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Tracking Down the Migration of Mouse Neural Crest Cells

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Abstract
During early embryonic development, cell migration is one of the most important morphogenetic processes. Neural crest cells arise from the dorsal part of the neural tube and migrate along different pathways to numerous locations where they differentiate into a variety of tissues. In the mouse, studies of neural crest cell migration have been difficult partly because of the absence of specific markers which can label neural crest cells throughout their migration from their origin to the site of differentiation. Nevertheless, the use of different experimental strategies involving extrinsic, intrinsic or genetic cell markers has already led to a good understanding of this migration. In our studies, extrinsic markers such as wheat germ agglutinin-gold conjugates and DiI and genetic markers including *Hoxb2-lacZ* and green fluorescent protein have been employed in tracing migrating neural crest cells. The labelling procedures and the strength and weaknesses of the tracing methods are reviewed herein.

Introduction

Cell migration is a major feature of morphogenesis in animals. Cells leave their place of origin and move over long distances along different migratory pathways to their final location where they undergo differentiation. This kind of directed movement of cells from one location to another can be involved in the rearrangement of cell layers, changes in the shape of a developing structure, assignment of cell fate and tissue patterning. Examples of migrating cells during early embryonic development include the haematopoietic stem cells, pigment precursor cells, primordial germ cells and ingressing ectodermal cells through the primitive streak.

During the early development of the central nervous system, cell migration is also a very important morphogenetic process. In humans, from about the 8th week of gestation onwards, postmitotic neuroblasts derived from the

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ventricular zone start their radial migration along radial glial cells to form the cortical plate between the subplate and the marginal zone in an inside-out gradient [1–5]. It has been shown that the pyramidal neurons of the cortex mostly originate in the ventricular zone, whereas the majority of the cortical interneurons appear to be derived from the ganglionic eminence, which is an enlarged structure on the basolateral floor of the lateral ventricle. These interneurons first migrate tangentially through the intermediate zone and then move along the radial glial fibers to reach their cortical locations [6].

Another group of migrating cells in the developing nervous system are the neural crest cells, which originate from the dorsal part of the neural tube. They are capable of migrating over long distances to colonize different regions of the embryo where they give rise to a variety of tissues including dorsal root ganglia, parasympathetic ganglia, adrenal medulla, pigment cells and craniofacial structures [7]. Investigations of migrating neural crest cells in mammals have been difficult, partly because migrating neural crest cells usually do not exhibit special morphological features which can allow them to be distinguished from their neighboring cells and partly because specific cell markers which can label migrating cells throughout their migration from their origin to their final location are not available. This contrasts with the situation in the avian embryo, where use of the technique of quail-chick chimaeras [8] has provided a wealth of information on neural crest migration. Hence, different experimental strategies have been devised to trace migrating cells at different developmental stages of mammalian development.

In this short review, we have no intention of providing an exhaustive summary of tracing strategies, but, instead, we focus on the methods that have been used in our laboratories to track down the migration of neural crest cells in the mouse in the hope that our experience can help to provide hints or clues for designing methods to trace other types of migrating cells.

Extrinsic, Intrinsic and Genetic Cell Markers

Experimental strategies for following cell migration usually require a cell-labelling method to identify the otherwise morphologically indistinguishable cell type. When a cell marker is employed, it should ideally be: (1) cell localized: the marker remains associated with the cell until the cell divides, and following cell division, the marker is passed on to the cell's mitotic descendants; (2) developmentally neutral: the marker does not perturb developmental processes in any way; (3) specific: the marker differentially labels the cell to be followed; (4) stable: the marker is stable during the time period of interest and is not diluted upon cell division; (5) easily and reliably detectable: the marker can be easily visualized in a variety of tissue preparations such as living cells, whole mount tissues and paraffin or frozen sectioned tissues; and (6) compatible with other markers: the marker can be simultaneously visualized with other markers.

For decades, extrinsic cell markers have been used for tracing migrating cells. Vital dyes such as Nile blue sulfate, neutral red and Bismark brown were used as cell markers by direct in situ application [9–11] or grafting an appropriately labelled piece of tissue to an unlabelled recipient [11, 12]. Useful information on neural crest cell migration has also been obtained by using tritiated thymidine to label embryos from which donor tissues were isolated and grafted to unlabelled embryos [13–18]. These cell markers, however, suffer from the problems of dilution as a result of cell division, a lack of specificity due to diffusion to neighbouring unlabelled cells [19] and, in some cases, a certain level of cytotoxicity. Later, more extrinsic markers emerged, which include lectin conjugates (e.g. wheat germ agglutinin-gold conjugates (WGA-Au) [20–23]), carbocyanine dyes (e.g. 1,1-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), DiO [24–28]), carboxyfluorescent diacetate (e.g. CFSE [29–33]) and lysinated rhodamine dextran [34, 35]. These markers are more cell localized, give a stronger signal, are easier to detect and are less toxic to cells. In addition, these markers are readily available, easy to prepare for labelling and relatively inexpensive.

Besides the extrinsic markers, the use of intrinsic markers has also been explored for many years. Early studies made use of cytological features such as cytoplasmic inclusions (e.g. pigments, yolk granules), RNA content, cell or nuclear size and differential staining properties [36, 37]. While these markers are useful in identifying clusters of neural crest cells and do not have the dilution problem over cell divisions, difficulties in localizing isolated cells arise when the cells are migrating within the mesenchyme. More recent advances in molecular technology have enabled the use of antibodies or probes to detect expression of neural crest-specific molecules, and these techniques have become the mainstay of studies of mammalian neural crest cell migration. Among the 'expression markers' of neural crest cells, HNK1/NC1 has contributed much to our understanding of the early steps of migration in avian and rat embryos. However, it is not expressed in mouse neural crest cells and is neither a per-

manent nor a specific marker for neural crest cells of other species. HNK1/NC1 is expressed in various other cell types, including the neural tube, perichondrium and heart [38–40]. Other molecules which are expressed by neural crest cells or their derivatives include vimentin-related 4E9R antigens [41], RhoB [42, 43], Pax 3 [44], Sox 10 [45, 46], Hoxa-3 [47], Foxd3 [48], CrabpI [49], Prx1 and 2 [50], c-met [51], MASH1 [52–55], Phox2a [56–58], Phox2b [57–59], AP-2 [60, 61], 5-HT2B receptor [62], tyrosinase [63], receptor tyrosine kinase Ret [64–68], neurotropin receptor $p75^{NTR}$ [53, 69, 70], endothelin receptor B [71] and tyrosine hydroxylase [72–74]. Detection of these molecules has yielded significant information on the migration and development of neural crest cells. The problems associated with these markers are as follows: (1) they may be expressed in non-neural crest tissues; (2) they may not be expressed in all of the migrating neural crest cells and (3) they may not be expressed in neural crest cells, throughout their migration from their origin to their site of differentiation.

Another approach to tracing migrating neural crest cells is genetic labelling. Replication-defective retroviruses carrying the marker gene *lacZ* (which encodes the protein β-galactosidase) have been used as markers by either direct introduction into the neural crest migratory pathway to label the migrating neural crest cells [75] or by infection of neural tube fragments which were then grafted isotopically into uninfected host embryos [76, 77]. Multiple lines of transgenic mice have also been generated that overexpress *lacZ* under the control of different types of promoters or enhancers with the aim of identifying transgenes that are expressed specifically in the neural crest cell population. The dopamine β-hydroxylase-*lacZ* transgenic marker which is expressed by enteric neural crest cells has been used to study the aberrant migration of neural crest cells in mutant mice [78], while a mouse line expressing a *lacZ* reporter in the neural crest cells under the control of the *Wnt-1* enhancer has been employed for studying the defect in neural crest development [79]. Recently, a powerful technique has been introduced, by which neural crest cells are caused to permanently express *lacZ* following Cre-mediated recombination under control of the *Wnt1* promoter [80]. Transgenic embryos which ubiquitously express a marker transgene (e.g. Rosa 26-hPAP and Rosa 26-EGFP [81], X-linked HMG-CoA-*lacZ* [82]) can also be potential sources of labelled cells for the production of chimaeric embryos in studies of neural crest cell migration.

In the following sections, two extrinsic markers, namely WGA-Au and DiI, and two genetic markers, namely a *Hoxb2-lacZ* construct and a green fluorescent protein (GFP) vector, are taken as examples to illustrate how they have been used as markers for neural crest cells.

WGA-Au and DiI

The lectin WGA, with a molecular mass of 35,000, is known to bind to N-acetyl glucosamine and sialic acid residues on the cell surface. After binding to the cell surface, it is quickly engulfed into the cytoplasm by absorptive endocytosis [83]. When tagged with gold particles, WGA can be easily localized intracellularly by electron microscopy or light microscopy using either dark field illumination [20] or following silver enhancement staining, where the gold particles are greatly enlarged by deposition of silver granules on their surface [22, 23, 84]. Alternatively, WGA can also be localized by immunohistochemical methods using an antibody against WGA [85, 86]. WGA is not recycled to the plasma membrane following endocytosis, even though the binding sugar residues may resurface [87], thus minimizing the chance of WGA being transferred to neighboring cells. Ultrastructural studies also show that WGA-Au particles remain within intracellular vacuoles and do not appear in the extracellular space [20, 22]. Studies of double-injected chimaeras, in which two separate populations of labelled neural crest cells, one labelled with WGA-Au and the other with thymidine, were injected into a single embryo, indicate no transfer of markers between populations even when the two populations were mixed together [86, 88]. At the concentration used for labelling, WGA-Au does not perturb the normal development of embryonic cells or other cell types such as neurons [22, 86, 88–92]. However, WGA-Au can only be used as a short-term marker because it becomes diluted in the rapidly dividing neural crest population. We find that the amount of WGA-Au within cells diminishes below detection level 24–48 h after labelling.

Another commonly used extrinsic cell marker is the fluorescent carbocyanine dye DiI. DiI is hydrophobic and lipophilic, and thus easily intercalates into almost all cell membranes that it contacts [93]. Diffusion of DiI from one labelled cell to another appears to be minimal [25, 94, 95]. At the labelling concentration, adverse effects on neural crest development and cellular toxicity usually observed at high DiI concentrations are not evident [25, 95–97]. Furthermore, dilution of DiI over cell divisions is not such a great problem as for WGA-Au, owing to the intense fluorescent signal of DiI.

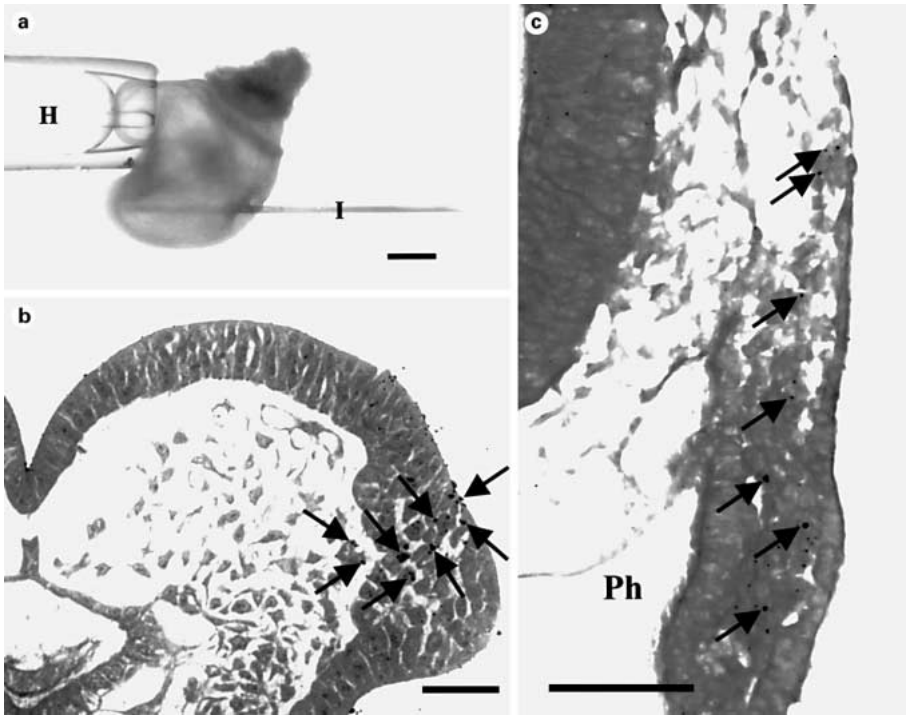

Fig. 1. a Photomicrograph showing labelling of a hindbrain neural crest region with WGA-Au solution using a micromanipulator. A mouse embryo with its yolk sac intact is held by the holding pipette (H) using slight suction, and the injection pipette (I) loaded with the WGA-Au solution (original colour: red) at its tip has been pushed through the yolk sac and amnion to enter the amniotic cavity. A small amount of the WGA-Au solution is being released in the vicinity of the neural crest of the hindbrain region. Bar: 250 μm. b Two hours after labelling, WGA-Au-labelled cells carrying dark intracellular granules (arrows) are found in the neural crest region. Bar: 50 μm. c Twenty-four hours after labelling, WGA-Au-labelled cells (arrows) can be detected migrating in the mesenchyme, while some have already arrived at regions lateral to the developing pharynx (Ph). Bar: 100 μm.

Microinjection and Whole Embryo Culture

Three techniques are commonly used for introducing WGA-Au or DiI into mouse neural crest cells. In blanket labelling, WGA-Au or DiI is injected into the lumen of the neural tube with the aid of a micromanipulator and the dye labels all neural tube cells, including pre-migratory neural crest [25, 95, 98]. A second technique is focal labelling, where a small amount of concentrated WGA-Au or DiI is placed directly to the neural crest region (fig. 1a). With focal labelling, a small population of neural crest cells can be labelled at a selected axial level and cell migration can be followed over time (fig. 1b, c). The third technique is grafting of WGA-Au- or DiI-labelled tissues into unlabelled host embryos. Neural tubes are first isolated from mouse embryos by microdissection following dispase or trypsin-pancreatin digestion [22, 99]. The neural tubes are then labelled with WGA-Au or DiI by immersion in the labelling solution for several minutes before tissue fragments are dissected from the neural crest region. The labelled neural crest fragments are then microinjected into unlabelled host embryos [22], and the migration of the labelled neural crest cells can be followed against the unlabelled background of the host embryo.

As the success rate of re-implanting post-implantation mouse embryos back into the uterus is extremely low [100], the most viable approach is to maintain embryos following labelling in vitro, using the method of whole embryo culture. Embryos whose visceral yolk sac and amnion are intact are grown in rolling bottles containing a culture medium in a thermostatic environment [101, 102]. This in vitro method enables rodent embryos explanted as early as the egg-cylinder stage to develop normally for up to 96 h, during which time the major organ rudiments are formed at a rate comparable to that in vivo [22, 101, 103–105]. The whole embryo culture method has been successfully employed in our laboratories in studies of teratogenic effects of drugs [106–109], limb bud regeneration [110, 111], neurulation [112–114], primordial germ cell migration [115] and neural crest cell migration [22, 23, 116].

Hoxb2-lacZ Construct

Genetic labelling is a powerful approach for tracing the migration of neural crest cells. *Hox* genes are known for their role in specifying regional characteristics along the anterior-posterior axis, and a combination of *Hox* genes

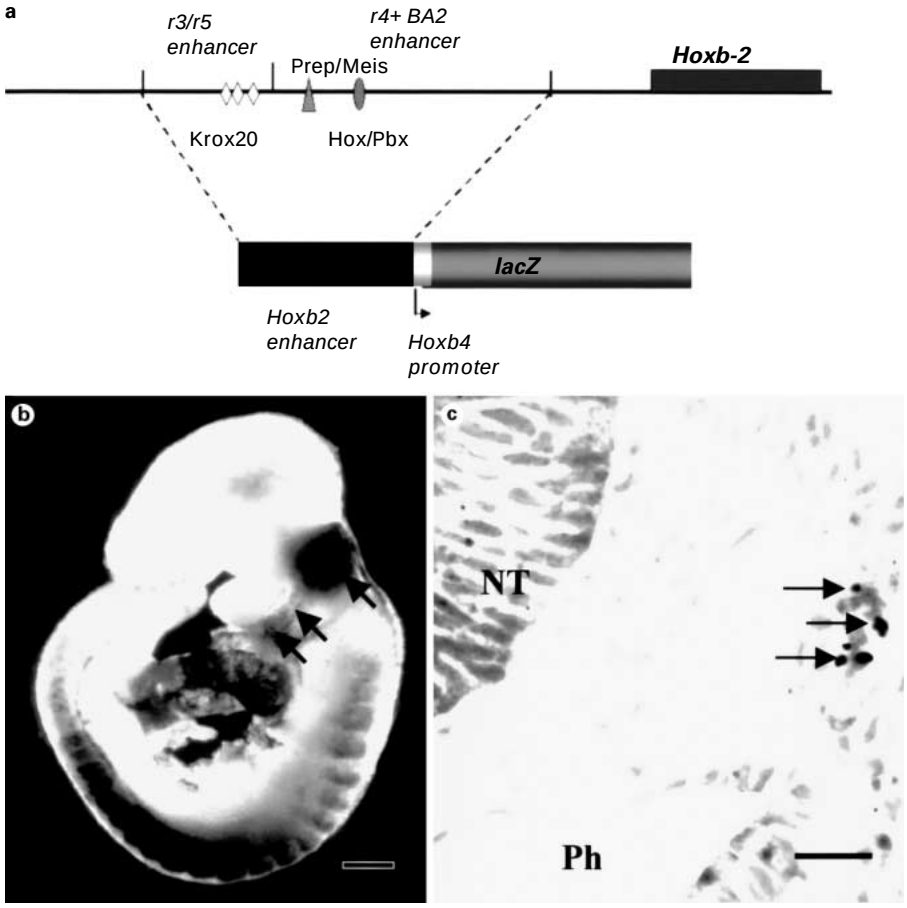

Fig. 2. a Diagram showing the *Hoxb2-lacZ* DNA construct used for generating transgenic embryos [for details of the construct, see ref. 121, 122, 135] [for vector information, see ref. 136]. b A transgenic embryo showing positive cells in the pre-otic hindbrain, cranial mesenchyme and branchial arch regions (arrows). Also note that somites and the heart are also positive. c A transverse section of a transgenic embryo at the pre-otic hindbrain level after WGA-Au labelling. The counter-staining (eosin) has been filtered out during photography and does not show up in this photomicrograph. Hence, only the *lacZ*-expressing cells show positive staining (light black, original colour was blue). Note that WGA-Au-positive cells (dark black, original colour was black) are also *lacZ* positive (arrows). NT = Neural tube; Ph = pharynx. Bar: 500 μm in b and 25 μm in c.

expressed in the various regions of the neural crest specifies aspects of its developmental fate [117–120]. In studies of the regulatory elements that mediate the patterns of Hox gene expression, a *Hoxb2-lacZ* construct (fig. 2a) was used to produce transgenic mouse lines [121, 122]. It was found that *Hoxb2-lacZ* is expressed in the pre-otic hindbrain neural tube and in the neural crest cells migrating from this region towards the branchial arches (fig. 2b). *Hoxb2-lacZ* is not expressed in other regions of the brain, although it is expressed in the somites and developing heart. When the pre-otic hindbrain neural crest of transgenic embryos is labelled with WGA-Au, most of the WGA-Au-labelled neural crest cells also express the transgene (fig. 2c), indicating that the *Hoxb2-lacZ* construct can be potentially used as a marker to specifically label the pre-otic hindbrain neural crest cells.

GFP Vector

GFP was originally introduced as a reporter gene for monitoring the cell-specific control of gene expression and protein localization in both prokaryotic and eukaryotic systems [123]. By microinjection of GFP mRNA into early blastomeres of mouse embryos, GFP was used as a marker for tracing the fate of embryonic stem cells in living mouse embryos [124]. Recently, GFP has been widely used as a reporter in various studies using different techniques for gene transfer [125–131]. Among these techniques, electroporation can generate a unidirectional current which enables targeted delivery of a GFP expression vector to a specific embryonic site [130–133]. Hence, using electroporation, the dorsal regions of the embryo, including the neural crest, can be specifically labelled with a GFP vector (fig. 3a, b). Although the labelling efficacy is only around 40%, successfully labelled embryos exhibit strong GFP signals, which may last for up to 8–10 days [130] and can be easily detected either in living tissues or

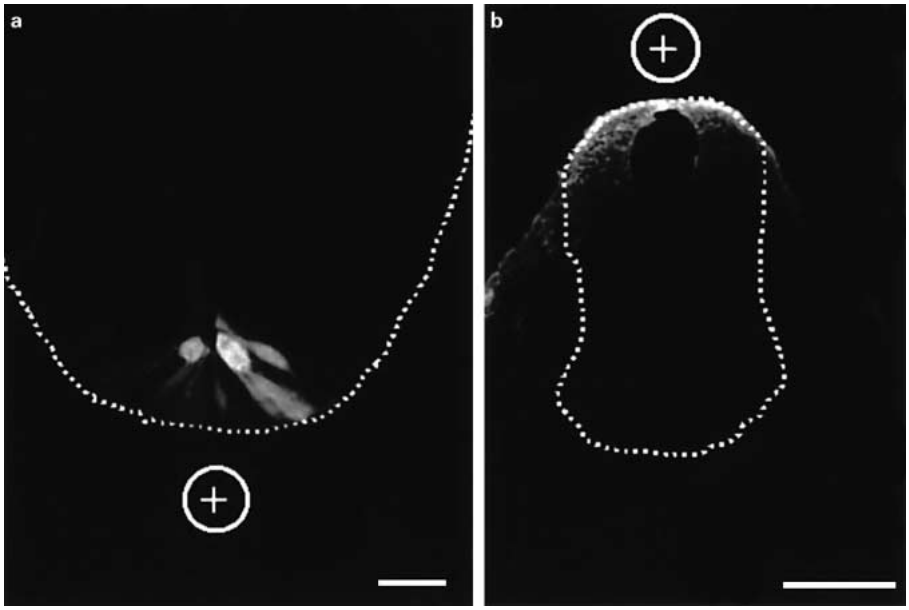

Fig. 3. Photomicrographs showing transverse sections of neural tubes after labelling with GFP by electroporation. Plasmid DNA encoding an enhanced GFP, driven by a CMV promoter, was microinjected into the lumen of the neural tube, and the positive electrode (+) was placed either close to the ventral side (a) or dorsal side (b) of the neural tube (outlined by the dotted line). Note that GFP-positive cells in both paraffin sections (a) and cryostat sections (b) are found on the same side as the positive electrode. Bar: 20 μm in a and 100 μm in b.

in tissue sections using epifluorescence microscopy or confocal microscopy. The GFP signals are well preserved after fixation in 4% paraformaldehyde, during standard procedures for both paraffin (fig. 3a) and cryostat sectioning (fig. 3b) [134]. Therefore, when coupled with whole embryo culture and tissue transplantation, electroporation of a GFP vector into the pre-migratory neural crest can provide an alternative method for tracing the migration of neural crest cells.

Concluding Remarks

An ideal marker to label mammalian neural crest cells throughout their migration has yet to be found, and different experimental strategies have been devised to follow the migration and fate of neural crest cells in different regions of the embryo. Depending on the objectives of the study, appropriate rather than ideal markers have been used. It is hoped that molecular techniques combined with various manipulations such as microinjection, tissue transplantation and whole embryo culture can help to improve our understanding of neural crest development.

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References

1 Copp AJ, Harding BN: Neuronal migration disorders in humans and in mouse models – an overview. Epilepsy Res 1999;36:133–141.
2 Chan WY, Kostovic I, Takashima S, Feldhaus C, Stoltenburg-Didinger G, Verney C, Yew D, Ulfig N: Normal and abnormal development of the human cerebral cortex. Neuroembryology 2002;1:78–90.
3 Chan WY, Lorke DE, Tiu SC, Yew DT: Proliferation and apoptosis in the developing human neocortex. Anat Rec 2002;267:261–276.
4 Rakic P: Mode of cell migration to the superficial layers of fetal monkey neocortex. J Comp Neurol 1972;145:61–83.
5 Caviness VS Jr, Sidman RL: Time of origin or corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: An autoradiographic analysis. J Comp Neurol 1973;148:141–151.
6 Parnavelas JG: The origin and migration of cortical neurons: New vistas. Trends Neurosci 2000;23:126–131.
7 Le Douarin NM, Kalcheim C: The Neural Crest. Cambridge, Cambridge University Press, 1999.
8 Le Douarin NM, Ziller C, Couly GF: Patterning of neural crest derivatives in the avian embryo: In vivo and in vitro studies. Dev Biol 1993;159:24–49.
9 Detwiler SR: Observations upon the migration of neural crest cells, and upon the development of the spinal ganglia and vertebral arches in amblystoma. Am J Anat 1937;61:63–94.

10 Stone LS: Selective staining of the neural crest and its preservation for microscopic study. Anat Rec 1932;51:267–273.
11 Hörstadius S: The Neural Crest: Its Properties and Derivatives in the Light of Experimental Research. London, Oxford University Press, 1950.
12 Hilber H: Experimentelle Studien zum Schicksal des Rumpfganglienleistenmaterials. Roux Arch Entwicklungsmech Org 1943;142:100–120.
13 Weston JA: A radioautographic analysis of the migration and localization of trunk neural crest cells in the chick. Dev Biol 1963;6:279–310.
14 Johnston MC: A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. Anat Rec 1966;156:143–156.
15 Noden DM: An analysis of migratory behavior of avian cephalic neural crest cells. Dev Biol 1975;42:106–130.
16 Noden DM: Cytodifferentiation in heterotopically transplanted neural crest cells. J Gen Physiol 1976:68:13a.
17 Noden DM: The control of avian cephalic neural crest cytodifferentiation. I. Skeletal and connective tissues. Dev Biol 1978;67:296–312.
18 Noden DM: The control of avian cephalic neural crest cytodifferentiation. II. Neural tissues. Dev Biol 1978;67:313–329.
19 Weston JA: Cell marking; in Wessels FH, Wilt NK (eds): Methods in Developmental Biology. New York, Crowell, 1967, pp 723–736.
20 Smits-Van Prooije AE, Poelmann RE, Dubbeldam JA, Mentink MMT, Vermeij-Keers C: Wheat germ agglutinin-gold as a novel marker for mesectoderm formation in mouse embryos cultured in vitro. Stain Technol 1986;61:97–106.
21 Smits-Van Prooije AE, Vermeij-Keers C, Dubbeldam JA, Mentink MMT, Poelmann RE: The formation of mesoderm and mesectoderm in presomite rat embryos cultured in vitro, using WGA-Au as a marker. Anat Embryol (Berl) 1987;176:71–77.
22 Chan WY, Tam PPL: A morphological and experimental study of the mesencephalic neural crest cells in the mouse embryo using wheat germ agglutinin-gold conjugate as the cell marker. Development 1988;102:427–442.
23 Chan WY, Lee KKH: The incorporation and dispersion of cells and latex beads on microinjection into the amniotic cavity of the mouse embryo at the early-somite stage. Anat Embryol (Berl) 1992;185:225–238.
24 Bronner-Fraser M: Manipulations of neural crest cells or their migratory pathways. Methods Cell Biol 1996;51:61–79.
25 Serbedzija GN, Burgan S, Fraser SE, Bronner-Fraser M: Vital dye labelling demonstrates a sacral neural crest contribution to the enteric nervous system of chick and mouse embryos. Development 1991;111:857–866.
26 Serbedzija GN, Bronner-Fraser M, Fraser SE: Vital dye analysis of cranial neural crest cell migration in the mouse embryo. Development 1992;116:297–307.
27 Sechrist J, Serbedzija GN, Fraser SE, Scherson T, Bronner-Fraser M: Segmental migration of the hindbrain neural crest does not arise from segmental generation. Development 1993;118:691–703.
28 Lumsden A, Sprawson N, Graham A: Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. Development 1991;113:1281–1291.
29 Garton HJL, Schoenwolf GC: Improving the efficacy of fluorescent labeling for histological tracking of cells in early mammalian and avian embryos. Anat Rec 1996;244:112–117.
30 Schoenwolf GC, Garcia-Martinez V, Dias MS: Mesoderm movement and fate during avian gastrulation and neurulation. Dev Dyn 1992;193:235–248.
31 Smith JL, Gesteland KM, Schoenwolf GC: Prospective fate map of the mouse primitive streak at 7.5 days of gestation. Dev Dyn 1994;201:279–289.
32 Schramm C, Solursh M: The formation of premuscle masses during chick wing bud development. Anat Embryol (Berl) 1990;182:235–247.
33 Sulik K, Dehart DB, Inagaki T, Carlson JL, Vrablic T, Gesteland K, Schoenwolf GC: Morphogenesis of the murine node and notochordal plate. Dev Dyn 1994;201:260–278.
34 Bronner-Fraser M, Fraser SE: Cell lineage analysis reveals multipotency of some avian neural crest cells. Nature 1988;335:161–164.
35 Artinger KB, Fraser S, Bronner-Fraser M: Dorsal and ventral cell types can arise from common neural tube progenitors. Dev Biol 1995;172:591–601.
36 Raven CP: Experiment on the origin of the sheath cells and sympathetic neuroblasts in Amphibia. J Comp Neurol 1937;67:220–240.
37 Triplett EL: The development of the sympathetic ganglia, sheath cells, and meninges in amphibians. J Exp Zool 1958;138:283–312.
38 Tucker GC, Aoyama M, Tursz T, Thiery JP: Identical reactivity of monoclonal antibodies HNK-1 and NC-1: Conservation in vertebrates on cells derived from the neural primordium and on some leukocytes. Cell Differ 1984;14:223–230.
39 Wenink AC, Symersky P, Ikeda T, DeRuiter MC, Poelmann RE, Gittenberger-de Groot AC: HNK-1 expression patterns in the embryonic rat heart distinguish between sinuatrial tissues and atrial myocardium. Anat Embryol (Berl) 2000;201:39–50.
40 Nakagawa M, Thompson RP, Terracio L, Borg TK: Developmental anatomy of HNK-1 immunoreactivity in the embryonic rat heart: Codistribution with early conduction tissue. Anat Embryol (Berl) 1993;187:445–460.
41 Kubota Y, Morita T, Ito K: New monoclonal antibody (4E9R) identifies mouse neural crest cells. Dev Dyn 1996;206:368–378.
42 Henderson DJ, Ybot-Gonzalez P, Copp AJ: RhoB is expressed in migrating neural crest and endocardial cushions of the developing mouse embryo. Mech Dev 2000;95:211–214.
43 Liu JP, Jessell TM: A role for rhoB in the delamination of neural crest cells from the dorsal neural tube. Development 1998;125:5055–5067.
44 Conway SJ, Henderson DJ, Copp AJ: Pax3 is required for cardiac neural crest migration in the mouse: Evidence from the splotch (Sp2H) mutant. Development 1997;124:505–514.
45 Herbarth B, Pingault V, Bondurand N, Kuhlbrodt K, Hermans-Borgmeyer I, Puliti A, Lemort N, Goossens M, Wegner M: Mutation of the Sry-related Sox10 gene in Dominant megacolon, a mouse model for human Hirschsprung disease. Proc Natl Acad Sci USA 1998;95:5161–5165.
46 Southard-Smith EM, Kos L, Pavan WJ: Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nat Genet 1998;18:60–64.
47 Manley NR, Capecchi MR: The role of Hoxa-3 in mouse thymus and thyroid development. Development 1995;121:1989–2003.
48 Dottori M, Gross MK, Labosky P, Goulding M: The winged-helix transcription factor Foxd3 suppresses interneuron differentiation and promotes neural crest cell fate. Development 2001;128:4127–4138.
49 Leonard L, Horton C, Maden M, Pizzey JA: Anteriorization of CRABP-I expression by retinoic acid in the developing mouse central nervous system and its relationship to teratogenesis. Dev Biol 1995;168:514–528.
50 Leussink B, Brouwer A, El Khattabi M, Poelmann RE, Gittenbergerde Groot AC, Meijlink F: Expression patterns of the paired related homeobox genes MHox/Prxl and S8/Prx2 suggest roles in development of the heart and the forebrain. Mech Dev 1995;52:51–64.
51 Tsarfaty I, Resau JH, Rulong S, Keydar I, Faletto DL, Vande Woude GF: The met protooncogene receptor and lumen formation. Science 1992;257:1258–1261.
52 Lo LC, Johnson JE, Wuenschell CW, Saito T, Anderson DJ: Mammalian achaete-scute homolog 1 is transiently expressed by spatially restricted subsets of early neuroepithelial and neural crest cells. Genes Dev 1991;5:1524–1537.
53 Lo L, Anderson DJ: Postmigratory neural crest cells expressing c-RET display restricted developmental and proliferative capacities. Neuron 1995;15:527–539.
54 Lo L, Sommer L, Anderson DJ: MASH1 maintains competence for BMP2-induced neuronal differentiation in post-migratory neural crest cells. Curr Biol 1997;7:440–50.
55 Greenwood AL, Tuner EE, Anderson DJ: Identification of dividing, determined sensory neuron precursors in the mammalian neural crest. Development 1999;126:3545–3559.
56 Tiveron MC, Hirsch MR, Brunet JF: The expression pattern of the transcription factor Phox2 delineates synaptic pathways of the autonomic nervous system. J Neurosci 1996;16:7649–7660.
57 Young HM, Hearn CJ, Ciampoli D, Southwell BR, Brunet JF, Newgreen DF: A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of Phox2b, ret, and p75 and by explants grown under the kidney capsule or in organ culture. Dev Biol 1998;202:67–84.

58 Young HM, Ciampoli D, Hsuan J, Canty AJ: Expression of Ret-, p75(NTR)-, Phox2a-, Phox2b-, and tyrosine hydroxylase-immunoreactivity by undifferentiated neural crest-derived cells and different classes of enteric neurons in the embryonic mouse gut. Dev Dyn 1999;216:137–152.
59 Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF: Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. Development 1997;124:4065–4075.
60 Schorle H, Meier P, Buchert M, Jaenisch R, Mitchell PJ: Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 1996;381:235–238.
61 Zhang JA, Hagopian-Donaldson S, Serbedzija G, Elsemore J, Plehn-Dujowich D, MacMahon AP, Flavell RA, Williams T: Neural tube, skeletal and body wall defects in mice lacking transcription factor AP-2. Nature 1996;381:238–241.
62 Fiorica-Howells E, Maroteaux L, Gershon MD: 5-HT2B receptors are expressed by neuronal precursors in the enteric nervous system of fetal mice and promote neuronal differentiation. Ann NY Acad Sci 1998;861:246.
63 Tief K, Schmidt A, Aguzzi A, Beermann F: Tyrosinase is a new marker for cell populations in the mouse neural tube. Dev Dyn 1996;205:445–456.
64 Pachnis V, Mankoo B, Costantini F: Expression of the c-ret proto-oncogene during mouse embryogenesis. Development 1993;119:1005–1017.
65 Pachnis V, Durbec P, Taraviras S, Grigoriou M, Natarajan D: Role of the RET signal transduction pathway in development of the mammalian enteric nervous system. Am J Physiol 1998;275:G183–G186.
66 Tsuzuki T, Takahashi M, Asai N, Iwashita T, Matsuyama M, Asai J: Spatial and temporal expression of the ret proto-oncogene product in embryonic, infant, and adult rat tissues. Oncogene 1995;10:191–198.
67 Durbec PL, Larsson-Blomberg LB, Schuchardt A, Costantini F, Pachnis V: Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. Development 1996;122:349–358.
68 Watanabe Y, Harada T, Ito T, Ishiguro Y, Ando H, Seo T, Kobayashi S, Takahashi M, Nimura Y: Ret proto-oncogene product is a useful marker of lineage determination in the development of the enteric nervous system in rats. J Pediatr Surg 1997;32:28–33.
69 Baetge G, Pintar JE, Gershon MD: Transiently catecholaminergic (TC) cells in the bowel of the fetal rat: Precursors of noncatecholaminergic enteric neurons. Dev Biol 1990;141:353–380.
70 Chalazonitis A, Rothman TP, Chen J, Lamballe F, Barbacid M, Gershon MD: Neurotrophin-3 induces neural crest-derived cells from fetal rat gut to develop in vitro as neurons or glia. J Neurosci 1994;14:6571–6584.
71 Gariepy CE, Williams SC, Richardson JA, Hammer RE, Yanagisawa M: Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of Hirschsprung disease. J Clin Invest 1998;102:1092–1101.
72 Cochard P, Goldstein M, Black IB: Ontogenetic appearance and disappearance of tyrosine hydroxylase and catecholamines. Proc Natl Acad Sci USA 1978;75:2986–2990.
73 Teitelman G, Joh TH, Reis DJ: Transient expression of a noradrenergic phenotype in cells of the rat embryonic gut. Brain Res 1978;158:229–234.
74 Jonakait GM, Wolf J, Cochard P, Goldstein M, Black IB: Selective loss of noradrenergic phenotypic characters in neuroblasts of the rat embryos. Proc Natl Acad Sci USA 1979;76:4683–4686.
75 Epstein ML, Mikawa T, Brown AMC, McFarlin DR: Mapping the origin of the avian enteric nervous system with a retroviral marker. Dev Dyn 1994;201:236–244.
76 Stocker KM, Brown AM, Ciment G: Gene transfer of lacZ into avian neural tube and neural crest cells by retroviral infection of grafted embryonic tissues. J Neurosci Res 1993;34:135–145.
77 Mikawa T, Fischman DA, Dougherty JP, Brown AMC: In vivo analysis of a new lacZ retrovirus vector suitable for cell lineage marking in avian and other species. Exp Cell Res 1991;195:516–523.
78 Kapur RP, Livingston R, Doggett B, Sweetser DA, Siebert JR, Palmiter RD: Abnormal microenvironmental signals underlie intestinal aganglionosis in dominant megacolon mutant mice. Dev Biol 1996;174:360–369.
79 Serbedzija GN, McMahon AP: Analysis of neural crest cell migration in splotch mice using a neural crest-specific lacZ reporter. Dev Biol 1997;185:139–147.
80 Jiang XB, Rowitch DH, Soriano P, McMahon AP, Sucov HM: Fate of the mammalian cardiac neural crest. Development 2000;127:1607–1616.
81 Kisseberth WC, Brettingen NT, Lohse JK, Sandgren EP: Ubiquitous expression of marker transgenes in mice and rats. Dev Biol 1999;214:128–138.
82 Tam PPL, Tan SS: The somitogenic potential of cells in the primitive streak and the tail bud of the organogenesis-stage mouse embryo. Development 1992;115:703–715.
83 Gonatas J, Stieber A, Olsnes S, Gonatas NK: Pathways involved in fluid phase and adsorptive endocytosis in neuroblastoma. J Cell Biol 1980;87:579–588.
84 Tam PPL, Beddington RSP: The metameric organization of the presomitic mesoderm and somite specification in the mouse embryo; in Bellairs R (ed): Somites in Developing Embryos. London, Plenum Press, 1987, pp 17–36.
85 Sofroniew MV: Direct reciprocal connections between the bed nucleus of the stria terminalis and dorsomedial medulla oblongata: Evidence from immunohistochemical detection of tracer proteins. J Comp Neurol 1983;213:399–405.
86 Tan SS, Morriss-Kay GM: The development and distribution of the cranial neural crest in the rat embryo. Cell Tissue Res 1985;240:403–416.
87 Gonatas NK, Stieber A, Hickey WF, Herbert SH, Gonatas JO: Endosomes and Golgi vesicles in adsorptive and fluid phase endocytosis. J Cell Biol 1984;99:1379–1390.
88 Trainor PA, Tam PPL: Cranial paraxial mesoderm and neural crest cells of the mouse embryo: Co-distribution in the craniofacial mesenchyme but distinct segregation in branchial arches. Development 1995;121:2569–2582.
89 Kleinschuster ST, Moscona AA: Interactions of embryonic and fetal neural retina cells with carbohydrate-binding phytoagglutinins: Cell surface changes with differentiation. Exp Cell Res 1972;70:397–410.
90 Harper CG, Gonatas JO, Stieber A, Gonatas NK: In vivo uptake of wheat germ agglutinin-horseradish peroxidase conjugates into neuronal GERL and lysosomes. Brain Res 1980;188:465–472.
91 Gonatas NK, Gonatas JO: Lectin and toxin-peroxidase conjugates as sensitive markers for the study of neuronal connectivity; in Feldman G, et al (eds): Proceedings of Second International Congress of Immunoenzymatic Techniques. Amsterdam, Elsevier, 1983, pp 111–120.
92 Trojanowski JQ, Gonatas NK: A morphometric study of the endocytosis of wheat germ agglutinin-horseradish peroxidase conjugates by retinal ganglion cells in the rat. Brain Res 1983;272:201–210.
93 Sims PJ, Waggoner AS, Wang CH, Hoffman JF: Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry 1974;13:3315–3330.
94 Honig MG, Hume RI: Fluorescent carbocyanine dyes allow living neurons of identical origin to be studied in long-term cultures. J Cell Biol 1986;103:171–187.
95 Serbedzija GN, Bronner-Fraser M, Fraser SE: A vital dye analysis of the timing and pathways of avian trunk neural crest cell migration. Development 1989;106:806–816.
96 Fukiishi Y, Morriss-Kay GM: Migration of cranial neural crest cells to the pharyngeal arches and heart in rat embryos. Cell Tissue Res 1992;268:1–8.
97 Yung RKM, Chan AOK, Chan WY: Migration of cardiac neural crest cells in the mouse embryo. Neurosci Lett 1997;47(suppl):S27.
98 Chan AOK, Cheung CS, Chan WY: Migration pathways of mouse secondary neural crest cells. Neurosci Lett 1999;53(suppl):S13.
99 Newgreen DF, Murphy M: Neural crest cell outgrowth cultures and the analysis of cell migration. Methods Mol Biol 2000;137:201–211.

100 Beddington RS: The development of 12th to 14th day foetuses following reimplantation of pre- and early-primitive-streak-stage mouse embryos. J Embryol Exp Morphol 1985;88: 281–291.
101 Cockroft DL: Dissection and culture of post-implantation embryos; in Copp AJ, Cockroft DL (eds): Postimplantation Mammalian Embryos: A Practical Approach. Oxford, Oxford University Press, 1990, pp 15–40.
102 Chan WY: Experimental techniques for studying tissue development and differentiation; in Cai W-Q, Li H-B (eds): Developmental Neurobiology. Beijing, Scientific Publishing, 1999, pp 384–396.
103 New DAT, Coppola PT, Cockroft DL: Improved development of head-fold rat embryos in culture resulting from low oxygen and modifications of the culture serum. J Reprod Fertil 1976;48:219–222.
104 New DAT, Coppola PT, Cockroft DL: Comparison of growth in vitro and in vivo of post-implantation rat embryos. J Embryol Exp Morphol 1976;36:133–144.
105 Sadler TW, New DAT: Culture of mouse embryos during neurulation. J Embryol Exp Morphol 1981;66:109–116.
106 Chan WY, Ng TB: Comparison of the embryotoxic effects of saporin, agrostin (type 1 ribosome-inactivating protein) and ricin (a type 2 ribosome-inactivating protein). Pharmacol Toxicol 2001;88:300–303.
107 Ng TB, Chan WY: Polysaccharopeptide from the mushroom *Coriolus versicolor* possesses analgesic activity but does not produce adverse effects on female reproductive or embryonic development in mice. Gen Pharmacol 1997;29:269–273.
108 Ng TB, Shaw PC, Chan WY: Importance of the Glu 160 and Glu 189 residues to the various biological activities of the ribosome inactivating protein trichosanthin. Life Sci 1996; 58:2439–2446.
109 Chan WY, Ng TB, Shaw PC: Mouse embryonic development and tumor growth under the influence of recombinant trichosanthin (a ribosome inactivating protein) and its muteins. Teratog Carcinog Mutagen 1995;15: 259–268.
110 Chan WY, Lee KKH, Tam PPL: The regenerative capacity of forelimb buds following amputation in the early-organogenesis-stage mouse embryo. J Exp Zool 1991;260:74–83.
111 Lee KKH, Chan WY: A study on the regenerative potential of partially excised mouse embryonic forelimb bud. Anat Embryol (Berl) 1991;184:153–157.
112 Brook FA, Shum ASW, Van Straaten HWM, Copp AJ: Curvature of the caudal region is responsible for failure of neural tube closure in the curly tail (ct) mouse embryo. Development 1991;113:671–678.
113 Copp AJ, Checiu I, Henson JN: Developmental basis of severe neural tube defects in the *loop-tail* (*Lp*) mutant mouse: Use of microsatellite DNA markers to identify embryonic genotype. Dev Biol 1994;165:20–29.
114 Ybot-Gonzalez P, Cogram P, Gerrelli D, Copp AJ: Sonic hedgehog and the molecular regulation of neural tube closure. Development 2002;129:2507–2517.
115 Copp AJ, Roberts HM, Polani PE: Chimaerism of primordial germ cells in the early postimplantation mouse embryo following microsurgical grafting of posterior primitive streak cells in vitro. J Embryol Exp Morphol 1986;95:95–115.
116 Mok SWF, Tse PS, Chan WY: Early migration of vagal and sacral neural crest cells in the mouse embryo. Neurosci Lett 2001; 56(suppl):S2.
117 Gendron-Maguire M, Mallo M, Zhang M, Gridley T: Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. Cell 1993; 75:1317–1331.
118 Rijli FM, Mark M, Lakkaraju S, Dierich A, Dolle P, Chambon P: A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. Cell 1993;75: 1333–1349.
119 Gavalas A, Studer M, Lumsden A, Rijli FM, Krumlauf R, Chambon P: Hoxa1 and Hoxb1 synergize in patterning the hindbrain, cranial nerves and second pharyngeal arch. Development 1998;125:1123–1136.
120 Studer M, Gavalas A, Marshall H, Ariza-McNaughton L, Rijli FM, Chambon P, Krumlauf R: Genetic interactions between Hoxa1 and Hoxb1 reveal new roles in regulation of early hindbrain patterning. Development 1998;125:1025–1036.
121 Sham MH, Vesque C, Nonchev S, Marshall H, Frain M, Gupta RD, Whiting J, Wilkinson D, Charnay P, Krumlauf R: The zinc finger gene Krox20 regulates HoxB2 (Hox2.8) during hindbrain segmentation. Cell 1993;72: 183–196.
122 Maconochie MK, Nonchev S, Studer M, Chan SK, Pöpperl H, Sham MH, Mann RS, Krumlauf R: Cross-regulation in the mouse HoxB complex: The expression of Hoxb2 in rhombomere 4 is regulated by Hoxb1. Genes Dev 1997;11:1885–1895.
123 Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC: Green fluorescent protein as a marker for gene expression. Science 1994; 263:802–805.
124 Zernicka-Goetz M, Pines J, McLean Hunter S, Dixon JPC, Siemering KR, Haseloff J, Evans MJ: Following cell fate in the living mouse embryo. Development 1997;124: 1133–1137.
125 Lim FY, Martin BG, Sena-Esteves M, Radu A, Crombleholme TM: Adeno-associated virus (AAV)-mediated gene transfer in respiratory epithelium and submucosal gland cells in human fetal tracheal organ culture. J Pediatr Surg 2002;37:1051–1057.
126 Shestopalov VI, Missey H, Bassnett S: Delivery of genes and fluorescent dyes into cells of the intact lens by particle bombardment. Exp Eye Res 2002;74:639–649.
127 Wendland M, Bumann D: Optimization of GFP levels for analyzing Salmonella gene expression during an infection. FEBS Lett 2002; 521:105–108.
128 Mori K, Gehlbach P, Yamamoto S, Duh E, Zack DJ, Li Q, Berns KI: AAV-mediated gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization. Invest Ophthalmol Vis Sci 2002;43:1994–2000.
129 Kreppel F, Luther TT, Semkova I, Schraemeyer U, Kochanek S: Long-term transgene expression in the RPE after gene transfer with a high-capacity adenoviral vector. Invest Ophthalmol Vis Sci 2002;43:1965–1970.
130 Swartz M, Eberhart J, Mastick GS, Krull CE: Sparking new frontiers: Using in vivo electroporation for genetic manipulations. Dev Biol 2001;233:13–21.
131 Osumi N, Inoue T: Gene transfer into cultured mammalian embryos by electroporation. Methods 2001;24:35–42.
132 Saito T, Nakatsuji N: Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. Dev Biol 2001;240:237–246.
133 Yaneza M, Gilthorpe JD, Lumsden A, Tucker AS: No evidence for ventrally migrating neural tube cells from the mid- and hindbrain. Dev Dyn 2002;223:163–167.
134 Walter I, Fleischmann M, Klein D, Müller M, Salmons B, Günzburg WH, Renner M, Gelbmann W: Rapid and sensitive detection of enhanced green fluorescent protein expression in paraffin sections by confocal laser scanning microscopy. Histochem J 2000;32: 99–103.
135 Ferretti E, Marshall H, Pöpperl H, Maconochie M, Krumlauf R, Blasi F: Segmental expression of Hoxb2 in r4 requires two separate sites that integrate cooperative interactions between Prep1, Pbx and Hox proteins. Development 2000;127:155–166.
136 Kwan CT, Tsang SL, Krumlauf R, Sham MH: Regulatory analysis of the mouse Hoxb3 gene: Multiple elements work in concert to direct temporal and spatial patterns of expression. Dev Biol 2001;232:176–190.