biological systems often require distinct transgenic manipulations of different cell populations. For this purpose, we recently developed the Q system, a second repressible binary expression system. We describe here the basic steps for performing a variety of Q system experiments in vivo. These include how to generate and use Q system reagents to express effector transgenes in tissues of interest, how to use the Q system in conjunction with the GAL4 system for the generation of intersectional expression patterns that precisely limit which tissues will be experimentally manipulated, and how to use the Q system to perform mosaic analysis. The protocol described here can be adapted to a wide range of experimental designs.

Keywords

MARCM; QF; QS; quinic acid; QUAS; transcription factor; coupled MARCM; reporter expression; fly; technique

INTRODUCTION

Binary expression is a powerful strategy for regulating expression of an effector transgene for the purpose of interrogating the development or function of cells and tissues in multicellular organisms. In such a strategy, one transgene contains a specific promoter driving an exogenous transcription factor, while the other transgene uses a promoter specifically activated only by that introduced transcription factor. An additional layer of control is afforded if the transcription factor itself can be specifically inhibited by an exogenous element. The yeast GAL4 system is such a repressible binary expression system, and has revolutionized experimental manipulations in flies[1,2]. The GAL4 transcription factor binds to an *Upstream Activation Sequence* (*UAS*) to induce expression of a reporter transgene (*UAS-geneX*). Only when GAL4 and *UAS-geneX* are in the same animal is *geneX* expressed in the GAL4 expression pattern. Thousands of GAL4 lines have been characterized for tissue and developmental expression patterns, and can be used in combination with thousands of effector lines. Effector lines range from cell markers (e.g.,

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AUTHOR CONTRIBUTIONS

C.J.P designed and performed the experiments and generated the figures and tables; C.J.P and L.L. wrote the paper.

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membrane tagged GFP) to signaling molecules (e.g., activated Ras) to inhibitory molecules (e.g., neurotoxins or RNAi constructs). Furthermore, GAL4 activity can be inhibited by GAL80, a natural suppressor of GAL4[3]. Thus, when GAL80 is co-expressed with GAL4, *UAS-geneX* reporters are silent. This allows for further effector refinement, including the Mosaic Analysis with a Repressible Cell Marker (MARCM) technique[3]. The combination of the three GAL4 components (GAL4, *UAS-geneX,* GAL80) allows for a rich diversity of experimental investigations.

Nonetheless, the GAL4 system has its limitations. *UAS-geneX* effectors can only be expressed in the one population of cells defined by GAL4. In complex cellular organisms, it is often desirable to express an effector in a fraction of a cellular population, and then examine the effects on the other population of cells. Likewise, one might want to differentially label and manipulate two different types of tissues- neurons labeled with GFP and glia labeled with RFP. Such techniques would be invaluable for determining non-cell autonomous effects (such as ligand/receptor interactions).

We have recently characterized the Q system for these and other purposes[4]. The Q system utilizes genes from the *qa* gene cluster of the filamentous fungus *Neurospora crassa*. This gene cluster, consisting of 7 genes, is required for the catabolism of quinic acid (quinate) under conditions of limited glucose levels[5–9]. This gene cluster contains two regulatory genes: *qa-1f* (encoding a protein of 816 aa) and *qa-1s* (encoding a protein of 918 aa). qa-1f (shortened as QF hereafter) is a transcription factor, and qa-1s (shortened as QS hereafter) is a repressor of QF. The other 5 genes in the *qa* gene cluster encode enzymes or cofactors required for the catabolism of quinic acid. The promoters for the 7 *qa* genes in *Neurospora* contain binding sites for QF, and expression of the *qa* genes can be induced by the QF transcription factor. The binding site for QF is the sequence “GGRTAA RYRY TTATCC” (R is A/G, Y is C/T). Under normal growth conditions where glucose is high, QS binds to and inhibits QF, and prevents expression of the *qa* gene cluster. However, when glucose is limiting and quinic acid is present, quinic acid binds to and inhibits QS. This releases QF from QS suppression, allowing QF now to induce expression from the *qa* gene cluster. This results in the expression of the factors required for the catabolism of quinic acid as an energy source. In effect, the catabolite (quinic acid) controls expression of the genes required for its catabolism.

Development of the Q system in *Drosophila*

The Q system introduced into *Drosophila* consists of three components: the QF transcription factor, a *QUAS-geneX* effector, and the QS suppressor (Fig. 1, Fig. 2). The *QUAS* element contains 5 QF binding sites, and allows for robust QF-dependent expression of the effector. As such, the Q system contains the same 3 basic components (QF, *QUAS,* QS) as the analogous GAL4 system (GAL4, *UAS,* GAL80). In addition, the molecule quinic acid can inhibit the QS suppressor in flies fed a diet containing quinic acid (Fig. 1, Fig. 2). This allows temporal control of the Q system upon treatment with this non-toxic molecule (Fig. 1).

Applications of the method

The Q system contains the same basic components as the GAL4 system, and so can be used for the same applications as the GAL4 system: binary expression in a subset of tissues, refinement of that expression by using the QS inhibitor, and MARCM analysis[1,3,10] (Fig. 2). In addition, temporal control of QF activity can be achieved by using quinic acid and QS expression (Fig. 1c, Fig. 4). However, a key experimental advantage is obtained when the Q repressible binary system is used in conjunction with the GAL4 repressible binary system. Figure 2 shows some of the possible applications achievable. Highlighted is the ability to

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define intersectional expression patterns whereby finer precision of tissue manipulations can be achieved (Figs. 5–8, Fig. 11). In addition, the Q system can be used for MARCM analysis (Fig. 9), which has a variety of in vivo applications[3,10–13]. Since the Q system and GAL4 system function independently in vivo[4], Q-MARCM and GAL4 MARCM can be coupled to the same mitotic event. As such, an unlabeled progenitor cell would give rise by mitosis to one cell that is positively labeled by the Q system (as it lacks the QS repressor), and a sister cell that is positively labeled by the GAL4 system (as it lacks the GAL80 repressor). This is called "coupled MARCM" as the segregation of the QS and GAL80 suppressor are coupled to the same mitotic event (Fig. 10). This allows for the differential marking and manipulation of all progeny from a single mitotic event. If segregation of the QS and GAL80 suppressor were not coupled to the same mitotic event, then the cell progeny could independently be labeled or unlabelled by the GAL4 or Q system. This is called "independent double MARCM" (Fig. 2).

Comparisons with other methods

Binary Expression Systems—The bacteria *LexA/LexAop* binary expression system has also been used to express effectors independently of GAL4[14]. LexA contains a DNA binding domain specific for the *LexA operator* (*LexAop*), yet does not contain a transcriptional activation domain. In *Drosophila,* LexA is either fused to the viral acidic activation domain VP16 or the GAL4 activation domain (GAD). The LexA-VP16 protein is insensitive to GAL80, whereas the LexA-GAD protein can be inhibited by GAL80. The LexA/LexAop system does not contain an endogenous suppressor, and so cannot be used to generate some intersectional expression patterns or for GAL4 independent MARCM analysis. The *LexAop-geneX* reporter also exhibits a higher basal level of expression compared to *UAS-gene*X or *QUAS-geneX* reporters[4]. Nonetheless, recent progress has been made to optimize the LexA/LexAop binary expression system for use in vivo[15,16].

Intersectional Expression Patterns—Limiting GAL4 expression patterns can also be achieved by expressing GAL80 in the tissue of interest[3,17]. However, GAL80 expression patterns are difficult to determine, and GAL80 levels need to be higher than GAL4 for effective suppression. This can make it difficult to precisely define the resulting GAL4 expression pattern. A better approach, as detailed in Figure 2 and **Step 7A,** is to use a binary expression system to drive GAL80 expression. Similarly, the LexA/LexAop system could be used to refine GAL4 expression patterns. In this case, *LexA-VP16* would be used to drive *LexAop-GAL80*. However, given the lack of an independent repressor of LexA, the reciprocal experiment (using GAL4 to limit *LexAop-geneX* reporter expression) is not possible. This approach is possible using the Q system (**Step 7B,** Fig. 5b).

Limiting expression patterns to overlapping subsets is also achieved by using the "split GAL4" method in which GAL4 is split into two halves- one half containing the DNA binding domain and the other half containing the activation domain[18]. The two GAL4 halves can be reconstituted *in vivo* by the addition of leucine zippers to the split GAL4 proteins. This technique can achieve precise intersectional expression patterns[18]. However, split GAL4 can not utilize existing characterized GAL4 lines for intersectional expression, the reconstituted GAL4 is not as robust as the original GAL4, and split GAL4 transgenes are not useful for many other purposes (in contrast to a new QF reagent that can be used for binary expression or MARCM experiments).

Mosaic labeling methods—Coupled MARCM allows for the labeling of all progeny from a single mitotic event. It can also be used for independent gain- and loss-of-function genetic manipulations of both progeny. A number of other techniques also allow for the marking of both sister progenies.

"Dual expression control MARCM" uses *LexA-GAD* in conjunction with GAL4 based MARCM to visualize progeny from a cell division[14]. This technique allows labeling of different populations of cells (one labeled by the LexA driver and the other by the GAL4 driver) that arise from a common progenitor. However, since both *LexA-GAD* and GAL4 are suppressed by GAL80, this prevents labeling and manipulation of all progeny from a cell division. This technique has been used successfully for lineage analysis of certain neuronal populations[14,19].

"Twin-spot MARCM" uses *UAS-Inverse Repeat* (*UAS-IR*) transgenes as the source of repressors against two different fluorescent proteins. Similar in design to coupled MARCM which utilizes the differential loss of *tubP-GAL80* and *tubP-QS,* twin-spot MARCM follows the coupled loss of the *UAS-IR* repressors[20]. This creates two sibling cells, each losing one of the *RNAi repressor* genes. Twin-spot MARCM is simpler in design than coupled MARCM (since it utilizes fewer transgenes). However, both progeny are labeled by the same GAL4 driver, which could miss labeling of a cell progeny that lies outside this expression pattern. In addition, since the system is GAL4 only based, cell progeny can not be independently manipulated. Nonetheless, this technique is a powerful method for resolving the lineage pattern of a GAL4 expression pattern[20,21].

"Twin-spot generator" (TSG) does not utilize a binary expression system but instead places two split chimeric fluorescent proteins on the same chromosome arm *in trans*[22]. Upon FLP/FRT-mediated recombination, the two fluorescent proteins are reconstituted and can be segregated to daughter cells. This is similar in design to the mouse MADM system for mosaic analysis[23]. The advantage of the TSG method over other methods that use a repressible binary system is the ability to examine clones shortly after clonal induction since there is no perdurance of a repressor molecule. However, a major limitation is low marker expression due to the lack of binary system-based amplification. In addition, both markers are driven by a ubiquitous promoter, which severely limits the utility for tracking complex lineages. And since TSG does not utilize a repressible binary system, cell progeny cannot be easily manipulated by effector transgene expression.

Limitations of the Q system—As the Q system has only been recently introduced, a number of Q reagents, such as *QUAS-geneX* effectors or *promoter-QF* lines, remain to be generated. However, as more studies use the Q system, the availability of useful reagents will grow. Alternatively, in cases where the GAL4 system is not sufficient, the LexA/LexAop system could be used if LexA system reagents have already been generated and validated for a tissue of interest, and experimental designs do not require an endogenous LexA suppressor.

Experimental Design

Generation of QF transgenic flies—The first step for many Q system studies is the generation of Q system reagents for the manipulation of target tissues. The most straightforward approach is to clone a previously characterized enhancer/promoter region of interest into a QF DNA construct. A number of suitable QF DNA constructs are shown in Supplementary Table 1. There are two basic choices for cloning QF constructs: *pattB-QF-hsp70* and *pattB-QF-SV40*. These differ in their 3' transcriptional terminators. SV40 terminators lead to increased mRNA stability and higher protein levels. We have found that in most cases this increased protein level is not necessary or desirable when generating QF constructs due to potential toxicity of high level QF expression in as-yet-unidentified tissues. *We therefore recommend that the pattB-QF-hsp70 construct be used for routine enhancer and promoter cloning*.

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There are three basic strategies for generating QF transgenics utilizing previously characterized expression patterns. The first involves the cloning of gene promoters. In many cases, an enhancer and promoter region will be the genomic region immediately upstream of the ATG start site of a gene up to the preceding gene[24]. A PCR reaction that introduces flanking BamHI and EcoRI restriction sites can be used to amplify this genomic region for placement into the *pattB-QF-hsp70* construct.

The second strategy to generate QF expression patterns of interest is to clone the genomic region associated with enhancer trap insertions. The expression pattern of an enhancer trap could be mimicked by cloning a large genomic region immediately preceding the insertion site of an enhancer trap[4,11,25]. In this case, a promoter would also need to be included, such as either the P-element promoter or the synthetic DSCP promoter[26] with the *QF-hsp70* cassette following the cloned genomic region.

When the above two approaches fail to recapitulate the expression pattern of interest, a third strategy is to clone a larger genomic region associated with the gene or enhancer trap insertion. BAC recombineering could be used to insert a *promoter-QF-hsp70* cassette into a larger genomic region (20kb or 80kb) to more likely recapitulate a complex regulatory locus[27]. These BAC resources are compatible with PhiC31 integration for the generation of transgenic animals[28,29].

In some cases, generating *promoter-QF* transgenic lines might be difficult, especially for constructs that would result in widespread expression of QF. This could be due to QF being more toxic than GAL4. To reduce *QF* expression (and potential QF toxicity), the QF cDNA has been codon non-optimized for *Drosophila* expression. This allows for the generation of *promoter-QF* constructs that were previously difficult to generate, such as a pan-neuronal *synaptobrevin-QF* (CJP, unpublished results).

In addition, success rates for generating QF transgenic lines can be improved when using P-element or piggyBac based vectors instead of attB vectors (CJP, unpublished results). Alternative QF coding variants and QF cloning vectors that use piggyBac or P-elements are available from the authors upon request.

Generation of QF enhancer trap lines—Enhancer trap lines can often give rise to an expression pattern that is difficult to reproduce by cloning. In addition, a small scale enhancer trap screen can quickly generate many new expression patterns in parallel. A number of suitable QF enhancer trap DNA constructs are shown in Supplementary Table 1. These constructs can be injected with P-element transposase to generate new QF enhancer trap lines. Note that the available QF enhancer traps are P-element based and use an SV40 terminator. Alternative QF enhancer traps that use hsp70 terminators or piggyBac vectors are available from the authors upon request.

In addition, existing QF enhancer trap lines (Supplementary Table 2) can be mobilized by crossing to a stable P-element transposase (e.g., Δ2–3, Bloomington Stock #1798) to generate additional QF lines exhibiting new expression patterns. A small screen of ~25 lines has already identified QF enhancer trap lines that label trachea (*ET14-QF*), glia (*ET31-QF*), imaginal discs (*ET40-QF*), and many tissues including neuronal and epithelial (*ET49-QF*).

QF enhancer traps (and occasionally *promoter-QF* transgenes) can exhibit tracheal expression, especially if the trapped enhancers are weak. This is likely due to a cryptic weak tracheal enhancer in the QF coding sequence. Constructs that use QF coding variants (and no longer contain the cryptic tracheal enhancer) exhibit decreased or no tracheal expression in enhancer traps (CJP, unpublished results). In addition, *tracheal-promoter-QS* transgenic

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lines can be used to inhibit tracheal QF induced reporter expression (CJP, unpublished results). These reagents are available from the authors upon request.

Generation of *QUAS-geneX* effector lines—Another important Q system reagent is the QF inducible reporter- *QUAS-geneX*. A number of *QUAS-geneX* transgenic flies are available (Supplementary Table 2). To simplify the generation of additional *QUAS-geneX* transgenic flies, the *pQUAST* vector (Supplementary Table 1) contains the same multicloning site as the *pUAST* vector (EcoRI-BglII-NotI-SacII-XhoI-KpnI-XbaI), which allows for easy exchange of inserts between *pUAST* and *pQUAST* Vectors. If the *pUAST-geneX* plasmid is not available, genomic DNA from flies containing the *UAS-geneX* transgene can be used as the source of the *geneX* insert[4].

By using P-element based transgenesis[30], many independent insertions of the same *QUAS-geneX* construct will be generated. It is often useful to keep a single transgenic line on each of the 3 major chromosomes (X, 2nd, 3rd). Each transgenic line should be tested for inducibility and for lack of position effect. Even though most *QUAS-geneX* insertions are silent without a QF inducer, occasionally a *QUAS-geneX* line might be expressed due to induction of the minimal *hsp70* promoter by local strong enhancer elements. Such lines should be discarded.

Generation of QS effector lines—QS expression can be used to limit QF reporter expression patterns. Similar to the approaches for cloning QF transgenic animals, a promoter region known to express in defined tissues can be cloned into a *QS-SV40* transformation vector (Supplementary Table 1). For example, the EcoRI/KpnI flanked tubulin promoter in *ptubP-QS-SV40* could be replaced with the promoter of choice. Alternatively, the *QS* coding region from *pBS-KS-QS* (that has restriction sites KpnI-ApaI-HindIII-EcoRI-**QS**-XbaI-NotI-EagI) could be cloned into an existing promoter-containing vector of choice.

For Q MARCM experiments (BOX 1), ubiquitous QS expression is required. Lines expressing ubiquitous QS (driven by the tubulin promoter) have been recombined with FRT sites for every chromosome arm as well as inserted onto the CyO and TM6B balancers (Supplementary Table 2). In addition, by using a *UAS-QS* transgenic animal (Supplementary Table 2), GAL4 patterns can be used to direct QS expression with the purpose of limiting QF expression patterns (see **Step 7B**; Fig. 5b).

Potential applications of the Q system—The Q system can be used for a variety of in vivo applications. In many cases, the experimental question will determine which *QUAS-geneX* effector is used. Table 1 presents a sampling of possible studies, the *geneX* effectors for *QUAS-geneX* constructs that might be used, and the method of detection or analysis.

MATERIALS

REAGENTS

- Q system cloning vectors: many Q system cloning vectors (Supplementary Table 1) are available from Addgene (http://www.addgene.org/pgvec1?identifier=Luo.p9EJQGBAq0qGJ7t4LCsvD2Yax9w&cmd=findpub)
- *Drosophila* fly stocks: many Q system fly stocks are available from the Bloomington Stock Center (Supplementary Table 2; http://flystocks.bio.indiana.edu/Browse/misc-browse/Qintro.htm)
- quinic acid (Sigma-Aldrich, cat. no. 138622)
- Active dry yeast (Red Star Active Dry Yeast, Flystuff.com, cat. no. 62-103)

- propionic acid (Flysuff.com, cat. no. 20-271)

EQUIPMENT

- Standard fly-culturing equipment
- Wide Polystyrene Vials (cat. no. 32-110, Flystuff.com)
- Fly Vial Plugs (Wide Flugs, cat. no. 49-101, Flystuff.com)
- Dissecting microscope (Stemi 2000, Zeiss).
- fluorescent dissecting microscope (Stereo Discovery V8 Pentafluar, Zeiss)
- RFP filter cube for V8 Pentafluar (KSC 295-834D DS RED, Zeiss)
- GFP filter cube for V8 Penatfluar (KSC 295-814D GFP CUBE, Zeiss)
- 37 °C water bath for heat-shock (if using heat-shock promoter for FLP expression during MARCM experiments)
- humidified 25°C incubator to maintain fly crosses (Environmental Chamber 3940, Forma Scientific)
- Imaging microscope and software (e.g., Zeiss LSM 510 confocal microscope)
- Sharp forceps for brain dissections (Ted Pella, Inc, cat. no. 503, Dumont Biology Grade Tweezers Style 3)
- Three-well glass dissection dishes (Fisher Scientific, cat. no. 21-379)

REAGENT SETUP

- **Quinic acid solution** Dissolve quinic acid in water to achieve desired concentration. Saturated concentration is ~ 300 mg/mL (roughly equivalent to 1.56 M). The solution might need to be incubated at 37°C for ~15 min to help dissolve the quinic acid. Solution can be stored as aliquots at −20°C. The aliquot size for making ~10 quinic acid vials is ~3.5 mL.
- **0.5% propionic acid (weight/vol)** In 1 L bottle, mix 5 grams of propionic acid with 999 mL of water.
- **Yeast Paste** In small container, mix approximately equal volumes of active dry yeast with 0.5% propionic acid. Mix with metal spatula until yeast paste has dissolved. Mix in additional dried yeast as needed to achieve creamy peanut butter consistency.

EQUIPMENT SETUP

- **Quinic acid containing vials** Poke ~10 holes into medium of standard fly vials with wooden sticks. Apply ~300 μl of *quinic acid solution* to medium, making sure all holes are covered. Cover vials with cotton plug, and allow to dry on benchtop overnight. Vials should be used fresh (within 3–4 days if stored at RT), but can be stored at 4°C for ~ 2 weeks.

PROCEDURE

Performing repressible binary expression experiments. •TIMING ~15 days

1| In a yeasted vial, cross 3–5 *promoter1-QF* transgenic animals to 3–5 transgenic animals containing the appropriate *QUAS-geneX* reporter (Fig. 1b; Fig. 2; Table 1; Supplementary Table 2).

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2. Depending on the goal of the experiment and the identity of *geneX,* determine the effect of binary expression on F1 progeny at an appropriate developmental stage using an appropriate method (see Table 1). Alternatively, if *promoter1-QF* and *QUAS-geneX* are on the same chromosome, you may wish to proceed directly to **Step 3** to generate a stable binary expression stock for subsequent analyses.

Generating a stable binary expression stock. •TIMING 2–3 generations

3| It is often convenient to recombine the *promoter1-QF* and *QUAS-geneX* reporter onto the same chromosome for future expression experiments. This requires that the *promoter1-QF* and *QUAS-geneX* are both located to the same chromosome. Common *QUAS-geneX* reporters are available with insertions on each of the 3 major chromosomes (Supplementary Table 2). Choose 5–10 virgin F1 females of genotype *promoter1-QF/QUAS-geneX* from the progeny in **Step 1** and cross to a balancer stock.

•CRITICAL STEP: To get a successful recombinant, it is essential to use F1 heterozygote females since meiotic recombination occurs only in females, and not males.

4| Select single male progeny that contain both copies of the selectable marker (usually two copies of the mini-white+ gene) and set up individual crosses to virgin females from an appropriate balancer stock. Carry out appropriate sib-crosses with the progeny to generate a balanced *promoter1-QF,QUAS-geneX* stock derived from each original male.

•CRITICAL STEP: Single males are used for establishing balanced recombinant stocks since recombination does not occur in males. The use of single male crosses ensures that a generated stock will be genetically homogeneous.

•CRITICAL STEP If the expression pattern of the *promoter1-QF + QUAS-geneX* reporter can be visualized in live animals, this expression activity can be used to select for recombinant animals (instead of scoring for both copies of the selectable marker).

5| If desired, use the balanced stocks to analyze the effects of binary expression. Alternatively, proceed to **Step 6** to repress or temporally control binary expression or to **Step 7** to carry out intersectional experiments in conjunction with the GAL4 system.

Repression and temporal control of QF induced binary expression. •TIMING 1 generation

6| QF induced *QUAS-geneX* expression can be effectively silenced by the presence of QS. To refine a QF expression pattern, for example to remove a subset of QF labeled tissues, follow option (A). To completely abolish QF expression, for example when performing quinic acid treatment experiments, follow option (B). QS suppression of QF induced reporters can be relieved by quinic acid treatment, resulting in temporal suppression of QF (Fig. 1, Fig. 2, Fig. 4). To relieve QS suppression of QF during larval development, follow option (C). To relieve QS suppression of QF only in adult animals, follow option (D). Ubiquitous expression of QS that is linked to a mitotic recombination event can also be used for mosaic analysis with a repressible cell marker (Q MARCM) (BOX 1). Coupling both GAL4 based MARCM and Q based MARCM to the same mitotic event can be used for coupled MARCM (BOX 2).

A. Expressing QS in a subset of tissues

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(i) Generate (or select an existing) *promoter2-QS* line that results in the desired expression pattern of QS. Cross *promoter2-QS* flies to *promoter1-QF,QUAS-geneX* flies (generated in **Step 4**) (Fig. 3) and maintain in standard fly food vials.

(II) Use an appropriate method to analyse the effects of QS in F1 progeny with the genotype *promoter1-QF,QUAS-geneX; promoter2-QS*. Where QS is expressed, the *QUAS-geneX* reporter will no longer be expressed even if *QF* is present. As a control, reporter expression without QS presence should also be examined *i.e.* in parental flies of genotype *promoter1-QF, QUAS-geneX*. Alternatively, raise F1 to adulthood and proceed to Step 6D to relieve QS-mediated suppression of QF using quinic acid.

•CRITICAL STEP *Promoter2-QS* transgenic lines should express QS in the same pattern as *promoter2-QF* transgenic animals that use the same promoter. This should be verified by crossing the *promoter2-QS* transgenic fly to a *promoter2-QF, QUAS-geneX* recombinant to confirm that the entire *promoter2-QF* reported expression pattern is silenced. Different insertions of the *promoter2-QS* might need to be tested to find a line that effectively suppresses *promoter2-QF*.

B. Expressing QS in all tissues.

i. Ubiquitous expression of QS can be achieved by using the tubulin promoter to drive QS (*tubP-QS*). Select an appropriate *tubP-QS* stock (Supplementary Table 2) and cross to stable *promoter1-QF, QUAS-geneX* lines (from **Step 4**); maintain on standard fly food.

•CRITICAL STEP It is highly recommended to use a *promoter1-QF, QUAS-geneX* recombinant for ubiquitous QS experiments. Since the outcome of *tubP-QS* experiments is lack of expression, it is vital to know with 100% certainty that both *promoter1-QF* and *QUAS-geneX* components are present. The lack of either of these components will appear identical to *tubP-QS* suppression.

ii. Examine the F1 progeny for suppression of QF using an appropriate method (Table 1). The effects of ubiquitous QS expression can be confirmed by the lack of signal from the *QUAS-geneX* reporter. As a control for effectiveness of *tubP-QS,* reporter expression of parental flies of genotype *promoter1-QF, QUAS-geneX* can be examined. Alternatively, raise F1 to adulthood and proceed to **Step 6D** to relieve QS-mediated suppression of QF using quinic acid.

C. Quinic acid treatment of developing animals.

i. Prepare fresh quinic acid containing food vials (see REAGENT SETUP).

•PAUSE POINT Quinic acid fly food can be stored for up to a week if kept at 4°C.

ii. Cross ~10 *tubP-QS* animals to ~10 *promoter1-QF, QUAS-geneX* animals (from **Step 4**) and let them lay eggs in quinic acid containing food for 6–12 hours. Transfer adults to fresh quinic acid food vials approximately every 12 hours to prevent overcrowding of progeny. The developing larval progeny will ingest sufficient quinic acid for suppression of QS and re-expression of the *QUAS-geneX* effector (Fig. 1c).

•CRITICAL STEP: Alternatively, to target a specific developmental period, crosses could be set up on standard fly food and larvae at the required developmental stages transferred to grape plates or food containing quinic acid.

•CRITICAL STEP Quinic acid suppression of QS occurs within ~2 hours of animals being placed on quinic acid containing plates[4]. However, different tissues might respond differently to quinic acid feeding due to variations in proliferation rates or the extent of exposure to quinic acid. To reduce the level of quinic acid suppression, lower concentrations of quinic acid solution can be used when generating quinic acid food vials.

iii. Analyse expression at the appropriate developmental stage using an appropriate technique (Table 1).

D. Quinic acid treatment of adult animals.

i. Place adults of genotype *tubP-QS+promoter1-QF, QUAS-geneX* (**Step 6Bii**) in a fresh food vial containing quinic acid solution (Fig. 4).

•CRITICAL STEP Although quinic acid mediated relief of ubiquitous QS expression is detailed here, tissue specific *promoter2-QS* expression can also be relieved by quinic acid treatments as described above by using flies generated as described in **Step 6A**.

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ii. Analyse adult flies for suppression of QS (as monitored by QF induced *QUAS-geneX* expression) using an appropriate method (Table 1). Weak suppression of QS is seen within 6 hrs of being transferred to quinic acid containing vials, but is most notable within 24 hrs[4].

iii. For continued suppression, transfer flies to fresh quinic acid containing food vials every 24–48 hours. Quinic acid is non-toxic to flies and can be supplemented in their diet with no adverse effects.

Performing intersectional expression experiments. • TIMING Variable, depending on generation of fly stocks. ~5 fly generations to generate fly stocks and perform intersectional experiments

7| There are 12 intersectional expression patterns possible by using GAL4 and QF systems together (examples are shown in Fig. 2, Fig. 5, Fig. 11). Each of these 12 intersectional expression patterns represent an effector expression profile that is a subset of the GAL4 and QF expression patterns used in the experiment. See **Figure S7** in **ref.** 4 for a full list of expression patterns possible, including required genotypes. Below are details for 3 of the intersectionals which illustrate the basic principles for performing these genetic experiments. Choose option (A) to use QF expression patterns to limit the extent of GAL4 expression patterns. Choose option (B) to use GAL4 expression patterns to limit the extent of QF expression patterns. Choose options (C) or (D) to limit expression of an effector to only tissues that express both GAL4 and QF transgenes.

• CRITICAL STEP Even though the strategies in (C) and (D) reflect the overlapping intersection between QF and GAL4, they are not equivalent. Whichever line is driving FLPase expression will capture the entire developmental profile of that expression pattern, which could be much broader than the expression pattern at the target stage (e.g., the adult stage). The final

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effector expression level is reflected by whichever transcription factor is driving the final effector transgene (e.g., QF driving *QUAS>geneX*).

? TROUBLESHOOTING

A. GAL4 NOT QF intersectional experiments

i. Recombine *promoter2-GAL4* and the *UAS-geneX* onto the same chromosome and generate a balanced stock (as described in **Steps 3** and **4** for *promoter1-QF* and *QUAS-geneX*).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

ii. To this *promoter2-GAL4, UAS-geneX* stock, cross in a *QUAS-GAL80* transgene and generate a balanced stock (Fig. 6). *QUAS-GAL80* transgenes are available on each chromosome (Supplementary Table 2).

• CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

iii. Cross a *promoter1-QF* to the *promoter2-GAL4, UAS-geneX; QUAS-GAL80* stock (Fig. 6). Select progeny that contain all four genetic components required (*promoter1-QF, promoter2-GAL4, UAS-geneX,* and *QUAS-GAL80*) (Fig. 6; Supplementary Table 2). As a control, also choose animals for analysis that do not contain the *QUAS-GAL80* transgene (e.g. select for Tubby animals in Fig. 6).

•CRITICAL STEP These genetic components may be located on any chromosome just as long as progeny contain all four components. The scheme above is designed to simplify the testing of many different *promoter-QF* lines on altering GAL4 expression patterns.

iv. Analyse *UAS-geneX* expression using an appropriate technique (Table 1)

• CRITICAL STEP *UAS-geneX* effector expression will be refined based on the expression pattern of the *promoter1-QF*. For example, if *promoter1-QF* overlaps a portion of the *promoter2-GAL4* expression pattern, than the overlapping tissues would no longer express the *UAS-geneX* effector.

B. QF NOT GAL4 intersectional experiments

i. Recombine *promoter1-QF* and the *QUAS-geneX* onto the same chromosome and generate a balanced stock (see **Steps 3 and 4**).

ii. To the *promoter1-QF, QUAS-geneX* stock, cross in a *UAS-QS* transgene (Supplementary Table 2) and generate a balanced stock (Fig. 7).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

iii. Cross a *promoter2-GAL4* to the *promoter1-QS, QUAS-geneX; UAS-QS* stock (Fig. 7). Select progeny that contain all four genetic components required (*promoter1-QF, promoter2-GAL4, QUAS-geneX,* and *UAS-QS*) (Fig. 5b; Fig. 7; Supplementary Table 2). As a control, also choose animals for imaging that do not contain the *UAS-QS* transgene (e.g. select for Tubby animals in Fig. 7).

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•CRITICAL STEP These genetic components may be located on any chromosome just as long as progeny contain all four components. The scheme shown in Fig. 7 is designed to simplify the testing of many different *promoter-GAL4* lines for their effects on QF expression patterns.

iv. Analyse *QUAS-geneX* expression using an appropriate technique (Table 1).

•CRITICAL STEP *QUAS-geneX* effector expression will be refined based on the expression pattern of the *promoter2-GAL4*. For example, if *promoter2-GAL4* is *tubulin-GAL4,* then there would be no expression of the *QUAS-geneX* effector. If *promoter2-GAL4* overlaps a portion of the *promoter1-QF* expression pattern, than only the overlapping tissues would no longer express the *QUAS-geneX* effector (Fig. 5b).

? TROUBLESHOOTING

C. QF AND GAL4 intersectional experiment that captures the developmental profile of *promoter-QF* expression

(i) Recombine *promoter1-QF* with a QUAS “FLP-out” reporter, such as *QUAS>stop>mCD8-GFP* (Supplementary Table 2) and generate a balanced stock.

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

(ii) To the *promoter1-QF, QUAS>stop>mCD8-GFP* stock, cross in a *UAS-FLP* transgene and generate a balanced stock (Fig. 8a).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

(iii) Cross a *promoter2-GAL4* animals to the *promoter1-QF, QUAS>stop>mCD8-GFP; UAS-FLP* stock. Select progeny that contain all four genetic components required for QUAS reporter expression (Fig. 8a). In this case, GAL4 will drive FLPase expression, which will excise the transcription stop from the *QUAS>stop>mCD8-GFP* effector. QF is then able to induce expression from the resulting *QUAS>mCD8-GFP* transgene (Fig. 5c). As a control, also select animals for imaging that do not contain the *UAS-FLP* transgene (e.g. select for Tubby animals in Fig. 8a).

•CRITICAL STEP These four genetic components may be located on any chromosome just as long as progeny contain all four components. The scheme shown in Fig. 8a is designed to simplify the testing of many different *promoter-GAL4* lines to determine their overlapping expression pattern with a *promoter1-QF* line. Unbalanced lines could be used for these experiments since only when all four components are together will there be any reporter expression. However, using unbalanced lines will reduce the efficiency of the cross and increase the number of animals that need to be processed to ensure a positive result.

(iv) Analyse *QUAS>mCD8-GFP* expression by immunohistochemistry or on live animals by fluorescent microscopy (Table 1).

D. QF AND GAL4 intersectional experiment that captures the developmental profile of *promoter-GAL4* expression.

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(xiii) Recombine *promoter1-QF* with a UAS “FLP-out” reporter, such as *UAS>stop>mCD8-GFP* (Supplementary Table 2) and generate a balanced stock.

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

(xiv) To the *promoter1-QF, QUAS>stop>mCD8-GFP* stock, cross in a *QUAS-FLPo* transgene (Supplementary Table 2) and generate a balanced stock (Fig. 8b).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

(xv) Cross *promoter2-GAL4* animals to the *promoter1-QF, UAS>stop>mCD8-GFP; QUAS-FLPo* stock. Select progeny that contain all four genetic components required for UAS reporter expression (Fig. 8b). In this case, QF will drive FLPase expression, which will excise the transcription stop from the *UAS>stop>mCD8-GFP* effector. GAL4 is then able to induce expression from the resulting *UAS>mCD8-GFP* transgene. As a control, also choose animals for imaging that do not contain the *QUAS-FLPo* transgene (e.g., select for Tubby animals in Fig. 8b).

•CRITICAL STEP These four genetic components may be located on any chromosome just as long as progeny contain all four components. The scheme shown in Fig. 8b is designed to simplify the testing of many different *promoter-GAL4* lines to determine their overlapping expression pattern with a *promoter1-QF* line. Unbalanced lines could be used for these experiments as only when all four components are together will there be any reporter expression. However, using unbalanced lines will reduce the efficiency of the cross and increase the number of animals that need to be processed to insure a positive result.

(xvi) Analyse *UAS>mCD8-GFP* expression by immunohistochemistry or by using a fluorescent dissecting scope (Table 1).

TROUBLESHOOTING

Troubleshooting advice is provided in Table 2.

• TIMING

Step 1, ~10 days (1 fly generation)

Step 2, ~5 days for immunohistochemistry and imaging

Step 3, 1 fly generation

Step 4, 2 fly generations (~20 days)

Step 5, Variable depending on experimental design; ~5 days if staining and imaging are required

Step 6A, 1 generation for cross; ~ 5 days if staining and imaging are required

Step 6B, 1 generation for cross; ~5 days if staining and imaging are required

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Step 6C, 1 generation for cross; variable depending on extent of quinic acid feeding during development.

Step 6D, 1 generation for cross; Adult feeding of quinic acid can continue as long as necessary for the experiment.

Step 7A, ~ 4 fly generations to generate required stocks; 1 fly generation to perform intersectional experiment; ~ 5 days for staining and imaging if required.

Step 7B, Variable depending on necessity to generate appropriate fly stocks: 1–5 fly generations, and ~ 5 days for immunohistochemistry and imaging if required.

Step 7C, Variable: 1–5 fly generations, and ~ 5 days for imaging.

Step 7D, Variable: 1–5 fly generations, and ~ 5 days for imaging.

BOX 1, Variable: 1–5 fly generations, and ~ 5 days for imaging.

BOX2, Variable: 1–5 fly generations, and ~ 5 days for imaging.

ANTICIPATED RESULTS

When a *promoter-QF* and *QUAS-geneX* are combined in the same fly, there will be induced expression of *geneX*. However, when the *QUAS-geneX* is alone, there will be no efector expression. Figure 1c shows adult flies that contain the *QUAS-mtDT-3xHA* reporter alone, or when combined with a QF enhancer trap line. When the QS suppressor is also introduced, this will block QF activity and keep *QUAS-geneX* reporters silent. Figure 1c also shows adult flies whose broad QF induced expression of *QUAS-mtdT-3xHA* has been silenced by ubiquitous expression of QS. QS mediated suppression can itself be inhibited by treating flies with quinic acid. Quinic acid can be fed to developing animals by supplementing their food with quinic acid, and larvae will ingest enough quinic acid for efficient QS suppression in many tissues. Figure 1c shows an adult fly that was previously suppressed by ubiquitous QS but was relieved from such QS suppression by developing on fly food containing quinic acid. Similar quinic acid mediated re-expression of QF induced genes can also be performed in adult animals.

By combining the GAL4 and Q systems together, more refined expression patterns can be achieved (Fig. 5, Fig. 11). These are called intersectional expression experiments since the final expression pattern depends on the intersection between the QF and GAL4 expression domains. Such intersectional expression experiments could be used to target expression of an effector to a carefully defined target tissue, bypassing confounding effects due to more widespread expression. The outcome of the intersectional experiment depends on the additional genetic components that are used with the *promoter1-QF* and *promoter2-GAL4* lines. By using a *UAS-QS* transgene, GAL4 expression can be used to effectively limit a QF expression pattern. An example of this QF NOT GAL4 intersection is shown in Figure 11d. Similarly, by using a *QUAS-GAL80* transgene, QF expression can be used to effectively limit a GAL4 expression pattern. An example of this GAL4 NOT QF intersection is shown in Figure 11e. A powerful expressional refinement approach is to limit effector expression only to tissues that express both QF and GAL4. An example of this GAL4 AND QF intersection is shown in Figure 11f. This approach can effectively limit effector expression to a very small subset of cells. Since the expression pattern of *promoter1-QF* and *promoter2-GAL4* can be easily determined, targeting expression to a desired population of cells only requires picking and choosing the right intersectional combination of GAL4 and QF lines.

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Ubiquitous QS expression can effectively silence QF induced reporter expression. By using mitotic recombination to differentially segregate a *tubP-QS* transgene, one population of cells will no longer have the *tubP-QS* transgene and so will be released from QS suppression. These cells that are positively labeled (e.g., marked by a *QUAS-CD8-GFP* reporter) can also be made homozygous mutant for a gene of interest. This technique is called Q MARCM and is a powerful approach to genetically manipulate and label a small number, or even single, cells. An example of a Q MARCM clone that labels a single olfactory projection neuron in shown in Figure 9c and 9d.

The MARCM technique was originally developed for the GAL4 system[3]. In this case, ubiquitous expression of the GAL4 suppressor, GAL80, is differentially segregated to cell progeny based on a mitotic recombination event. Since the GAL4 system and the Q system function independently, these two mosaic labeling techniques can be combined together in coupled MARCM (Fig. 10). An example of a coupled MARCM clone in the wing imaginal disc is shown in Figure 10c and 10d. A QF marked clone could be homozygous mutant for a gene of interest and/or express an effector gene. Similarly, the GAL4 marked clone could be homozygous for a different gene of interest, and/or express a different effector gene. Such experiments could prove useful in addressing cell-cell communication or cell non-autonomous effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BOX 1 Performing Q MARCM experiments. • TIMING Variable, depending on generation of fly stocks (1– 5 generations)

MARCM experiments can serve a variety of purposes including generating mosaic tissues that are mutant for a gene of interest, or for identifying the anatomy of a single neuron. Any QF driver line can be used for Q MARCM experiments (Fig. 9). The protocol below is adapted from a Nature Protocol for performing MARCM experiments[10].

Generate Q MARCM ready flies

(i) Use standard genetic techniques to introduce the following genetic components into a single fly: 1) *FLP recombinase* under the control of a heat-shock promoter, 2) a *QUAS-geneX* reporter to visualize the Q MARCM clone, such as *QUAS-mCD8-GFP,* 3) an FRT site and *tubP-QS* recombined onto the chromosome arm of interest (Fig 9b). *tubP-QS* insertions recombined with FRT sites are available for each of major chromosome arms (Supplementary Table 2).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

Generate a *promoter-QF* line that is Q MARCM ready

(ii) Use standard genetic techniques to combine a QF line (e.g., *GH146-QF*) with an FRT chromosome that uses the same FRT site as the Q MARCM ready flies generated in the previous step. For example, to be compatible with an *82B^{FRT}, tubP-QS* stock, an *82B^{FRT}* line with *GH146-QF* could be used. The *GH146-QF* insertion can be on any chromosome arm.

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

•CRITICAL STEP The *promoter-QF* insertion can be located distal to the desired FRT (for example, *82B^{FRT}, promoter-QF*). However, since this chromosome arm will become homozygous after the mitotic recombination event, it might affect the tissue of interest in cases where the transgene insertion disrupts proper gene functions. It is recommended instead to position the *promoter-QF* insertion on any other chromosome arm. If possible, recombine the *promoter-QF* onto the chromosome arm opposite to the utilized *FRT (e.g., promoter-QF, 82B^{FRT}*) which can simplify future MARCM experiments.

Perform Q MARCM cross and generate MARCM clones

(iii) Cross 5–10 *promoter-QF* MARCM ready males to 10–20 *Q MARCM* ready virgins in a freshly yeasted vial. Depending on the birth date of the tissues of interest, heat shock the progeny in a 37°C water bath for 30 minutes to 2 hours (see ref. 10 for additional details). For example, to generate olfactory projection neuron clones, a 1.5 hr heat shock can be performed from embryonic to third instar stages. For imaginal wing disc MARCM clones, a 30 minute heat shock is performed at 48 hrs after egg laying.

•CRITICAL STEP The developmental time point and extent of the heat shock needs to be experimentally determined for each target tissue. The Q MARCM ready flies often contain a *hsFLP* insertion on the X chromosome

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(e.g., Fig. 9b). Using females of these flies for the Q MARCM cross will ensure that both males and female progeny will contain Q MARCM clones.

Analyze and examine Q MARCM clones

(iv) Analyse Q MARCM clones using an appropriate technique[10]; live or fixed tissues can be used.

? TROUBLESHOOTING

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BOX 2 Performing coupled MARCM experiments. •TIMING Variable, depending on generation of fly stocks (1– 6 generations)

To label or manipulate all progeny of a mitotic division, coupled MARCM experiments can be used (Fig. 10). This involves combining both Q MARCM and GAL4 MARCM techniques.

Generate coupled MARCM ready flies containing *tubP-QS*

(i) Use standard genetic techniques to introduce the following genetic components into a single fly: 1) *FLP recombinase* under the control of a heat-shock promoter, 2) a *QUAS-geneX* reporter to visualize the Q MARCM clone, such as *QUAS-mtdT-3xHA,* 3) a *UAS-geneX* reporter to visualize GAL4 MARCM clones, such as *UAS-mCD8-GFP,* 4) an FRT site and *tubP-QS* recombined onto the chromosome arm of interest (Fig. 10b). *tubP-QS* insertions recombined with FRT sites are available for each major chromosome arm (Supplementary Table 2).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments. This fly line could also be used for Q MARCM experiments.

Generate coupled MARCM ready flies containing *tubP-GAL80*

(ii) Use standard genetic techniques to introduce the following genetic components into a single fly: 1) *tubP-GAL80* recombined distally to an FRT chromosome that is the same FRT site as the coupled MARCM ready flies generated in the previous step, 2) *promoter2-GAL4,* 3) *promoter1-QF* (Fig. 10b).

•CRITICAL STEP This balanced stock is a valuable reagent and should be maintained for future experiments.

•CRITICAL STEP. The *promoter2-GAL4* and *promoter1-QF* insertions can technically be located on any chromosome arm to generate coupled MARCM clones. However, as mentioned for Q MARCM in Box 1, it is best to avoid recombining these reagents distal to the FRT site being used in case these lines, when homozygous, disrupt endogenous gene functions. The crossing scheme diagramed in Fig. 10b allows for different promoter-GAL4 or promoter-QF lines to be used with the same coupled MARCM ready flies. However, *promoter2-GAL4* and/or *promoter1-QF* could also be combined to other components in the previous step. The positioning of such components depends on the simplicity in generating a compatible coupled MARCM stock.

Perform coupled MARCM cross and generate coupled MARCM clones

(iii) In a freshly yeasted vial, cross 5–10 coupled MARCM ready males containing *tubP-GAL80* to 10–20 *Q MARCM* ready virgins containing *tubP-QS*. Depending on the birth date of the tissues of interest, heat shock the progeny in a 37°C water bath for 30 minutes to 2 hours.

•CRITICAL STEP The developmental time point and extent of the heat shock needs to be experimentally determined for each target tissue. The coupled MARCM ready flies with the *tubP-QS* often contain a *hsFLP* insertion on the X chromosome (e.g., Fig. 10b). Using females of these flies

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for the Q MARCM cross will ensure that both males and female progeny will contain Q MARCM clones.

Analyze and examine coupled MARCM clones

(iv) Analyse coupled MARCM clones using an appropriate technique[4,10]; live or fixed tissues can be used.

? TROUBLESHOOTING

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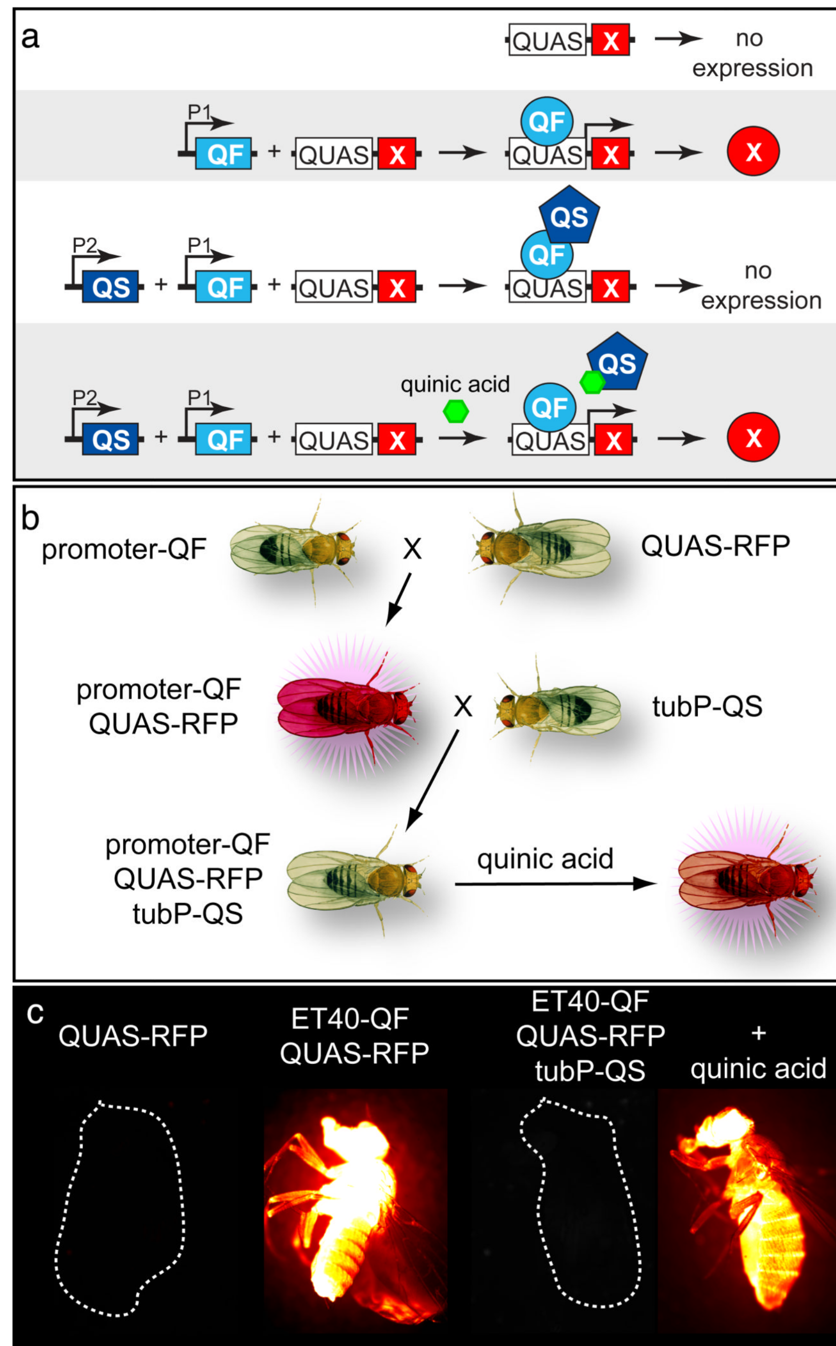

Figure 1. Schematic and example of Q system components in *Drosophila*

(a) Schematic representing the function of the Q system components. P1 and P2 indicate Promoter 1 and Promoter 2.

(b) Diagram illustrating a crossing scheme for Q system transgenic flies. tubP indicates the tubulin promoter.

(c) Transgenic *Drosophila* examples of the genotypes shown in (b). Transgenic flies not expressing the RFP reporter are outlined by a dashed white circle. The quinic acid treated flies developed on quinic acid containing fly food (see **Step 6C**). Images and schematics reprinted with permission from ref. 4.

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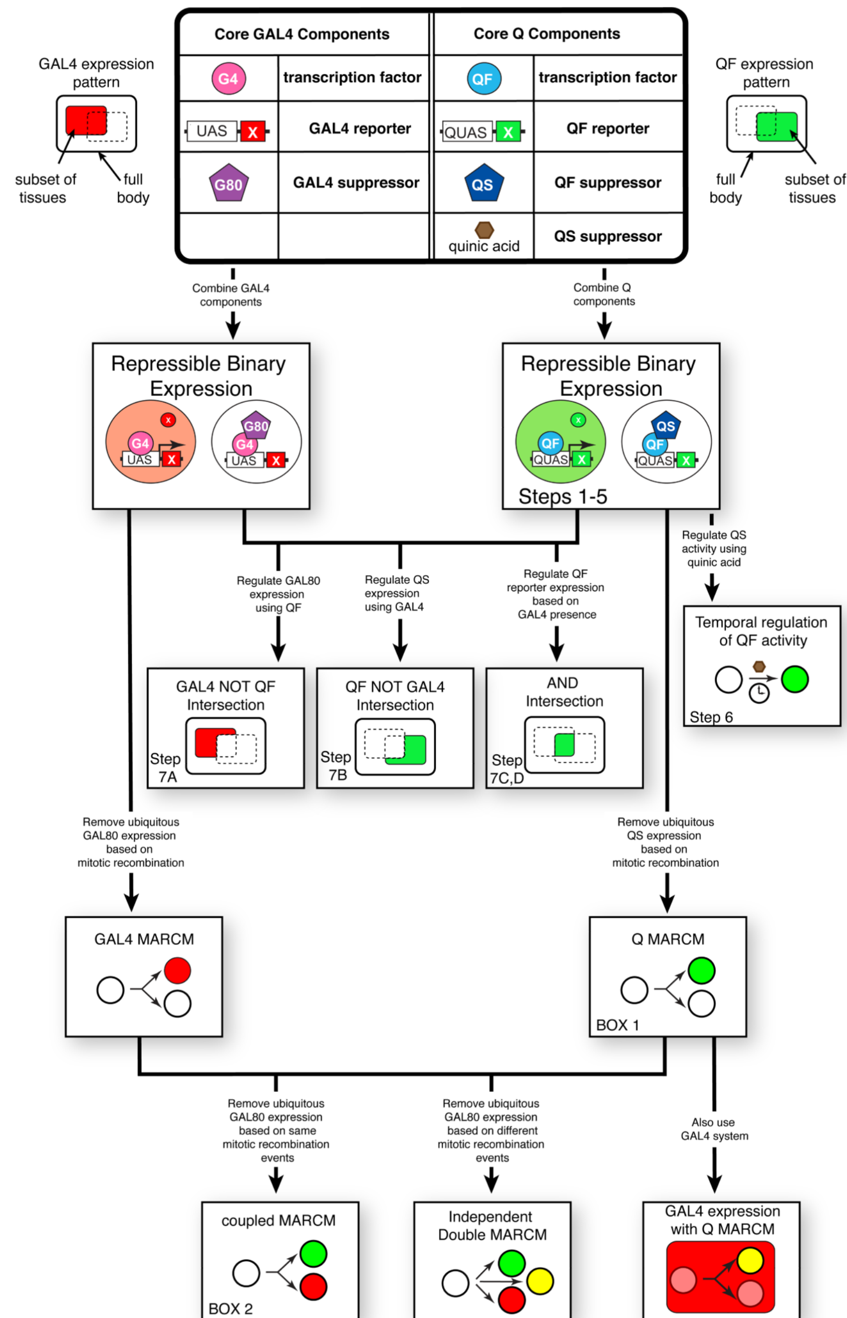

Figure 2. Flowchart of example GAL4 and Q system applications

The main box illustrates the basic GAL4 and Q system components: the transcription factors GAL4 and QF, the GAL4 and QF reporters *UAS-geneX* and *QUAS-geneX,* and the GAL4 and QF suppressors GAL80 and QS. In addition, the Q system includes a small drug inhibitor of QS (quinic acid). The manipulation and combination of these core components (arrows) allows for a number of in vivo applications. The Procedure Step describing the application is listed.

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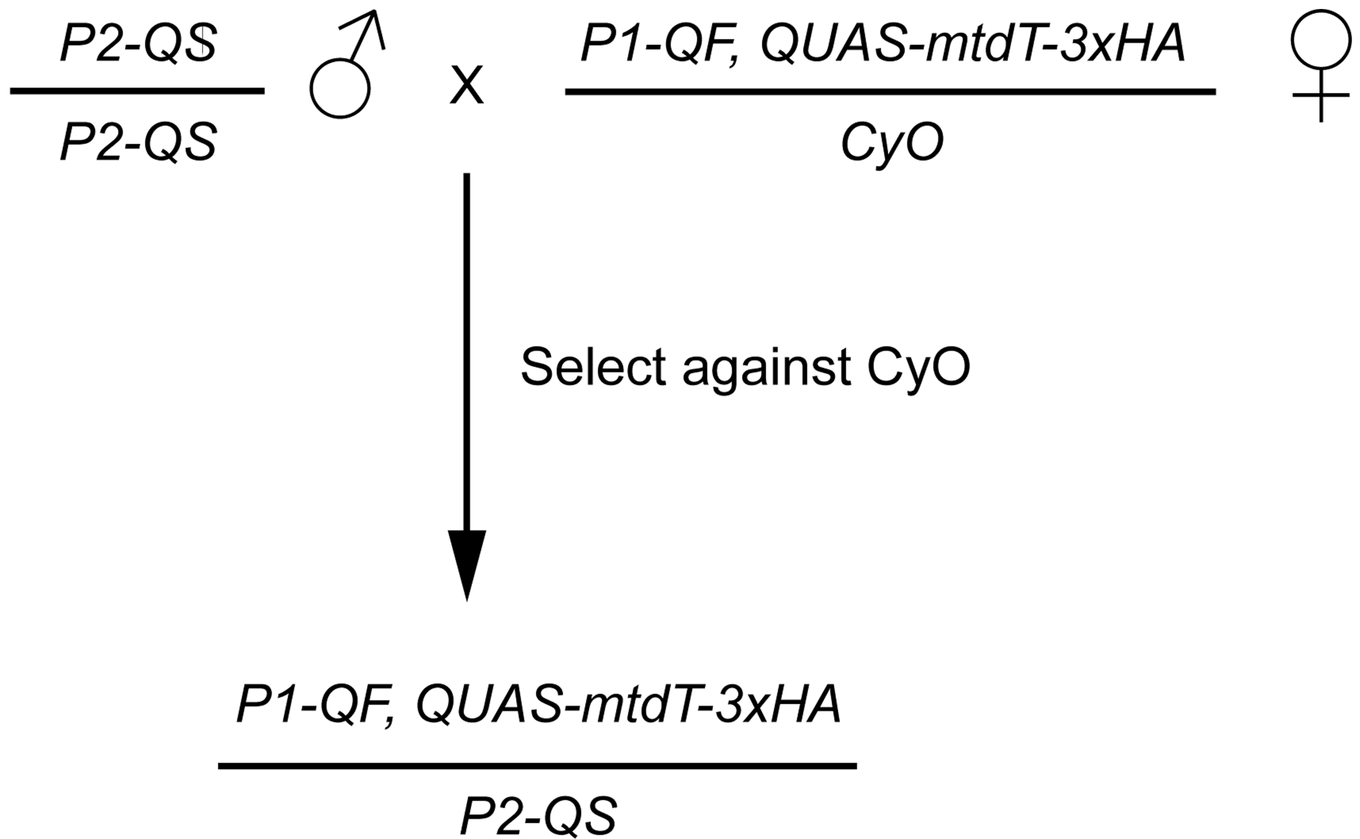

Figure 3. Crossing scheme for tissue specific QS suppression of QF

To simplify analysis of QS suppression on a QF induced expression pattern, the *QUAS-geneX* reporter (*QUAS-mtdT-3xHA*) is recombined with *P1-QF*. Crossing this stable expression line to a *P2-QS* fly and selecting against the CyO balancer will result in progeny that have a subset of tissues no longer expressing the *QUAS-geneX* reporter. This can be directly compared to the original expression pattern.

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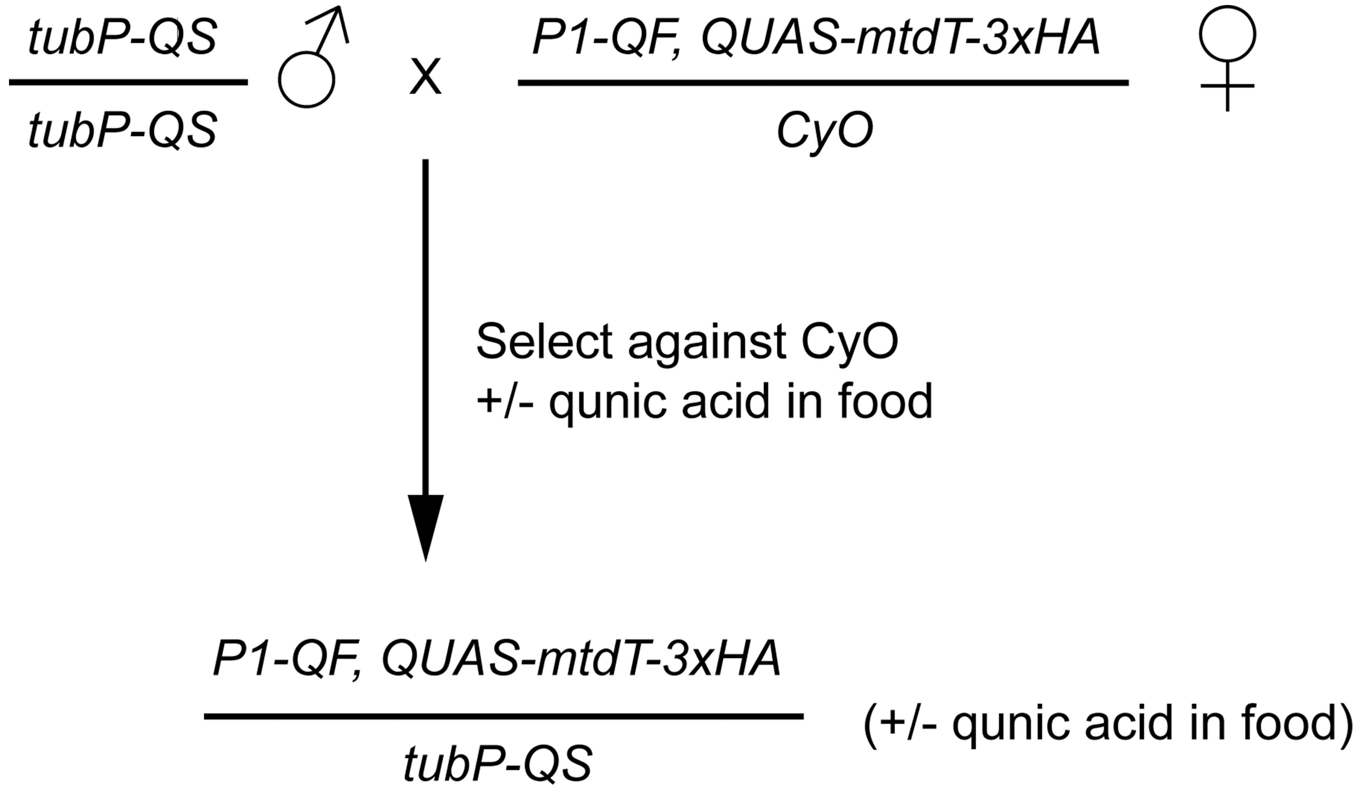

Figure 4. Crossing scheme for ubiquitous QS mediated suppression of QF coupled with quinic acid treatment

Ubiquitous QS expression is achieved by using a tubulin promoter to drive QS (*tubP-QS*). Crossing *tubP-QS* to a *P1-QF, QUAS-geneX* recombinant and selecting against the *CyO* balancer will result in progeny that no longer express the *QUAS-geneX* effector in any tissues. This QS mediated suppression can be inhibited by feeding developing flies quinic acid, or by feeding adult flies quinic acid. If treated with quinic acid, the *QUAS-geneX* reporter induced by *P1-QF* will be expressed. Differing levels of QS suppression can be achieved by altering the concentration of quinic acid fed to the flies.

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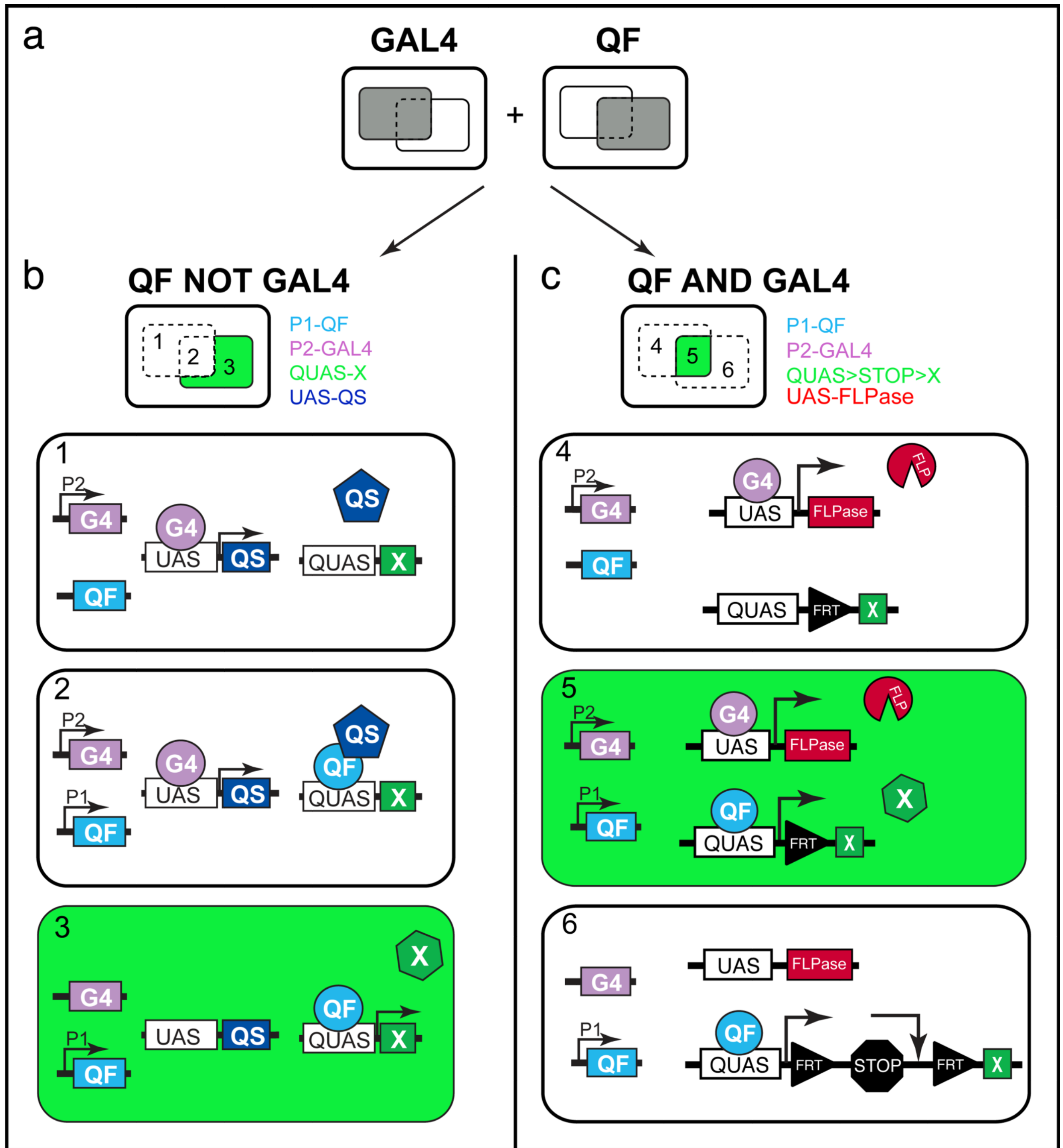

Figure 5. Using the Q system with the GAL4 system for generating intersectional expression patterns

(a) The gray squares represent the extent of the GAL4 or QF expression pattern.

(b) In the "QF NOT GAL4" example, a GAL4 line (*P2-GAL4*) is used to drive expression of the QS suppressor (*UAS-QS*) to restrict *QUAS-geneX* expression. This results in a final expression pattern reflecting where QF is expressed but not where GAL4 is also expressed. Region 1 does not express the *QUAS-geneX* since *P1-QF* is not expressed in this region. Region 2 expresses both *P2-GAL4* and *P1-QF* but does not express the *QUAS-geneX* due to expression of *QS*. Only region 3 expresses the *QUAS-geneX*. See **Step 7B**.

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(c) In the “QF AND GAL4” example, *QUAS-geneX* expression is limited to regions where both QF and GAL4 are expressed. The *QUAS-geneX* contains a ‘*FRT-transcription stop-FRT*’ cassette (>*stop*>) between the *QUAS* promoter and the reporter gene. This cassette can be excised by the activity of the FLPase recombinase. Region 4 does not express the *QUAS>stop>geneX* since QF is not expressed in this region. Region 5 expresses the *QUAS>stop>geneX* since *P2-GAL4* induces *UAS-FLPase* expression, which removes the transcription stop cassette, allowing for *P2-QF* induced expression. Region 6 does not express the *QUAS>stop>geneX* since *P2-GAL4* is not expressed in this region. ‘>’ indicates *FRT*. See **Step 7C**.

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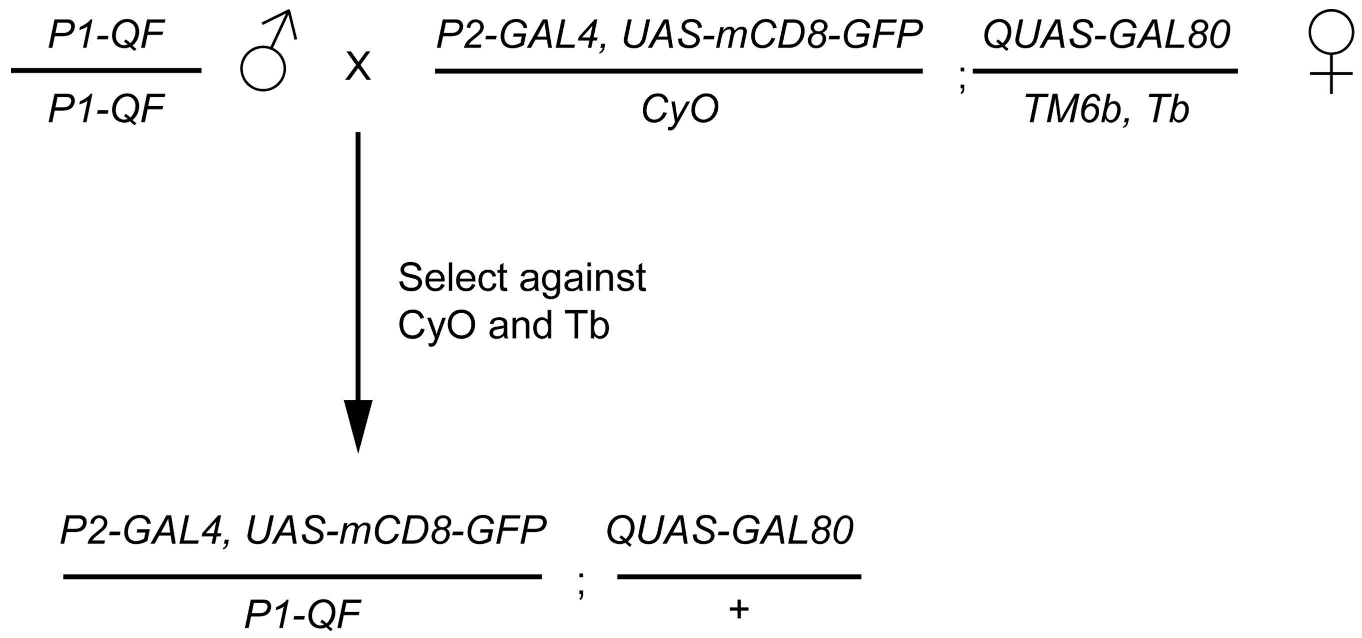

Figure 6. Crossing scheme for GAL4 NOT QF intersectional experiments

For this NOT intersectional strategy to work, four components (*P1-QF, P2-GAL4, UAS-geneX,* and *QUAS-GAL80)* need to be combined into a fly. In this example, a GAL4 NOT intersectional ready female fly is diagramed. This fly contains a *P2-GAL4* line recombined with a *UAS-mCD8-GFP* marker, as well as the *QUAS-GAL80* transgene on the third chromosome. Crossing this stock to any QF line and selecting against the balancers will result in progeny that have reduced GAL4 expression based on the QF expression pattern. This simplifies the experimental setup for testing the intersectional results for many different QF lines.

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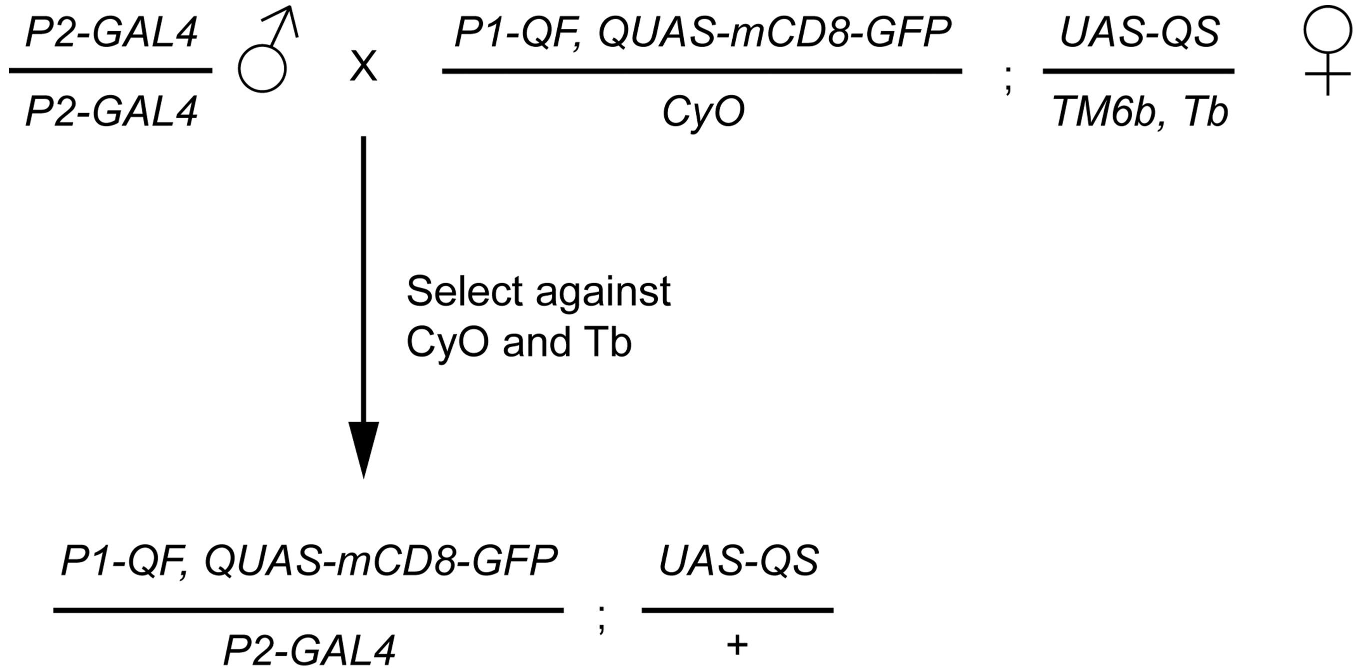

Figure 7. Crossing scheme for QF NOT GAL4 intersectional experiments

For the NOT intersectional strategy to work, four components (*P1-QF, P2-GAL4, QUAS-geneX,* and *UAS-QS*) need to be combined into a fly. In this example, a QF NOT intersectional ready female fly is diagramed. This fly contains a *P1-QF* line recombined with a *QUAS-mCD8-GFP* marker, as well as the *UAS-QS* transgene on the third chromosome. Crossing this stock to any GAL4 line and selecting against the balancers will result in progeny that have reduced QF expression based on the GAL4 expression pattern. This simplifies the experimental setup for testing the intersectional results for many different GAL4 lines.

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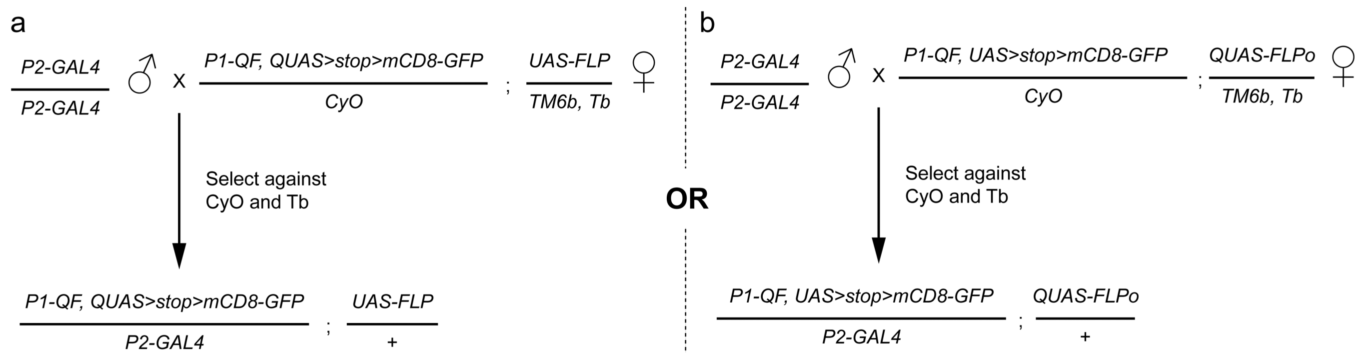

Figure 8. Crossing scheme for QF AND GAL4 intersectional experiments

There are two strategies to perform an AND intersectional cross. Both strategies require 4 components to be combined: *P1-QF* and *P2-GAL4* along with **(a)** *UAS-FLP, QUAS>stop>mCD8-GFP* or **(b)** *QUAS-FLPo, UAS>stop>mCD8-GFP*. In these examples, QF AND intersectional ready flies are shown for each strategy. These AND intersectional ready females flies contain all the necessary components except for the *P2-GAL4*. Crossing these stocks to any GAL4 line and selecting against the balancers will result in progeny that only have expression where both QF and GAL4 are expressed. These crossing schemes simplify the experimental design required to quickly test many different GAL4 lines for their intersection with a characterized QF line. Although both strategies limit expression to only where GAL4 and QF are expressed, they are not equivalent. In **(a)** the resulting expression pattern is determined by the developmental expression pattern of the GAL4 line, and the final expression pattern of the QF line. Conversely, in **(b)** the resulting expression pattern is determined by the developmental expression pattern of the QF line, and the final expression pattern oftheGAL41ine.

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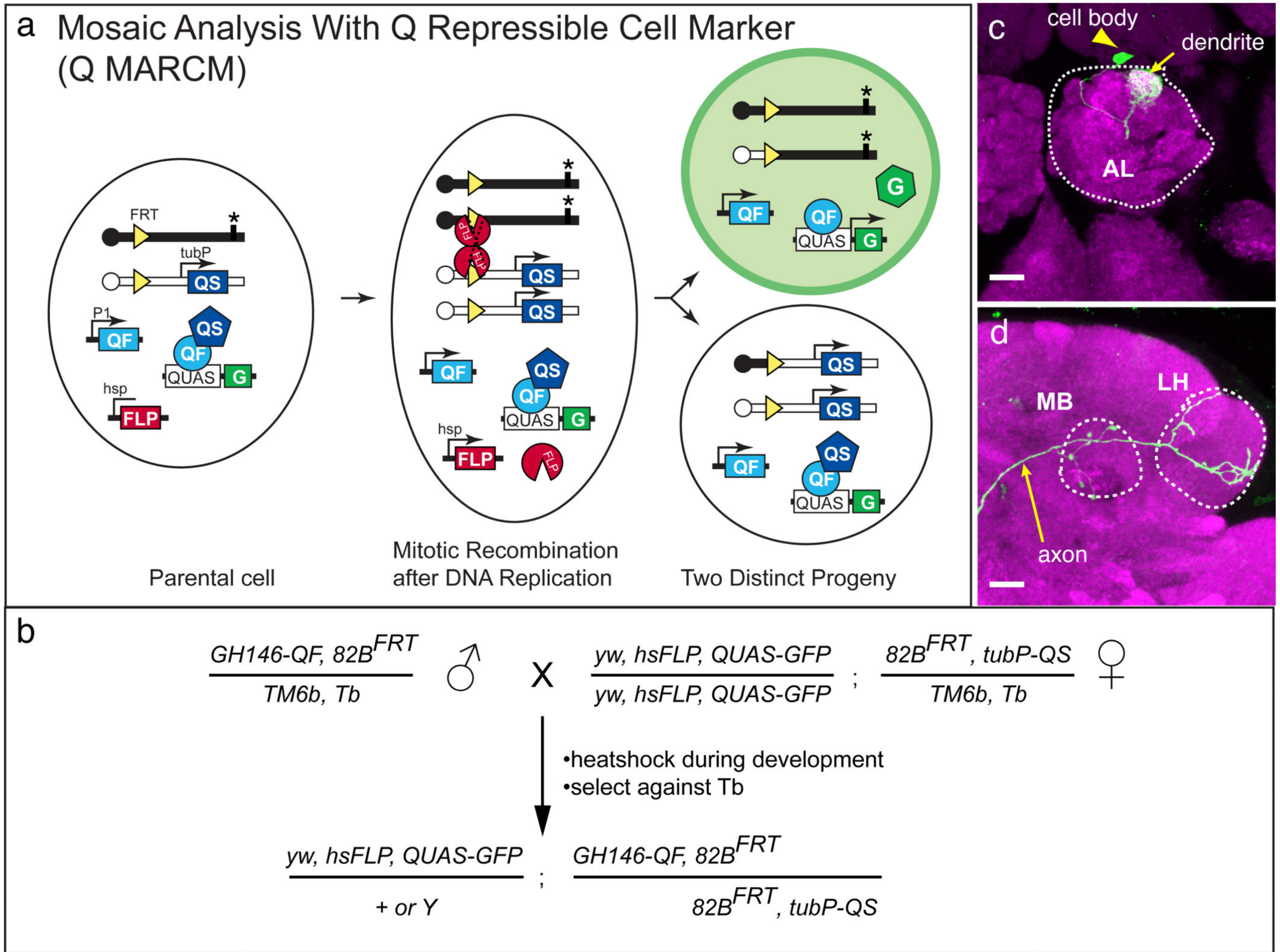

Figure 9. Schematic and example of Q-based Mosaic Analysis with a Repressible Cell Marker (Q MARCM)

(a) In a MARCM experiment, ubiquitous expression of the QS suppressor (driven by the tubulin promoter) is removed by a mitotic recombination event mediated by the FLP/FRT system, which allows for QF to activate *QUAS-geneX* reporters in a subset of cells. The parental cell contains sister chromosomes (black bar and white bars) containing the same *FRT* insertion (yellow triangle) distal to the centromere (circles). Distal to one of the *FRT* sites is the *tubP-QS* transgene. The other sister chromosome could contain a mutation of interest (*). *FLPase* expression is under control of a *heat shock promoter* (*hsp*). A heat shock pulse induces FLPase expression (red pacman) at or before mitosis. FLP/FRT mediated mitotic recombination at the G2 phase of the cell cycle (dotted black cross) followed by the chromosome segregation shown causes the top cell progeny to lose both copies of *tubP-QS,* restore QF activity, and become capable of expressing the *QUAS-GFP* marker (G). It also becomes homozygous for a mutation (*). *hsFLP, QF,* and *QUAS-geneX* transgenes can be located on any other chromosome arm. Schematic modified from ref. 4.
(b) Example crossing strategy for the Q MARCM experiment in (**c–d**).
(**c–d**) Example of a single DL1 olfactory projection neuron labeled by Q MARCM. The antennal lobe (AL), mushroom body calyx (MB), and lateral horn (LH) are outlined. Reprinted with permission from ref. 4. Scale bars: 20 μm.

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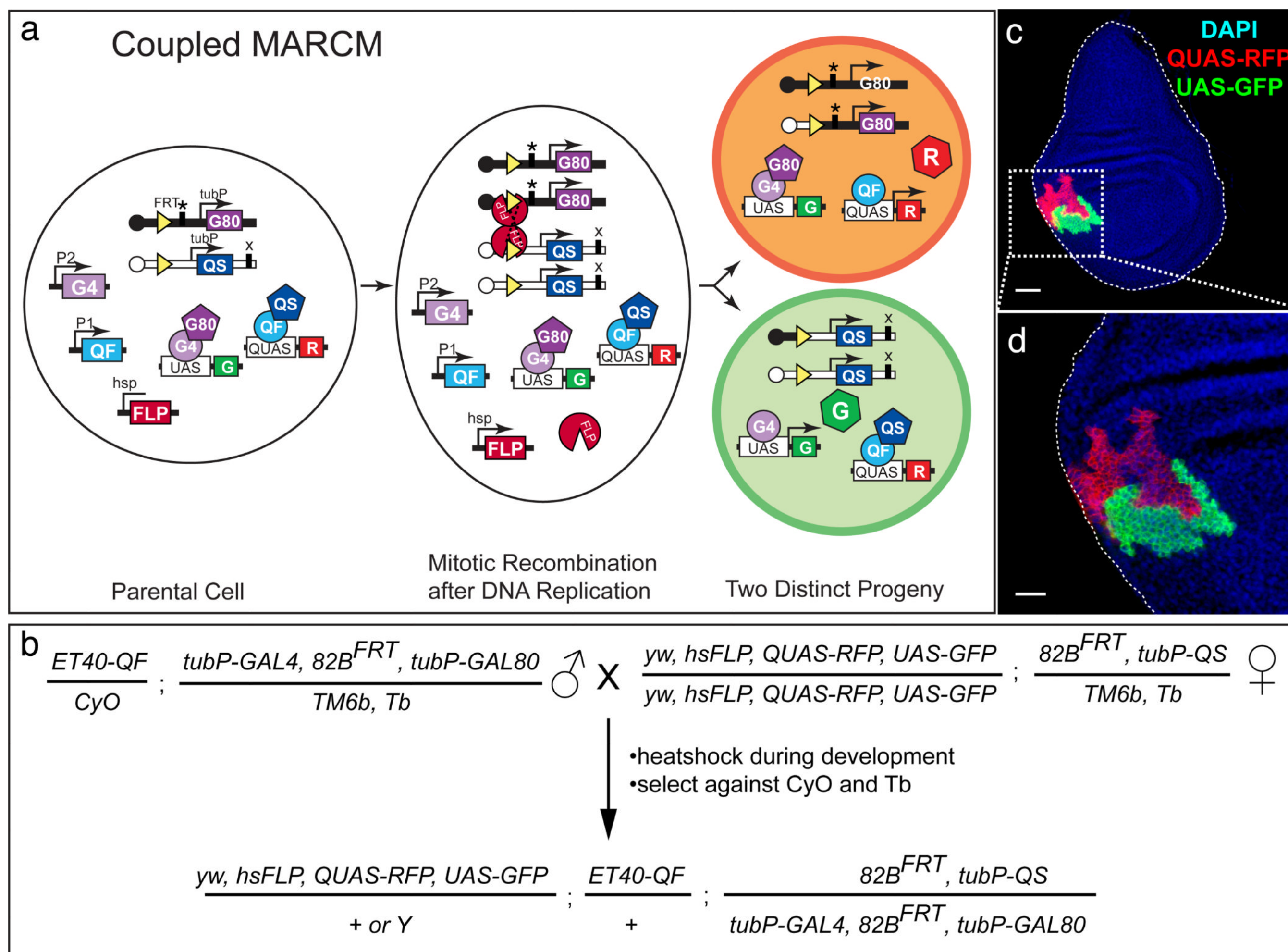

Figure 10. Schematic and example of coupled MARCM

(a) In a coupled MARCM experiment, ubiquitous expression of the QS and GAL80 suppressors (driven by the tubulin promoter) are simultaneously segregated to different progeny by an experimentally induced mitotic recombination event. This results in two distinct progeny- one that has an active QF (due to loss of the QS suppressor) and the other that has an active GAL4 (due to loss of the GAL80 suppressor). See Figure 9 for additional details. '*' and 'x' designate two independent mutations that can be rendered homozygous in sister progeny. *hsFLP, QF, GAL4, UAS-geneX,* and *QUAS-geneX* transgenes can be located on any other chromosome arm. Schematic modified from ref. 4.

(b) Example crossing strategy for the coupled MARCM clone shown in (c–d). *ET40-QF* is a QF enhancer trap on the second chromosome which expresses QF in imaginal discs.

(c–d) Example coupled MARCM clone in a third instar larval wing imaginal disc. Cell nuclei are labeled with DAPI. Larvae were heat shocked for 30 minutes at 48 hrs after egg laying. Scale bars: 20 μm.

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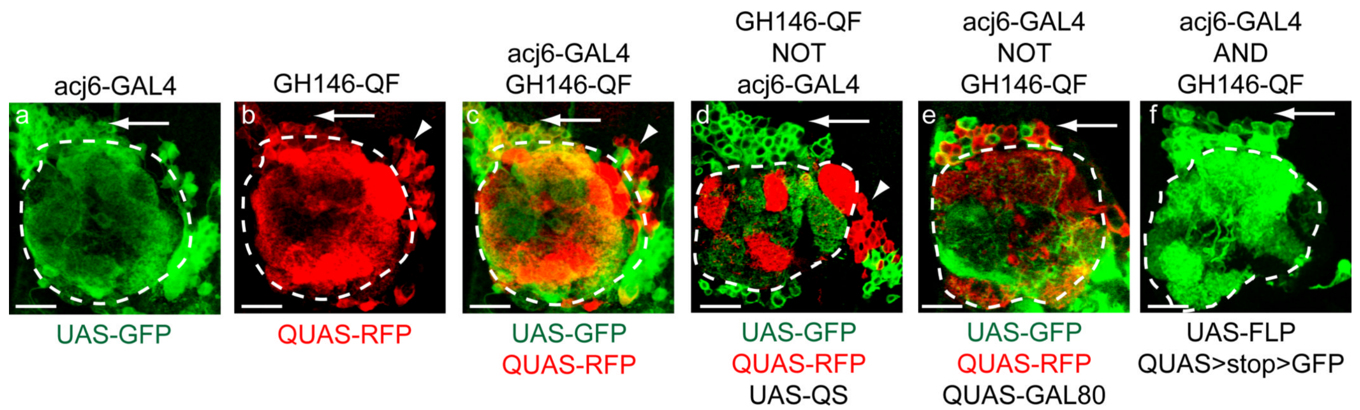

Figure 11. Example intersectional expression experiments between GAL4 and QF olfactory projection neuron lines

(a) Shown is the antennal lobe innervation of *acj6-GAL4* projection neurons labeled by *UAS-mCD8-GFP*. The antennal lobe is circled. The arrow in all panels points to the dorsal population of projection neuron cell bodies, **(b)** Shown is the antennal lobe innervation of *GH146-QF* labeled by *QUAS-mtdt-3xHA*. The arrowhead in all panels points to a GH146$^+$ lateral population of projection neuron cell bodies. **(c)** *GH146-QF* expresses in a subset of *acj6-GAL4* expressing dorsal projection neurons (labeled in yellow). *GH146-QF* and *acj6-GAL4* do not express in the same population of lateral projection neurons. **(d)** Example of the GH146-QF NOT acj6-GAL4 intersectional expression pattern. *QUAS-mtdT-3xHA* is no longer expressed in any of the dorsal projection neurons (arrow) due to *acj6-GAL4* expression (green) driving *UAS-QS*. The lateral *GH146-QF* projection neurons remain labeled (arrowhead) since they do not express *acj6-GAL4*. **(e)** Example of the acj6-GAL4 NOT GH146-QF intersectional expression pattern. *UAS-mCD8-GFP* is no longer expressed in a subset of dorsal projection neurons due to *GH146-QF* expression (red) driving *QUAS-GAL80*. **(f)** Example of the acj6-GAL4 AND GH146-QF intersectional expression pattern. The *QUAS>GFP* reporter is only expressed in a subset of dorsal projection neurons that coexpress both *acj6-GAL4* and *GH146-QF* (arrow). The lateral projection neurons are not labeled. GFP, mCD8-GFP; RFP, mtdT-3xHA; Scale bars: 20 μm. Panels **(d)** and **(f)** reprinted with permission from ref. 4.

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Table 1

Example Applications of the Q system

Application	***geneX* for *QUAS-geneX***	**Detection/analysis method**	**Reference**
Labeling tissues	**mCD8-GFP** **mtDT-3xHA** **CD2-HRP**	Live imaging Immunohistochemistry Electron microscopy	3,4,31,32
Marking different cellular compartments	**EYFP-Mito** (mitochondria) **EYFP-Golgi** (golgi) **DenMark** (dendrites) **synaptotagmin-HA** (pre-synaptic termini) **nuclearLacZ** (nucleus) **GFP-α-tubulin** (microtubules)	Live imaging Immunohistochemistry	12,33–35
Ectopically expressing a gene of interest	**Tsc1/Tsc2** (cell growth/proliferation) **Akt** (cell growth) **TβH** (enzyme for synthesis of octopamine)	Live imaging Electron Microscopy Immunohistochemistry Behavior	36–38
Cell ablation	**reaper** **hid** **grim**	Immunohistochemistry	39–41
Report cell activity	**GCAMP3** (neural activity) **tGPH** (PIP3 signaling)	2-photon microscopy Immunohistochemistry	42 43
Gene knockdown	**Interfering DNA against *geneX*** (RNAi) **microRNA against *geneX***	Behavior Live imaging Immunohistochemistry	44 45
Neuronal activation	**Channel Rhodopsin** (blue light activation) **TRPA1** (high temperature activation) **TRPM8** (low temperature activation)	Behavior Calcium imaging	46–49
Neuronal inactivation	**$shibire^{ts1}$** (inhibits vesicle recycling) **Kir2.1** (hyperpolarizes neuron) **tetanus toxin** (cleaves synaptobrevin)	Behavior	50–53
Mosaic analysis	**reporter** (to label clones and/or mutant tissue)	Immunohistochemistry	3,10,54

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Table 2

Troubleshooting

Step	Problem	Possible reason	Possible solution
2	No reporter expression with *promoter-QF* line.	QF line not expressed.	Try *promoter-QF* insertion at different genomic locus.
		QF killing expressing cells.	Verify cells are dying by co-labeling cells with antibody marker or GAL4/UAS marker. Try weaker *promoter-QF* line.
		Reporter expression is low.	Use two copies of reporter or *promoter-QF* line. Use different reporter.
6A	QS expression can not inhibit QF	QS expression too low.	Use extra copies of QS transgenic lines.
6B		QS not expressed in same cells as QF.	Use different QS transgene.
6C	Quinic acid not inhibiting QS	Quinic acid solution too old.	Make fresh quinic acid solution.
6D		Quinic acid solution not concentrated enough.	Make saturated 300 mg/mL quinic acid solution.
		QS expression too high.	Try different QS transgenic line (e.g., *tubP-QS#9B,* Bloomington Stock # 30022).
7C	Intersection of QF AND GAL4 shows no expression.	FRT-STOP-FRT reporter weak	Use extra copies of the FRT-STOP-FRT reporter.
7D		QF and GAL4 are not expressed in the same cells.	Try a different QF or GAL4 line.
		Expression of QFor GAL4 is weak at examined stage	There are two approaches for the “AND” intersectional. They differ by which transcription factor is the final readout, and which is the developmental readout. The final readout might be weak at the examined stage. Try the alternative method.
		All four required genetic components are not in same fly	Check crossing strategy to ensure that selected progeny contain all four components.
7C	Intersection of QF AND GAL4 shows stochasticity in labeled tissues.	Low FLPase expression.	Use extra copy of FLPase or reporter.
7D			Use codon optimized FLPase for higher expression.
		Low expression of GAL4 enhancer trap.	Perform intersection using different components (e.g., different *UAS-FLPase* line; or use *UAS-FLPase, QUAS>stop>reporter* instead of *QUAS-FLPase, UAS>stop>reporter).*
BOX 1	Little or no Q MARCM clones	MARCM stocks broken down	Check that all components (e.g., *hsFLP,* FRT sites) are still present.
		Heat shock time at wrong developmental period or too short.	Try heat shocking at earlier developmental time points. Try heat shocking for longer (e.g., 1.5–2 hr at 37°C)