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Data Article

Transcriptome dataset of trunk neural crest cells migrating along the ventral pathway of chick embryos

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ABSTRACT

We present a transcriptome dataset generated from migratory chick trunk neural crest cells, which are destined to form components of the peripheral nervous system. Using the Sox10E1 enhancer, which specifically labels neural crest cells migrating on the trunk ventral pathway, we performed fluorescence activated cell sorting (FACS) of electroporated embryos to obtain a pure population of these cells for library preparation and Illumina sequencing. The results provide a list of genes that are enriched in the trunk neural crest. To validate the data, we performed *in situ* hybridization to visualize expression of selected transcripts.

Specifications table

Subject area	Biology
More specific subject area	Developmental Biology
Type of data	Figures, Graphs, Tables

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How data was acquired	Data was obtained with Illumina HiSeq. 2000. RNA in situ hybridization was used to validate gene expression
Data format	Analyzed
Experimental factors	Embryos were electroporated to label migrating neural crest cells, which were then isolated by fluorescent activated cell sorting (FACS).
Experimental features	Electroporated, sorted embryonic cells
Data source location	Pasadena, USA
Data accessibility	Data are within the article and provided as Supplementary files. Raw sequencing data have been deposited into the NCBI sequence read archive (SRA) under BIO Project PRJNA494045
Related research article	Simões-Costa et al. [1]

Value of the data

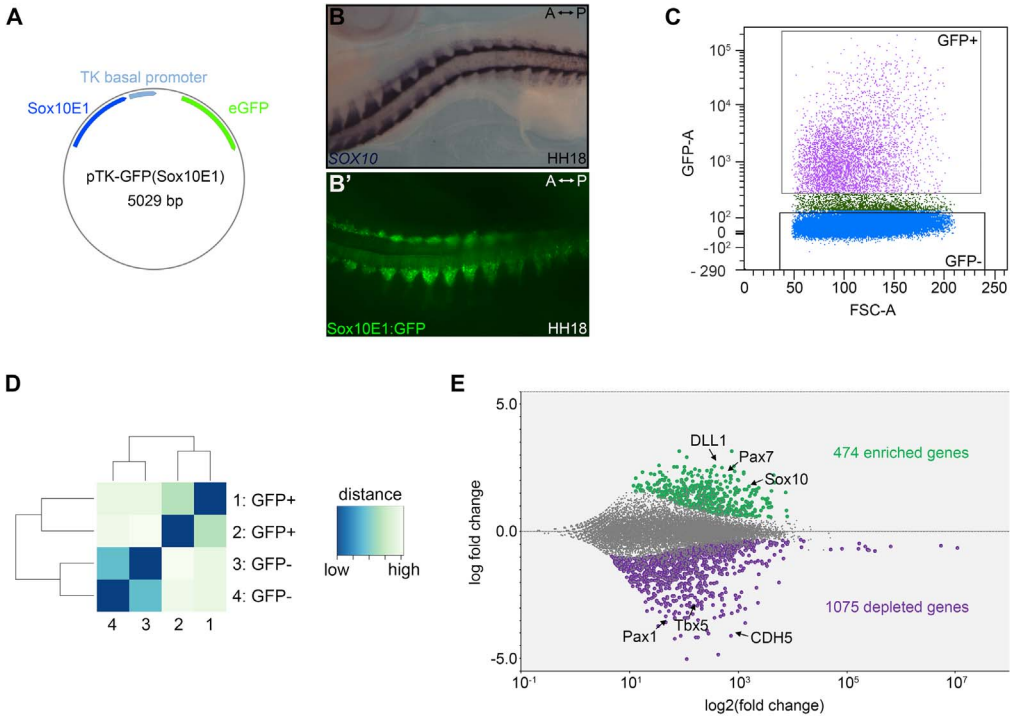
- The data will be useful for other researchers in the field to determine specific gene expression levels in the ventrally migrating trunk neural crest population.
- The data allow comparison with previously published datasets and provides gene expression patterns within subpopulations of neural crest cells.
- This screen could contain new targets for the treatment of diseases arising from the trunk neural crest (e.g. neuroblastoma, Hirschsprung disease).

1. Data

The neural crest is a multipotent embryonic cell population that gives rise to many different cell types in vertebrates. Initially specified in the dorsal neural tube, these cells soon detach by undergoing an epithelial to mesenchymal transition and start to migrate throughout the embryonic body. The developmental potential of neural crest cells differs according to their axial level of origin and the migratory route they follow. Hence, neural crest cells on each pathway exhibit distinct gene expression profiles [2].

Our group has utilized enhancer based cell sorting together with next generation sequencing to generate genome-wide expression profiles of specific neural crest subpopulations in the chicken embryo [1,3,4]. Here, we present the dataset we have generated from neural crest cells migrating along the trunk ventral pathway. To label these cells, we have used a GFP reporter driven by the Sox10E1 enhancer that is specifically active in neural crest cells following this pathway [5,6]. The cells were isolated by FACS and sequenced on an Illumina HiSeq platform. By comparing the sorted GFP+ cell population with unlabeled cells from the rest of the trunk region (GFP-), we have obtained a list of transcripts that are enriched in the neural crest.

The data show enrichment of genes associated with neurogenesis and cell migration as well as a number of signaling pathways including Wnts and BMPs (bone morphogenetic protein) (Fig. 1 and Supplementary lists). We have further validated the data by performing whole mount *in situ* hybridization of selected transcripts (Fig. 2, Fig. S1 and Table 1). For that purpose, we selected transcripts that already had known neural crest expression (e.g. *TFAP2A)*, transcripts that have not been analyzed in the neural crest but have been shown to play important roles in processes that may be relevant to neural crest migration or differentiation or are part of enriched signaling pathways (e.g. *DTX4*), and transcripts that have not been described in any related context so far (e.g. *AGPAT4*).



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Biological Process and Molecular Function	Genes
neurogenesis	129
cell migration	71
cytoskeletal protein binding	42
transcription factor activity	34
TM receptor protein tyrosine phosphatase activity	5

Signaling Pathway	Genes
Wnt signaling pathway	38
smoothened signaling pathway	13
BMP signaling pathway	13
semaphorin-plexin signaling pathway	5
Notch signaling pathway	11
Cadherin signaling pathway	11

Disease	Genes
Schizophrenia	79
Neuroblastoma	73
Hirschsprung Disease	15
Craniofacial Abnormalities	17

Fig. 1. Generation of a transcriptome dataset from the trunk neural crest. Plasmid map of pTK-GFP (Sox10E1) (A). Dorsal view of HH18 embryo (embryonic day 3) subjected to *in situ* hybridization for *SOX10* (B) or electroporated with Sox10E1-driven GFP (B'). The trunk region from the wing bud until the leg bud level was dissected for FACS. Neural crest cells (GFP+) and GFP negative cells from surrounding tissues (GFP-) were sorted based on GFP fluorescence and the two populations were compared with RNA-seq. Plot showing GFP+ and GFP- subpopulations selected based on GFP fluorescence activated cell sorting (C). Heatmap of the distance between RNA-seq datasets obtained for duplicate samples of GFP positive (+) and GFP negative (-) trunk cells (D). MA-plot showing differential expression data for all genes. Dots representing enriched genes are green and depleted genes are magenta (E). List of all enriched and depleted genes can be found in Supplementary data. Functional classification terms of genes enriched in trunk neural crest cells (F).

2. Experimental design, materials, and methods

2.1. Embryos

Fertilized chicken eggs were obtained from McIntyre Poultry & Eggs (Lakeside, CA) and incubated at 37 °C until embryos reached the desired developmental stage. Embryos were harvested in Ringer's solution and staged according to the criteria of Hamburger and Hamilton [7]. Embryos for *in situ* hybridization were fixed in 4% paraformaldehyde (PFA) overnight at 4 °C, washed in phosphate buffered saline (PBS), dehydrated in a methanol series and stored at -20 °C until further processing.

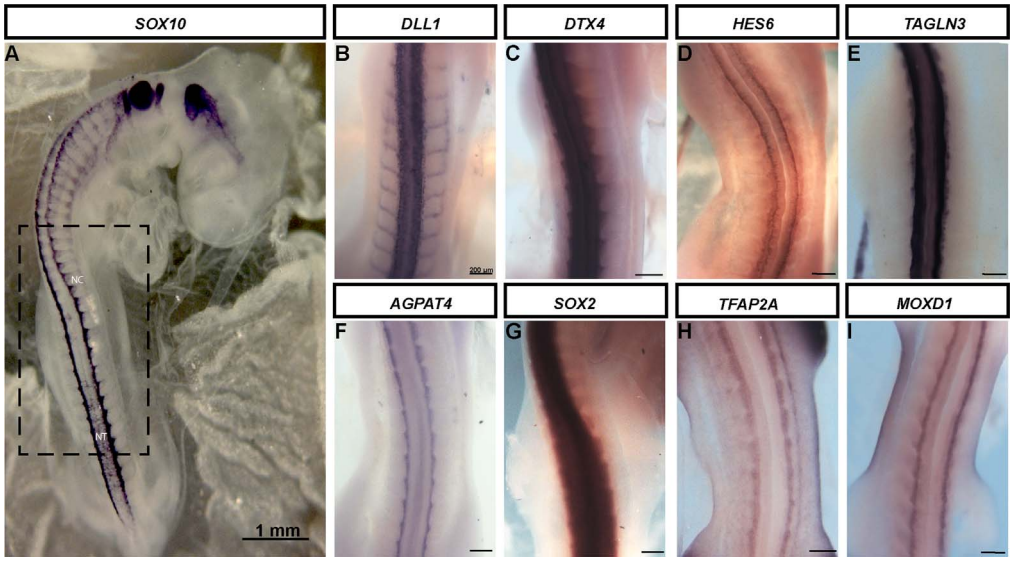

Fig. 2. *in situ* verification of transcripts enriched in the trunk neural crest. Whole mount *in situ* hybridized embryos showing trunk neural crest expression of selected transcripts enriched in the transcriptome dataset. Overview of a whole chick embryo at stage HH18 showing expression of *SOX10* (A). "NC" indicates migrating neural crest cells; "NT" indicates dorsal neural tube and refers to premigratory neural crest cells. Dashed lines mark the region of the trunk that is magnified in B-I showing embryos hybridized with the following probes: *DLL1* at stage HH17 (B); *DTX4* at stage HH18 (C), *HES6* at stage HH 18 (D), *TAGLN3* at stage HH18 (E), *AGPAT4* at stage HH18 (F), *SOX2* at stage HH19 (G), *TFAP2A* at stage HH19 (H), *MOXD1* at stage HH19 (I). The difference in embryonic development shown for individual probes reflects the different time points where each transcript was expressed at highest levels/most distinguishable.

2.2. Electroporation

Embryos were electroporated *in ovo* as previously described [8]. A pTKGFP vector containing the Sox10E1 enhancer sequence (described in 6) was injected at 2 µg/µl into the neural tube lumen of HH13–14 (2 days old) embryos, followed by electroporation with platinum wire electrodes for 3 pulses of 20 V for 30 ms, with an interval of 100 ms between pulses. This was followed by another round of electroporation with reversed polarity to achieve DNA uptake on both sides of the neural tube. Eggs were then sealed and reincubated until the embryos reached stage HH18–19 (3 days).

2.3. Cell dissociation and FACS

Electroporated embryos that exhibited robust GFP expression were harvested and washed in sterile PBS. Using a fluorescent stereoscope, the trunk was cut from the level of the wing bud until the leg buds. Tissue samples were pooled together and cells were dissociated with Accumax (Accutase SCR006). Clumps of cells were removed by passing through a 40 µm cell strainer (BD Biosciences). GFP+ and GFP- cells were sorted using a BD FACS ARIA Cell Sorter (BD Bioscience). The 7- AAD viability dye (Thermo Fisher) was used to exclude dead and damaged cells. We manually choose the cut off for sorting the GFP+ cell population at stringent conditions to minimize contaminations with false positive auto-fluorecent cells (e.g. blood cells).

2.4. Library preparation and sequencing

We used the RNAqueous Micro kit (Ambion) to isolate RNA from ~50,000 sorted cells per replicate from each GFP+ and GFP- populations. RNA quality was assayed in an Agilent 2100 Bioanalyzer, and only

Table 1
Transcripts analyzed by *in situ* hybridization.

Gene name	Gene ID	P-adj	log2(FC)	in situ
DLL1	ENSGALG00000011182	7,04122E-22	2,546658444	NC
SOX2	ENSGALG00000043460	8,32583E-15	2,275654915	NC
AGPAT4	ENSGALG00000011571	0,007149007	1,345032181	NC
ANKFN1	ENSGALG00000003105	5,59869E-08	1,959835002	NC
HES5	ENSGALG00000001141	0,007426558	1,556181559	NC
TAGLN3	ENSGALG00000015379	0,012212793	1,62649238	NC
DTX4	ENSGALG00000010835	1,43041E-09	1,833313017	NC
PAX7	ENSGALG00000043204	8,07397E-16	2,169404336	NC
HES6	ENSGALG00000028415	2,331E-16	2,309646573	NC
DRAXIN	ENSGALG00000004631	8,07397E-16	2,409119463	NC
PAX3	ENSGALG00000030944	3,09434E-20	2,046728588	NC
SOX10	ENSGALG00000012290	2,24326E-14	1,64523744	NC
MOXD1	ENSGALG00000002911	0,000159845	1,01172748	NC
TFAP2A	ENSGALG00000012775	0,041892446	0,860921861	NC
SCRT2	ENSGALG00000028912	2,78475E-08	2,52286606	NC
RHBDL3	ENSGALG00000003426	4,77513E-13	2,36586812	NC
GDF10	ENSGALG00000005985	1,93411E-07	2,296980425	NC
HS3ST6	ENSGALG00000005413	0,015486417	1,570936264	NC
PROX1	ENSGALG00000009791	6,35622E-10	2,114807597	NT
NOTCH1	ENSGALG00000002375	2,74837E-15	1,572570986	NC
DBX1	ENSGALG00000003965	2,78759E-44	3,150177224	NT
ISM1	ENSGALG00000009042	1,62958E-12	2,913884059	NC
SOX3	ENSGALG00000040383	3,87094E-10	2,221184752	NT
FAM222A	ENSGALG00000029944	0,001777813	1,799084864	NT
RFX4	ENSGALG00000012647	1,26809E-14	2,027190365	NT
SOX13	ENSGALG00000000583	7,59841E-09	1,93016488	NT
PAX6	ENSGALG00000012123	4,70928E-23	2,502697167	NT
WNT4	ENSGALG00000041708	3,74752E-30	2,544362001	NT
CHL1	ENSGALG00000037856	4,29E-21	2,35611689	NT
FZD3	ENSGALG00000042308	2,12E-13	1,847532482	NT
LRRC4C	ENSGALG00000007948	2,28E-07	1,693090154	NT
PTPRN2	ENSGALG00000030054	2,10E-10	1,817486577	NT

Summary of all transcripts that were verified by *in situ* hybridization. Abbreviations: NT: dorsal neural tube (premigratory neural crest); NC: migrating neural crest.

samples with an RNA integrity number (RIN) >8 were further processed. Concentration of the samples was measured with a Qubit fluorometer. 50 ng of RNA were used for RNA amplification and cDNA synthesis with the Ovation RNA-Seq System V2 (NuGEN). 2 µg of cDNA were then used for generating SR50 datasets with at least 40 million reads depth per sample in a HiSeq. 2000 Illumina instrument. 2 biological replicates were used for each condition (GFP+ and GFP-).

2.5. RNA-seq analysis

Sequenced reads were aligned to the *Gallus gallus* genome (galGal5.91) with HISAT2 [9], counted with featureCounts [10] and compared with DESeq. 2 [11] to identify differentially expressed transcripts. Statistical significance of genes that are enriched in the neural crest population is based on a 0.05 false discovery rate (Benjamini-Hochberg method). Functional classification of genes enriched in the neural crest was performed with the ToppGene suite [12] and data from the Gene Ontology Consortium [13], PantherDB [14], and DisGeNET [15].

2.6. Probe preparation and in situ hybridization

Dioxigenin labeled antisense probes were generated by *in vitro* transcription using Promega RNA Polymerases. Probes were either generated from linearized template DNAs or directly amplified by PCR and addition of T7 recognition sites to the antisense primers. The following probes were used:

DLL1: described in [16];
HES6: linearized template made from ChEST clone 62d7;
SOX2: described in [17];
TFAP2A: linearized template made from ChEST clone 401g22;
MOXD1: described in [18].

The following probes were amplified by PCR using gene specific primers:

DTX4: Fwd: CATCGGCTTCTGCTACGTGA, Rev: taatacgactcactataggAGACCAGTCGGGATGTACCA;
AGPAT4: Fwd: TGGACATCGTTGGCTTTCTGA, Rev: taatacgactcactataggATAGGCACTGCTGGGTAGGT
TAGLN3: Fwd: GGCAAGCATTAGAGATGGCT, Rev: taatacgactcactataggCGCCTCAGAGCACTAACTAT

Whole mount RNA *in situ* hybridization was performed as described previously [19]. Post hybridization washes were carried out using MABT (maleic acid buffer containing 0.1% Tween-20) and target-specific probe binding was visualized using NBT/BCIP (nitro-blue tetrazolium chloride/ 5-bromo-4-chloro-3'-indolylphosphate p-toluidine salt) as the color substrate. The time to develop the color reaction was probe specific and varied from 2 h to several days. A mixture of embryos at developmental stages between HH16 and HH20 was used for each probe to assess the spatio-temporal gene expression pattern of each transcript during the course of migration (e.g. from premigratory/delaminating to migratory and coalescence of dorsal root ganglia).

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.11.109.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.11.109.

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