Cre-driver lines used for genetic fate mapping of neural crest cells in the mouse: An overview

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Abstract: The neural crest is one of the embryonic structures with the broadest developmental potential in vertebrates. Morphologically, neural crest cells emerge during neurulation in the dorsal folds of the neural tube before undergoing an epithelial-to-mesenchymal transition (EMT), delaminating from the neural tube, and migrating to multiple sites in the growing embryo. Neural crest cells generate cell types as diverse as peripheral neurons and glia, melanocytes, and so-called mesectodermal derivatives that include craniofacial bone and cartilage and smooth muscle cells in cardiovascular structures. In mice, the fate of neural crest cells has been determined mainly by means of transgenesis and genome editing technologies. The most frequently used method relies on the Cre-loxP system, in which expression of Cre-recombinase in neural crest cells or their derivatives genetically enables the expression of a Cre-reporter allele, thus permanently marking neural crest-derived cells. Here, we provide an overview of the Cre-driver lines used in the field and discuss to what extent these lines allow precise neural crest stage and lineage-specific fate mapping.

DOI: https://doi.org/10.1002/dvg.23105
Cre-driver lines used for genetic fate mapping of neural crest cells in the mouse: An overview

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Funding information
Swiss National Science Foundation

Summary
The neural crest is one of the embryonic structures with the broadest developmental potential in vertebrates. Morphologically, neural crest cells emerge during neurulation in the dorsal folds of the neural tube before undergoing an epithelial-to-mesenchymal transition (EMT), delaminating from the neural tube, and migrating to multiple sites in the growing embryo. Neural crest cells generate cell types as diverse as peripheral neurons and glia, melanocytes, and so-called mesectodermal derivatives that include craniofacial bone and cartilage and smooth muscle cells in cardiovascular structures. In mice, the fate of neural crest cells has been determined mainly by means of transgenesis and genome editing technologies. The most frequently used method relies on the Cre-loxP system, in which expression of Cre-recombinase in neural crest cells or their derivatives genetically enables the expression of a Cre-reporter allele, thus permanently marking neural crest-derived cells. Here, we provide an overview of the Cre-driver lines used in the field and discuss to what extent these lines allow precise neural crest stage and lineage-specific fate mapping.

KEYWORDS
Cre/LoxP system, lineage tracing, neural crest

1 | A BRIEF SYNOPSIS OF METHODS USED FOR NEURAL CREST CELL FATE MAPPING

The astonishing variety of neural crest derivatives has long been recognized, mainly through the pioneering work by Le Douarin and co-workers who used interspecies transplantation in avian embryos to determine normal fates as well as the developmental potential of neural crest cell populations from different axial levels of the embryo (Bronner & Simões-Costa, 2016; Le Douarin & Dupin, 2003). Together with assays involving dye labeling and retroviral infection of neural crest cells, this approach led to the establishment of comprehensive fate maps and revealed that certain neural crest derivatives (such as peripheral glia, sensory neurons, and melanocytes) are produced from all axial levels, whereas other neural crest cell lineages (such as cartilage, bone, smooth muscle, connective tissue, endocrine cells as well as, for instance, neurons and glia from the enteric and parasympathetic nervous system) originate from discrete levels along the neuraxis.

Given the limited accessibility of mammalian embryos, lineage-tracing experiments as performed in avian embryos have not been widely used for fate mapping of mammalian neural crest cells. Rather, various genetic tools have been established that allow noninvasive and long-term tracing of neural crest cells in mice in vivo (Zurkirchen & Sommer, 2017). By far the most frequently used approach in the field is Cre-loxP-based conditional genetic recombination that, when combined with a Cre-reporter line, results in inheritable and irreversible expression of a marker gene in Cre-recombinase-expressing cells and in all of their progeny (Woodworth, Girskis, & Walsh, 2017). Furthermore, inducible forms of Cre-recombinase have been applied to perform stage-dependent fate mapping of neural crest cells and their derivatives or fate mapping at low recombination density for in vivo single cell tracing (Baggiolini et al., 2015; Kaucka et al., 2016). Apart from confirming in mice many of the findings obtained by fate mapping of avian neural crest cells, genetic lineage tracing of murine neural crest cells led, for instance, to the identification of minor neural crest-derived cell populations present in tissues of nonneural crest origin, to the
establishment of novel lineage trees (revealing, in particular, the broad developmental potential of peripheral glial cells), and to the demonstration of in vivo multipotency of single premigratory and migratory neural crest cells. While these studies have recently been covered elsewhere (Petersen & Adameyko, 2017; Zurkirchen & Sommer, 2017), in the present review we aim to focus on the tool set used in the field, summarizing the findings made with and discussing specific properties of various Cre lines that have been utilized to trace neural crest cells.

2 TRACING OF PREMIGRATORY AND MIGRATORY NEURAL CREST CELLS USING NONINDUCIBLE CRE-DRIVER LINES

A well-established Cre-driver line for neural crest lineage tracing is Wnt1-Cre (Danielian, Muccino, Rowitch, Michael, & McMahon, 1998) (Table 1). This transgenic mouse line expresses Cre initially in the midbrain and, after closure of the neural tube, in the midlines of the midbrain and the caudal diencephalon, in the midbrain–hindbrain junction, and in the dorsal spinal cord, where it recombines premigratory neural crest cells. By crossing Wnt1-Cre mice with the ROSA26 (R26R) Cre-reporter line (that drives β-galactosidase expression upon Cre-mediated recombination) (Soriano, 1999), it was shown that Wnt1-Cre is a highly efficient Cre-driver line, resulting in recombination of approximately 96% of all migratory neural crest cells (Hari et al., 2012).

Because Wnt1 is not expressed in migratory neural crest cells and Wnt activity rapidly decreases in neural crest cells after their delamination from the neural tube (Kl/Cre cell et al., 2005; Rabadan et al., 2016; Zervas, Millet, Ahn, & Joyner, 2004), it can be assumed that most neural crest cells are very efficiently targeted by Wnt1-Cre before or at the time of their delamination. Intriguingly, however, despite the early activity of Wnt1-Cre in the dorsal neural tube, recombination apparently occurs too late to allow investigation of mechanisms regulating epithelial-to-mesenchymal transition (EMT) or delamination of neural crest cells. Indeed, Wnt1-Cre-mediated ablation of signaling pathways shown in other animal models to be crucial for neural crest EMT, such as canonical Wnt signaling and signaling by TGFβ superfamily factors, did not affect early stages of neural crest development (Brault et al., 2001; Buchmann-Müller et al., 2009; Hari et al., 2002; Jia et al., 2007). In contrast, the differentiation potential of neural crest cells along all axial levels could readily be monitored using Wnt1-Cre, both during development and at postnatal stages (Chai et al., 2000; Jiang, Rowitch, Soriano, McMahon, & Sucov, 2000; Zurkirchen & Sommer, 2017). A potential caveat of this line is, however, that, at least in the midbrain, Wnt1 is ectopically expressed from the Wnt1-Cre transgene, which could lead to ectopic activation of canonical Wnt signaling (Lewis, Vasudevan, O'Neill, Soriano, & Bush, 2013). Although it is not known whether such ectopic Wnt1 expression also affects the neural crest, the use of a new driver line termed Wnt1-Cre2 should be considered (Lewis et al., 2013).

### Table 1

| Premigratory neural crest drivers | Official Name | Original study |
|----------------------------------|---------------|---------------|
| P3Pro-Cre                        | Tg(Pax3-cre)1Joe | Li et al. (2000) |
| Wnt1-Cre                         | H2afvTg(Wnt1-cre)11Rth | Danielian et al. (1998) |
| Wnt1-Cre2                        | E2f1Tg(Wnt1-cre)2Sor | Lewis et al. (2013) |
| Wnt1-CreER                       | Tg(Wnt1-cre/ERT1)Alj | Zervas et al. (2004) |
| Wnt1-Flpe                        | Tg(Wnt1-FLP1)1Dym | Dymecki et al. (1998) |
| Wnt1-FlpeER<sup>T2</sup>         | Tg(Wnt1-flpe/ERT2)94Sor | Hunter et al. (2005) |

| Migratory neural crest drivers   | Official Name | Original study |
|----------------------------------|---------------|---------------|
| AP2a-IRESCre                     | Tfap2a<sup>tm1(cre)Moen</sup> | Macatee et al. (2003) |
| Ht-PA-Cre                        | Tg(PLAT-cre)116Sdu | Pietri et al. (2003) |
| Mef2c-F10N-Cre                   | Tg(Mef2c-cre)5Patr | Aoto et al. (2015) |
| PO-Cre                           | Tg(Mpz-cre)94Ims | Yamauchi et al. (1999) |
| Plp-CreERT<sup>1</sup>           | Tg(Plp1-cre/ERT1)3Pop/J | Doerflinger et al. (2003) |
| Plp-CreERT<sup>2</sup>           | Tg(Plp1-cre/ERT2)1Ueli | Leone et al. (2003) |
| Sox10-Cre                        | Tg(Sox10-cre)1Ueli | Matsuoka et al. (2005) |
| Sox10ERT<sup>2</sup>CreERT<sup>2</sup> (SECE) | Tg(Sox10-cre/Ert2)17Sor | He and Soriano (2015) |
| Sox10-icCreERT<sup>2</sup>       | Tg(Sox10-icre/ERT2)1Ldm | Simon et al. (2012) |
| Sox10-icCreERT<sup>2</sup>       | Tg(Sox10-icre/ERT2)26Vpa | Laranjeira et al. (2011) |
| TEC1                             | Tg(Tyr-cre)1Gf | Tonks et al. (2003) |
fact, in studies addressing the role of fibronectin in cardiac neural crest development, considerable phenotypic variances have been reported upon Wnt1-Cre vs. Wnt1-Cre2-mediated recombination, although this could have been due to differences between the two transgenic lines other than aberrant Wnt1 expression (Wang & Astrof, 2016).

Apart from the Cre/loxP system, another site-specific recombinase system has also been established to trace the fate of neural crest cells. To this end, two transgenic mouse lines (termed Wnt1-Flpe mice) were independently generated that express Flp recombinase from the Wnt1 promoter (Dymecki & Tomasiwicz, 1998; Hatzistergos et al., 2015). Although the recombination efficiency and the extent of neural crest lineages traceable by these lines have not been described in detail, these lines were instrumental to perform intersectional lineage tracing of cells that concurrently express two distinct promoters. When combined with either the RC::FrePe (Engleka et al., 2012) or RC::Fela (Jensen et al., 2008) dual reporter alleles (which report dual Flp and Cre recombination), a fraction of cKit-CreERT²™-tagged cardiac progenitors was shown to derive from the cardiac neural crest (traced by Wnt1-Flpe) (Hatzistergos et al., 2015). Likewise, intersectional fate-mapping with the RC::FrePe allele was used to demonstrate that Isl1 is not an exclusive marker for second heart field cardiac progenitors, as previously suggested, but also marks a subpopulation of cardiac neural crest cells (Engleka et al., 2012).

Another mouse line expressing Cre in the dorsal neural tube and premigratory neural crest is P3Pro-Cre, in which Cre expression is driven from a Pax3 promoter fragment (Li, Chen, & Epstein, 2000). Although Pax3 is expressed in the neural plate border before bona fide neural crest specification (Bronner & Simões-Costa, 2016), Cre-mediated conditional inactivation of pathways controlling EMT/delamination did not affect neural crest cell production and early migration in P3Pro-Cre embryos (Buchmann-Moller and Sommer, unpublished). Thus, we are not aware of a Cre-driver line suitable for the study of early events in neural crest development, including neural crest specification, EMT, and delamination. Fate mapping experiments with P3Pro-Cre have demonstrated efficient labeling of postmigratory neural crest derivatives, such as the enteric nervous system, the mesenchyme in pharyngeal arches, and cardiovascular structures. In contrast to the Wnt1-Cre line, however, P3Pro-Cre-mediated recombination appears to be less specific for neural crest lineage tracing as it also marks noncrest neuroepithelial cells and several mesodermal tissues, including cartilaginous portions of the ribs and a large part of the skeletal musculature (Jarad & Miner, 2009; Li et al., 2000; Liu et al., 2006; Lang et al. 2000).

Several Cre-driver lines have been generated that, unlike the Wnt1-Cre or P3Pro-Cre lines, express Cre-recombinase in neural crest cells not before they undergo an EMT in the dorsal neural tube, but only as the cells begin to migrate. For instance, transgenic Ht-PA-Cre mice express Cre under the control of a human tissue plasminogen activator (Ht-PA) promoter fragment specifically in migratory neural crest cells (Pietri, Eder, Blanche, Thiery, & Dufour, 2003). A detailed comparison with Wnt1-Cre/R26R mice revealed very efficient labeling of neural crest derivatives by Ht-PA-Cre, including neuronal, glial, melanocytic, and mesenchymal cell populations during development and in adult structures (Pietri et al., 2003; Wong et al., 2006). Ht-PA-Cre/R26R mice were also reported to label a fraction of nonneural epithelial cells lateral to cranial neural folds that were suggested to contribute to mesectodermal structures in the head (Breau, Pietri, Stemmler, Thiery, & Weston, 2008).

Additional Cre-driver lines mostly used to study cranial and cardiac neural crest development are the transgenic line Me2c-F10N-Cre (Aoto et al., 2015) and the AP2α-IRESCre line generated by knock-in of an IRESCre cassette into the 3′ untranslated region of the AP2α transcription factor locus (Macatee et al., 2003). A detailed analysis of Me2c-F10N-Cre/R26R embryos at different stages confirmed the neural crest origin of various neural and nonneural structures and emphasized the neural crest origin of olfactory ensheathing glial cells, cells in the meninges surrounding the forebrain, and cells of the choroid plexus. Apart from marking migratory neural crest cells only after delamination from the neural tube, Me2c-F10N-Cre/R26R presented a neural crest fate map highly similar to the one obtained with the Wnt1-Cre line, with the exception of some differences apparent in the calvarial bones (Aoto et al., 2015).

Tyrosinase (Tyr) is a key enzyme of the melanin biosynthetic pathway and, accordingly, a transgenic mouse line, in which Tyr enhancer and promoter elements drive Cre expression, was found to mark the melanocytic lineage, but not other neural crest derivatives (Delmas, Martinuzzi, Bourgeois, Holzenberger, & Larue, 2003). In contrast, an independently produced Tyr-Cre line termed TEC1 also marks neural crest lineages other than melanoblasts, such as craniofacial structures, dorsal root ganglia (DRG), and sympathetic cephalic ganglia, albeit at a seemingly low recombination efficiency and at a relatively late stage in neural crest development (embryonic day (E) 10.5 onwards) (Tonks et al., 2003). Whether this reflects activity of tyrosinase transcriptional elements in a subset of undifferentiated migratory neural crest cells or aberrant transgene expression remains to be determined.

Although P0 was originally identified as a marker of the peripheral glial lineage, the expression of P0-Cre in transgenic mice was not lineage restricted, but detected already in migratory neural crest cells (Yamauchi et al., 1999). Consequently, P0-Cre/R26R mice displayed β-galactosidase expression in multiple neural and nonneural tissues originating from the neural crest. However, at least in some structures such as the DRG, the recombination efficiency appeared to be considerably lower than the one achieved by Wnt1-Cre-mediated recombination. Furthermore, cranial neural crest cell populations are differentially marked in Wnt1-Cre vs. P0-Cre mice: Wnt1-Cre preferentially recombines midbrain as opposed to hindbrain neural crest cells, while hindbrain neural crest cells are efficiently targeted by P0-Cre (Chen et al., 2017). Intriguingly, fate-mapping experiments by means of the P0-Cre line also allowed the identification of neural crest-derived cells in structures previously not known to harbor such cells. Specifically, Nagoshi and colleagues demonstrated that neural crest cells give rise to vascular endothelial and smooth muscle cells present in the adult bone marrow, which was later confirmed in Wnt1-Cre mice (Nagoshi et al., 2008; Wislet-Gendebien et al., 2012). Likewise, between 5 and 15% of all hormone-producing cells in the anterior lobe of the pituitary turned out to originate from the neural crest based on P0-Cre lineage tracing (Uehara et al., 2017).
Yet another transgenic mouse line expressing Cre in neural crest cells only once they have engaged in migration is Sox10-Cre (Hari et al., 2012; Matsuoka et al., 2005). Fate mapping in Sox10-Cre/R26R embryos, combined with immunostaining for Cre protein, demonstrated targeting of approximately 78% of all Sox10-positive neural crest cells after their emigration from the neural tube (Hari et al., 2012). Thanks to this high recombination efficiency it was possible to address stage-specific functions of canonical Wnt signaling upon constitutive activation in premigratory neural crest (using Wnt1-Cre) vs. migratory neural crest cells (using Sox10-Cre). Furthermore, the Sox10-Cre line was used together with the Wnt1-Cre line to show an unexpected contribution of neural crest cells in the murine neck region to muscle connective tissue, cartilage and bone, including endochondral bones that were till then believed to exclusively originate from the mesoderm (Matsuoka et al., 2005). However, unlike Wnt1-Cre, Sox10-Cre activity leads to recombination of some adult structures that are not of neural crest origin, such as subpopulations of epithelial cells present in hair follicles (Figure 1).

3 | NEURAL CREST LINEAGE TRACING USING INDUCIBLE CRE-DRIVER LINES

The above-described Cre lines allow in vivo fate mapping or functional analysis of a gene of interest only at the first time the Cre-driving promoter is active. To temporally control Cre activity, inducible forms of Cre have been developed, for instance by fusion with a mutant ligand-binding domain of the human estrogen receptor (CreER) that bind to the synthetic estrogen receptor ligand 4-hydroxytamoxifen (4-OHT) or tamoxifen (TM), but not to endogenous estradiol (Feil et al., 1996). Subsequently, a refined version with a higher TM sensitivity was constructed (termed CreERT2) (Feil, Wagner, Metzger, & Chambon, 1997). Although some mouse lines expressing inducible forms of Cre display leakiness, CreER- and CreERT2-expressing lines have become very valuable tools to determine stage-specific roles of genes of interest, to carry out lineage tracing experiments at different stages of neural crest development, or to perform clonal analyzes of neural crest cells in vivo by choosing conditions enabling low recombination frequencies (Zurkirchen & Sommer, 2017).

Because Wnt1 is only expressed in premigratory neural crest, but not once neural crest cells have emigrated from the neural tube, Wnt1-CreER and Wnt1-FlpeERT2 mice (generated to study lineage relationships of cells at the mid/hindbrain boundary (Hunter, Awatramani, Farley, & Dymecki, 2005; Zervas et al., 2004)) (Table 1) are not suitable for neural crest lineage tracing at different time points. However, the Wnt1-CreER line has been successfully used in conjunction with the multicolor Cre-reporter allele R26R-Confetti (Snippert et al., 2010) to show that premigratory Wnt1-expressing neural crest cells are multipotent in vivo (Bagnoli et al., 2015). In this study, low dose TM treatment of pregnant mice led to low density recombination of the multicolor Cre-reporter in neural crest cells homozygous for R26R-Confetti, resulting in clones of cells expressing either nuclear green, cytoplasmic yellow, cytoplasmic red, membrane-bound blue, or rare combinations thereof. Intriguingly, around 20% of all the clones derived
from single Wnt1-CreER-traced neural crest cells not only contributed to multiple neural crest cell lineages, but also contained daughter cells in the dorsal neural tube. These data are consistent with the idea that at least some premigratory neural crest cells self-renew in vivo. The assumption that multipotent neural crest cells can self-renew during a given time window was further supported by clonal analysis of migratory neural crest cells, traced shortly after emigration by means of a Sox10-iCreERT2 driver line (Baggiolini et al., 2015; Simon, Lickert, Götz, & Dimou, 2012). By using R26R-Confetti as Cre-reporter in Sox10-iCreERT2 mice treated with low TM doses, the vast majority of migratory neural crest cells were shown to maintain multipotency, and the Sox10-iCreERT2-expressing cells did not display any higher degree of fate restriction as compared to their premigratory counterparts (Baggiolini et al., 2015). The only structure labeled by Wnt1-CreER—but not Sox10-iCreERT2—lineage tracing was the dorsal neural tube, confirming that Sox10-iCreERT2 is not expressed in the premigratory neural crest, but only once the cells start to migrate. R26R-Confetti-based clonal analysis of neural crest cells has also been performed with another, independently produced Sox10-iCreERT2 line (Kaukua et al., 2014). Such studies might also be possible with yet another independently generated Sox10-iCreERT2 line (McKenzie et al., 2014) or with a Sox10ERT2CreERT2 line termed SECE (He & Soriano, 2015). Of note, while high dose TM treatment in SECE/R26R embryos resulted in labeling of multiple neural and nonneural neural crest derivatives, low dose application of TM was found to affect the reporter gene expression pattern, allowing the tracing specifically of cranial as opposed to trunk neural crest cells (He & Soriano, 2015).

Another genetic tool suitable for neural crest cell fate mapping at different developmental stages is a Plp-CreERT2 mouse line generated by Leone and colleagues (Leone et al., 2003). Plp is a glia-specific...
marker, and the Plp gene regulatory elements used to drive CreERT2 expression in these mice were reported to direct specific transgene expression in oligodendrocytes and Schwann cells. Accordingly, TM-induced recombination during embryonic development and in adult mice led to very efficient labeling of peripheral glia in Plp-CreERT2/R26R double transgenic mice (approximately 80% upon TM injection of adult mice) (Leone et al., 2003). Moreover, using this line, an unexpectedly broad developmental potential of peripheral glial cells was revealed, with Plp-CreERT2-traced cells along peripheral nerves giving rise to cell types as diverse as melanocytes, parasympathetic neurons, mesenchymal cells in teeth, and chromaffin cells of the adrenal medulla (Adameyko et al., 2009; Dyachuk et al., 2014; Furlan et al., 2017; Kaukua et al., 2014; Petersen & Adameyko, 2017). The glial origin of at least some melanocytes was also suggested by fate mapping experiments using another inducible Plp-Cre-driver line, Plp-CreER (Doerflinger, Macklin, & Popko, 2003). Using this line, it was reported that melanocytes in hair follicles, but not in the interfollicular epidermis of the tail, originate from Plp-Cre-positive glial cells at E11.5 in the mouse (Deo, Huang, Fuchs, de Angelis, & Van Raamsdonk, 2013). The potential of peripheral glia to generate nonglial cell types was also demonstrated by alternative approaches, involving fate mapping with other lineage-specific Cre-driver lines or single cell RNA sequencing (Espinosa-Medina et al., 2014; Furlan et al., 2017; Uesaika, Nagashimada, & Enomoto, 2015). The latter allowed generation of “pseudo-time” lineage trajectories and revealed an intermediate cellular state in between the states defining Schwann cell precursors and differentiated nonglial cells, respectively (Furlan et al., 2017). Finally, the Plp-CreERT2 line (Leone et al., 2003) was instrumental to demonstrate by fate mapping that adult peripheral glia become activated upon skin wounding, detach from axons, and colonize the wound bed to support wound healing in a paracrine manner, without notable differentiation into other, nonglial cell types (Parfejevs et al., 2018).

However, inducible Cre activity in the Plp-CreERT2 line turned out not to be specific for the glial lineage (Hari et al., 2012; Leone et al., 2003). Indeed, in Plp-CreERT2/R26R embryos, TM treatment at early stages of neural crest development (E9.5) resulted in prominent labeling of peripheral neurons, glia, and melanocytes, i.e., a fate map highly reminiscent of multipotent neural crest cells (Hari et al., 2012). The expression pattern of β-galactosidase became gradually restricted upon TM injection at later stages. However, at all stages examined, the melanocytic lineage was marked with considerable efficiency upon Plp-CreERT2-mediated recombination. Likewise, induction of Plp-CreERT2-driven recombination in adult mice marked a substantial fraction of skin melanocytes, independently of the Cre-reporter allele used (Parfejevs et al., 2018). Some of this expression might be due to CreERT2 leakiness during early neural crest development, given that in adult skin of both Plp-CreERT2/R26R-tdTomato and Sox10-iCreERT2/R26R-tdTomato mice, about 25% of all hair follicles contain recombined melanocytes even in the absence of any TM treatment (Figure 2) (Parfejevs and Sommer, unpublished). However, TM injection in the adult significantly induced Cre-reporter expression in both peripheral glia and melanocytes in the skin (Parfejevs et al., 2018), demonstrating persistent activity of Plp-CreERT2 and Sox10-iCreERT2 in these adult tissues.

Thus, as with Sox10-CreERT2-expressing mice, different neural crest derivatives can be traced by means of the PLP-CreERT2-line, albeit with an apparently lower recombination efficiency. Recently, Kaucka and colleagues made use of this feature to carry out clonal analysis of cranial neural crest cells in Plp-CreERT2/R26R-Confetti embryos to confirm data obtained with a Sox10-iCreERT2 line (Kaucka et al., 2016).

In conclusion, distinct mouse lines are available for fate mapping premigratory and migratory neural crest cells. Together with Cre-driver lines specific for fate-restricted precursor cells that we have not covered in the present review, there is an increasing tool set available to the community to study the neural crest lineage tree and the molecular mechanisms shaping it. The finding that several Cre and CreERT2 driver lines expected to exhibit lineage-specific expression appear to mark multipotent neural crest cells (although with quite divergent recombination efficiencies) could simply reflect unfaithful transgene expression. Alternatively, however, migratory neural crest cells as well as, for instance, cells in peripheral nerves might comprise distinct subpopulations expressing supposedly lineage-specific markers together with multipotency markers. Conceivably, such cell populations may be more or less ready to respond to the activity of cues controlling fate decisions during development or upon injury. To address such issues, the genetic approaches for prospective lineage tracing of neural crest cells described herein will have to be complemented with other methods, notably including retrospective lineage tracing by single cell transcriptome analysis.

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