**Supplementary data**

**sTable 1**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Forward Primer | Reverse Primer | Amplicon Size (bp) |
| **Degenerate Primer Sequence** |
| *Foxl2* | DCSCAGAARCCMCCSTACTC | TAGTTGCCCTTKCGCTCRCC | 227 |
| *Aromatase* | GATGATGATHGCTGCHCCBG | TGCACAGARCMGWGGDGATC  | 1100 |
| **Gene specific primers** |
| *CvFoxl2* | CCAGTACATCATCGGCAAGTT | GGCACCTTGATGAAGCACTC | 105 |
| *CvRspo1* | GGTCCTCCCTCTCTGCTTCTTC | CCTCCTTCCGCTCACCTT | 233 |
| *CvSox9* | CCGTCAGCCAGTTCTTGAAG | TTCTCCTCCGTGGCTGATAC | 312 |
| *CvArom* | ACGCAGGATTTCCACAGAAG | TAGGGTGCTTTGCAATGAGA | 191 |
| *CvWnt4* | CAACTGGCTGTACCTGGCCAA | GAAGGCTACGCCATAGGCGAT | 427 |
| *CvßActin* | CGACTTGACCGACTACCTCA | TCGTAGCTCTTCTCCAGGGA | 174 |

**sTable 2**

|  |  |  |
| --- | --- | --- |
| **CvActin**940C- 5min940C-30 Sec600C-40 Sec 35 Cycles720C-4 0Sec720C-10min | **CvFoxl2**940C- 5min940C-30 Sec600C-30 Sec 40 Cycles720C-30Sec720C-10min | **CvRspo1**940C- 5min940C-30 Sec600C-1min 40 Cycles720C-1min720C-10min |
| **CvWnt4**940C- 5min940C-30 Sec600C-1min 40 Cycles720C-1min720C-10min | **CvArom**940C- 5min940C-30 Sec560C-40 Sec 40 Cycles720C-40Sec720C-10min | **CvSox9**940C- 5min940C-35 Sec550C-35 Sec 40 Cycles720C-35Sec720C-10min |

**S Figure 1**

**Probe Sequences:**

**CvFoxl2**

ACGCAGAAGCCACCCTACTCGTACGTGGCGCTGATCGAAAGGCCATCCGCGAGAGCGCCGAGAAGCGGCTGACGCTGTCGGGCATCTACCAGTACATCATCGGCAAGTTCCCCTTCTACGAGAAGAACAAGAAGGGCGGGCAGAACAGCATCCGCCACAACCTGAGCCTCAACGAGTGCTTCATCAAGGTGCCGCGAGAGGGCGGCGGCGAGCGAAAGGGCAACTAA

**mRspo1 (Riboprobe)**

ATTCTGCTGGAGAGGAACGACATCCGCCAGGTGGGCGTCTGCCTGCCGTCCTGCCCACCTGGATACTTTGATGCCCGCAACCCCGACATGAACAAATGCATCAAATGCAAGATCGAGCACTGTGAGGCCTGCTTCAGCCACAACTTCTGCACCAAGTGTCAGGAGGCCTTGTACTTACACAAGGGCCGCTGCTATCCAGCCTGCCCTGAGGGCTCTACAGCCGCTAACAGCACCATGGAGTGCGGCAGTCCTGCACAATGTGAAATGAGCGAGTGGTCCCCGTGGGGACCCTGCTCCAAGAAGAGGAAGCTGTGCGGTTTCCGGAAGGGATCGGAAGAGCGGACACGCAGAGTGCTCCATGCTCCCGGGGGAGACCACACCACCTGCTCCGACACCAAAGAGACCCGCAAGTGTACCGTGCGCAGGACGCCCTGCCCAGAGGGGCAGAAGAGGAGGAAGGGGGGCCAGGGCCGGAGGGAGAATGCCAACAGGCATCCGGCCAGGAAGAACAGCAAGGAGCCGAGGTCCAACTCTCGGAGACACAAAGGGCAACAGCAGCCACAGCCAGGGACAACAGGGCCACTCACATCAGTAGGACCTACCTGGGCACAGTGACCGGTCTCCAGATACCTGTGGAAGAGTACAGTGCTGTACTGTATAATGAGAACTTTCCAGAACTGGAGCATCTGGGAGAGTCCACACATACCCCATCCACCCACCCATCCAACTATCCATCCATCCATCCATGCACACATATGGCCACATCTGAAAACGTCAACACACACACACACACACACACACACACACACACACATTCTTGAGGTCACTGAAGACACTTCTATTCTGTGGCCCAGCTGTATATTCAGTCTTTAATGCTCTTGGAAGACATATCTGAGAGAACCTTTCCCAGCATCTGAAACTAAGGAGTGGAACCTTCTGGAGGAACTTCTGGGACAGCATCTGACAG

**CvRspo1**

GGTCCTCCCTCTGCTTCTTCCTCCCGCAGCCCAATGTGAGATGAGCGAGTGGGGGCCCTAGGGGCCCTGCTCCAAGAGGAGGAGGCAGTGCGGCTTCCGGAAGGGCAACGAGGAGCGCGCCCGGACGGTCCTGCAGGTGCTCTCGGGAGAGGCGCCTGCCTGCCCGCCCACCACCGAGTCCCGGAGATGCACCGTGCAGAGACGCCCGTGTCCGGAAGGTGAGCGGAAGGAGG

**CvAromatase**

GGCAAGCTCTCCTCATCAAACCAGACATCTTCTTTAAGATTTCTTGGCTATACAAAAAGCATGAGAAGTCTGTCAAGGATTTGAAAGATGCCATAGAAGTTCTGATAGCAGAAAAAAGACGCAGGATTTCCACAGAAGAGAAACTGGAAGAATGTATGGACTTTGCCACTGAGTTGATTTTAGCAGAGAAACGTGGTGACCTGACAAGAGAGAATGTGAACCAGTGCATATTGGAAATGCTGATCGCAGCTCCTGACACCATGTCTGTCTCTTTGTTCTTCATGCTATTTCTCATTGCAAAGCACCCTAATGTTGAAGAGGCAATAATAAAGGAAATCCAGACTGTTATTGGTGAGAGAGACATAAAGATTGATGATATACAAAAATTAAAAGTGATGGAAAACTTCATTTATGAGAGCATGCGGTACCGGCCTGTCGTGGACTTGGTCATGCGCAAAGCCTTAGAAGATGATGTAATCGATGGCTACCCAGTGAAAAAGGGGACAAACATTATCCTGAATATTGGAAGGATGCACAGACTCGAGTT

**S Figure 2 Nucleotide sequence alignment of different genes**

***2a. CvFoxl2***

Homo GCGG------------------CGGGAC---AGCCCCGGAGAAGCCGGACCCGGCGCAGA

Mus ----------------------CGGGAC---AACACCGGAGAAACCAGACCCCGCGCAGA

Gallus -----------------------GGC---------CCCGAGAAGCCGGACCCGTCGCAGA

Chrysemys -----------------------GGG---------GCCGAGAAGCCGGACCCCTCTCAGA

Alligator -----------------------GGC---------GCCGACAAGCCGGACCCGTCGCAGA

Danio -----------------------GGG---------CCGGACAAATCCGACCCGACCCAGA

Anolis CCTCTTCCTCGTCCTCTGTCTCGGCGGC---GGCGGCGGAGAAAGGCGACCCTTCGCAGA

Gekko GAGC-----CGGCGGCGGCGGCG-CCTC---GTCGTCGGAGAAGGGCGACCCGTCGCAGA

Calotes -----------------------------------------------------ACGCAGA

Pogona CCTCGGCCGTGGCGGCGGCGGCGGCGGCCGCGTCGTCGGAGAAAGGCGACCCTTCGCAGA

 \* \*\*\*\*

Homo AGCCCCCGTACTCGTACGTGGCGCTCATCGCCATGGCGATCCGCGAGAGCGCGGAGAAGA

Mus AGCCCCCGTACTCGTACGTGGCGCTCATCGCCATGGCGATCCGCGAGAGCGCCGAGAAGA

Gallus AGCCCCCCTACTCCTACGTGGCCCTGATCGCCATGGCCATACGGGAGAGCGCCGAGAAGA

Chrysemys AGCCCCCCTACTCCTACGTAGCTCTGATCGCCATGGCCATCCGGGAGAGCGCCGAGAAGC

Alligator AGCCGCCCTACTCGTACGTGGCGCTGATCGCCATGGCCATCCGCGAGAGCGCCGAGAAGC

Danio AACCACCGTACTCATACGTGGCGTTAATCGCGATGGCAATCCGCGAGAGCTCGGAGAAGC

Anolis AGCCTCCCTACTCCTACGTGGCCCTGATCGCCATGGCCATCCGCGAGAGCACCGAGAAGC

Gekko AACCGCCCTACTCGTACGTGGCGCTGATCGCCATGGCCATCCGCGAGAGCGCCGAGAAGC

Calotes AGCCACCCTACTCGTACGTGGCGCTGATCG-AAAGGCCATCCGCGAGAGCGCCGAGAAGC

Pogona AGCCGCCCTACTCGTACGTGGCGCTGATCGCCATGGCCATCCGCGAGAGCGCCGAGAAGC

 \* \*\* \*\* \*\*\*\*\* \*\*\*\*\* \*\* \* \*\*\*\* \* \*\*\* \*\* \*\* \*\*\*\*\*\* \* \*\*\*\*\*\*

Homo GGCTCACGCTGTCCGGCATCTACCAGTACATCATCGCGAAGTTCCCGTTCTACGAGAAGA

Mus GGCTCACTCTGTCCGGCATCTACCAGTACATCATAGCCAAGTTCCCGTTCTACGAGAAGA

Gallus GGCTCACGCTGTCCGGGATCTACCAGTACATCATCAGCAAGTTCCCTTTCTACGAGAAGA

Chrysemys GGCTCACGCTGTCCGGCATCTACCAGTACATCATCAGCAAGTTCCCCTTCTACGAGAAGA

Alligator GGCTCACGCTGTCGGGCATCTACCAGTACATCATCAGCAAGTTCCCCTTCTACGAGAAGA

Danio GTCTTACGCTGTCCGGTATATACCAGTACATCATCAGTAAGTTTCCATTCTACGAGAAGA

Anolis GCCTCACGCTGTCGGGCATCTACCAGTACATCATCGGCAAGTTCCCTTTCTACGAGAAGA

Gekko GGCTGACGCTGTCGGGCATCTACCAGTACATCATCGGCAAGTTCCCCTTCTACGAGAAGA

Calotes GGCTGACGCTGTCGGGCATCTACCAGTACATCATCGGCAAGTTCCCCTTCTACGAGAAGA

Pogona GGCTGACCCTGTCGGGCATCTACCAGTACATCATCAGCAAGTTCCCTTTCTACGAGAAGA

 \* \*\* \*\* \*\*\*\*\* \*\* \*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\* \*\*\*\*\* \*\* \*\*\*\*\*\*\*\*\*\*\*\*\*

Homo ATAAGAAGGGCTGGCAAAATAGCATCCGCCACAACCTCAGCCTCAACGAGTGCTTCATCA

Mus ACAAGAAGGGCTGGCAGAATAGCATCCGCCACAACCTCAGCCTCAACGAGTGCTTCATCA

Gallus ACAAGAAGGGCTGGCAGAACAGCATCCGCCACAACCTCAGCCTCAACGAGTGCTTCATCA

Chrysemys ACAAGAAGGGCTGGCAGAACAGCATCCGCCACAACCTGAGCCTCAACGAGTGCTTCATCA

Alligator ACAAGAAGGGCTGGCAGAACAGCATCCGCCACAACCTCAGCCTCAACGAGTGCTTCATCA

Danio ACAAGAAGGGATGGCAGAACAGCATCCGACACAACCTGTCACTCAACGAGTGCTTCATCA

Anolis ACAAGAAGGGCTGGCAGAACAGCATCCGGCACAACCTGAGCCTCAACGAGTGCTTCATCA

Gekko ACAAGAAGGGCTGGCAGAACAGCATCCGCCACAACCTGAGCCTCAACGAGTGCTTCATCA

Calotes ACAAGAAGGGCGGGCAGAACAGCATCCGCCACAACCTGAGCCTCAACGAGTGCTTCATCA

Pogona ACAAGAAGGGCTGGCAGAACAGCATCCGCCACAACCTGAGCCTCAACGAGTGCTTCATCA

 \* \*\*\*\*\*\*\*\* \*\*\*\* \*\* \*\*\*\*\*\*\*\* \*\*\*\*\*\*\*\* \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Homo AGGTGCCGCGCGAGGGCGGCGGCGAGCGCAAGGGCAACTACTGGACGCTGGACCCGGCCT

Mus AGGTGCCGCGCGAGGGCGGCGGCGAGCGCAAGGGCAACTACTGGACGCTCGACCCGGCCT

Gallus AGGTGCCCCGCGAGGGCGGCGGCGAGCGCAAGGGCAACTACTGGACGCTGGACCCCGCCT

Chrysemys AGGTGCCCCGCGAGGGGGGCGGCGAGCGCAAGGGCAACTACTGGACACTGGACCCGGCCT

Alligator AGGTGCCCCGCGAGGGCGGCGGCGAGCGCAAGGGCAACTACTGGACGCTGGACCCCGCCT

Danio AGGTGCCTCGGGAGGGCGGCGGTGAGCGAAAGGGCAACTACTGGACCCTGGATCCTGCAT

Anolis AGGTGCCGCGCGAGGGCGGCGGGGAGCGCAAGGGCAACTACTGGACGCTGGACCCGGCCT

Gekko AGGTGCCGCGAGAGGGCGGCGGGGAGCGCAAAGGCAACTACTGGACCCTCGACCCGGCCT

Calotes AGGTGCCGCGAGAGGGCGGCGGCGAGCGAAAGGGCAACTA--------------------

Pogona AGGTGCCGCGAGAGGGCGGCGGAGAGCGCAAAGGCAACTACTGGACCCTCGACCCGGCCT

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**2b. *CvArom***

Gallus CTTTGATGCTTGGCAAGCTCTTCTATTAAAGCCCGACATATTTTTTAAGATTTCTTGGCT

Pogona CTTTGACGCTTGGCAGGCCCTCCTGCTGAAACCCGACATCTTCTTCAAGTTTTCTTGGCT

Anolis CTTTGATGCCTGGCAGGCACTTCTGCTGAAACCCGATATCTTCTTTAAGTTTTCTTGGCT

Calotes -----------GGCAAGCTCTCCTCATCAAACCAGACATCTTCTTTAAGATTTCTTGGCT

Homo TTTTGATGCATGGCAAGCTCTCCTCATCAAACCAGACATCTTCTTTAAGATTTCTTGGCT

Mus CTTTAACGCCTGGCAAGCTCTCCTCATCAAACCAAACATCTTCTTTAAGATTTCTTGGCT

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Gallus TTGCAAGAAGTATGAAGAGGCAGCCAAAGACTTGAAAGGAGCAATGGAAATCTTAATAGA

Pogona GTACAGGAAATACGAGAAGTCTGCGAATGACTTGAAGGAGGCCATTGAAGTCTTGATCCA

Anolis GTACAAGAAATACGAAAAGTCCGCGAACGATCTGAAAGAAGCCATCGAGGACCTAATAGA

Calotes ATACAAAAAGCATGAGAAGTCTGTCAAGGATTTGAAAGATGCCATAGAAGTTCTGATAGC

Homo ATACAAAAAGTATGAGAAGTCTGTCAAGGATTTGAAAGATGCCATAGAAGTTCTGATAGC

Mus CTACAGAAAGTATGAACGATCCGTCAAGGACTTGAAAGACGAGATAGCTGTTTTGGTGGA

 \* \*\* \*\* \* \*\* \* \* \*\* \*\* \*\*\*\* \* \* \*\* \* \* \*

Gallus ACAGAAACGACAAAAGCTTTCCACTGTTGAAAAGTTGGATGAGCACATGGATTTTGCATC

Pogona GCAGAAGCGGCAGAAGCTTTCCCTGCTGGAGAAACTGGAGGAGCCCATGGATTTCACCTC

Anolis GCAGAAGCGGCAAAAGCTTTCCACGATTGACAAACTGGAGGACCATATGGATTTTGCATC

Calotes AGAAAAAAGACGCAGGATTTCCACAGAAGAGAAACTGGAAGAATGTATGGACTTTGCCAC

Homo AGAAAAAAGACGCAGGATTTCCACAGAAGAGAAACTGGAAGAATGTATGGACTTTGCCAC

Mus AAAGAAGAGACACAAAGTTTCCACAGCTGAGAAACTGGAAGACTGTATGGATTTTGCAAC

 \* \*\* \* \* \* \*\*\*\*\* \*\* \*\* \*\*\*\* \*\* \*\*\*\*\* \*\* \* \*

Gallus TCAGCTGATTTTTGCACAGAACAGAGGAGATCTGACTGCTGAGAATGTGAACCAGTGTGT

Pogona CCAGCTGATTTTTGCACAGAGCCGTGGGGAACTCACCGGTGAGAATGTAAACCAGTGTGT

Anolis CCAGTTGATTTTTGCGCAGAGCCGCGGGGAGCTGACTGCTGAGAACGTCAACCAGTGCGT

Calotes TGAGTTGATTTTAGCAGAGAAACGTGGTGACCTGACAAGAGAGAATGTGAACCAGTGCAT

Homo TGAGTTGATTTTAGCAGAGAAACGTGGTGACCTGACAAGAGAGAATGTGAACCAGTGCAT

Mus TGATTTGATTTTCGCTGAGAGACGTGGAGACCTGACGAAAGAGAACGTGAATCAGTGCAT

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Gallus GCTGGAGATGATGATTGCTGCTCCTGATACTCTGTCCGTGACACTCTTCATTATGCTAAT

Pogona TCTGGAGATGCTGATTGCTGCCCCTGATACTCTCTCCGTGACACTGTTTTTCATGCTGGT

Anolis TTTGGAGATGCTCATCGCTGCCCCGGACACCCTTTCGGTCACTCTGTTCTTCATGCTGGT

Calotes ATTGGAAATGCTGATCGCAGCTCCTGACACCATGTCTGTCTCTTTGTTCTTCATGCTATT

Homo ATTGGAAATGCTGATCGCAGCTCCTGACACCATGTCTGTCTCTTTGTTCTTCATGCTATT

Mus ACTGGAGATGCTGATTGCAGCCCCTGACACCATGTCGGTCACTCTGTACTTCATGTTACT

 \*\*\*\* \*\*\* \* \*\* \*\* \*\* \*\* \*\* \*\* \* \*\* \*\* \* \* \* \* \*\*\* \* \*

Gallus ACTGATTGCAGAGCACCCCACAGTGGAGGAGAAGATGATGAGAGAAATTGAAACTGTTAT

Pogona GCTAATTGCAGAACACCCTCAGGTGGAAGACGCAATGATGAAGGAAATACAGGCACAGAT

Anolis GCTGATCGCAGAGCACCCCCAAGTCGAAGAGGCCATGATGAAGGAAATACAGACCGTGAT

Calotes TCTCATTGCAAAGCACCCTAATGTTGAAGAGGCAATAATAAAGGAAATCCAGACTGTTAT

Homo TCTCATTGCAAAGCACCCTAATGTTGAAGAGGCAATAATAAAGGAAATCCAGACTGTTAT

Mus TCTCGTCGCAGAGTATCCAGAGGTCGAAGCAGCAATCCTGAAGGAGATCCACACTGTTGT

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Gallus GGGTGACAGAGATGTACAGAGTGATGACATGCCAAACCTCAAAATTGTGGAGAATTTTAT

Pogona AGGTAACAGGGAGATCCAAGGTGAGGACATGCCCAAACTTAAAGTGGTGGAGAATTTTAT

Anolis AAGTGACAGAGACATCCAAAGCGAGGACATGCCGAAGCTGAAGGTGGTTGAGAATTTCAT

Calotes TGGTGAGAGAGACATAAAGATTGATGATATACAAAAATTAAAAGTGATGGAAAACTTCAT

Homo TGGTGAGAGAGACATAAAGATTGATGATATACAAAAATTAAAAGTGATGGAAAACTTCAT

Mus GGGTGACAGAGACATAAAGATCGAGGATATCCAAAATTTAAAAGTGGTGGAAAACTTCAT

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Gallus TTATGAGAGCATGAGATACCAGCCAGTTGTGGACTTAATCATGAGAAAAGCTTTACAAGA

Pogona CCTCGAGAGCATGAGATACCAGCCGGTGGTGGACCTGGTCATGCGCAAAGCCTTGCGGGA

Anolis TCTGGAGAGCATGAGATACCAGCCGGTCGTGGACTTGGTCATGCGCAAAGCTCTGCAAGA

Calotes TTATGAGAGCATGCGGTACCGGCCTGTCGTGGACTTGGTCATGCGCAAAGCCTTAGAAGA

Homo TTATGAGAGCATGCGGTACCAGCCTGTCGTGGACTTGGTCATGCGCAAAGCCTTAGAAGA

Mus TAATGAAAGCATGCGGTACCAGCCTGTTGTGGACTTGGTCATGCGCAGAGCTCTGGAGGA

 \*\* \*\*\*\*\*\* \* \*\*\*\* \*\*\* \*\* \*\*\*\*\*\* \* \*\*\*\*\* \* \* \*\*\* \* \*\*

Gallus TGATGTAATTGATGGATATCCTGTGAAAAAGGGAACAAACATTATTCTCAACATTGGACG

Pogona TGACGTGATCGATGGCTATCCAGTGAAGAAAGGAACCAACATCATTCTGAACATTGGCCG

Anolis TGATGTGATTGATGGCTATCCGGTGAAAAAAGGCACCAACATCATTCTCAACATCGGGCG

Calotes TGATGTAATCGATGGCTACCCAGTGAAAAAGGGGACAAACATTATCCTGAATATTGGAAG

Homo TGATGTAATCGATGGCTACCCAGTGAAAAAGGGGACAAACATTATCCTGAATATTGGAAG

Mus TGACGTAATTGACGGCTACCCGGTTAAAAAGGGAACTAACATCATTCTGAACATCGGAAG

 \*\*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\* \*\*\*\*\* \*\* \*\* \*\* \*\* \*\* \*

Gallus CATGCACAAGCTTGAATTCTTCCCAAAACCGAATGAGTTTTCTCTTGAAAACTTTGAGAA

Pogona CATGCATAAGCTAGAGTTTTTCCCGAAACCTAATGAATTCTCCCTTGAGAATTTTGAGAA

Anolis CATGCATCGGCTTGAGTTCTTCCCAAAGCCCAATGAGTTCTCGCTCGAAAATTTTGAAAA

Calotes GATGCACAGACTCGAGTT------------------------------------------

Homo GATGCACAGACTCGAGTTTTTCCCCAAACCCAATGAATTTACTCTTGAAAATTTTGCAAA

Mus AATGCACAGGCTCGAGTACTTCCCTAAGCCCAATGAATTTACCCTTGAAAACTTTGAGAA

 \*\*\*\*\* \*\* \*\* \*

**2c *CvRspo1***



**Figure 2: Multiple sequence alignment of *CvFoxl2 (a), CvArom (b) & CvRspo1 (c)* mRNA sequence**. Sequences of *Calotes* and other vertebrates were aligned using clustalW multiple sequence alignment program. Stars (\*) indicates conserved sequence. Missing nucleotide/sequences are indicated by hyphen (-).

**S Figure 3 Amino acid alignment with proteins of different vertebrates:**

**3a (*CvFoxl2*)**



**3b (*CvArom*)**



**3c (*CvRspo1*)**



**Figure 3: Multiple sequence alignment of *CvFoxl2 (a), CvArom (b) & CvRspo1 (c)* amino acid sequence**. Sequences of *Calotes* and other vertebrates were aligned using clustalW multiple sequence alignment program. Stars (\*) indicates conserved sequence. Missing nucleotide/sequences are indicated by hyphen (-).