Chapter 8

Transcriptome Analysis of *Drosophila* Neural Stem Cells

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Abstract

In *Drosophila*, the central nervous system is populated by a set of asymmetrically dividing neural stem cells called neuroblasts. Neuroblasts are derived from epithelial or neuroepithelial precursors, and divide along their apico-basal axes to produce a large apical neuroblast and a smaller basal ganglion mother cell. The ganglion mother cell will divide once again to produce two post-mitotic neurons or glia. In this chapter we outline a method for labeling different types of neural precursors in the *Drosophila* central nervous system, followed by their extraction and processing for transcriptome analysis. This technique has allowed us to capture and compare the expression profiles of neuroblasts and neuroepithelial cells, resulting in the identification of key genes required for the regulation of self-renewal and differentiation.

Key words: *Drosophila*, Neural stem cell, GAL4/UAS, In situ cell picking, RT-PCR, Transcriptome analysis

1. Introduction

Understanding how a limited set of neural precursors can give rise to the complexity and diversity of the fully formed central nervous system is one of the major research topics in developmental neurobiology. A powerful tool for investigating this question is lineage analysis, in which the number and cell fate of the progeny arising from a single stem cell are determined. Lineage analysis can be combined with molecular genetic and bioinformatic techniques to elucidate the genetic networks that regulate stem cell behavior and decide cell fates.

There are two main approaches to labeling neural stem cell lineages in *Drosophila*: progenitors can either be physically labeled, or genetically marked. Methods for the former usually involve the injection of lineage tracers, such as horseradish peroxidase (1, 2), or the application of lipophilic vital dyes, such as DiI (3). More recently, a number of genetic labeling methods for lineage analysis have been developed and are still evolving (e.g., MARCM (4),

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GTRACE (5), twin spot generator (6), twin spot MARCM (7); see also (8) for a brief review of some new techniques).

Lineage analysis often relies on antibody staining of fixed tissue samples. This approach has led to the identification of molecular markers for most embryonic neuroblasts, and detailed descriptions of many of their lineages (9–13). The advent of increasingly sophisticated time lapse microscopy techniques (14–18) means these kinds of data can now be complemented by live imaging to capture more dynamic aspects of stem cell behavior.

Research in our lab is focused on understanding the genetic regulation of neural stem cell self-renewal and differentiation (19–21). To this end, we have devised a method for isolating single or small groups of neural stem cells from living *Drosophila* brains, extracting total RNA, and synthesizing cDNA libraries for transcriptome analysis (22, 23). We developed this approach by combining a technique for cell extraction using a microcapillary needle (see Note 1), originally pioneered for embryonic neuroblast transplantation assays (24, 25), with a reverse transcription PCR protocol optimized for single cells (22, 26–29).

Recently, we used this method to investigate how the switch from symmetric to asymmetric neural stem cell division is regulated in the larval optic lobe (23). Symmetrically dividing neuroepithelial cells in the outer proliferation center of the optic lobe transform into asymmetrically dividing neuroblasts during larval development (Fig. 1) (30, 31). We reasoned that, as neuroepithelial cells and neuroblasts are clonally related, there might be a limited but crucial number of transcriptional changes governing the switch in division mode. In order to uncover these changes, we extracted around 50 GFP-labeled neuroepithelial cells or neuroblasts from individual late-third instar larval brains and isolated total RNA, which was reverse transcribed, amplified, and then hybridized to whole transcriptome oligonucleotide arrays (Fig. 2). Directly comparing the neuroepithelial cell and neuroblast transcriptomes enabled us to identify the Notch pathway as a key regulator of the transition from symmetric to asymmetric division (23).

The optic lobe is particularly suitable for investigating neural stem cell regulation in this manner because independent GAL4 driver lines exist for labeling the two populations of neural precursors, and the cells lie close to the brain surface, so they are easier to extract. However, although the method outlined below refers to the larval optic lobe, the technique is versatile and can easily be modified to isolate neural stem cells in different locations or at different developmental stages. For example, we have also isolated and carried out transcriptome analysis on neuroblasts from the embryonic ventral nerve cord (22) (see Note 2). In principal it could be further adapted for any progenitor, provided the cell type of interest is identifiable (either morphologically distinct or strongly and specifically labeled by a reporter gene), and accessible in situ (see Note 3).

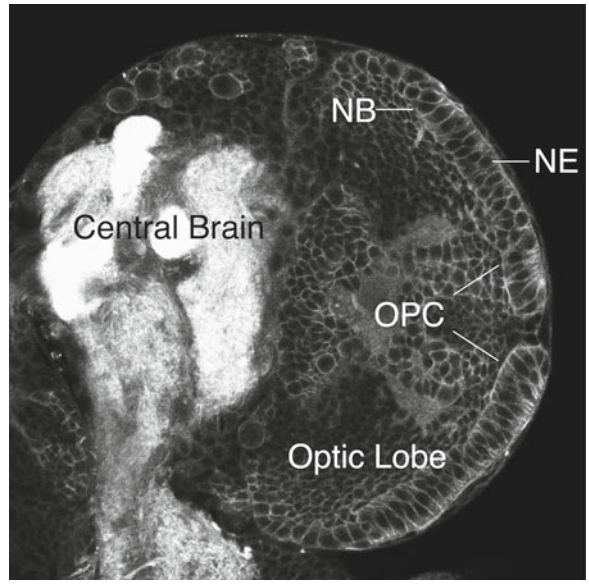

Fig. 1. Third larval instar brain lobe, consisting of the central brain and optic lobe. Cells are outlined by Dlg (Discs large) antibody staining. The outer proliferation center (OPC) of the optic lobe contains two populations of neural stem cells: symmetrically dividing neuroepithelial cells and asymmetrically dividing neuroblasts. Neuroepithelial cells (NE) transform into neuroblasts (NB) at the medial edge of the neuroepithelium. These neuroblasts give rise to the neurons of the medulla cortex, one of the visual integration centers of the adult brain. Adapted from (23).

2. Materials

2.1. Needle Preparation: Microcapillary Pulling and Beveling

1. Borosilicate glass microcapillaries, 1.0 mm outside diameter × 0.78 mm inside diameter (GC100TF-10, Harvard Apparatus, Edenbridge, UK).
2. Commercial micropipette puller (Flaming/Brown P-87 with 2.5 × 2.5 mm box filament, Sutter Instrument Company, Novato, USA).
3. Micropipette beveller (Bachhofer, Reutlingen, Germany) for sharpening microcapillaries.
4. Air-filled syringe and polyethylene tubing for controlling pressure in the microcapillary when extracting and expelling cells (25).
5. DEPC-treated ddH_2O.
6. 70% ethanol.

2.2. Drosophila Strains

Multiple GAL4 lines drive GAL4 expression in neural stem cells in the embryo and larva. There are also a variety of fluorescent UAS reporter lines for visualizing cells in living brain. Many of these can be ordered from the major fly stock centers such as the Bloomington

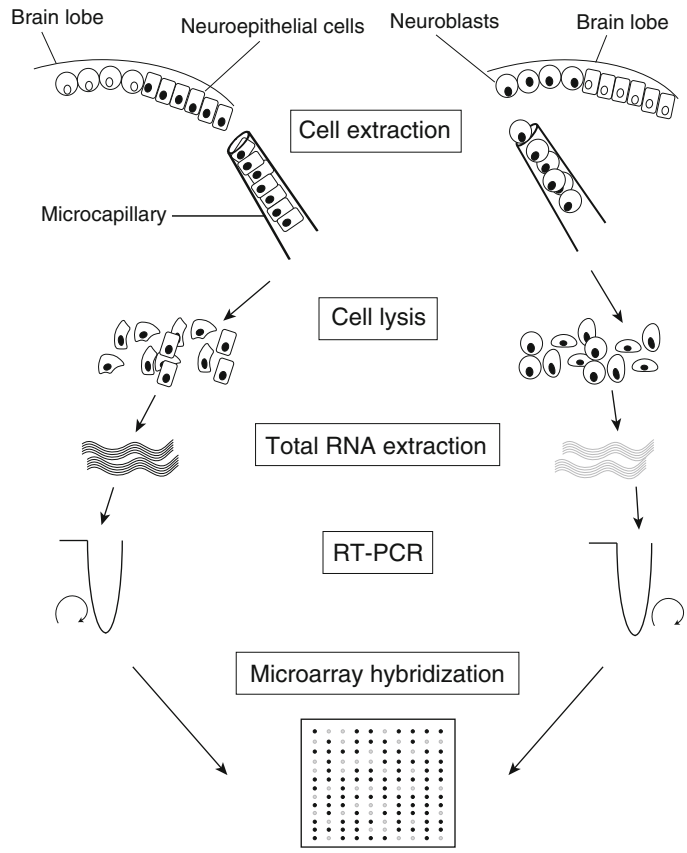

Fig. 2. Scheme showing major steps for optic lobe neural stem cell isolation and transcriptome analysis. Neuroepithelial cells or neuroblasts are genetically labeled, using different GAL4 lines. Cells are extracted from third instar larval brains using a beveled glass microcapillary. Cells are lysed in order to extract total RNA, which is reverse transcribed and amplified by PCR. The neuroepithelial and neuroblast cDNA libraries are fluorescently labeled and directly compared by hybridization to a full genome microarray. Adapted from (23).

Stock Centre (http://flystocks.bio.indiana.edu/) and Kyoto Stock Center (http://www.dgrc.kit.ac.jp/en/index.html). In order to visualize neural precursors in the optic lobe, we use two driver lines, which label the neuroepithelial and neuroblast populations distinctly: *GAL4*^{c855a} and *GAL4*^{1407} (*inscuteable-GAL4*) (see Note 4). We have used these to drive the expression of membrane-tethered mCD8-GFP and Histone-2B-mRFP1, so that both cell membranes and nuclei were labeled.

2.3. Staged Larval Collections

1. Fly cages.
2. Apple juice plates with wet yeast.
3. Fly food plates (35 mm Petri dishes filled with fly food), or fly food vials, with wet yeast.

2.4. Drosophila Larval Brain Dissection

1. Cold phosphate-buffered saline (PBS), pH 7.4.
2. Sharp, fine forceps (Dumont no. 5, Fine Science Tools).
3. Dissection needles (e.g., 0.4×13 mm syringe needles, BD Microlance, mounted on cotton buds).
4. Lids from plastic cell culture Petri dishes (e.g., Nunc).
5. 22×50 mm poly-L-lysine-coated coverslips (no. 1.5, VWR): prepare 20% (v/v) poly-L-lysine solution in ddH_2O from 0.1%w/v stock (Sigma-Aldrich). Pipette a 5 µl drop onto the center of each coverslip and leave to dry on a hot plate. Store in a coverslip rack in a dust-free container.

2.5. Cell Extraction and Lysis

1. Cell lysis buffer (945 µl): prepare stock solution in advance by mixing 100 µl 10× PCR buffer with $MgCl_2$ (Invitrogen), 10 µl NP-40 (American Bioanalytical), 50 µl 0.1 M DTT (Dithiothreitol, Invitrogen), 785 µl ddH_2O treated with DEPC (Diethylpyrocarbonate). Store at –20°C.
2. Cell lysis mix (50 µl): 1 µl RNase inhibitor mix (1:1 mixture of RNasin, Promega, and Stop, Flowgen Bioscience), 1 µl 10 ng/µl Anchor T amplification oligonucleotide primer (HPLC grade and resuspended in ddH_2O, sequence TAT AGA ATT CGC GGC CGC TCG CGA 24(T)), 1 µl 2.5 mM dNTPs (Takara), 47 µl cell lysis buffer (see Item 1 of Section 2.5). Keep on ice. This should be prepared on the day of cell extraction (see Step 3 of Section 3.3).
3. Mineral oil.
4. Inverted fluorescence microscope (Olympus 1X71, Olympus, Japan) with micromanipulator (MN-151 Joystick Micromanipulator, Narishige, Tokyo, Japan) and UV light source.
5. Glass microscope slides.

2.6. Reverse Transcription PCR

1. Reverse transcription (RT) working mix (2.5 µl): 0.3 µl Superscript II (Invitrogen), 0.1 µl RNase inhibitor mix (see Item 2 of Section 2.5), 2.1 µl lysis buffer (see Item 1 of Section 2.5). Keep on ice.
2. Poly(A) tailing reaction mix (5 µl): 0.15 µl 100 mM dATP (Promega), 0.5 µl 10× PCR buffer with $MgCl_2$ (Invitrogen), 3.85 µl DEPC-treated ddH_2O, 0.25 µl TdT (terminal deoxynucleotidyl transferase, Roche), and 0.25 µl RNaseH (Roche). Keep on ice.
3. PCR mix (50 µl): 5 µl 10× ExTaq buffer (Takara), 5 µl 2.5 mM dNTPs (Takara), 1 µl 1 µg/µl Anchor T primer (see Item 2 of Section 2.5), 38.5 µl DEPC-treated H_2O, 0.5 µl ExTaq polymerase (Takara). Keep on ice.
4. Commercial kit for PCR purification (e.g., Qiagen, Sigma).
5. Equipment for running standard DNA agarose gels.
6. Spectrophotometer.

3. Methods

3.1. Needle Preparation: Microcapillary Pulling and Beveling

One of the most important steps in this technique is the preparation of suitable needles for cell isolation. There is really no substitute for trial and error optimization, but we have laid out a few key principles below. Please note that gloves should be worn at all times when handling the microcapillaries.

1. Take borosilicate glass microcapillaries and pull them on a commercial micropipette puller (see Item 2 of Section 2.2).
2. The aim is to produce a needle with a long, narrow taper. It should be fine enough not to cause too much damage during insertion into the brain and wide enough to take up cells. The taper should not be so long that it bends rather than piercing and entering the brain (see Note 5).
3. Once the needle has been pulled, the tip should be cut back with fine forceps under a dissecting microscope. The needle diameter should be slightly larger than that of the cells you are isolating. For example, it should have an internal diameter of 12–15 μm to accommodate optic lobe neuroepithelial cells and neuroblasts.
4. Mount the needle on a commercial micropipette beveller and set the beveling angle to between 10 and 12° from the vertical. Mark the posterior upper surface of the needle with a pen, so that the needle has the correct angle when it is being fixed to the micromanipulator for cell extraction.
5. Clean the needles, first with DEPC-treated H_2O and then 70% ethanol, to remove glass shards. Store them on plasticine in a dust-free plastic container.

3.2. Staged Larval Collections

1. Set up fly cross in a cage at 25°C to obtain progeny with the appropriate genotype.
2. Collect embryos in 4-h time windows on apple juice plates with fresh yeast.
3. Remove any hatched larvae and yeast from apple juice plates at around ~22–23 h after egg laying.
4. Collect freshly hatched larvae 4 h later. Transfer from apple juice plates to fly food plates or vials.
5. Rear at 25°C to the desired stage.

3.3. Preparation of Cell Lysis Buffer and Microscope Setup

1. Fresh cell lysis mix should be prepared in an eppendorf tube before dissection begins (see Item 2 of Section 2.5), and should be kept on ice next to the microscope.
2. Take a clean, sharpened microcapillary and attach it to an air-filled syringe using polyethylene tubing.

3. Mount the microcapillary on the micromanipulator and fill with mineral oil. The easiest way to do this is to place a large drop of mineral oil onto a glass slide, lower the microcapillary into the oil, and slowly draw up the syringe plunger to take up the oil.

3.4. Drosophila Larval Brain Dissection

1. Pick third instar larvae from vials or food plates at the appropriate time point, using forceps.
2. Transfer the larvae to a Petri dish containing tissue paper soaked in water. Leave the larvae to crawl for a few minutes, so that they are clean of fly food and yeast.
3. Place a clean larva in a drop of PBS on a Petri dish lid.
4. Tear the larva in half using the forceps and discard the posterior half.
5. Grip the anterior end of the larva (at the mouth hooks) with one pair of forceps. Use the other pair of forceps to peel the body wall back over the mouth hooks, turning the larva inside out. Identify the brain and release it from the body wall by cutting the nerves and esophagus. Then slice off the imaginal discs using the dissecting needles, being especially careful not to damage the brain lobes when removing the eye discs.
6. Move the dissected brain to a clean drop of PBS and continue dissecting to obtain 2–3 brains in total.
7. Place a drop of PBS onto a poly-L-lysine-coated coverslip. Transfer dissected brains into the drop, using the forceps to hold them by the nerves. Orient the brains so that they are dorsal side up and push them down gently onto the coverslip. Check that they adhere securely to the poly-L-lysine.

3.5. Cell Extraction and Lysis

1. Place the coverslip on the inverted microscope stage.
2. Using Nomarski optics, focus on one of the dissected brains. We use either the 20× or 40× objectives in conjunction with 10× eyepieces.
3. Bring the needle into the same plane of focus. Take up a small volume of PBS by drawing up the syringe plunger, so that you can see a clear oil/PBS interface in the microcapillary.
4. Insert the needle into brain lobe. The easiest point of insertion is usually at the ventral-most level, since this is where the brain adheres most firmly to the coverslip (see Note 6). Once the outer glial sheath has been penetrated, slowly move the microcapillary tip to the region of interest. In the case of the optic lobe, this is the lateral portion of the brain, lying just underneath the surface.
5. Open the UV filter to visualize the exact location of the cells of interest (see Note 7). Position the needle tip next to the GFP-positive cells, and slowly draw up the syringe plunger to extract them (Fig. 3, see Note 8).

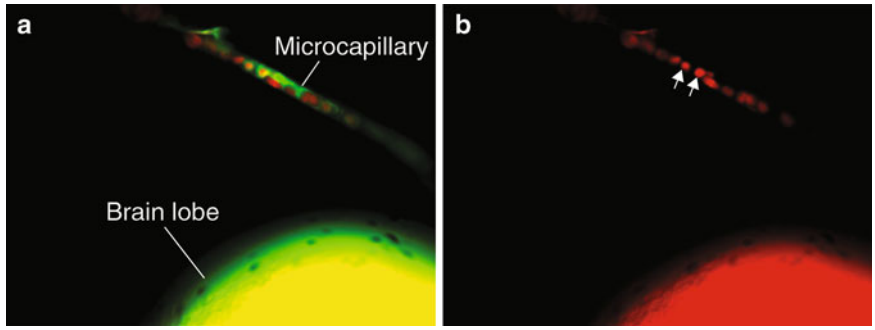

Fig. 3. Neural stem cell extraction in situ. (**a**) Neuroepithelial cells that have just been extracted from the optic lobe can be visualized inside the glass microcapillary. The cells are expressing membrane-tethered GFP and histone-bound RFP, driven by *GAL4*^{c855a}. (**b**) Individual cell nuclei, marked with Histone-2B-mRFP1, can be distinguished under UV illumination (see white *arrows*). Picture courtesy of Boris Egger.

6. Remove needle from the brain and remove coverslip from microscope.
7. Pipette 2.5 µl of ice-cold lysis mix from the eppendorf onto a clean glass slide and mount slide on microscope.
8. Expel cells from needle into the drop of cold lysis mix (see Note 9).
9. Take up 2.5 µl of the lysed material with a pipette and transfer to a PCR tube.
10. Incubate PCR tube with lysis mix at 65°C for 2 min to denature RNA.
11. After 2 min, snap cool on ice.

3.6. Reverse Transcription

1. Make up the reverse transcription mix on ice (see Item 1 of Section 2.6).
2. Carry out reverse transcription by adding 2.5 µl of RT mix to the lysed cells and incubating at 37°C for 90 min.
3. Terminate the RT reaction by heating to 65°C for 10 min, and then cool the samples to 4°C.
4. The first DNA strands synthesized by reverse transcription must be polyadenylated to allow second strand synthesis and PCR amplification. Make up TdT mix for poly(A) tailing on ice (see Item 2 of Section 2.6).
5. Add 5 µl of TdT mix to the RT reaction mixture. Place the tube in a PCR machine and incubate the reaction for 20 min at 37°C, followed by 10 min at 65°C.

3.7. PCR Amplification

1. Make up PCR mix on ice (see Item 3 of Section 2.6).
2. Add the 10 µl aliquot of polyA-tailed cDNA to the PCR mix.

3. Amplify the reverse transcribed and tailed samples using the following PCR program:
   (a) One cycle of 95°C for 1 min, 37°C for 5 min, and 72°C for 20 min.
   (b) 30–34 cycles of 95°C for 30 s, 67°C for 1 min, and 72°C for 6 min with a 6 s extension per cycle.
   (c) 72°C for 10 min.
   (d) Hold at 4°C, and store amplified samples at –20°C.
4. Run out 5 μl of the sample on an agarose gel to check quality. A homogenous smear of DNA should be visible.
5. Purify cDNA with a commercial kit, according to the manufacturer's instructions.
6. Measure cDNA concentration and check sample purity ($A_{260/280}$) with a spectrophotometer. The cDNA is now ready to be used for expression profiling (see Note 10).

4. Notes

1. We have experimented with other methods of neuroblast isolation in *Drosophila* embryos, in particular magnetic bead sorting and FACS. However, although enrichment of neuroblasts from the total cell fraction can be achieved with these methods, the mechanical shear and stress involved lead to widespread cell lysis (K. Edoff, personal communication). We find that extracting cells in situ using a microcapillary needle is faster and results in less cell death.
2. Embryonic neuroblasts can be identified by their size, shape, and position within the ventral nerve cord, in addition to reporter gene expression.
3. The proliferation of reporter lines (32, 33) and gene targeting systems in *Drosophila* (e.g., the Q system (34), LexA (35)) makes it increasingly likely that most cell types can be reproducibly identified and manipulated. Another possibility is to combine cell extraction with the MARCM system in order to isolate clones of wild-type or mutant cells.
4. Perdurance of GFP, as well as GAL4 protein, needs to be considered when choosing a driver line to label progenitors. In many cases, perdurance of these proteins can lead to the labeling of more differentiated cells further along the stem cell lineage, resulting in a less pure sample.
5. The heat, pulling strength, velocity, delay, and time are all variables which can be manipulated to produce needles with the

right kind of taper. A good guide to micropipette pulling can be found on the Sutter Instruments website (http://www.sutter.com/contact/faqs/pipette_cookbook.pdf). Prokop and Technau's chapter on cell transplantation also contains detailed information on needle preparation (25).

6. The glial sheath surrounding the larval brain can be tough and difficult to penetrate. It is worth experimenting with the taper dimensions and beveling angle to make sharper needles.
7. Try to reduce any UV-induced cell damage by extracting cells as quickly as possible to limit their exposure.
8. We extract around 50 cells per sample from the optic lobe, but our lab has also carried out single cell amplification and analysis using this protocol. However single cell data must be processed and analyzed with care, since there can be significant transcriptional variability between phenotypically identical cells (Subkhankulova et al. 2008).
9. It is possible to monitor the progress of cell lysis using the UV illumination to ensure that all the cells lyse.
10. Diagnostic PCRs can be carried out at this point to assess the likelihood of cDNA library contamination by other cell types. We recommend testing both positive and negative markers for the cell type of interest, using a low number of PCR cycles. For example, neuroblasts express genes such as *asense* and *deadpan*, but not the glial marker *repo*, while neuroepithelial cells should express epithelial markers such as *PatJ*.

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References

1. Technau GM, Campos-Ortega JA (1985) Fate-mapping in wild-type Drosophila melanogaster. Dev Genes Evol 194:196–212
2. Technau GM (1987) A single cell approach to problems of cell lineage and commitment during embryogenesis of Drosophila melanogaster. Development 100:1–12
3. Bossing T, Technau GM (1994) The fate of the CNS midline progenitors in Drosophila as revealed by a new method for single cell labelling. Development 120:1895–1906
4. Lee T, Luo L (1999) Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461
5. Evans CJ, Olson JM, Ngo KT, Kim E, Lee NE, Kuoy E, Patananan AN, Sitz D, Tran P, Do MT, Yackle K, Cespedes A, Hartenstein V, Call GB, Banerjee U (2009) G-TRACE: rapid Gal4-based cell lineage analysis in Drosophila. Nat Methods 6:603–605
6. Griffin R, Sustar A, Bonvin M, Binari R, del Valle Rodriguez A, Hohl AM, Bateman JR, Villalta C,

Heffern E, Grunwald D, Bakal C, Desplan C, Schubiger G, Wu CT, Perrimon N (2009) The twin spot generator for differential Drosophila lineage analysis. Nat Methods 6:600–602

7. Yu HH, Chen CH, Shi L, Huang Y, Lee T (2009) Twin-spot MARCM to reveal the developmental origin and identity of neurons. Nat Neurosci 12:947–953
8. Lee T (2009) New genetic tools for cell lineage analysis in Drosophila. Nat Methods 6:566–568
9. Doe CQ (1992) Molecular markers for identified neuroblasts and ganglion mother cells in the Drosophila central nervous system. Development 116:855–863
10. Broadus J, Skeath JB, Spana EP, Bossing T, Technau G, Doe CQ (1995) New neuroblast markers and the origin of the aCC/pCC neurons in the Drosophila central nervous system. Mech Dev 53:393–402
11. Bossing T, Udolph G, Doe CQ, Technau GM (1996) The embryonic central nervous system lineages of Drosophila melanogaster. I. Neuroblast lineages derived from the ventral half of the neuroectoderm. Dev Biol 179:41–64
12. Schmidt H, Rickert C, Bossing T, Vef O, Urban J, Technau GM (1997) The embryonic central nervous system lineages of Drosophila melanogaster. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Dev Biol 189:186–204
13. Spindler SR, Hartenstein V (2010) The Drosophila neural lineages: a model system to study brain development and circuitry. Dev Genes Evol 220:1–10
14. Urbach R, Schnabel R, Technau GM (2003) The pattern of neuroblast formation, mitotic domains and proneural gene expression during early brain development in Drosophila. Development 130:3589–3606
15. Urbach R, Technau GM (2003) Segment polarity and DV patterning gene expression reveals segmental organization of the Drosophila brain. Development 130:3607–3620
16. Urbach R, Technau GM (2003) Molecular markers for identified neuroblasts in the developing brain of Drosophila. Development 130:3621–3637
17. Megason SG, Fraser SE (2003) Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in to imaging of development. Mech Dev 120: 1407–1420
18. Walter T, Shattuck DW, Baldock R, Bastin ME, Carpenter AE, Duce S, Ellenberg J, Fraser A, Hamilton N, Pieper S, Ragan MA, Schneider JE, Tomancak P, Heriche JK (2010) Visualization of image data from cells to organisms. Nat Methods 7:S26–41
19. Southall TD, Egger B, Gold KS, Brand AH (2008) Regulation of self-renewal and differentiation in the Drosophila nervous system. Cold Spring Harb Symp Quant Biol 73:523–528
20. Egger B, Chell JM, Brand AH (2008) Insights into neural stem cell biology from flies. Phil Trans R Soc Lond 363:39–56
21. Doe CQ (2008) Neural stem cells: balancing self-renewal with differentiation. Development 135:1575–1587
22. Choksi SP, Southall TD, Bossing T, Edoff K, de Wit E, Fischer BE, van Steensel B, Micklem G, Brand AH (2006) Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. Dev Cell 11: 775–789
23. Egger B, Gold KS, Brand AH (2010) Notch regulates the switch from symmetric to asymmetric neural stem cell division in the Drosophila optic lobe. Development 137:2981–2987
24. Technau GM (1986) Lineage analysis of transplanted individual cells in embryos of Drosophila melanogaster. Dev Genes Evol 195: 389–398
25. Prokop A, Technau GM (1993) Cell transplantation. In: Hartley D (ed) Cellular interactions in development: a practical approach. Oxford University Press, p 33–57
26. Iscove NN, Barbara M, Gu M, Gibson M, Modi C, Winegarden N (2002) Representation is faithfully preserved in global cDNA amplified exponentially from sub-picogram quantities of mRNA. Nat Biotechnol 20:940–943
27. Osawa M, Egawa G, Mak SS, Moriyama M, Freter R, Yonetani S, Beermann F, Nishikawa S (2005) Molecular characterization of melanocyte stem cells in their niche. Development 132:5589–5599
28. Saito H, Kubota M, Roberts RW, Chi Q, Matsunami H (2004) RTP family members induce functional expression of mammalian odorant receptors. Cell 119:679–691
29. Subkhankulova T, Livesey FJ (2006) Comparative evaluation of linear and exponential amplification techniques for expression profiling at the single-cell level. Genome Biol 7:R18
30. Egger B, Boone JQ, Stevens NR, Brand AH, Doe CQ (2007) Regulation of spindle orientation and neural stem cell fate in the Drosophila optic lobe. Neural Dev 2:1
31. Hofbauer A, Campos-Ortega JA (1990) Proliferation pattern and early differentiation of the optic lobes in Drosophila melanogaster. Dev Genes Evol 198:264–274
32. Pfeiffer BD, Jenett A, Hammonds AS, Ngo TT, Misra S, Murphy C, Scully A, Carlson JW, Wan KH, Laverty TR, Mungall C, Svirskas R,

Kadonaga JT, Doe CQ, Eisen MB, Celniker SE, Rubin GM (2008) Tools for neuroanatomy and neurogenetics in Drosophila. Proc Natl Acad Sci USA 105:9715–9720

33. Pfeiffer BD, Ngo TT, Hibbard KL, Murphy C, Jenett A, Truman JW, Rubin GM (2010) Refinement of tools for targeted gene expression in Drosophila. Genetics 186:735–755
34. Potter CJ, Tasic B, Russler EV, Liang L, Luo L (2010) The Q system: a repressible binary system for transgene expression, lineage tracing, and mosaic analysis. Cell 141:536–548
35. Diegelmann S, Bate M, Landgraf M (2008) Gateway cloning vectors for the LexA-based binary expression system in Drosophila. Fly 2:236–239