Supplemental data (Three figures and four tables)

**Fig. S1** Brief flow chart of the primer design

 

**Table S1 Number of genes, exons**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | chicken | turkey | zebra finch | flycatcher |
| Number of genes | 15,262 | 11,610 | 12,622 | 14,264 |
| Number of exons | 158,376 | 150,092 | 144,565 | 153,091 |
| Number of common exons | 59,274 |
| Number of common exons with a　downstream intron | 53,381 |
| Number of common exons with under 1kbp downstream intron | 25,123 |
| Number of common exons with under 1kbp-downstream-polymorphic intron | 19,821 |
| Percentages of exons (exon sets) adopted for EPIC analysis (%) | 12.52 | 13.21 | 13.71 | 12.95 |

**Table S2**　Designed primers for PCR experiment

|  |  |  |
| --- | --- | --- |
| Primer name | Forward | Reverse |
| TEAD3\* | CTCGAGTACTCYGCCTTCATGGAGG | CAAAACTTGACGAGRAAGAAGGAGT |
| TBC1D22B\* | CAGTTTGCCTTTCGYTGGATGAACA | GATCTCCTTCCGCCACTTGATCARG |
| CACNA1S\_1 | CAGGGATGTGGTGAARTCCAAGTTC | TGTTCATGCCCTCAATTTCATACAR |
| CACNA1S\_2 | TGAAGATCCTSCGGGTGCTGAG | AGGAGCCTTTGTGCAGGAAAGCA |
| KIF21B\_1 | GAGTTCAAAGTCAGCGCCCARTTC | ACGTGTCCARGTCAAAGACAAAGTC |
| KIF21B\_2 | CCACBGCCAGCACCCAGATGAA | ATTTTGATGTTGGATTTGCGGTGRC |
| KIF21B\_3 | CAAGTTTCACTTTGTGGACCTGG | ATCTGGGTGCTGGCVGTGGTGC |
| IPO9\_1 | TCCTKCTTTACATGCAGATCACAGA | TCRGAATCCACAGGATCCTCCACCT |
| IPO9\_2 | GTTGTTGCTTCTCTTGCCCARGATA | GGGTGCTCTCRGAGATCTTCAGCTG |
| IPO9\_3 | CAGTGCAGCYTGAACACAGATGACA | CAGGCTTCTATYTGAGCTAGCTCCT |
| IPO9\_4 | RGATGCATTAAAGGACCCTCTTTAC | ATCTGAYAAGAACTGTCCTGCTAAG |
| TMEM183A | GCTTTTCTGGTGCAAAAAGATTGWA | TGAAGTTRAAGGTGGTGATCTGCA |
| DENND2D | GATGGCAGCAGGAAGATTGGYTACT | CRGGGAAGCAGAAGAGGGGGATGG |
| LOC395100\* | CTCCAAGGGCAGCTGYTGTGGAG | CYTCCCAGCTGATCCAAAGGTCCC |
| ADIPOR1\_1 | CTGGCGTGTGATCCCVTACGATGT | ACACRAACTCTTCCATCTTCTCCAT |
| ADIPOR1\_2 |  CACTCAGAGAAGGTCTCRCGGACTT | GTABGGGATCACACGCCAGCGY |
| AHCYL1\_1\* | TACGTCACVGAGATCGACCCCATCT | TTCRCTCAGCTTCACCACCCGAAAT |
| AHCYL1\_2\* | AACATCTACTCCACCCAGAACGARG | ACCACCAGAARTCATCCTCTGACTC |
| ETV7 | GAGAAGATGTCMCGAGCGCTCAGAC | CTCCCACYTGATGTAGGGCTCGTAG |
| STK38 | AGAGACCTGCTGCRATTTCAATAGA | ACTCTTTATTTCCTCCACACCAGAT |
| PHTF1\_1\* | TGTTTTTCTTYATGATGTGTGTTGC | GACTGAARAGCTTGGCAAACAAAA |
| PHTF1\_2\* | GAATGACTGYAAGAAGGTGGACATG | GCAACACACATCATRAAGAAAAACA |
| RSBN1\_1\* | TCCCMACAGCTGATATGCCAAAATC | GGTCCGAGGTAGGTACTGSAGRT |
| RSBN1\_2 | GTACTGCTGTGGMACCTACAGAG | TATCAGCTGTKGGGATCATCTGCTC |
| HIPK1\_1 | AGGGACYGACATGTTGGCAGAGAAG | CGCCATATCATCCAAACAGTTGAAT |
| HIPK1\_2 | CAAGCACAAAYCTGACAATGAGCTT | CTTCTCTGCCAACATGTCRGTCCCT |
| HIPK1\_3 | ATTCYACTGCTGCTGCTACTCTTTC | TTTGTGCTTGTRTTAGGAGCAACGT |
| HIPK1\_4 | AATGCTGTCCCAATTGTRCCACAAG | AAGAGTAGCAGCAGCAGTRGAATTG |
| AMPD1\* | AGAAATGAGCCAAAGGACCTGCCYT | TCTCCTCCATCTCATTKAGCATTTC |
| CSDE1\* | CTTCKGATCGCCGAACTGGAAAACC | CTCTCTAAATTGTGAGGAACAGCAC |
| PPARD | CAAGCACATCTACAAYGCCTACCTG | ARCGAGAGGCATTTCTGGAAGCG |
| PDXDC1\* | TTAGAAAAGGGATACAGGAAGCACA | GAAKGGAGAAAACCAGTTCAGCACA |
| RRN3\_1 | CGAGAGGTAGTGGAAGAATATCTGG | TTCATGCAGCCARTTAATGATCTGG |
| RRN3\_2 | TCAGAGAAGATGATGTGGAYATTTCA | CCAGATATTCTTCCACTACCTCTCG |
| RRN3\_3 | ATCTGCAGAGTTTRAATTTTGAGCG | GCCCATGGAGAGCTACATCACARTA |
| GSPT1\_1 | AATGATGGAAGAGGAGGAGGAAATR | TGAAATTTCCATGTTAACAGCAGCA |
| GSPT1\_2 | TGAAACTGGATTTGARAAAGGTGGA | CCAGGAGCATCTARAATAGTGAAGT |
| GSPT1\_3 | GCCAAACTTCAACCGTTCAGYTGAT | TGTTCTTTAAGGTTTGCTCCAGTSA |
| GSPT1\_4 | CAAAGGACARCAGCTTGTGATGATG | CCATCARCTGAACGGTTGAAGTTTG |
| GSPT1\_5 | ATTGAGCACAAATCCATTATCTGCC | ATCAGAAAGRATTCCAAGAACTTCCA |
| GSPT1\_6 | AGATCAAGTMTGCATTGCCCGTTTA | GGCAGATAATGGATTTGTGCTCAAT |
| PARN | TCCAAAACCCTTTAACAGAARTTCACC | TTAAAAGTGCAAAGGCCAAACTGAA |
| BFAR\* | YCCAGGAAAATCACTCTTTCTT | AAGGAGAATAATTCCAGAACAGAGAG |
| CCZ1\_1 | ACCTTTAGYCCAACAAAACCTGCAA | ACTTCATTTGGGTGATAGAAGAGAA |
| CCZ1\_2 | TGGAAAGCCAGTCTATGAATAYCAGG | YTCTTCAGGTTCATGGAAAAACTGYC |
| OCM | TATTTCCTTCAGAGGTTTGAGTGTG | CCTTGAGCTGGCTRCTACTCTTTTT |
| LOC396531 | AAAGTTTTTGGRATCCTTGATCAGG | AGCTGGAGAGAGCAGATTCAATATC |
| BAIAP2L1 | ATCMGAAGGAAAAGCCAAGGAGCAC | GGTCTGTTTTCTTCTCCAGTTCAGA |
| TMEM130 | CAGATTACCAAGGARCCCTTTGTCT | TCTGCTGTCTTCTATCTGGGCWATG |
| FAM20C\_1\* | ACATGGATCGACATCACTAYGAAACC | AAGGCACCAATATGGAAAGTTCATC |
| FAM20C\_2\* | AGGAAAACCTGGAGAAACCCYTGGA | GGGTGTTTGTTTAACCTCTTCACAA |
| LOC427656 | ATCAAATCAAAGRGCCTGGCAGAAC | CATTTTCACACGTGCTGTTTCAATT |
| GET4\_1\* | ACAGTRTTGTGTGAACAGTATCAACC | RAAGAAAAGCTGTCCTATTCTATCT |
| GET4\_2 | CCACTGCTAAACTTCATCTGGTTTC | GGTTGATACTGTTCACACAAYACTG |
| GET4\_3\* | GCTGATCTGTCCATGYTGGTTTTGG | TTCCACAGGGTAATRGCTAGTAACTG |

\*: PCR products over 10 species found

**Table S3**　Exons where the designed primers are localized in each reference species

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | gallus | ficedulla | meleagris | taeniopygia |
| primer\_name | forward | reverse | forward | reverse | forward | reverse | forward | reverse |
| TBC1D22B | chr26|TBC1D22B|9 | chr26|TBC1D22B|10 | chr1A|TBC1D22A|11 | - | chr28|TBC1D22B|10 | chr28|TBC1D22B|11 | chr26|TBC1D22B|12 | chr26|TBC1D22B|13 |
| CACNA1S\_1 | chr26|CACNA1S|10 | chr26|CACNA1S|9 | chr26|CACNA1S|10 | chr26|CACNA1S|9 | chr28|CACNA1S|8 | chr28|CACNA1S|7 | chr26|CACNA1S|12 | chr26|CACNA1S|11 |
| TEAD3 | chr26|TEAD3|7 | chr26|TEAD3|8 | chr26|TEAD3|9 | chr26|TEAD3|10 | chr28|TEAD3|9 | chr28|TEAD3|10 | chr26|TEAD3|8 | chr26|TEAD3|9 |
| CACNA1S\_2 | chr26|CACNA1S|21 | chr26|CACNA1S|20 | chr26|CACNA1S|21 | chr26|CACNA1S|20 | chr28|CACNA1S|19 | chr28|CACNA1S|18 | chr26|CACNA1S|23 | chr26|CACNA1S|22 |
| KIF21B\_1 | chr26|KIF21B|3 | chr26|KIF21B|2 | chr26|KIF21B|3 | chr26|KIF21B|2 | chr28|KIF21B|3 | chr28|KIF21B|2 | chr26|KIF21B|3 | chr26|KIF21B|2 |
| KIF21B\_2 | chr26|KIF21B|5 | chr26|KIF21B|4 | chr26|KIF21B|5 | chr26|KIF21B|4 | chr28|KIF21B|5 | chr28|KIF21B|4 | chr26|KIF21B|5 | chr26|KIF21B|4 |
| KIF21B\_3 | chr26|KIF21B|6 | chr26|KIF21B|5 | chr26|KIF21B|6 | chr26|KIF21B|5 | chr28|KIF21B|6 | chr28|KIF21B|5 | chr26|KIF21B|6 | chr26|KIF21B|5 |
| IPO9\_1 | chr26|IPO9|10 | chr26|IPO9|9 | chr26|IPO9|10 | chr26|IPO9|9 | chr28|LOC100549138|3 | chr28|LOC100549138|2 | chr26|IPO9|10 | chr26|IPO9|9 |
| IPO9\_3 | chr26|IPO9|17 | chr26|IPO9|16 | chr26|IPO9|17 | chr26|IPO9|16 | chr28|LOC100549138|10 | chr28|LOC100549138|9 | chr26|IPO9|17 | chr26|IPO9|16 |
| IPO9\_2 | chr26|IPO9|16 | chr26|IPO9|15 | chr26|IPO9|16 | chr26|IPO9|15 | chr28|LOC100549138|9 | chr28|LOC100549138|8 | chr26|IPO9|16 | chr26|IPO9|15 |
| IPO9\_4 | chr26|IPO9|23 | chr26|IPO9|22 | chr26|IPO9|23 | chr26|IPO9|22 | chr28|LOC100549138|16 | chr28|LOC100549138|15 | chr26|IPO9|23 | chr26|IPO9|22 |
| TMEM183A | chr26|TMEM183A|6 | chr26|TMEM183A|7 | chr26|TMEM183A|12 | chr26|TMEM183A|13 | chr28|LOC100551434|5 | chr28|LOC100551434|6 | chr26|TMEM183A|6 | chr26|TMEM183A|7 |
| ADIPOR1\_1 | chr26|ADIPOR1|3 | chr26|ADIPOR1|2 | chr26|ADIPOR1|3 | chr26|ADIPOR1|2 | chr28|ADIPOR1|2 | chr28|ADIPOR1|1 | chr26|ADIPOR1|3 | chr26|ADIPOR1|2 |
| ADIPOR1\_2 | chr26|ADIPOR1|4 | chr26|ADIPOR1|3 | chr26|ADIPOR1|4 | chr26|ADIPOR1|3 | chr28|ADIPOR1|3 | chr28|ADIPOR1|2 | chr26|ADIPOR1|4 | chr26|ADIPOR1|3 |
| AHCYL1\_1 | chr26|AHCYL1|11 | chr26|AHCYL1|12 | chr26|AHCYL1|9 | chr26|AHCYL1|10 | chr28|AHCYL1|9 | chr28|AHCYL1|10 | chr26|AHCYL1|9 | chr26|AHCYL1|10 |
| LOC395100 | chr26|LOC395100|5 | chr26|LOC395100|6 | chr26|LOC101812664|5 | chr26|LOC101812664|6 | chr28|LOC100551128|5 | chr28|LOC100551128|6 | chr26|LOC100231703|5 | chr26|LOC100231703|6 |
| DENND2D | chr26|DENND2D|4 | chr26|DENND2D|3 | chr26|DENND2D|4 | chr26|DENND2D|3 | chr28|DENND2D|4 | chr28|DENND2D|3 | chr26|DENND2D|4 | chr26|DENND2D|3 |
| ETV7 | chr26|ETV7|6 | chr26|ETV7|5 | chr26|ETV7|9 | chr26|ETV7|8 | chr28|ETV7|5 | chr28|ETV7|4 | chr26|ETV7|5 | chr26|ETV7|4 |
| AHCYL1\_2 | chr26|AHCYL1|6 | chr26|AHCYL1|7 | chr26|AHCYL1|4 | chr26|AHCYL1|5 | chr28|AHCYL1|4 | chr28|AHCYL1|5 | chr26|AHCYL1|4 | chr26|AHCYL1|5 |
| PHTF1\_1 | chr26|PHTF1|12 | chr26|PHTF1|13 | - | chr26|PHTF1|12 | chr28|PHTF1|9 | chr28|PHTF1|10 | - | chr26|PHTF1|13 |
| STK38 | chr26|STK38|12 | chr26|STK38|11 | chr26|STK38|12 | chr26|STK38|11 | chr28|LOC100542873|7 | chr28|LOC100542873|6 | chr26|STK38|12 | chr26|STK38|11 |
| PHTF1\_2 | chr26|PHTF1|11 | chr26|PHTF1|12 | chr26|PHTF1|10 | - | chr28|PHTF1|8 | chr28|PHTF1|9 | chr26|PHTF1|11 | - |
| RSBN1\_1 | chr26|RSBN1|4 | chr26|RSBN1|5 | chr26|RSBN1|3 | chr26|RSBN1|4 | chr28|RSBN1|3 | chr28|RSBN1|4 | chr26|RSBN1|4 | chr26|RSBN1|5 |
| RSBN1\_2 | chr26|RSBN1|3 | chr26|RSBN1|4 | chr26|RSBN1|2 | chr26|RSBN1|3 | chr28|RSBN1|2 | chr28|RSBN1|3 | chr26|RSBN1|3 | chr26|RSBN1|4 |
| HIPK1\_1 | chr26|HIPK1|5 | chr26|HIPK1|4 | chr26|HIPK1|5 | chr26|HIPK1|4 | chr28|HIPK1|6 | chr28|HIPK1|5 | chr26|LOC100229690|5 | chr26|LOC100229690|4 |
| HIPK1\_3 | chr26|HIPK1|7 | chr26|HIPK1|6 | chr26|HIPK1|7 | chr26|HIPK1|6 | chr28|HIPK1|8 | chr28|HIPK1|7 | chr26|LOC100229690|7 | chr26|LOC100229690|6 |
| HIPK1\_2 | chr26|HIPK1|6 | chr26|HIPK1|5 | chr26|HIPK1|6 | chr26|HIPK1|5 | chr28|HIPK1|7 | chr28|HIPK1|6 | chr26|LOC100229690|6 | chr26|LOC100229690|5 |
| HIPK1\_4 | chr26|HIPK1|8 | chr26|HIPK1|7 | chr26|HIPK1|8 | chr26|HIPK1|7 | chr28|HIPK1|9 | chr28|HIPK1|8 | chr26|LOC100229690|8 | chr26|LOC100229690|7 |
| CSDE1 | chr26|CSDE1|2 | chr26|CSDE1|3 | chr26|CSDE1|2 | chr26|CSDE1|3 | chr2|LOC104909591|2 | chr2|LOC104909591|3 | chr26|CSDE1|2 | chr26|CSDE1|3 |
| PPARD | chr26|PPARD|4 | chr26|PPARD|3 | chr26|PPARD|4 | chr26|PPARD|3 | chr28|PPARD|4 | chr28|PPARD|3 | chr26|PPARD|4 | chr26|PPARD|3 |
| AMPD1 | chr26|AMPD1|6 | chr26|AMPD1|7 | chr26|AMPD1|6 | chr26|AMPD1|7 | chr28|AMPD1|6 | chr28|AMPD1|7 | chr26|AMPD1|4 | chr26|AMPD1|5 |
| PDXDC1 | chr14|PDXDC1|20 | chr14|PDXDC1|21 | chr14|PDXDC1|19 | chr14|PDXDC1|20 | chr16|PDXDC1|18 | chr16|PDXDC1|19 | chr14|PDXDC1|20 | chr14|PDXDC1|21 |
| RRN3\_1 | chr14|RRN3|4 | chr14|RRN3|3 | chr14|RRN3|4 | chr14|RRN3|3 | chr16|RRN3|4 | chr16|RRN3|3 | chr14|RRN3|4 | chr14|RRN3|3 |
| RRN3\_2 | chr14|RRN3|5 | chr14|RRN3|4 | chr14|RRN3|5 | chr14|RRN3|4 | chr16|RRN3|5 | chr16|RRN3|4 | chr14|RRN3|5 | chr14|RRN3|4 |
| RRN3\_3 | - | chr14|RRN3|13 | chr14|RRN3|14 | chr14|RRN3|13 | chr16|RRN3|14 | chr16|RRN3|13 | chr14|RRN3|14 | chr14|RRN3|13 |
| GSPT1\_3 | chr14|GSPT1|8 | chr14|GSPT1|7 | chr14|GSPT1|10 | chr14|GSPT1|9 | chr16|GSPT1|8 | chr16|GSPT1|7 | chr14|GSPT1|8 | chr14|GSPT1|7 |
| GSPT1\_2 | chr14|GSPT1|6 | chr14|GSPT1|5 | chr14|GSPT1|8 | chr14|GSPT1|7 | chr16|GSPT1|6 | chr16|GSPT1|5 | chr14|GSPT1|6 | chr14|GSPT1|5 |
| GSPT1\_1 | chr14|GSPT1|2 | chrZ|ZSWIM6|10 | chr14|GSPT1|4 | chr14|GSPT1|3 | chr16|GSPT1|2 | chrZ|LOC100538555|1 | chr14|GSPT1|2 | - |
| GSPT1\_4 | chr14|GSPT1|9 | chr14|GSPT1|8 | chr14|GSPT1|11 | chr14|GSPT1|10 | chr16|GSPT1|9 | chr16|GSPT1|8 | chr14|GSPT1|9 | chr14|GSPT1|8 |
| GSPT1\_5 | chr14|GSPT1|11 | chr14|GSPT1|10 | chr14|GSPT1|13 | chr14|GSPT1|12 | chr16|GSPT1|11 | chr16|GSPT1|10 | chr14|GSPT1|11 | chr14|GSPT1|10 |
| GSPT1\_6 | chr14|GSPT1|12 | chr14|GSPT1|11 | chr14|GSPT1|14 | chr14|GSPT1|13 | chr16|GSPT1|12 | chr16|GSPT1|11 | chr14|GSPT1|12 | chr14|GSPT1|11 |
| PARN | chr14|PARN|2 | chr14|PARN|1 | chr14|PARN|5 | chr14|PARN|4 | chr16|PARN|1 | - | chr14|PARN|5 | chr14|PARN|4 |
| CCZ1\_1 | chr14|CCZ1|3 | chr14|CCZ1|2 | chr14|CCZ1|3 | chr14|CCZ1|2 | chr1|LIPI|3 | chr11|AMER3|1 | chr14|CCZ1|3 | chr14|CCZ1|2 |
| BFAR | chr14|BFAR|5 | chr14|BFAR|6 | chr14|BFAR|5 | chr14|BFAR|6 | chr16|BFAR|5 | chr16|BFAR|6 | chr14|BFAR|5 | chr14|BFAR|6 |
| CCZ1\_2 | chr14|CCZ1|4 | chr14|CCZ1|3 | chr14|CCZ1|4 | chr14|CCZ1|3 | chr16|CCZ1|2 | chr23|PERM1|1 | chr14|CCZ1|4 | chr14|CCZ1|3 |
| OCM | chr14|OCM|3 | chr14|OCM|2 | chr14|LOC101813769|3 | chr14|LOC101813769|2 | chr16|LOC100545184|3 | chr16|LOC100545184|2 | chr14|LOC100230782|3 | chr14|LOC100230782|2 |
| LOC396531 | chr14|LOC396531|2 | chr14|LOC396531|1 | chr14|LOC101813301|2 | chr14|LOC101813301|1 | chr16|LOC100545341|2 | chr16|LOC100545341|1 | chr14|LOC100221179|2 | chr14|LOC100221179|1 |
| TMEM130 | chr14|TMEM130|4 | chr14|TMEM130|3 | chr14|TMEM130|4 | chr14|TMEM130|3 | chr16|TMEM130|4 | chr16|TMEM130|3 | chr14|TMEM130|3 | chr14|TMEM130|2 |
| FAM20C\_1 | chr14|FAM20C|8 | chr14|FAM20C|9 | chr14|FAM20C|9 | chr14|FAM20C|10 | chr16|FAM20C|5 | chr16|FAM20C|6 | chr14|FAM20C|9 | chr14|FAM20C|10 |
| FAM20C\_2 | chr14|FAM20C|6 | chr14|FAM20C|7 | chr14|FAM20C|7 | chr14|FAM20C|8 | chr16|FAM20C|3 | chr16|FAM20C|4 | chr14|FAM20C|7 | chr14|FAM20C|8 |
| BAIAP2L1 | chr14|BAIAP2L1|6 | chr14|BAIAP2L1|5 | chr14|BAIAP2L1|6 | chr14|BAIAP2L1|5 | chr16|BAIAP2L1|3 | chr16|BAIAP2L1|2 | chr14|BAIAP2L1|6 | chr14|BAIAP2L1|5 |
| GET4\_1 | chr14|GET4|7 | chr14|GET4|8 | chr14|GET4|7 | chr14|GET4|8 | chr16|GET4|6 | chr16|GET4|7 | chr14|GET4|6 | chr14|GET4|7 |
| GET4\_2 | chr14|GET4|6 | chr14|GET4|7 | chr14|GET4|6 | chr14|GET4|7 | chr16|GET4|5 | chr16|GET4|6 | chr14|GET4|5 | chr14|GET4|6 |
| GET4\_3 | chr14|GET4|3 | chr14|GET4|4 | chr14|GET4|3 | chr14|GET4|4 | chr16|GET4|2 | chr16|GET4|3 | chr14|GET4|2 | chr14|GET4|3 |
| LOC427656 | chr14|LOC427656|2 | - | chr14|LOC101820237|2 | - | chr16|LOC100545806|2 | - | chr14|LOC100219310|2 | - |

**Table S4**　PCR results and sizes from tried primers

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Primer name | chicken | black kite | jungle crow | carrion crow | brown-eared bulbul | turtle dove | rock dove | sparrow | swallow | martin | starling | black-backed wagtaiｌ |
| TEAD3\* | 260 | 280 | 270 | 270 | 290 | 270 | 270 | 280 | 265 | 265 | 260 | 290 |
| TBC1D22B\* | 365 | 400 | 380 | 380 | 350 | 320 | 320 | 370 | 360 | 355 | 360 | 380 |
| CACNA1S\_1 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| CACNA1S\_2 | M | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| KIF21B\_1 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| KIF21B\_2 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| KIF21B\_3 | M | ― | M | M | ― | M | M | M | ― | ― | M | ― |
| IPO9\_1 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| IPO9\_2 | ― | ― | ― | ― | ― | 800 | ― | ND | ND | ND | ND | ND |
| IPO9\_3 | ― | ― | ― | 700 | ― | ― | ― | ND | ND | ND | ND | ND |
| IPO9\_4 | ― | ― | ― | - | ― | ― | ― | ND | ND | ND | ND | ND |
| TMEM183A | 700 | ― | 750 | 750 | ― | 800 | 700 | 800 | ― | ― | 850 | ― |
| DENND2D | M | ― | M | M | ― | M | M | M | ― | ― | M | ― |
| LOC395100\* | M | M | M | M | M | M | M | M | 800 | 800 | M | ― |
| ADIPOR1\_1 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| ADIPOR1\_2 | ― | ― | M | M | ― | M | M | ― | ― | ― | M | ― |
| AHCYL1\_1\* | 450 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 |
| AHCYL1\_2\* | 260 | 480 | 860 | 860 | 1000 | 460 | 460 | 1000 | 960 | ― | 790 | 860 |
| ETV7 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| STK38 | ― | ― | ― | ― | ― | ― | ― | ND | ND | ND | ND | ND |
| PHTF1\_1\* | 450 | 220 | 450 | 450 | 500 | 210 | 220 | 450 | 500 | 500 | 450 | 500 |
| PHTF1\_2\* | 530 | 700 | 700 | 700 | 700 | M | M | 700 | 700 | 700 | 690 | 700 |
| RSBN1\_1\* | 680 | 990 | 990 | M | 1000 | 1000 | M | M | 1000 | 1000 | M | M |
| RSBN1\_2 | M | ― | M | M | ― | M | M | M | ― | ― | M | ― |
| HIPK1\_1 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| HIPK1\_2 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| HIPK1\_3 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| HIPK1\_4 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| AMPD1\* | 660 | M | 430 | 430 | 440 | 390 | 390 | 450 | 420 | 420 | 390 | 455 |
| CSDE1\* | M | 330 | M | M | 350 | 320 | 320 | 350 | 370 | 370 | 370 | 350 |
| PPARD | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| PDXDC1\* | 190 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| RRN3\_1 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| RRN3\_2 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| RRN3\_3 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_1 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_2 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_3 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_4 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_5 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GSPT1\_6 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| PARN | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| BFAR\* | 860 | 860 | 860 | 860 | 600 | 840 | 840 | 800 | M | 600 | 730 | 800 |
| CCZ1\_1 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| CCZ1\_2 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| OCM | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| LOC396531 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| BAIAP2L1 | ― | ― | ― | ― | ― | 800 | 800 | ― | ― | ― | ― | ― |
| TMEM130 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| FAM20C\_1\* | 510 | 200 | 200 | 200 | 200 | 200 | 200 | 190 | 200 | 200 | 200 | 190 |
| FAM20C\_2\* | 600 | ― | 600 | 600 | 360 | 700 | 600 | 550 | 550 | 550 | ― | 550 |
| LOC427656 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GET4\_1\* | 380 | 235 | M | M | M | 235 | 240 | M | 230 | 230 | M | M |
| GET4\_2 | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― | ― |
| GET4\_3\* | ― | 1260 | 430 | 420 | 435 | 1260 | ― | 200 | 435 | 435 | M | 430 |

\*PCR products over 10 species found

M: Multiple bands, ND: Not done

**Fig S2** Chip gel electrophoresis images of EPIC pcr products

　　

　　

 

 

 

 

 

 

**Legend to Fig. S2** MultiNA chip electrophoresis of TBC1D22B-primer amplified PCR. Primers which amplify more than 10 species were selected.

Species are rock dove (a), turtle　dove (b), brown-eared bulbul (c), martin (d), swallow (e), starling (f), chicken (g), sparrow (h), carrion crow (i), jungle crow (j), black-backed wagtail (k) and black kite (l). Molecular sized were predicted from internal markers listed in left-side of the graphical view.

**Fig. S3** MultiNA chip electrophoresis of PCR product of TBC1D22B gene from feather DNAs.

**A**.TBC1D22B-primer amplified PCR



**B.**TBC1D22B\_i-primer amplified PCR

 

Species are rock dove (a), turtle dove (b), brown-eared bulbul (c), martin (d), swallow (e), starling (f), chicken (g), sparrow (h), carrion crow (i), jungle crow (j), black-backed wagtail (k) and black kite (l).

Molecular sized were predicted from internal markers listed in left-side of the graphical view.

**Fig. S4** A phylogenetic trees on species picked up in this study



Legened to Fig.S3 　Species targeted for species identification in this study are indicated by blue frames and species with standard genome constructed by red frames.

Fig. S5 Sequence alignment of PCR products form each species of birds.

'Motacilla\_alba\_lugens' ---TTTGCCTTTCGYTGGATGAACAACCTGCTGATGAGGGAGCTGCCCCTGCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAGGCTCCACTGACCTCCCT-CTTGGCTTGTCACTGTCACTGGTGTCCCTGTCCCCA---C

'Corvus\_corone' CAGTTTGCCTTTCGYTGGATGAACAACCTGCTCATGAGGGAGCTGCCCCTCCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAGGCTCCACTGACC-CCCTGCCTGGCTTGTCACTGTCACTTGTGTCCCTGGCCCCA---A

'Corvus\_macrorhynchos' CAGTTTGCCTTTCGYTGGATGAACAACCTGCTCATGAGGGAGCTGCCCCTCCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAGGCTCCACTGACC-CCCTGCCTGGCTTGTCACTGTCACTTGTGTCCCTGTCCCCA---A

'Passer\_montanus' --GTTTGCCTTTCGYTGGATGAACAACCTGCTCATGAGGGAGCTGCCCCTGCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAG-CTCCACTG-CCTCCCT-CTTGCCTTGTCCCTGGCACCTGTGTCCCTGTCCCCA---C

'Gallus\_gallus\_domesticus' CAGTTTGCCTTTCGYTGGATGAACAATCTGCTCATGAGGGAGCTGCCTCTCCGCTGCACAATCCGCCTCTGGGACACCTACCAGGTACGTGGACTT-GCTGCCATCCCTCCTTGGCTGCTTGCTGT-----AGGGCCAACTCCCCACGCA

'Sturnus\_cineraceus' ---TTTGCCTTTCGYTGGATGAACAACCTGCTCATGAGGGAGCTGCCCCTCCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAG---------GCTCCACTGGCCTCCCTGTCACTGTCCCTCGTGTCCCTGTCCCCAGTAC

'Hirundo\_rustica' CAGTTTGCCTTTCGYTGGATGAACAATCTGCTCATGAGGGAGCTGCCCCTGCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAC-------TGCCCTCCCTGCTCGGCTTGTCACTGCCACTCGTGTCCCTGTCCCCA---C

'Hypsipetes\_amaurotis' --GTTTGCCTTTCGYTGGATGAACAACCTGCTCATGAGGGAGCTGCCCCTGCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTATGCAG-------TGACC-CCCTGCTTGGCTTGTCTCTGTCACCTGTGTCCCTGTCCCCA---C

'Columba\_livia' CAGTTTGCCTTTCGYTGGATGAACAATCTGCTGATGAGGGAGCTGCCGCTTCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTA--CAG---------------------GGGCTCCCGCTGCCTTCCCTGCTTTACTGGTCA----

'Streptopelia\_orientalis' CAGTTTGCCTTTCGYTGGATGAACAATCTGCTGATGAGGGAGCTGCCGCTTCGCTGCACCATCCGCCTCTGGGACACCTACCAGGTA--CAG---------------------GGGCTCCCGCTGCCTTCCCTGCTTTAATGGTCA----

'Motacilla\_alba\_lugens' GGGG--ACAGAGGTCTCCTGGCACCAGCCGGGGTGCTGAGGGAAACAAAGCAGTGTGGTCAC-AGGATGTTTACAGCNAGCTG--GGCGATGGGTGGCCCAGTAGGACGCCTGTGTCTGGGG--CTTGTTCTCCAGCTGAGCTGCAGGAG

'Corvus\_corone' GGGG--ACAGAGGTTTCCTGGCACCA--------GCTGAGGGGCACAAAGCAGTGTGGCCCC-AGGATGTTTACAGCCACCTG--GGTGATGGGTGACCCAGTGGGACATCTGTGTCTGGGGAAGTTGTTCTCCAGCTGAGCTGCAGGAG

'Corvus\_macrorhynchos' GGGG--ACAGAGGTTTCCTGGCACCA--------GCTGAGGGGCACAAAGCAGTGTGGCCCC-AGGATGTTTACAGCCAGCTG--GGTGATGGGTGACCCAGTAGGACATCTGTGTCTGGGGAAGTTGTTCTCCAGCTGAGCTGCAGGAG

'Passer\_montanus' GGGG--ACAGAGGGCTCCTGGCACCAGCTGGG-TGCTGAGGGAAACAAAGCAGAGTGGCCCC-AGGATGTTTGCAGCT----G--GGTGATGGGTGGCCCAGTAGGACATCTGTGTCTGGGG-----GTTCTCCAGCTGAGCTGCAGGAG

'Gallus\_gallus\_domesticus' GGGA--GATGAGGCACCATG----CAGCTGGGGAGATAAGAGGAACAA---AGTGTGGCCTCTGGGGTGTTTATAGACAGATGAAGGTGACACATGG-----------ACATGTGTCTGGGGGAGCTGTGCTCCAGCAGAGCCATAGGAG

'Sturnus\_cineraceus' AGACCCACAGAGGTCTCCTGGCACCAGCTGGGGTGCTGAGGGAAACAAAGCAGTGTGGGC-C-AGGATGTTTGCAGGCAGCTG--GGTGGAGGGTGGAC---------ATCTGTGGCTGGGG--GTTGTTCTCCAGCTGAGCTGCAGGAG

'Hirundo\_rustica' GGGG--ACAGAGGG-------------------TGCTCAGGGAAGCAAAGCAGTGTGGCCCC-AGGGTGTTCACAGCCAGCTG--GGTGCTGGGTGGCCTCCCAGGACATCTGTGTCTGGGG--GTCGTTCTCCAGCTGTGCTGCAGGAG

'Hypsipetes\_amaurotis' GGGG--ACAGAGGA-------------------TGCTGAGGGAAACAAAGCAATGTGGCCCC-AGGATGTTTATAGGCAGCTG--GGTGCTAGGTGGCCCC-------ATCTGTGTCTGGGG--GTTGTTCTCCAGCTGAGCTGCAGGGG

'Columba\_livia' -------C----------------------------TGTGGGGA------CAATT---CCAT-AGGACGTTTATAGCCACTTG--GGTGATGGGTGGCCCAGTAGGACGTGGGTTTCTGGGG------AGCTCCAGTGGAGCTGCAGGAG

'Streptopelia\_orientalis' -------C----------------------------TGTGGGGA------CAATT---CCAN-AGGACGTTTATAGCCACTTG--GGTGACGGGTGGCCTAGTAGGACATGGGTTTCTGGGG------AGCTCCAGTGGAGCTGCAGGAG

'Motacilla\_alba\_lugens' TTCACCCAGAGTTTTGCTGTCCACAGTCAGAGCCAGAGGGATTCTCCCACTCCACACCTGTACGTCTGTGCTGCCTTCYTGATCAAGTGGCNRAAGGAGATC

'Corvus\_corone' CTCACTCAGAGTTTTGCTGTCCACAGTCAGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGCGCTGCCTTCYTGATCAAGTGGCGNAAGGAGATC

'Corvus\_macrorhynchos' CTCACTCAGAGTTTTGCTGTCCACAGTCAGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGCGCTGCCTTCYTGATCAAGTGGCGRNNGGAGATC

'Passer\_montanus' CTCACCCAGANTTTTGCTGCCCACAGTCAGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGTGCTGCCTTCYTGATCAAGTGGCGRNAGGAGATC

'Gallus\_gallus\_domesticus' CTGACTCCGAGTTTTGTTGCTTACAGTCAGAGCCAGAGGGGTTCTCGCACTTC-CACCTCTATGTCTGTGCCGCCTTCYTGATCAAGTGGCGGAAGGAGATC

'Sturnus\_cineraceus' CTCACCCAGAGTTTTGCTGTCCACAGTCAGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGTGCTGCCTTCYTGATCAAGTGGCGNNNGGAGATC

'Hirundo\_rustica' TTCACCCAGAGTTTTGCTGTCCACAGTCAGAGCCGGAAGGATTCTCCCATTTCACACCTGTACGTCTGCGCTGCCTTCYTGATCAAGTGGCGGAAGGAGATC

'Hypsipetes\_amaurotis' TTCACCCAGAGTTTC-CTGTCCACAGTCAGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGTGCTGCCTTCYTGATCAAGTGGCGGAAGGAGATC

'Columba\_livia' CTGATCCAGAGTTTTGCTACTTGCAGTCGGAGCCAGAAGGATTCTCCCATTNC-CACCTGTACGTCTGCGCCGCATTCYTGATCAAGTGGCGGAAGGAGATC

'Streptopelia\_orientalis' CTGATCCGGAGTTTTGCTACTTGCAGTCGGAGCCAGAAGGATTCTCCCATTTC-CACCTGTACGTCTGCGCCGCCTTCYTGATCAAGTGGCGGNAGGAGATC

\* Sequences of PCR products from black kite (*Milvus migrans)* and martin (*Delichon urbica)* could not determined.

Nine sequences were newly identified and registered on DDBJ. Entry IDs on DDBJ: 54079b500fd0bf1a99000211.Motacilla\_alba\_lugens (black-backed wagtail), 54079b500fd0bf1a99000211.Corvus\_corone (carrion crow), 54079b500fd0bf1a99000211.Corvus\_macrorhynchos (jungle crow), black kite (*Milvus migrans*), 54079b500fd0bf1a99000211.Hypsipetes\_amaurotis (brown-eared bulbul), 54079b500fd0bf1a99000211.Streptopelia\_orientalis (turtle dove), 54079b500fd0bf1a99000211.Columba\_livia (rock dove), 54079b500fd0bf1a99000211.Passer\_montanus (sparrow), 54079b500fd0bf1a99000211.Hirundo\_rustica (swallow), martin (*Delichon urbica*), 54079b500fd0bf1a99000211.Sturnus\_cineraceus (starling)

Fig. S6 Program searching combinations that obtain the greatest results with the smallest number of genes

require 'csv'

def create\_wholepair()

 whole\_pair=Array.new

 (0..11).to\_a.each{|i1|

 (0..11).to\_a.each{|i2|

 if i1 < i2 then

 whole\_pair<< [i1,i2]

 end

 }

 }

 return whole\_pair

end

start\_time=Time.now

a\_bsps=Array.new

b\_electrph=Hash.new

i=0

CSV.foreach("size\_divergency\_birds.csv") do |row|

 if i==0 then

 a\_bsps=row

 else

 b\_electrph[row[0].sub("\*","")]=row[1..-1].map{|x| x.to\_i}

 end

 i+=1

end

wset=create\_wholepair

diff\_pair=Hash.new

b\_electrph.each{|gene,gdata|

 diff\_pair[gene]=Array.new

 gdata.each\_with\_index{|gs1,idx1|

 gdata.each\_with\_index{|gs2,idx2|

 if idx1 < idx2 then

 if gs1>0 && gs2>0 && (gs1-gs2).abs > 19 then

 diff\_pair[gene] << [[idx1,idx2],(gs1-gs2).abs]

 wset.delete([idx1,idx2])

 end

 end

 }

 }

 # p "#{gene} #{undistinguish[gene]}"

}

p "all primers #{wset}"

undistinguish=Hash.new

diff\_pair=Hash.new

b\_electrph.each{|gene,gdata|

 diff\_pair[gene]=Array.new

 undistinguish[gene]=create\_wholepair

 # p undistinguish[gene]

 gdata.each\_with\_index{|gs1,idx1|

 gdata.each\_with\_index{|gs2,idx2|

 if idx1 < idx2 then

 if gs1>0 && gs2>0 && (gs1-gs2).abs > 19 then

 diff\_pair[gene] << [[idx1,idx2],(gs1-gs2).abs]

 undistinguish[gene].delete([idx1,idx2])

 end

 end

 }

 }

 # p "#{gene} #{undistinguish[gene]}"

}

undist\_double=Hash.new

undistinguish.each{|gene1,udpair1|

 undistinguish.each{|gene2,udpair2|

 if gene1 != gene2 then

 undist\_double[[gene1,gene2].sort]=udpair1&udpair2

 end

 }

}

undist\_triple=Hash.new

undistinguish.each{|gene1,udpair1|

 undistinguish.each{|gene2,udpair2|

 undistinguish.each{|gene3,udpair3|

 if [gene1,gene2,gene3].uniq.length > 2 then

 undist\_triple[[gene1,gene2,gene3].sort]=udpair1&udpair2&udpair3

 end

 }

 }

}

CSV.open("undist\_double.csv","w") do |csv|

 undist\_double.sort\_by{|k,v| v.length}[0,10].each{|set|

 row=[]

 row << set[0]

 set[1].each{|pair|

 row << [pair,""].flatten

 }

 csv << row.flatten

 }

end

CSV.open("undist\_triple.csv","w") do |csv|

 undist\_triple.sort\_by{|k,v| v.length}[0,10].each{|set|

 row=[]

 row << set[0]

 set[1].each{|pair|

 row << [pair,""].flatten

 }

 csv << row.flatten

 }

end

p "Treatment time #{Time.now - start\_time}s"