**Supplementary Table 3. Markers used to define the scRNAseq cell clusters**

|  |  |  |
| --- | --- | --- |
| **Cluster #** | **Cluster Identification** | **Cluster Marker, reference** |
| 1 | r3-r7 mitotic progenitors (rhombomere 3 – rhombomere 7 mitotic progenitors) | *Rest*[*4*](https://sciwheel.com/work/citation?ids=10399356&pre=&suf=&sa=0)  *Id3*[*5*](https://sciwheel.com/work/citation?ids=4515131&pre=&suf=&sa=0)  *Hoxb1*[*6*](https://sciwheel.com/work/citation?ids=1960&pre=&suf=&sa=0)  *Isl1*[*7*](https://sciwheel.com/work/citation?ids=9716465&pre=&suf=&sa=0)  *Nr2f1*[*8*](https://sciwheel.com/work/citation?ids=1140743&pre=&suf=&sa=0)  *Nr2f2* (this study)  *Top2a*[*9*](https://sciwheel.com/work/citation?ids=5646976&pre=&suf=&sa=0) |
| 2 | Mitotic r4MN progenitors (mitotic rhombomere 4-derived motor neuron progenitors) | *Hoxb1*[*6*](https://sciwheel.com/work/citation?ids=1960&pre=&suf=&sa=0)  *Isl1*[*7*](https://sciwheel.com/work/citation?ids=9716465&pre=&suf=&sa=0)  *Nr2f1*[*8*](https://sciwheel.com/work/citation?ids=1140743&pre=&suf=&sa=0)  *Nr2f2* (this study)  *Top2a*[*9*](https://sciwheel.com/work/citation?ids=5646976&pre=&suf=&sa=0) |
| 3 | r4MN precursors (rhombomere 4-derived motor neuron precursors) | *Hoxb1*[*6*](https://sciwheel.com/work/citation?ids=1960&pre=&suf=&sa=0)  *Isl1*[*7*](https://sciwheel.com/work/citation?ids=9716465&pre=&suf=&sa=0)  *Nr2f1*[*8*](https://sciwheel.com/work/citation?ids=1140743&pre=&suf=&sa=0)  *Neurod4*[*10*](https://sciwheel.com/work/citation?ids=14184588&pre=&suf=&sa=0)  *Mybpc1* (this study) |
| 4 | Biopotent bipotent r4MNs (biopotent rhombomere 4-derived motor neurons) | *Isl1*[*7*](https://sciwheel.com/work/citation?ids=9716465&pre=&suf=&sa=0)  *Nr2f1*[*8*](https://sciwheel.com/work/citation?ids=1140743&pre=&suf=&sa=0)  *Nr2f2* (this study) |
| 5 | IEEs (inner ear efferent neurons) | *Gata2*[*11*](https://sciwheel.com/work/citation?ids=889128&pre=&suf=&sa=0)  *Gata3*[*12*](https://sciwheel.com/work/citation?ids=436349&pre=&suf=&sa=0)  *Gad2* (this study)  *Chm2* (this study)  *Il1rap* (this study)  *Gpr149* (this study) |
| 6 | FBMNs (facial branchial motor neurons) | *Lingo2*[*13*](https://sciwheel.com/work/citation?ids=330601&pre=&suf=&sa=0)  *Tmeff2*[*14*](https://sciwheel.com/work/citation?ids=14184592&pre=&suf=&sa=0)  *Alk* (this study)  *Shox2*[*15*](https://sciwheel.com/work/citation?ids=10519022&pre=&suf=&sa=0)  *Cdh8*[*16*](https://sciwheel.com/work/citation?ids=320092&pre=&suf=&sa=0)  *Slc44a5* (this study)  *Cbln2* (this study)  *Zfpm2*[*17*](https://sciwheel.com/work/citation?ids=14184595&pre=&suf=&sa=0)  *Syt4* (this study) |
| 7 | CN V, CN X, CN XII precursors (trigeminal, ambiguous, hypoglossal nuclei) | *Hoxa3*[*18*](https://sciwheel.com/work/citation?ids=5286512&pre=&suf=&sa=0) |
| 8 | CN V, CN X, CN XII (trigeminal, ambiguous, hypoglossal nuclei) | *Hoxa3*[*18*](https://sciwheel.com/work/citation?ids=5286512&pre=&suf=&sa=0)  *Dgkb* (this study)  *Kcnip4* (this study)  *Blc2* (this study)  *Kcna5* (this study)  *Runx1*[*19*](https://sciwheel.com/work/citation?ids=8088442&pre=&suf=&sa=0) |
| 9 | FVMNs (facial visceral motor neurons) | *Mgat4c* (this study)  *Irx1* (this study)  *Irx2* (this study) |
| 10 | CN V (trigeminal motor nucleus) | *Sema3d* (this study)  *Sox1* (this study) |
| 11 | CN VI (abducens) | *Mnx1*[*20*](https://sciwheel.com/work/citation?ids=10519024&pre=&suf=&sa=0)  *Rasgef1c* (this study)  *Isl2*[*21*](https://sciwheel.com/work/citation?ids=1007001&pre=&suf=&sa=0) |
| 12,13 | Vestibuloacoustic ganglion precursors and ganglion | *Neurod1*[*22*](https://sciwheel.com/work/citation?ids=12686672&pre=&suf=&sa=0)  *Sct* (this study)  *Tlx3*[*22*](https://sciwheel.com/work/citation?ids=12686672&pre=&suf=&sa=0)  *Tlx2*[*22*](https://sciwheel.com/work/citation?ids=12686672&pre=&suf=&sa=0)  *Six1*[*23*](https://sciwheel.com/work/citation?ids=14184596&pre=&suf=&sa=0) |
| 14 | Dorsal hindbrain interneurons | *Nrn1* (this study)  *Slc17a6*[*24*](https://sciwheel.com/work/citation?ids=14184597&pre=&suf=&sa=0)  *Prrxl1*[*25*](https://sciwheel.com/work/citation?ids=14184598&pre=&suf=&sa=0)  *Lbx1*[*26*](https://sciwheel.com/work/citation?ids=885383&pre=&suf=&sa=0) |
| 15 | Neural crest | *Gpc3* (this study)  *Twist1*[*27*](https://sciwheel.com/work/citation?ids=7039461&pre=&suf=&sa=0) |
| 16 | Hair cell precursors? | *Krt8*[*28*](https://sciwheel.com/work/citation?ids=3287491&pre=&suf=&sa=0)  *Clnd7*[*28*](https://sciwheel.com/work/citation?ids=3287491&pre=&suf=&sa=0)  *Epcam*[*29*](https://sciwheel.com/work/citation?ids=9745456&pre=&suf=&sa=0) |
| n/a | n/a | *Dnajb8*[*30*](https://sciwheel.com/work/citation?ids=14184602&pre=&suf=&sa=0) |

**Supplementary Table 4. Primer and Oligo Sequences**

|  |  |  |
| --- | --- | --- |
|  | **Forward Primer** | **Reverse Primer** |
| **Screening (Sanger)** | | |
| Chr3 cRE2 conserved region | GGCAACAGGAAGGAAGCAGA | CTGCATCTTGAGGCTCGGG |
| **Screening (ddPCR)** | | |
| DNAJB8 probe: CTGTCCCAGAACTCAA | ACCGGCTACACCTTCCGTAAC | CCACCACGGTCACTATTGAATG |
| Chr3 conserved region probe: AGCCACAGCTGGCCA | CTGTGCTAAGCAGACCCACTTG | GCCCCCACTACAGCATTAACC |
| hTERT | TaqMan™ Copy Number Reference Assay, human, TERT | Cat# 4403316 |
| RNaseP | TaqMan™ Copy Number Reference Assay, human, RNase P | Cat# 4403326 |
| **Breakpoint spanning PCR primers** | | |
| Fam1 | GCTCAGTCTGAAGCCCATTG | TCTCCCTGGGATGTTCTGTC |
| Fam2 | TGCAAACAGAGGGAAGAAGG | GGCCTGTTTCTCACAAGGAG |
| Fam10 | TCCTCAGTTACTCGAAGCCTC | GAAGAGTGGCTAGGAGGTCAG |
| Fam11 | GAAGAGTGGCTAGGAGGTCAG | ATGGAATGTTGTGCTGGTCC |
| Fam12 | CTGGCCTTTAAGTCCTGAGC | GTGCTGTGGGGCTTGTATTC |
| **Haplotyping primers** | | |
| **Fam2** | | |
| Chr3:128246197, 128246228 | TCCCAATGAGAGATGGCCTA | GGGGTGATAAGAGGGGTGAT |
| Chr3:128251841, 128251885 | GGTCTTGGTCCAAATACCACA | CACCACCATGCTTGGCTAAT |
| Chr3:128253165, 128253309, 128253509 | GGAGCCTACACTTCAGACCAA | AAGAGCTTGAGGGGGAGGT |
| Chr3:128257374, 128257544 | TGCAATGCCTAGAAGACCTG | TGACTTGTTGGGTAGGTGGT |
| Chr3:128316909, 128316965 | ACCAACCGGTGACAGATGTT | AGCTGACTGGGGATCTGTGA |
| Chr3:128055511 | CCATGTCTCCAACCTGCTG | TGTGGGTAGTCAGGGAGGAC |
| Chr3:128168598 | CACCCACCTATGCAGGTTCT | ATCAGACTGGGATGGGAGTG |
| **Fam7 and Fam8** | | |
| chr3:127256437 | CCTGAGGGTTGGGAATGGAA | GCATGACCACAGGAACGATG |
| chr3:127291274 | AGTTGGCTGTGTCCTGGATG | AGCACTGGGATCATAAGGCA |
| chr3:127514425 | CATGTTCTCACAAGTGGGAGC | TTGTGCAATGACCCATATTCCT |
| chr3:127881362 | CTGGAGTTGCTTGCCTTCAC | TTGCCTCAAGAAGCCAGAGT |
| chr3:127942573 | CCTGAACTGGTAGCTGGTAGC | AGGGATAAGCCAACCTATTT |
| chr3:128034859, 128034860 | GAAGTCCCTGCACACTGTCA | TGAATAAATACCACGCTCTTTGA |
| chr3:128185191 | AAAGCTTCTGTTGGGCCATG | CCTCCTATCCCATCACCACC |
| chr3:128191414 | GCCTCCACTCTCCTAACTCC | GTTACTGGCAGAGCTCACCT |
| chr3:128205486 | AAGATCAGGGTAGGCAGAGC | ACGAGGTGGACGTCTTCTTC |
| chr3:128510561 | ATTCACCTTCCTTGGCCTCC | AGTGGCAGGTCATCAGCAG |
| chr3:128739889 | CTGAGATCACGCCACTGCAT | CCACCCTTCCAGTCACTGAG |
| chr3:129072890 | AGGGAGTGTGGAGAGTCAGG | TCGAAAGCACTCCAAGGTCA |
| **In situ probes (**T7 RNA polymerase recognition sequence bolded) | | |
| *Dnajb8* | GGGGACTGGACCCTTTTTCC | **GCGTAATACGACTCACTATAGGG**TCGCTTGGTGGTCACCTTAC |
| *Gata2* | CCAGGATGGGTGGAACATAC | **GCGTAATACGACTCACTATAGGG**GACCCAAGAACCACTCAAA |
| *Isl1* | CACTGTGGACATTACTCCCTC | **GCGCTAATACGACTCACTATAGGG**AACATCTGAATGAATGTTCCTCATGCC |
| **EMSA probes** | | |
| pWT | ACAGAGGTCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAGTTAGTC | GACTAACTTCATCAAAGCCCCCACTACAGCATTAACCACCTGACCTCTGT |
| p3 | ACAGACGTCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAGTTAGTC | GACTAACTTCATCAAAGCCCCCACTACAGCATTAACCACCTGACCTCTGT |
| p4 | ACAGAGATCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAGTTAGTC | GACTAACTTCATCAAAGCCCCCACTACAGCATTAACCACCTGATCTCTGT |
| p5 | ACAGAGGCCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAGTTAGTC | GACTAACTTCATCAAAGCCCCCACTACAGCATTAACCACCTGGCCTCTGT |
| p7-8 | ACAGAGGTCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAGGTTAGTC | GACTAACCTCATCAAAGCCCCCACTACAGCATTAACCACCTGACCTCTGT |
| p9 | ACAGAGGTCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAATTAGTC | GACTAATTTCATCAAAGCCCCCACTACAGCATTAACCACCTGACCTCTGT |
| p5-p9 | ACAGAGATCAGGTGGTTAATGCTGTAGTGGGGGCTTTGATGAAATTAGTC | GACTAATTTCATCAAAGCCCCCACTACAGCATTAACCACCTGGCCTCTGT |
| **LacZ mutagenesis and sequencing primers** | | |
| cRE1 | ATGCTTGCTGGGATAAGTTAGAGG | AGCTTTGTGGAACACTCAGGAGT |
| cRE2 | CAGGCTTTCACAGAATCCTTCTGG | CTGCCTTTAAAATGGTGGTGATGA |
| cRE3 | AGAGACAAAGGACCTAGTATGAGA | GATGTACCTGGGTTGGGATGG |
| cRE2\*A site-directed mutagenesis primer | CAGCATTAACCACCTGGTGTCTGTCCC | GGGACAGACACCAGGTGGTTAATGCTG |
| cRE2\*B site-directed mutagenesis primer | CCTGGGACTAATCTAATCAAAGCCCCC | GGGGGCTTTGATTAGATTAGTCCCAGG |
| ***IslMN-GFP* genotyping** | | |
|  | TATATCATGGCCGACAAGCA | TCAGGAGAGCACACACTTGC |
| ***Gata2KO/flox* genotyping** | | |
| Gata2 flox allele | CCAGGATGGGTGGAACATAC | GAAGGACCCCAAGAACACAA |
| Gata2 KO allele | TGCAACTGGAGACAGCAACT | GAAGGACCCCAAGAACACAA |
| ***Gata3TLZ/flox* genotyping** | | |
| *Gata3* wildtype allele | CAGGAGTCCGCGGACCTCC | CGTTGAGGACCGCGGGGTG |
| *Gata3* TLZ allele | CAGGAGTCCGCGGACCTC C | TGCCTTTACTGACCATGCGAG |
| *Gata3* flox allele | [GTGCAGCAGAGCAGGAAACTCTCAC](https://urldefense.com/v3/__https:/www.thermofisher.com/order/catalog/product/10336022?tsid=Email_POE_OC_OrderConfirm*20*20_SKULINK__;JSU!!NZvER7FxgEiBAiR_!5C58Qhs9E9aDm4MjsuhYt9b71EF3i3y9Dx3HqH58CIFyJeSL3XY99yejhj37nCvWnskVvKr3fYSxQg$) | [CAGTCTCTGGTATTGATCTGCTTCTT](https://urldefense.com/v3/__https:/www.thermofisher.com/order/catalog/product/10336022?tsid=Email_POE_OC_OrderConfirm*20*20_SKULINK__;JSU!!NZvER7FxgEiBAiR_!5C58Qhs9E9aDm4MjsuhYt9b71EF3i3y9Dx3HqH58CIFyJeSL3XY99yejhj37nCvWnskVvKr3fYSxQg$) |
| ***Phox2bCre* genotyping** | | |
|  | CACCGTCTCCACATCCATC | CGGTTATTCAACTTGCACCA |
| ***Fam5SNV* mouse genotyping** |  |  |
| Taqman assay, ThermoFisher | Assay ID = AN7DWV6 | Order assay using the assay ID |
| ***cRE1dup* mouse genotyping** | | |
| For *cRE1dup* wild type allele | ACCCTTTCCTCTGACCCTGT | CCTGCCGAGGAAAGAGGCTG |
| For *cRE1dup* mutant allele | GTGAGGGGTGGAGATGGAG | GGGGAGGTTGTGCAGTAGG |