genesis
The Journal of Genetics and Development

Characterization of a novel transgenic mouse line expressing Cre recombinase under the control of the Cdx2 neural specific enhancer

Journal:	*genesis*
Manuscript ID:	gen-2013-0055.R1
Wiley - Manuscript type:	Technology Report
Date Submitted by the Author:	n/a
Complete List of Authors:	Coutaud, Baptiste; Université du Québec à Montréal, Sciences biologiques Pilon, Nicolas; Université du Québec à Montréal, Sciences biologiques;
Keywords:	Neural < Tissue, Neural crest < Tissue, Neurulation < Process, Mammal < Organism

Characterization of a novel transgenic mouse line expressing Cre recombinase under the control of the *Cdx2* neural specific enhancer

Baptiste Coutaud and Nicolas Pilon*

Molecular Genetics of Development Laboratory, Department of Biological Sciences and BioMed Research Center, Faculty of Sciences, University of Quebec at Montreal (UQAM)

***To whom correspondence should be addressed**: Nicolas Pilon, Department of Biological Sciences, University of Quebec at Montreal (UQAM), 141 President-Kennedy Ave, Montreal, PQ, Canada, H2X 3Y7; Tel.: 514-987-3000 x3342; Fax: 514-987-4647; Email: pilon.nicolas@uqam.ca

Running title: A Cre-driver line for the caudal neuroectoderm

Key words: Cre recombinase, Cdx2, neural specific enhancer, posterior neural plate, neural tube, neural crest cells.

ABSTRACT

Several genetically-modified mouse models have been generated in order to drive expression of the Cre recombinase in the neuroectoderm. However, none of them specifically targets the posterior neural plate during neurulation. To fill this gap, we have generated a new transgenic mouse line in which Cre expression is controlled by a neural specific enhancer (NSE) from the *Caudal-related homeobox 2* (*Cdx2*) locus. Analyses of Cre activity via breeding with R26R-YFP reporter mice have indicated that the *Cdx2*NSE-Cre mouse line allows for recombination of LoxP sites in most cells of the posterior neural plate as soon as from the head fold stage. Detailed examination of double-transgenic embryos has revealed that this novel Cre-driver line allows targeting the entire posterior neural tube with an anterior limit in the caudal hindbrain. Of note, the *Cdx2*NSE regulatory sequences direct Cre expression along the whole dorso-ventral axis (including pre-migratory neural crest cells) and, accordingly, YFP fluorescence has been also observed in multiple non-cranial neural crest derivatives of double-transgenic embryos. Therefore, we believe that the *Cdx2*NSE-Cre mouse line represents an important novel genetic tool for the study of early events occurring in the caudal neuroectoderm during the formation of both the central and the peripheral nervous systems.

INTRODUCTION

In vertebrates, early development of the nervous system involves the concerted action of numerous tightly regulated processes in order to properly shape and pattern the neuroectoderm. Formation of a closed neural tube (NT) has to be coordinated with acquisition of positional identity (along both the antero-posterior and dorso-ventral axes) as well as with posterior elongation and induction of neural crest cells (NCC). Such synchronization is required for establishing proper interconnections between the NT-derived central nervous system and the NCC-derived peripheral nervous system. It is also noteworthy that the multipotent and migratory NCC generate in addition multiple non-neural derivatives such as melanocytes, cranio-facial chondrocytes and a subset of conotruncal heart cells, to name a few. Abnormal early nervous system development can thus result in NT defects (such as *spina bifida* and craniorachischisis) and/or neurocristopathies (NCC-associated congenital anomalies), which are amongst the most prevalent group of human congenital malformations (Bolande, 1997; Copp *et al.*, 2003; Nye *et al.*, 1999). This clinical relevance warrants a better understanding of nervous system ontogeny and the mouse has prove to be a very useful animal model in this regard.

Several mouse models based on the Cre-LoxP system have been generated to study early development of the nervous system. Genetically-modified mouse models have been developed for expressing the Cre recombinase in either the whole NT (e.g. *Nestin*-Cre or *Sox1*-Cre) (Dubois *et al.*, 2006; Takashima *et al.*, 2007) or more restricted domains along the dorso-ventral axis (e.g. *Pax3*-Cre or *Ngn1*-Cre) (Li *et al.*, 2000; Quinones *et al.*, 2010). Some other Cre-driver lines with restricted expression along the antero-posterior axis have also been generated (e.g. *Foxg1*-Cre or *Krox20*-Cre) (Hebert and McConnell, 2000; Voiculescu *et al.*, 2001). However, none of the currently existing models directs Cre expression specifically in the posterior neural plate (i.e. the prospective spinal cord territory) at the onset of neurulation.

Antero-posterior patterning of the neuroectoderm is achieved by transformation of a default anterior fate in response to posteriorizing signals. Such posteriorizing cues consist of FGF (fibroblast growth factor), RA (retinoic acid) and canonical Wnt (Wingless/int1-

related) signaling pathways, which together regulate *Hox* gene expression via both direct and indirect means (Forlani *et al.*, 2003; Kudoh *et al.*, 2002; Lumsden and Krumlauf, 1996). *Hox* gene products will then form the so-called Hox code, which is involved in the conversion of antero-posterior positional information into morphological outcomes (Deschamps and van Nes, 2005). Members of the Cdx (Caudal-related homeobox) transcription factors (Cdx1/2/4) are also known to play a prominent role in this regulatory cascade by acting as intermediaries between posteriorizing signals and *Hox* genes (Lohnes, 2003). Murine *Cdx* genes are expressed in all three germ layers of the caudal embryo during gastrulation and neurulation (Beck *et al.*, 1995; Gamer and Wright, 1993; Meyer and Gruss, 1993), being notably under the direct regulation of canonical Wnt signals (Pilon *et al.*, 2006; Pilon *et al.*, 2007; Sanchez-Ferras *et al.*, 2012). The most anterior limit of *Cdx* expression is found in the neuroectoderm, being located around the prospective hindbrain/spinal cord boundary for *Cdx1* and *Cdx2* (Sturgeon *et al.*, 2011).

Functional analyses of cis-regulatory elements have revealed that *Cdx* gene expression is controlled by multiple modular enhancers located in upstream, downstream or intronic regions (Benahmed *et al.*, 2008; Gaunt *et al.*, 2003; Gaunt *et al.*, 2005; Lickert and Kemler, 2002; Rankin *et al.*, 2004; Wang and Shashikant, 2007). Interestingly, one of these studies identified a neural specific enhancer (NSE) located in *Cdx2* intron 1 (Wang and Shashikant, 2007). We thus took advantage of this previous work and use a 1 kb fragment containing this NSE in order to generate a mouse line that specifically drives Cre expression in the caudal neuroectoderm. Characterization of this mouse line – called *Cdx2*NSE-Cre – revealed that it allows recombination of LoxP sites in the caudal neuroectoderm, targeting both the NT and NCC posterior to the hindbrain.

RESULTS AND DISCUSSION

In order to generate a Cre-driver mouse line that specifically targets the posterior neural plate, we first cloned a 852bp fragment from *Cdx2* intron 1 previously reported to contain a neural specific enhancer (NSE) (Wang and Shashikant, 2007). This fragment was then subcloned upstream of a Cre recombinase expression cassette consisting of the *Hsp68*

minimal promoter, Cre open reading frame and SV40 polyA sequences. Standard oocyte microinjections of this construct yielded multiple transgenic animals from which only a single male was used for establishing the *Cdx2*NSE-Cre transgenic line. Both heterozygous and homozygous animals are viable, fertile and do not show any overt morphological defects nor any abnormal behavior.

Cre recombinase expression and activity was monitored by breeding *Cdx2*NSE-Cre animals with R26R-YFP reporter mice. In this particular background, Cre-dependent expression of YFP (yellow fluorescent protein) is under the control of the ubiquitous ROSA26 promoter and thus occurs in cells expressing Cre as well as in their progeny (Srinivas *et al.*, 2001). Using this strategy, the first sign of Cre recombinase activity was traced back to the head fold stage, at which time a significant number of cells exhibited YFP fluorescence in the caudal neuroectoderm of double transgenic embryos (Fig.1A-B'). By the 4-somite stage, robust Cre activity was detected in almost all cells of the posterior neural plate (Fig. 1C, C'). At this stage, the anterior limit of YFP fluorescence was found extending in the forming neural tube (NT) as far as in the prospective caudal hindbrain territory. As expected, transverse sections of the tailbud showed that YFP fluorescence was restricted to neural cells (Fig. 1D, D').

At embryonic day (E) 8.5, YFP fluorescence was still observed in the whole posterior neural plate and also in the closed NT with an anterior limit located between the first and second somite (Fig. 2A, A'). Transverse sections at trunk level revealed that YFP-labeled cells were exclusively neural, being found along the whole dorso-ventral axis of the NT (Fig. 2B-B'). However, more caudally, transverse sections of the tailbud suggested an expansion of Cre activity in a subset of presumptive mesoderm cells (Fig. 2C-E'). Immunofluorescent co-labeling with an antibody against the neural marker Sox2 was thus used to confirm the non-neural identity of these cells (Fig. 3). This analysis clearly revealed that a sub-population of YFP-positive cells was indeed also Sox2-negative at the most caudal end of *Cdx2*NSE-Cre::R26R-YFP embryos (Fig. 3D-E").

At E9.5, the pattern of YFP fluorescence was consistent with observations made at previous stages. While YFP-labeled cells were exclusively neural in the caudal hindbrain and anterior spinal cord regions, a subset of mesoderm cells were also YFP-positive more

posteriorly (Fig. 4). These YFP-labeled mesoderm cells were displayed along a rostro-caudal gradient in the most caudal quarter of the embryo, culminating in the tailbud. In addition, observations at this stage revealed that *Cdx2*NSE-Cre allows targeting migratory neural crest cells (NCC) (Fig. 4D'). Given that *Cdx2* is not expressed in these cells, this most likely reflected a cell lineage effect subsequent to Cre activity in the pre-migratory NCC population – which is located in the dorsal NT.

Characterization at later stages (between E11.5 and E15.5) further demonstrated that *Cdx2*NSE-Cre allows targeting every structure derived or colonized by non-cranial NCC (Fig. 5). Indeed, in addition to the NT, YFP-labeled cells were found in derivatives of trunk (dorsal root ganglia, peripheral nerves, sympathetic ganglia and melanoblasts; Fig. 5A, A' and F), cardiac (outflow tract; Fig. 5E) and vagal (enteric neural progenitors; Fig. 5B-D') NCC. At these later stages, the rostro-caudal gradient of YFP-labeled cells in the mesoderm was found spreading from around the posterior limb down to the tip of the tail (data not shown).

On the other hand, it is also noteworthy that some *Cdx2*NSE-Cre::R26R-YFP double transgenic embryos exhibited non-specific widespread YFP fluorescence in a genetic background-dependent manner (data not shown). Interestingly, we noticed that this effect occurred much less frequently when the FVB background contribution is increased. With a 50% FVB contribution, it was observed in 17% of fluorescent embryos whereas with a 87.5% FVB contribution, it was not observed. This outcome most likely reflects the fact that the regulatory sequences (NSE and *Hsp68* promoter) used to generate the *Cdx2*NSE-Cre transgene were cloned from FVB genomic DNA. Consequently, it should be noted that a *Cdx2*NSE-Cre mouse line having a nearly pure FVB background (backcrossed for at least 5 generations) will be available to the scientific community.

Taken together, our analyses have demonstrated that the *Cdx2*NSE-Cre mouse line targets the whole posterior neuroectoderm (both NT and NCC) with the caudal hindbrain as anterior limit. However, we have also found that this transgene is not fully neural-specific since it is also active in a subset of mesoderm cells of the most caudal quarter of the embryo. Given that such activity has been also observed with multiple transgenic founders (5 out of 8; data not shown), this suggests that it is a true inherent property of

the cloned sequences. Such hypothesis is supported by the fact that in the original description of the *Cdx2* NSE, E9.5 transient transgenic embryos did not only display reporter gene expression in the NT but apparently also in the whole tailbud (Wang and Shashikant, 2007). Therefore, the YFP labeling of mesoderm cells in *Cdx2*NSE-Cre::R26R-YFP double transgenic embryos was most likely indicative of a cell lineage effect resulting from Cre activity in tailbud mesoderm precursors. Further functional dissection of the NSE sequences might thus eventually lead to an improved version (i.e. fully neural specific) of the *Cdx2*NSE-Cre transgene. Nonetheless, we believe that the current *Cdx2*NSE-Cre mouse model represents a valuable novel genetic tool for the study of early NT and NCC development specifically in the caudal embryo.

MATERIALS AND METHODS

Ethics Statement

Experiments involving mice were performed following Canadian Council of Animal Care (CCAC) guidelines for the care and manipulation of animals used in medical research. Protocols involving the manipulation of animals were approved by the institutional ethics committee of the University of Quebec at Montreal (comité institutionnel de protection des animaux (CIPA)); Reference number 0512-R3-648-0513).

Construction of the *Cdx2*NSE-Cre transgene

The neural specific enhancer was cloned by PCR as previously described (Wang et Shashikant, 2007). Genomic DNA from a FVB mouse was used to amplify a 852-bp fragment using a forward primer containing a *Apa*I site (5'-GGGCCCGTGCTCTAAGAGCAGCATCCGTTC-3') and a reverse primer bearing a *Kpn*I site (5'-GGTACCGAGGCAACTATCCTGACCAAGTGA-3'). The minimal *Hsp68* promoter (315-bp) was similarly amplified with a forward primer containing a *Kpn*I site (5'-GGTACCTTGGTACCCCTCCTCCGGCT-3') and a reverse primer containing a *Bgl*II site (5'- AGATCTTTAGATCTGGCGCCGCGCTC -3'). Cre recombinase coding sequences (1032pb) were amplified from another plasmid (Pilon *et*

al., 2008) using a forward primer bearing a *Sac*I site (5'-GAGCTCATGTCCAATTTACTGACCGTACAC-3') and a reverse primer containing a *Spe*I site (5'- ACTAGTCTAATCGCCATCTTCCAGC-3'). All amplifications were done using the Platinum Taq DNA polymerase kit (Invitrogen) in accordance with manufacturer's instructions. PCR products were cloned in pGEMT-easy vector (Promega) and verified by sequencing. Restriction sites contained in primers were then used to assemble the *Cdx2*NSE-*Hsp68*p-Cre sequences in a vector containing SV40 polyA sequences (pIRES-EGFP2; Clontech). The *Cdx2*NSE-*Hsp68*p-Cre-SV40pA transgene was finally excised with *Nhe*I and *Pvu*II to obtain the 3150-bp fragment that was used for microinjections.

Generation and analysis of the *Cdx2*NSE-Cre transgenic mouse line

*Cdx2*NSE-Cre transgene DNA was microinjected in B6C3 oocytes in accordance with standard methods. Offspring was screened by PCR-based genotyping of tail DNA using specific Cre recombinase primers (forward: 5'-GATGAGGTTCGCAAGAACCTGATG-3' and reverse: 5'-AACAGCATTGCTGTCACTTGGTCG-3'). Thirteen F0 transgenic animals were identified, of which only males (eight) were tested for Cre activity as described below. A single male (R337) was then kept to generate the *Cdx2*NSE-Cre mouse line via breeding with a C57BL/6 female. *Cdx2*NSE-Cre transgenic animals are available upon request.

Cre recombinase activity was evaluated by crossing F1 *Cdx2*NSE-Cre mice with R26R-YFP animals (initially obtained from Frank Costantini and then maintained on a FVB background) as previously described (Pilon *et al.*, 2008; Srinivas *et al.*, 2001). Timed pregnancies were set up using standard vaginal plug detection, noon of the day of plug detection being considered as E0.5.

Microscopy and imaging

Embryos were dissected and fixed with 4% paraformaldehyde in PBS. Fixation time was adjusted in accordance with embryonic stages. For transverse sections, embryos were

embedded in 3% agarose and 100µm transverse sections were realized using a vibrating blade microtome Microm HM 650V (Thermo Scientific).

Bright field and YFP images were obtained with a Leica DFC 495 camera mounted on a Leica M205 FA microscope (Leica Microsystems). Exposure time was adjusted for optimal imaging, and kept constant throughout the experimental time course.

Immunostaining

Following dissection and fixation, whole embryos were first incubated in blocking solution (10% FBS and 0,1% triton in PBS) for 2h at room temperature. Embryos were then incubated overnight at 4°C with goat anti-SOX2 primary antibody (R&D Systems, #AF2018) diluted 1:200 in blocking solution. After washing, embryos were incubated 2h at room temperature with bovine anti-goat Alexa Fluor 647 secondary antibody diluted 1:500 in blocking solution (Jackson ImmunoResearch). After final washing, transverse sections were realized as described above and fluorescence from both YFP and immunostaining was captured with a laser-scanning confocal microscope (Nikon A1R).

REFERENCES

Beck F, Erler T, Russell A, James R. 1995. Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. Dev Dyn 204: 219-227.

Benahmed F, Gross I, Gaunt SJ, Beck F, Jehan F, Domon-Dell C, Martin E, Kedinger M, Freund JN, Duluc I. 2008. Multiple regulatory regions control the complex expression pattern of the mouse Cdx2 homeobox gene. Gastroenterology 135: 1238-1247, 1247 e1231-1233.

Bolande RP. 1997. Neurocristopathy: its growth and development in 20 years. Pediatr Pathol Lab Med 17: 1-25.

Copp AJ, Greene ND, Murdoch JN. 2003. The genetic basis of mammalian neurulation. Nat Rev Genet 4: 784-793.

Deschamps J, van Nes J. 2005. Developmental regulation of the Hox genes during axial morphogenesis in the mouse. Development 132: 2931-2942.

Dubois NC, Hofmann D, Kaloulis K, Bishop JM, Trumpp A. 2006. Nestin-Cre transgenic mouse line Nes-Cre1 mediates highly efficient Cre/loxP mediated recombination in the nervous system, kidney, and somite-derived tissues. Genesis 44: 355-360.

Forlani S, Lawson KA, Deschamps J. 2003. Acquisition of Hox codes during gastrulation and axial elongation in the mouse embryo. Development 130: 3807-3819.

Gamer LW, Wright CV. 1993. Murine Cdx-4 bears striking similarities to the Drosophila caudal gene in its homeodomain sequence and early expression pattern. Mech Dev 43: 71-81.

Gaunt SJ, Drage D, Cockley A. 2003. Vertebrate caudal gene expression gradients investigated by use of chick cdx-A/lacZ and mouse cdx-1/lacZ reporters in transgenic mouse embryos: evidence for an intron enhancer. Mech Dev 120: 573-586.

Gaunt SJ, Drage D, Trubshaw RC. 2005. cdx4/lacZ and cdx2/lacZ protein gradients formed by decay during gastrulation in the mouse. Int J Dev Biol 49: 901-908.

Hebert JM, McConnell SK. 2000. Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. Dev Biol 222: 296-306.

Kudoh T, Wilson SW, Dawid IB. 2002. Distinct roles for Fgf, Wnt and retinoic acid in posteriorizing the neural ectoderm. Development 129: 4335-4346.

Li J, Chen F, Epstein JA. 2000. Neural crest expression of Cre recombinase directed by the proximal Pax3 promoter in transgenic mice. Genesis 26: 162-164.

Lickert H, Kemler R. 2002. Functional analysis of cis-regulatory elements controlling initiation and maintenance of early Cdx1 gene expression in the mouse. Dev Dyn 225: 216-220.

Lohnes D. 2003. The Cdx1 homeodomain protein: an integrator of posterior signaling in the mouse. Bioessays 25: 971-980.

Lumsden A, Krumlauf R. 1996. Patterning the vertebrate neuraxis. Science 274: 1109-1115.

Meyer BI, Gruss P. 1993. Mouse Cdx-1 expression during gastrulation. Development 117: 191-203.

Nye JS, McLone DG, Charrow J, Hayes EA. 1999. Neural crest anomaly syndromes in children with spina bifida. Teratology 60: 179-189.

Pilon N, Oh K, Sylvestre JR, Bouchard N, Savory J, Lohnes D. 2006. Cdx4 is a direct target of the canonical Wnt pathway. Dev Biol 289: 55-63.

Pilon N, Oh K, Sylvestre JR, Savory JG, Lohnes D. 2007. Wnt signaling is a key mediator of Cdx1 expression in vivo. Development 134: 2315-2323.

Pilon N, Raiwet D, Viger RS, Silversides DW. 2008. Novel pre- and post-gastrulation expression of Gata4 within cells of the inner cell mass and migratory neural crest cells. Dev Dyn 237: 1133-1143.

Quinones HI, Savage TK, Battiste J, Johnson JE. 2010. Neurogenin 1 (Neurog1) expression in the ventral neural tube is mediated by a distinct enhancer and preferentially marks ventral interneuron lineages. Dev Biol 340: 283-292.

Rankin EB, Xu W, Silberg DG, Suh E. 2004. Putative intestine-specific enhancers located in 5' sequence of the CDX1 gene regulate CDX1 expression in the intestine. Am J Physiol Gastrointest Liver Physiol 286: G872-880.

Sanchez-Ferras O, Coutaud B, Djavanbakht Samani T, Tremblay I, Souchkova O, Pilon N. 2012. Caudal-related homeobox (Cdx) protein-dependent integration of canonical Wnt signaling on paired-box 3 (Pax3) neural crest enhancer. J Biol Chem 287: 16623-16635.

Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 1: 4.

Sturgeon K, Kaneko T, Biemann M, Gauthier A, Chawengsaksophak K, Cordes SP. 2011. Cdx1 refines positional identity of the vertebrate hindbrain by directly repressing Mafb expression. Development 138: 65-74.

Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, Nishikawa S. 2007. Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell 129: 1377-1388.

Voiculescu O, Taillebourg E, Pujades C, Kress C, Buart S, Charnay P, Schneider-Maunoury S. 2001. Hindbrain patterning: Krox20 couples segmentation and specification of regional identity. Development 128: 4967-4978.

Wang WC, Shashikant CS. 2007. Evidence for positive and negative regulation of the mouse Cdx2 gene. J Exp Zool B Mol Dev Evol 308: 308-321.

ACKNOWLEDGEMENTS

The authors thank Denis Flipo (UQAM) for assistance with the confocal analyses as well as Qinzhang Zhu (IRCM) for microinjections. This work was supported by a grant from the Canadian Institute for Health Research to NP (CIHR grant number MOP-111130). BC is supported by a UQAM scholarship and NP by a career award (chercheur-boursier Junior 2) from the Fonds de la Recherche du Québec – Santé (FRQS).

FIGURE LEGENDS

Figure 1: Characterization of *Cdx2*NSE-Cre at the onset of neurulation. Analyses of embryos obtained from crosses between *Cdx2*NSE-Cre and R26R-YFP reporter mice at head fold (A-B') and 4-somite (C-D') stages. Images were taken under bright field (A, B, C, D) or YFP detection mode (A', B', C', D'). Lateral (A,B), dorsal (C-F) and transverse (G-H) views reveal that YFP fluorescence is restricted to the caudal neuroectoderm. The dashed line in C-C' indicates the level at which the cross section shown in D-D' was made. Abbreviations: P, posterior; A, anterior; PNP, posterior neural plate; NT, neural tube.

Figure 2: Characterization of *Cdx2*NSE-Cre during early neurulation. Detailed analysis of a 7-somite stage embryo obtained from a cross between *Cdx2*NSE-Cre and R26R-YFP reporter mice. Images were taken under bright field (A, B, C, D, E) or YFP detection mode (A', B', C', D', E'). YFP fluorescence is observed in the posterior neural plate (PNP) and the closed neural tube (NT) with an anterior limit between the first and second somite (A-A'). The dashed lines in A-A' indicates the level at which the cross sections shown in B to E' were made. These transverse sections suggest that the neural specificity of YFP fluorescence is decreasing caudally, being also found in the presumptive mesoderm territory.

Figure 3: The *Cdx2*NSE-Cre transgene is not fully neural-specific. Immunofluorescent detection of Sox2 in a E8.5 *Cdx2*NSE-Cre::R26R-YFP double-transgenic embryo. The dashed lines in A indicate the level at which the cross sections shown on the right were made. Confocal imaging of these transverse sections show that a subset of YFP-labeled cells (green) are not labeled by an antibody against the neural marker Sox2 (red) in the most caudal end of the embryo, supporting the assumption that these cells are mesodermal. Note also that the number of these YFP-positive but Sox2-negative cells appears to form a gradient along the antero-posterior axis.

Figure 4: Characterization of *Cdx2*NSE-Cre during late neurulation. Analysis of E9.5 embryos obtained from crosses between *Cdx2*NSE-Cre and R26R-YFP reporter mice. Images were taken under bright field (A, B, C, D) or YFP detection mode (A', B', C', D'). Frontal (A, A') and lateral (B, B') views show YFP fluorescence in the closed neural tube (NT). Blue arrowheads point to progressive mesodermal expression in the caudal end of the embryo (A', B'). The dashed lines in B-B' indicate the level at which the cross sections shown in C to D' were made. Note that while the tailbud is almost completely labeled with YFP fluorescence (C, C'), this labeling is neuroectoderm-specific in the trunk (D, D'). Note also that YFP fluorescence is observed in migratory neural crest cells (NCC) of the trunk (D').

Figure 5: Characterization of *Cdx2*NSE-Cre activity in the neural crest lineage. Analyses of E11.5 (A, A'), E13.5 (B-E) and E15.5 (F) embryos obtained from crosses between *Cdx2*NSE-Cre and R26R-YFP reporter mice. Bright field (A, D), YFP fluorescence (A', B, C, D', F) and composite images (E) are shown. Transverse views of E11.5 embryos at trunk level (A, A') indicate that YFP is expressed in the neural tube (NT) as well as multiple derivatives of neural crest cells such as melanoblasts (M), dorsal root ganglia (DRG), peripheral nerves (PN) and sympathetic ganglia (SG). In E13.5 embryos, YFP-labeled neural crest cells are also observed colonizing the cardiac outflow tract (E) as well as the gut (B-D'). The dashed line in C indicates the level at which the cross section shown in D-D' was made; this section shows that YFP fluorescence marks enteric neural precursors (ENP). In E15.5 embryos, YFP-labeled melanoblasts can be observed spreading over the whole body except the head (F).

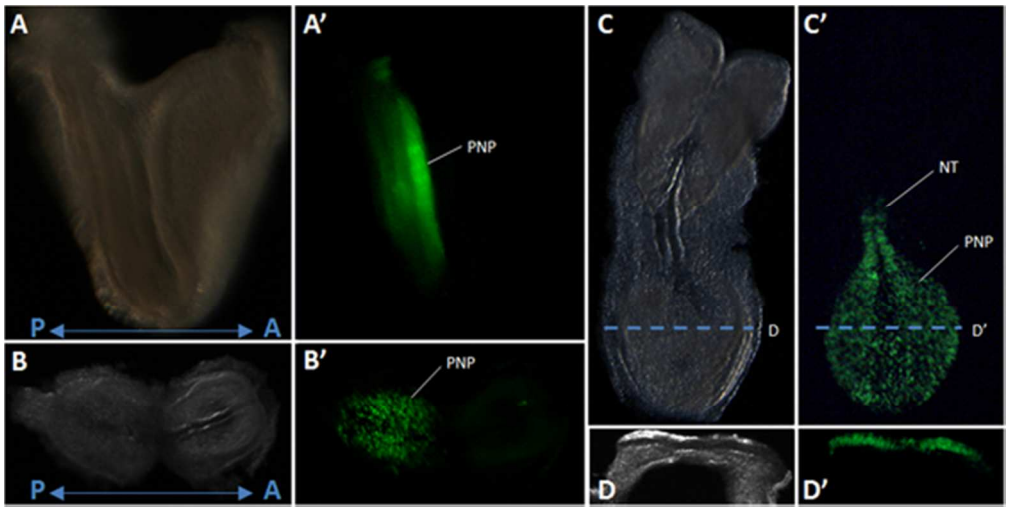

Figure 1: Characterization of Cdx2NSE-Cre at the onset of neurulation. Analyses of embryos obtained from crosses between Cdx2NSE-Cre and R26R-YFP reporter mice at head fold (A-B') and 4-somite (C-D') stages. Images were taken under bright field (A, B, C, D) or YFP detection mode (A', B', C', D'). Lateral (A,B), dorsal (C-F) and transverse (G-H) views reveal that YFP fluorescence is restricted to the caudal neuroectoderm. The dashed line in C-C' indicates the level at which the cross section shown in D-D' was made. Abbreviations: P, posterior; A, anterior; PNP, posterior neural plate; NT, neural tube.
50x25mm (300 x 300 DPI)

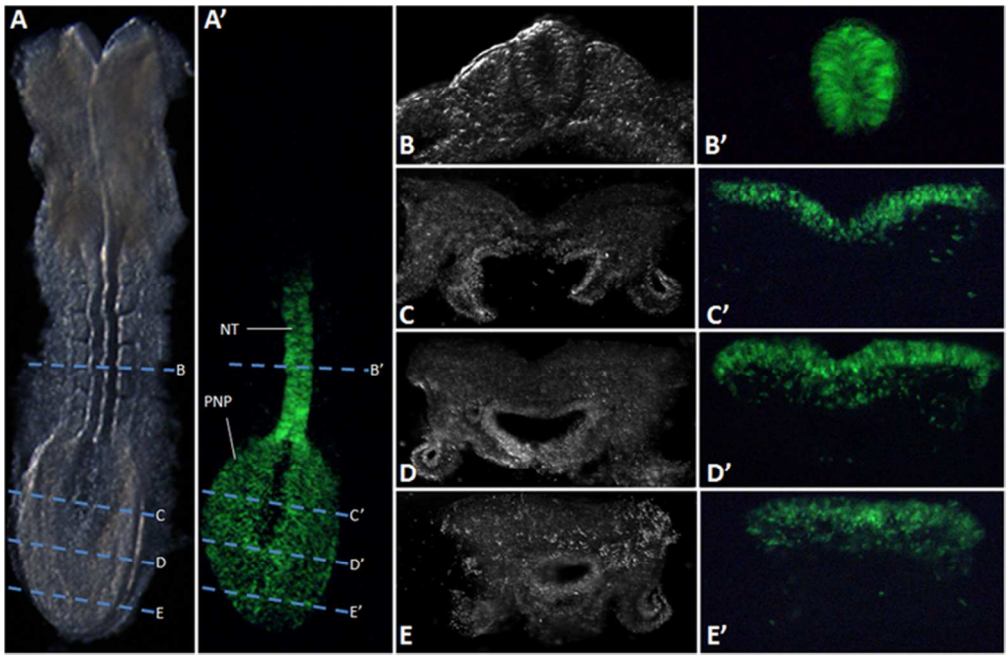

Figure 2: Characterization of Cdx2NSE-Cre during early neurulation. Detailed analysis of a 7-somite stage embryo obtained from a cross between Cdx2NSE-Cre and R26R-YFP reporter mice. Images were taken under bright field (A, B, C, D, E) or YFP detection mode (A', B', C', D', E'). YFP fluorescence is observed in the posterior neural plate (PNP) and the closed neural tube (NT) with an anterior limit between the first and second somite (A-A'). The dashed lines in A-A' indicates the level at which the cross sections shown in B to E' were made. These transverse sections suggest that the neural specificity of YFP fluorescence is decreasing caudally, being also found in the presumptive mesoderm territory.
64x41mm (300 x 300 DPI)

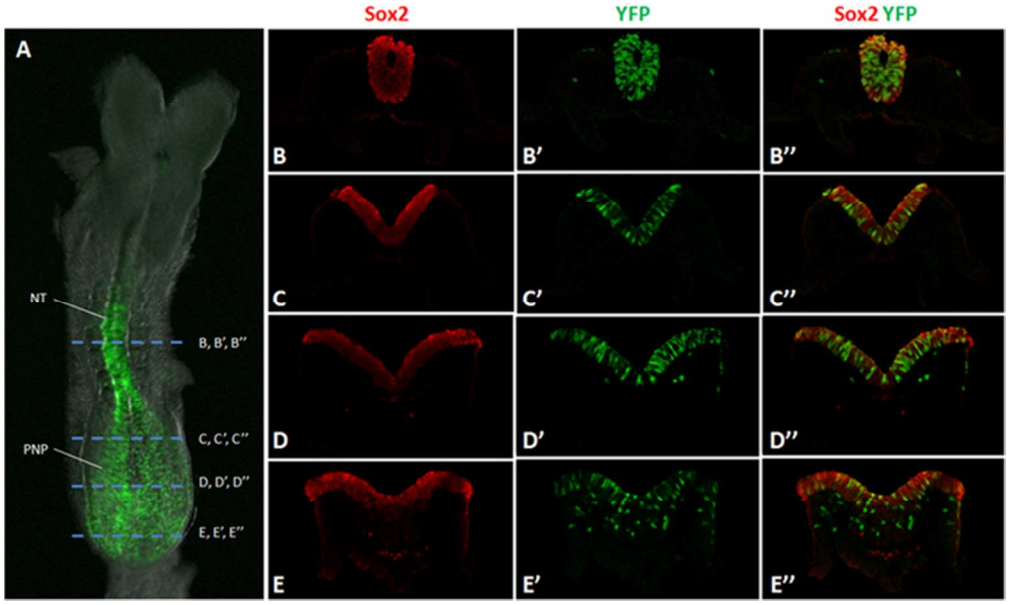

Figure 3: The Cdx2NSE-Cre transgene is not fully neural-specific. Immunofluorescent detection of Sox2 in a E8.5 Cdx2NSE-Cre::R26R-YFP double-transgenic embryo. The dashed lines in A indicate the level at which the cross sections shown on the right were made. Confocal imaging of these transverse sections show that a subset of YFP-labeled cells (green) are not labeled by an antibody against the neural marker Sox2 (red) in the most caudal end of the embryo, supporting the assumption that these cells are mesodermal. Note also that the number of these YFP-positive but Sox2-negative cells appears to form a gradient along the antero-posterior axis.
59x35mm (300 x 300 DPI)

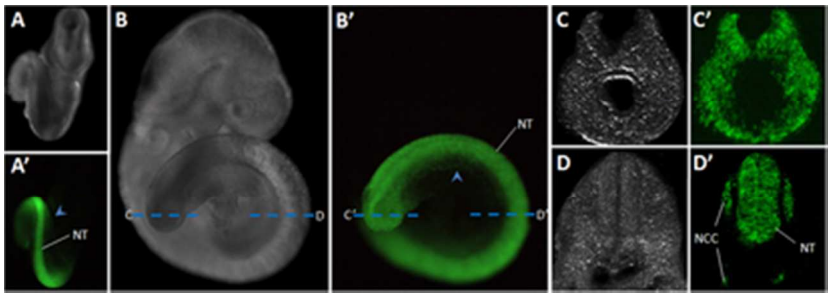

Figure 4: Characterization of Cdx2NSE-Cre during late neurulation. Analysis of E9.5 embryos obtained from crosses between Cdx2NSE-Cre and R26R-YFP reporter mice. Images were taken under bright field (A, B, C, D) or YFP detection mode (A', B', C', D'). Frontal (A, A') and lateral (B, B') views show YFP fluorescence in the closed neural tube (NT). Blue arrowheads point to progressive mesodermal expression in the caudal end of the embryo (A', B'). The dashed lines in B-B' indicate the level at which the cross sections shown in C to D' were made. Note that while the tailbud is almost completely labeled with YFP fluorescence (C, C'), this labeling is neuroectoderm-specific in the trunk (D, D'). Note also that YFP fluorescence is observed in migratory neural crest cells (NCC) of the trunk (D').
34x12mm (300 x 300 DPI)

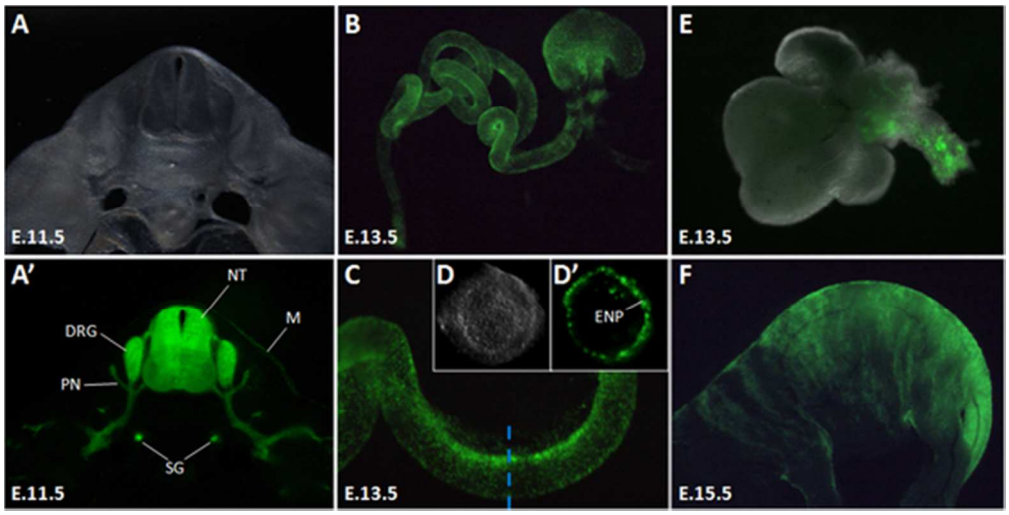

Figure 5: Characterization of Cdx2NSE-Cre activity in the neural crest lineage. Analyses of E11.5 (A, A'), E13.5 (B-E) and E15.5 (F) embryos obtained from crosses between Cdx2NSE-Cre and R26R-YFP reporter mice. Bright field (A, D), YFP fluorescence (A', B, C, D', F) and composite images (E) are shown. Transverse views of E11.5 embryos at trunk level (A, A') indicate that YFP is expressed in the neural tube (NT) as well as multiple derivatives of neural crest cells such as melanoblasts (M), dorsal root ganglia (DRG), peripheral nerves (PN) and sympathetic ganglia (SG). In E13.5 embryos, YFP-labeled neural crest cells are also observed colonizing the cardiac outflow tract (E) as well as the gut (B-D'). The dashed line in C indicates the level at which the cross section shown in D-D' was made; this section shows that YFP fluorescence marks enteric neural precursors (ENP). In E15.5 embryos, YFP-labeled melanoblasts can be observed spreading over the whole body except the head (F).
50x25mm (300 x 300 DPI)