# CHAPTER 2 Profiling of the Family of Sox Genes by PCR

## Introduction

The mouse foetal cDNA panel created in Chapter I was then tested by PCR with primers for members of the *Sox* gene family. A selection of these *Sox* genes, which showed differing expression patterns, was then taken for analysis by *in-situ* hybridization, as described in Chapter 3.

### 2.1 Special Equipment and Suppliers

| | |
|---|---|
| Computer | Sun workstation |
| Computer | Apple Mac. |
| Software | Mac Draw, Adobe photoshop, Microsft Office, Blixem, AceDB, Internet Explora |

### 2.2 Materials and Solutions

As described in Chapter 1

### 2.3 Establishment of the *Sox*-related sequences for study

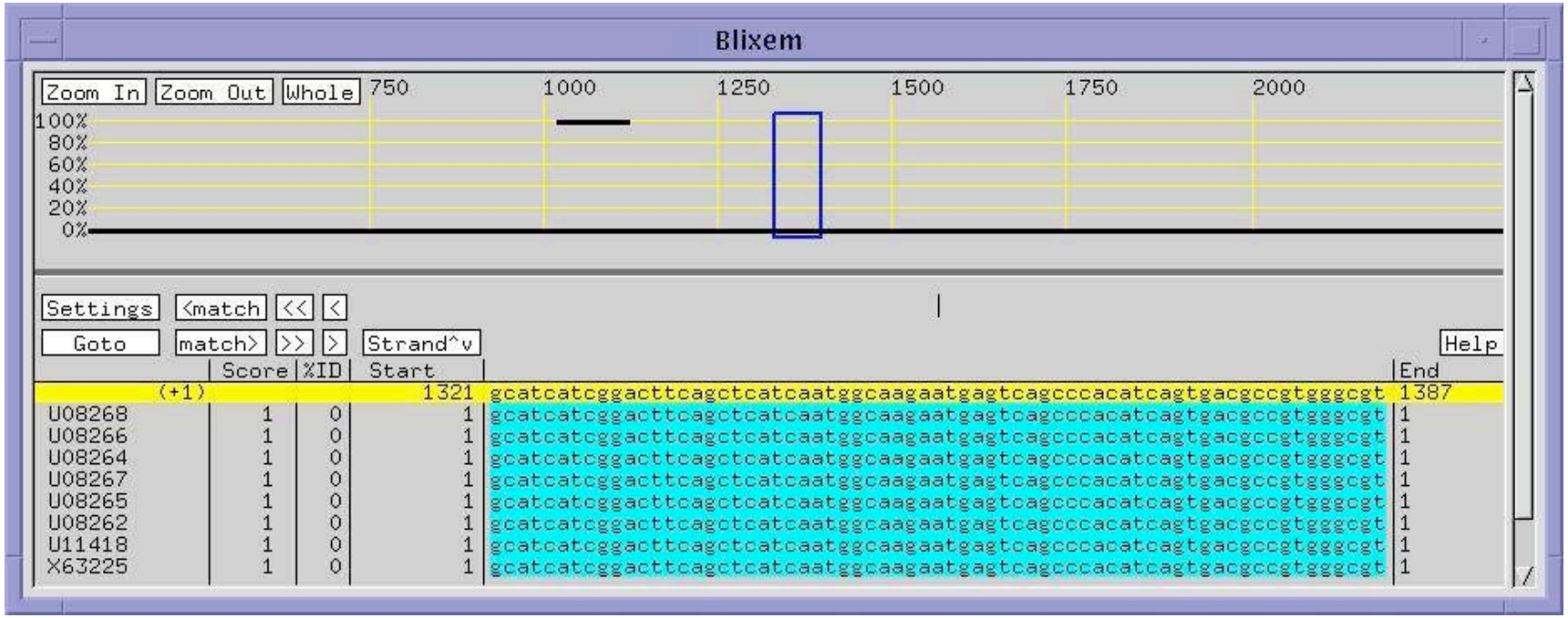
All known *Sox*-related sequences were obtained from the embl website and interrogated for primer design. This was done using the AceDB software designed by Durbin and Thierry-Mieg (1991; http://www.sanger.ac.uk/Software/Acedb/) and adapted as a repository for *Sox*-related sequences. Using the query form from http://www.sanger.ac.uk/SRS and the fields 'organism' and 'all text', for *Mus musculus* and Sox, respectively, a list of sequencing information was imported from

the embl and embl-new databases into this "Soxace database", with the kind assistance of Adam Butler.

It was necessary to determine how many of these genes were represented in this dataset. Therefore, the 274 collected sequences were clustered using the Clustal W and Phrap packages to ensure a 98% homology over a 100 base-pair stretch of sequence, resulting in 16 distinct cluster groups. Tight clustering stringency ensured that each of the *Sox* genes separated into different groups. The *Sry* group held 28 sequences, *Sox 2, 4, 5, 6, 7, 9, 11, 13* and *18* all contained a number of sequences. *Sox 1, 3, 8, 14, 16* and *19*, together with 63 other *Sox*-related sequences failed to group during this exercise. This study highlighted the current interest in the *Sox* genes as illustrated by the cluster sizes. Interestingly, at the time of data collection (July 1999), the *Sox 4* related gene showed the greatest number with 43 and 48 sequences in two separate groups. There are currently 687 *Sox*-related sequences held in the embl database (November 2003).

Blixem software ("BLast matches In an X-window Embedded Multiple alignment") was used to examine the clustered sequences, see Figure 32. This software was developed at the Sanger Institute by Dr. R. Durbin and provides a graphics window, to view sequence alignments of up to 1.2 megabases [1]. Those sequences with greatest homology to all others in their cluster, incorporating the longest 3' region, were chosen for primer design (section 1.3.10).

## Figure 32: Blixem Software




Blixem software used to view sequence alignment and identify similarities at the nucleotide level.

Of the primer pairs designed, seven (for *Sox 3, 13, 16, 17, 18, LZ* and *Sry*) failed at the prescreen stage (1.4.5.) and were redesigned. Despite a number of trials at prescreen, we failed to obtain successful primer pairs for *Sry, Sox 3* and *Sox18*. A list of all the primers used and the expected amplicon product sizes are in Figure 33 with further details in Figure 34 [NOTE: the final primer sets that failed the prescreen for *Sry, Sox 3* and *Sox18* are also included in brackets]. Failed PCRs were generally attributed to primer design, after one redesign of primers were usually designated as fails and no longer pursued.

## Figure 33: Expected amplicon sizes for PCR

| | |
|---|---|
| Sox 1 | 224 |
| Sox2 | 184 |
| [Sox3 | 211] |
| Sox4 | 210 |
| Sox5 | 239 |
| Sox6 | 238 |
| Sox7 | 113 |
| Sox8 | 106 |
| Sox9 | 104 |
| Sox11 | 151 |
| Sox12 | 151 |
| Sox13 | 333 |
| Sox14 | 100 |
| Sox15 | 198 |
| Sox16 | 151 |
| Sox17 | 175 |
| [Sox18 | 278] |

| | |
|---|---|
| Sox19 | 164 |
| Sox21(Sox10) | 203 |
| SoxLZ | 327 |
| Slc17a2 | 175 |
| Hox5b | 164 |
| Fsbpi | 126 |
| Adora1 | 179 |
| Csna | 128 |
| Fabph1 | 157 |
| Si-S | 203 |
| Calb1 | 187 |
| Rsp29 | 134 |
| Cab45 | 170 |
| [Sry] | |
| bac 573K1b | 265 |
| bac 573K1a | 261 |

## Figure 34: Primer Sequences

| Sanger ID | Forward Primer | Reverse Primer | Symbol | Accession number |
|---|---|---|---|---|
| St95_22 | ATGTGTGTGTGTGTGCACATG | TACACCCGCACTAATGGTCA | Slc17a2 | L33878 |
| st95_35 | GCTTCTATCTGGCGGAAGG | TGTCATCTGGCTACCTTCCC | Calb1 | M21531 |
| st95_42 | GCTTCTATCTGGCGGAAGG | TGTCATCTGGCTACCTTCCC | Calb1 | M23663 |
| st95_43 | CTCTTTTCTCCCACCTCATCC | CTACCAGGCAGCAGGAGTTC | Hox5b | M26283 |
| st95_58 | AAGTCAACTTCTCAGAGCCTGG | GCTTTGACAAGGCTGGAGAC | Fsbpi | M65034 |
| st95_69 | CCCAGAAGTACTACGGGAAGG | CGAGTTGCCGTGTGTGAG | Adora1 | U05671 |
| st96_128 | GCCAATGATTCATCTTGAGTTG | CCTTGATTCTCTCCGCTCAG | Csna | M36780 |
| st96_153 | CTCATGGTTTTCCCCTCTGA | GGTTCTGCTTTATTGACCTTGG | Fabph1 | U02883 |
| st96_273 | GGGGAAGTGGAACACACGG | AGCAGGAGTTGGCTGGAATG | Si-s | X15546 |
| st96_310 | CTGATCCGCAAATACGGG | GCATGATCGGTTCCACTTG | Rps29 | L31609 |
| st96_428 | GAAGAGTTCTGAGCATGCCC | TTCTTGGGGCCTATGGAAG | Cab45 | U45978 |
| st97_595 | AAAAAAAAATGCCCATGCAG | TACGGAAAATAAAAGGGGGG | Sox2 | U31967 |
| st97_596 | TATGGTATGAAGATGGACGGC | CATGCGGGCTCTTTAAGAAC | Sox6 | U32614 |
| st97_613 | TTGAAGAAGCCCTGTCCG | TGGCACTGTTTAACCCATAGC | Sox5 | X65657 |
| st97_614 | ACGTCTGGCAGTGCAGAAC | CTGCCTCATCCACATAGGGT | Sox7 | X65660 |
| st97_618 | TTTCAGCTCCTCATCGGC | CTCCTCTCCTGCCTCTTGG | Sox4 | X70298 |
| st97_625 | AATCCCCTCTCAGACGGTG | TTGATGCATTTTGGGGGTAT | Sox1 | X94126 |
| st97_627 | GTACACAACTACCCAAGGGAGC | GTGGAAGAGTCTGGGGATAGG | Sox15 | X98369 |
| st97_629 | TAACGCAGAGCTCAGCAAGA | CTTGTGCTGCACACGGAG | Sox8 | Z18957 |
| st97_630 | AGGAAGCTGGCAGACCAGTA | ACGAAGGGTCTCTTCTCGCT | Sox9 | Z18958 |
| st97_632 | CGAGCGCAGGAAGATCAT | ATCAGCCATGTGCTTGAGG | Sox11 | Z18960 |
| st97_635 | GATGGCCCAGGAAAATCC | GATGTAAGGCCGCTTCTCTG | Sox14 | Z18963 |
| st97_760 | ATGGCTTGATTGGTACCAGTG | GACAAGTGGAAAAACAGGAAGC | bac 573K1a | |
| st97_761 | TGCAGGCAGAGATGCTACTG | CGCTCAGAGAGAAAAAAATTGG | bac 573K1b | |
| st97_787 | TCAAGGGAAAAGAAGAGGGC | TTCAGCGTATCCACCACATCG | SoxLZ | D61689 |
| st97_785 | CACGGAGCCATTTTTAGCTC | TAGGGTCAACAGCGGTGA | Sox13 | AJ000740 |
| st97_786 | TGTCTGCCACTTGAACAGTTG | TGAGAAAACACGCATGACAA | Sox17 | D49473 |
| st97_788 | AGGCTGGACACTAAACCCCT | AAGTTAATCGGGGCTGGAGT | Sox21 | D87031 |
| [st97_789 | GGATGACTGACTGGCCATCT | GGCTGCTATTTTCTTCCAACC | Sry | E11536] |
| st97_790 | CATGGTGTGGAGCTCTGCTC | ACGAAGACGCTTAGCCTCCT | Sox16 | L29084 |
| [st97_791 | CAGGCTAGACACTGTCCTTGC | GAACATAAATGGCAGAAAAGCC | Sox18 | L35032] |
| [st97_792 | ATGGGCTCCGTGGTGAAGT | TTCCATTGACCGCAGTCC | Sox3 | X94125] |
| st97_793 | CATGGTCTGGTCGCAAATC | CCATGTGCTTTAGCCGAAGT | Sox19 | X98368 |
| st97_794 | GAGCGGAGAAAAATCATGGA | AGTCCGCCATGTGCTTGAG | Sox12 | Z18961 |

### 2.4.1 Results of the PCR for the Sox Genes

The tissue profiles of the PCR products, together with a summary of the PCR conditions, for each of the Sox gene primer sets used are shown as Figures 36-62. In order to view these figures graphically, a number of the original images were digitized as described in Chapter 1, section 1.5. Figure 35 is a list of the tissues examined with identification numbers for samples to accompany the graph images of tissue profiles for the *Sox* genes 1, 2, 4, 9 and 15. Each of the *Sox* genes will be discussed in the light of the expression profiles and existing knowledge in the published literature.

Legend for all gel images Figures 36 – 62 is:

Ethidium bromide stained 2.5% agarose gels, run at 125 volts, 30 milliamps, 2 hours and photographed with a Polaroid camera. Photographs were scanned on an Epson scanner, opened in Adobe Photoshop, where defined regions were then cut and pasted into MacDraw and scored through viewing the original photographs.

Gene/Est: Gene or EST name
Symbol: Acronym for gene/est
EMBL AcNo: Accession number from EMBL database
Primers: Sanger Centre identifying number

PCR: Polymerase Chain Reaction
Date: date of lab. work
$MgCl_2$: final concentration
Temp: annealing temperature
Cycles: number of cycles

Amplification signal: four catagories
Strong – dark blue – major signal from both duplicates.
Moderate-weak – blue – broadest range of signal.
Trace – green – faint signal.
None – yellow – no signal from either duplicate.
Discrepancy – red – signal from one only, of the duplicates.

## Figure 35: Tissue identification of graph position

| ID | tissue |
|---|---|
| 1 | whole conceptuses 8.5d |
| 2 | whole conceptuses 9.5d |
| 3 | whole embryo 10.5d |
| 4 | whole embryo 11.5d |
| 5 | whole embryo 12.5d |
| 6 | whole embryo 13.5d |
| 7 | whole embryo 15.5d |
| 8 | whole embryo 17.5d |
| 9 | forebrain 12.5d |
| 10 | forebrain 13.5d |
| 11 | forebrain 15.5d |
| 12 | forebrain 17.5d |
| 13 | midbrain 12.5d |
| 14 | midbrain 13.5d |
| 15 | midbrain 15.5d |
| 16 | midbrain 17.5d |
| 18 | hindbrain 12.5d |
| 19 | hindbrain 13.5d |
| 20 | hindbrain 15.5d |
| 21 | hindbrain 17.5d |
| 22 | spinal cord 12.5d |
| 23 | spinal cord 13.5d |
| 24 | spinal cord 15.5d |
| 25 | spinal cord 17.5d |
| 26 | head 12.5d |
| 27 | head 13.5d |
| 28 | head 15.5d |
| 29 | head 17.5d |
| 30 | heart 12.5d |
| 31 | heart 13.5d |
| 32 | heart 15.5d |
| 33 | heart 17.5d |
| 35 | lung 12.5d |
| 36 | lung 13.5d |
| 37 | lung 15.5d |
| 38 | lung 17.5d |
| 39 | liver 12.5d |
| 40 | liver 13.5d |
| 41 | liver 15.5d |
| 42 | liver 17.5d |
| 43 | gut 12.5d |
| 44 | intestine 13.5d |
| 45 | oes/stom 13.5d |
| 46 | intestine 15.5d |
| 47 | oes/stom 15.5d |
| 48 | intestine 17.5d |
| 49 | urogenital/kidney 12.5d |
| 50 | urogenital /kidney 13.5d |

| ID | tissue |
|---|---|
| 52 | urogenital/gonads 15.5d |
| 53 | urogenital male gonads 17.5d |
| 54 | urogenital female 17.5d |
| 55 | bladder 15.5d |
| 56 | kidney 15.5d |
| 57 | bladder 17.5d |
| 58 | kidney 17.5d |
| 59 | spleen 17.5d |
| 60 | forelimbs 12.5d |
| 61 | forelimbs 13.5d |
| 62 | forelimbs 15.5d |
| 63 | forelimbs 17.5d |
| 64 | rest of body 12.5d |
| 65 | rest of body 13.5d |
| 66 | rest of body 15.5d |
| 67 | rest of body 17.5d |
| 69 | extra embryonic 10.5d |
| 70 | extra embryonic 11.5d |
| 71 | placenta 12.5d |
| 72 | yolk sac 12.5d |
| 73 | placenta 13.5d |
| 74 | yolk sac 13.5d |
| 75 | placenta 15.5d |
| 76 | yolk sac 15.5d |
| 77 | placenta 17.5d |
| 78 | yolk sac 17.5d |
| 79 | whole brain - adult |
| 80 | spinal cord - adult |
| 81 | skeletal muscle - adult |
| 82 | heart - adult |
| 83 | liver - adult |
| 84 | kidney - adult |
| 86 | fundus - adult |
| 87 | caecum - adult |
| 88 | testis - adult |
| 89 | ovary - adult |
| 90 | one day old mouse |
| 91 | glycogen |
| 92 | mouse genomic |
| 93 | human genomic |
| 94 | rat genomic |
| 90 | glycogen |

**Figure 36: Expression Profile of *Sox1* in Mouse Foetal Panel**

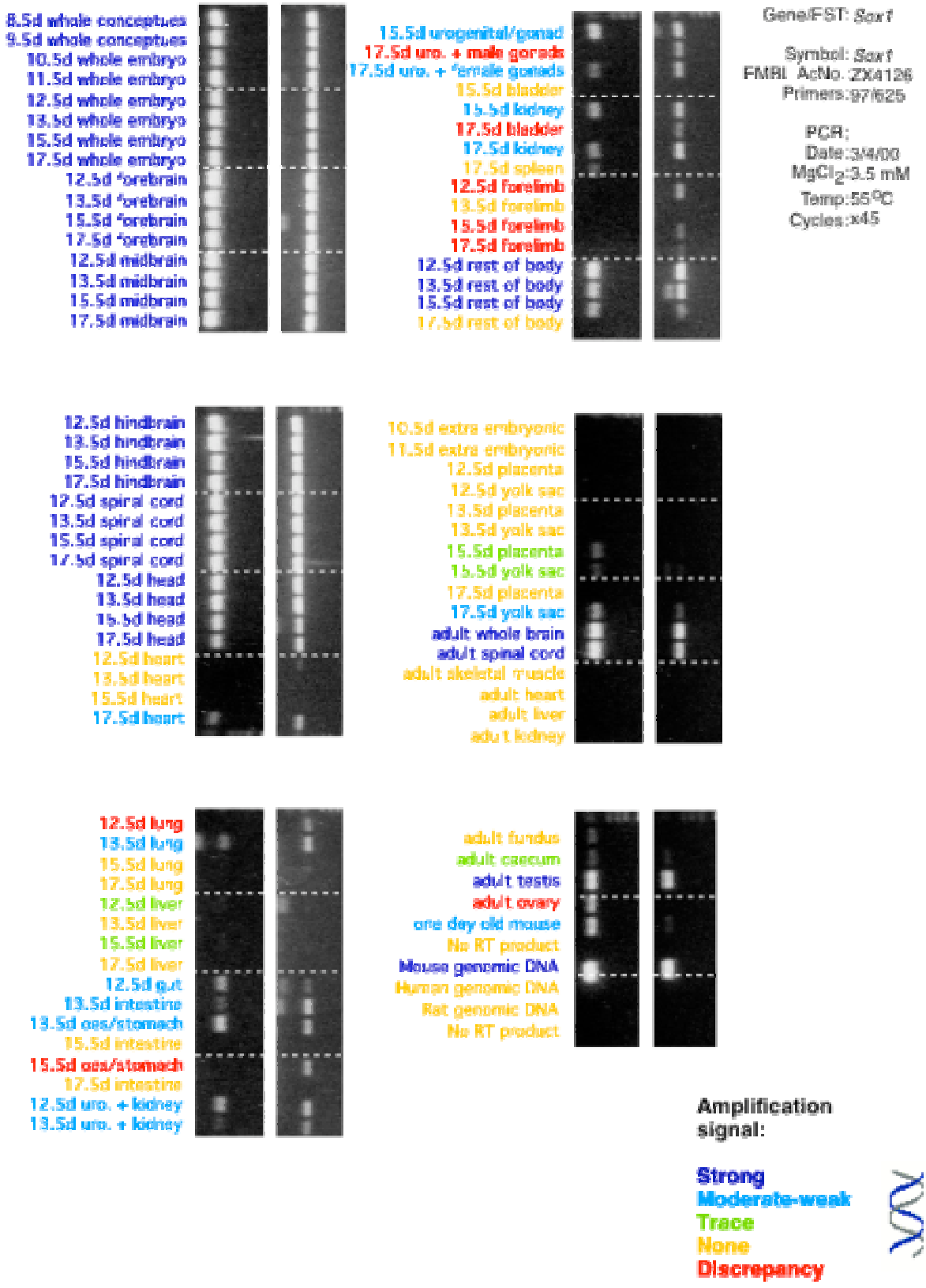
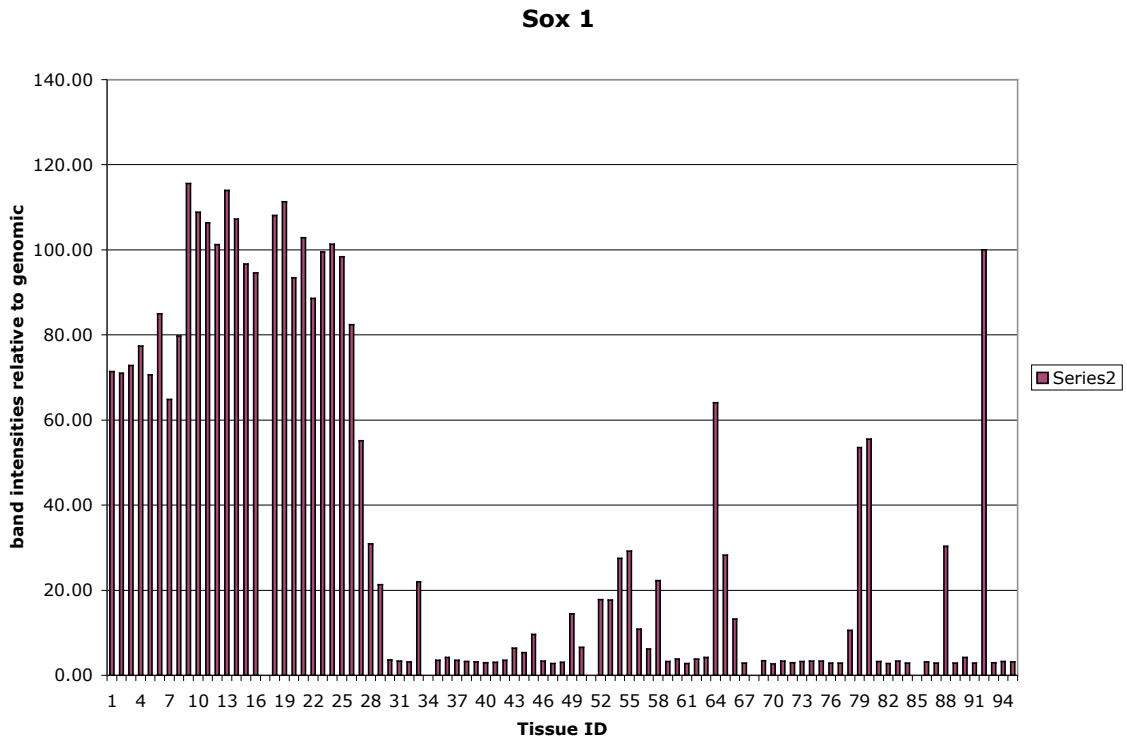


**Figure 37: Graphical representation of gel image in Figure 36.**




Densitometric measurements of gel band fluorescence displayed as a percentage of the mouse genomic fluorescent band intensity in position 92. Tissue identifications are located in Figure 35.

***Sox1***

Figure 36: *Sox 1* is noticeably strong in the whole organism, most probably as a result of the strong gene expression found in the brain and spinal cord. Internal organs show a much lower expression.

The graph Figure 37, of *Sox 1* highlights the overall pattern, clearly illustrating highest expression in the whole embryo, brain, spinal cord and head (1-29), plus rest of body13.5d – 15.5d (64-66); with strong expression also in the adult tissues brain (79), spinal cord (80), testis (88). Expression also noted in heart 17.5d (33) – but not in heart for 12.3d to 15.5d; and some of the internal organs - urogenital 15.5d, 17.5d, plus bladder 15.5d (52-55), and spleen 17.5d (58).

The adult tissues mirror expression found [2] earlier and confirm the importance of *Sox 1* in the brain, spinal cord and testis. Early studies have implicated *Sox 1*as an important gene in mouse brain [3], eye development [4] and it has also been shown to be involved in the onset of mouse neural determination [5].

***Sox2***

Figure 38: *Sox 2* expression in this study is shown through out the developing foetus with significantly lower levels in the liver as shown by the graph of the gel band intensities in Figure 38, and extra embryonic regions of early stages plus placenta and yolk sac of the later stages. The adult stages show no expression in the liver or kidney with strong expression in the neuronal tissues and fundus.

From the *Sox 2* graph, Figure 39, tissues with low expression are clearly identifiable as 12.5d heart (30), 15.5d heart (32), liver 12.5d – 17.5d (38-42), 17.5d intestine (48), and, although not obvious from the gel, spleen 17.5d (59).

The adult pattern of expression confirms earlier studies [2]. In mice, maternal *Sox2* is operational until the late morula stage, when zygotically transcribed *Sox 2* becomes essential for normal epiblast growth at post implantation stages [6]. Knockout *Sox2* mice are embryonic lethals. *Sox 2* (together with *Pax6*) are required for murine lens differentiation [7], [8], [9]. In other species, *Sox 2* is implicated in ovine developing gonads and germ cell formation [10].

In *Drosophila*, Dichaete, a protein related to *Sox 2*, is found throughout the developing CNS (central nervous system) [11], and from studies in chicken, *cSox2* expression increases in the CNS as neural ectoderm is established [12].

**Figure 38: Expression Profile of *Sox2* in Mouse Foetal Panel**

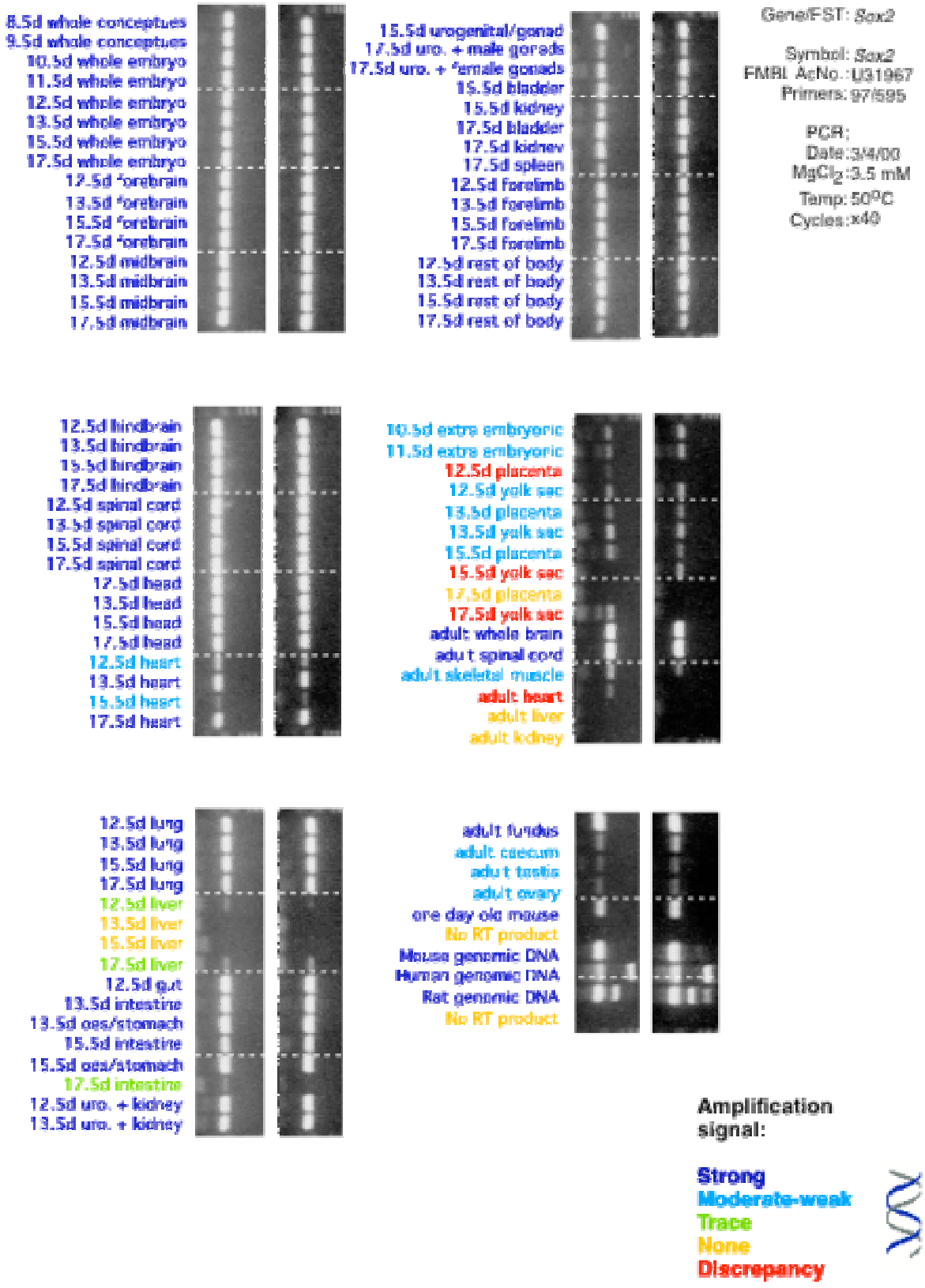
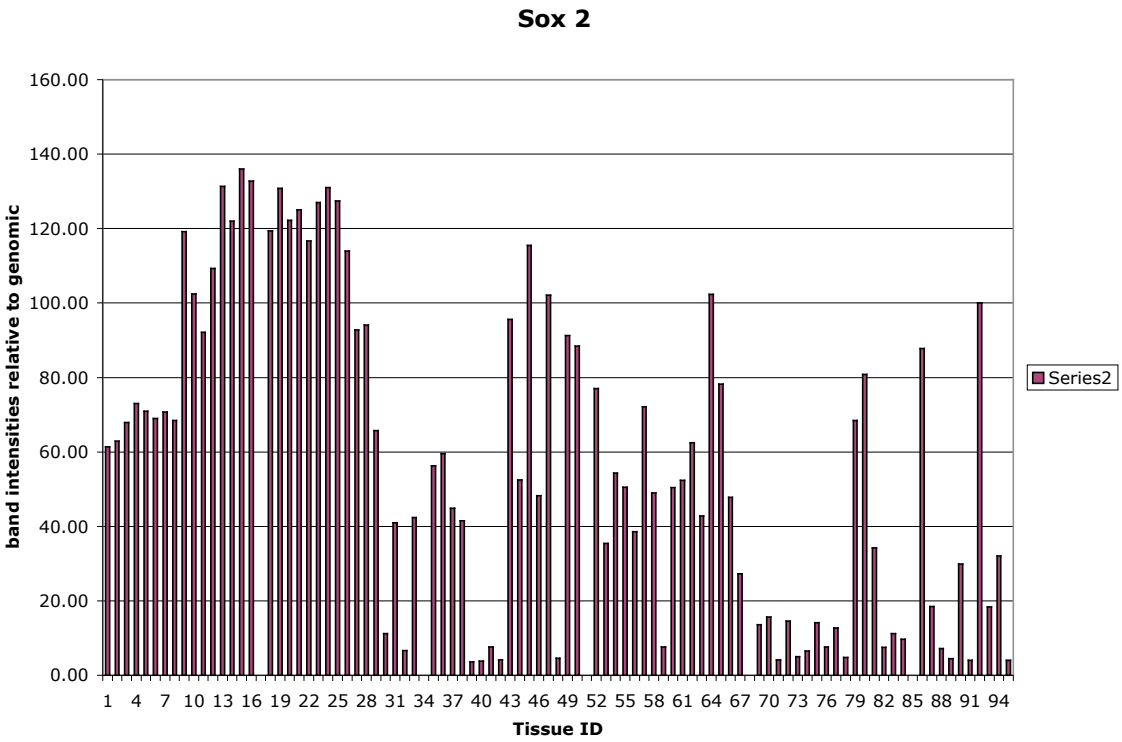


**Figure 39: Graphical representation of gel image in Figure 38**




Densitometric measurements of gel band fluorescence displayed as a percentage of the mouse genomic fluorescent band intensity in position 92. Tissue identifications are located in Figure 35.

***Sox4***

Figure 40: *Sox 4* expression is found throughout the developing and adult mouse in this study and previously [2]. The graph, Figure 41 of the gels for *Sox 4* highlights areas with lower levels of expression as head 17.5d (29), heart 15.5d (32) and liver tissues (39-42). *Sox4* has been reported in a wide range of tissues and knockout mice have been shown to die of heart defects during embryogenesis with malformed CNS [13].

**Figure 40: Expression Profile of *Sox4* in Mouse Foetal Panel**

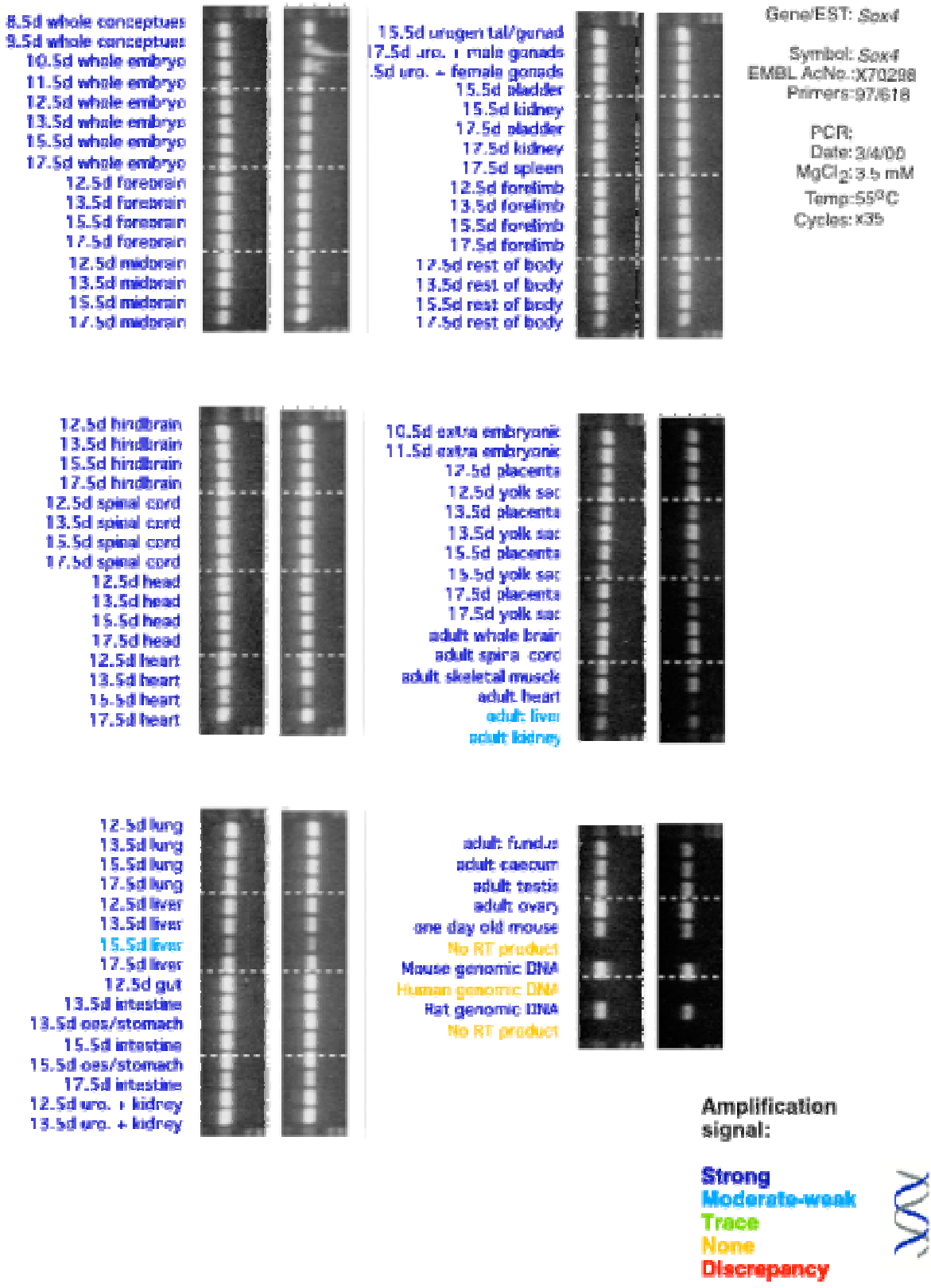
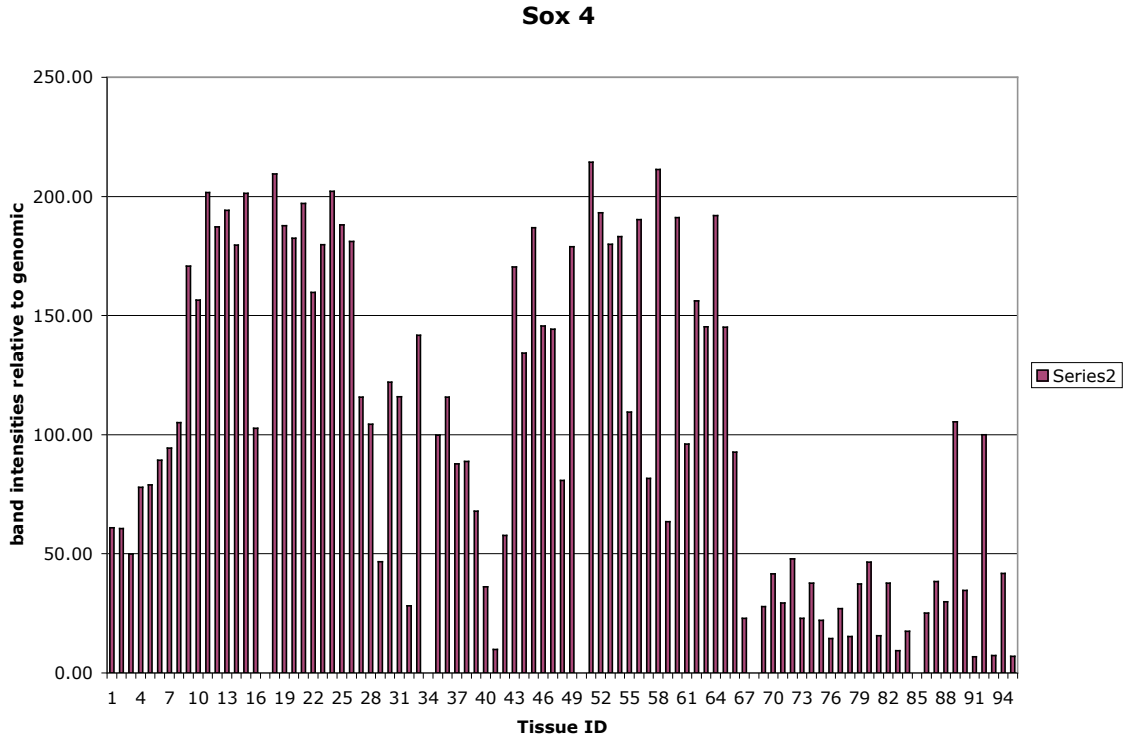


**Figure 41: Graphical representation of gel image in Figure 40.**




Densitometric measurements of gel band fluorescence displayed as a percentage of the mouse genomic fluorescent band intensity in position 92. Tissue identifications are located in Figure 35.

## ***Sox5***

Figure 42: *Sox 5* expression in the mouse foetal panel is clearly greater than that found in the adult panel [2]. *Sox 5* has been reported to be important during chondrogenesis [14] [15], cartilage formation being a major activity during development, and this would fit with the high levels found throughout this foetal panel. Post-meiotic spermatids in adult testis [16] have also been identified as a major site for *Sox5* expression, hence the large amount found here in adult testis. It has been reported that the human form of *SOX5* exists as different transcripts in the fetal brain and adult testis [17], which may explain the difference in levels between adult and foetal development found in this study.

**Figure 42: Expression Profile of *Sox5* in Mouse Foetal Panel**

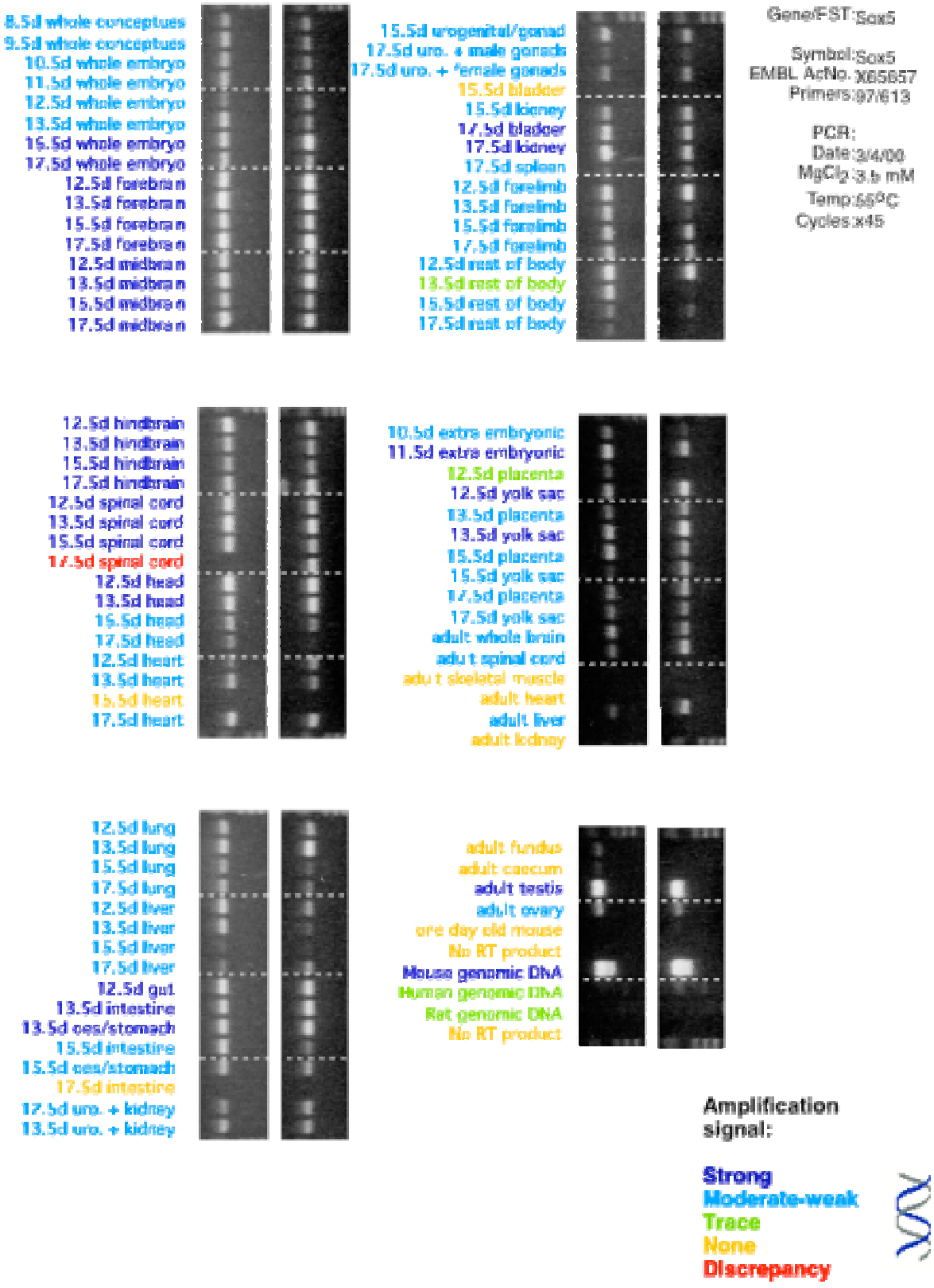
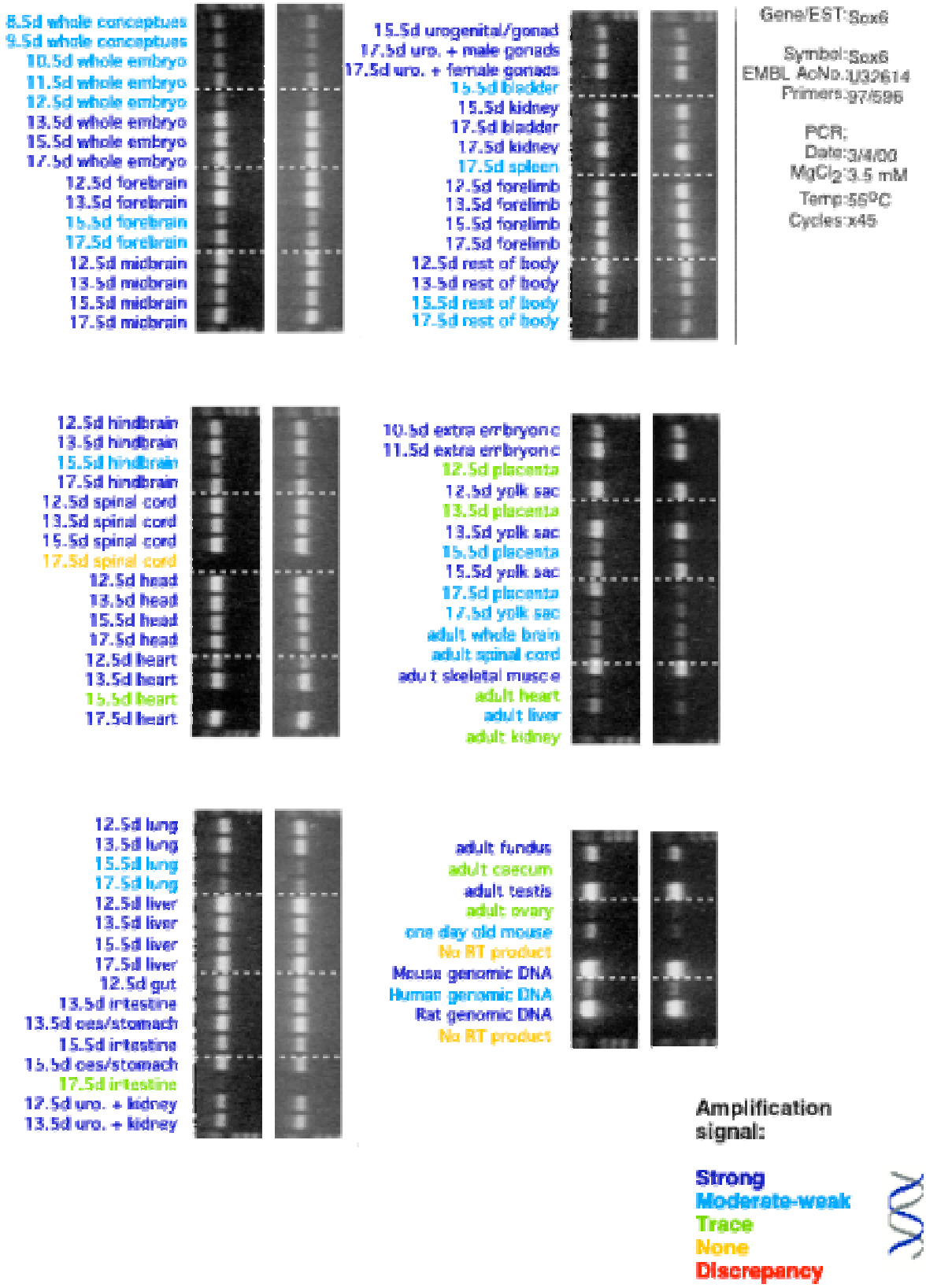


**Figure 43: Expression Profile of *Sox6* in Mouse Foetal Panel**



***Sox6***

Figure 43: *Sox6* pattern of expression in this foetal panel is not dissimilar to that found for *Sox5* (Figure 42), with the major exception of the adult skeletal muscle (*Sox6,* but not *Sox5)*. *Sox5* and *Sox6* are very similar at the sequence level, clustering in the B group [18]. Like *Sox5, Sox6* is required for chondrogenesis [14] and would explain the similarity in expression found in this study.

***Sox7***

Figure 44: *Sox7* shows a wider expression profile in this study relative to the expression profile found earlier [2] in adult tissues alone. Northern analysis of three adult tissues found ovary and heart positive but not skeletal muscle [19], confirming the expression pattern found in the adult tissues by RTPCR. From *in-situ* hybridization and Northern blot analyses, it has now been shown to be present in a variety of adult and fetal tissues, including skeletal muscle and is now thought to be involved in the development of the vascular system [20].

***Sox8***

Figure 45: *Sox8* expression in this study is found throughout the developing foetus in all tissues, with significantly less in both 15.5d and 17.5d lung and liver. Studies in *Sox8*-deficient mice, show this gene is not essential for development, suggesting that it's role may be taken by one of the other group E family members, either *Sox9* or *Sox10* [21]. It has also been suggested that this gene may substitute for *Sox9* during the formation of Sertoli cells. *Sox8* has been shown to be expressed between 12dpc in mouse testis and beyond 16dpc in the Sertoli cells [22]. From

work with cartilage development, all three E group *Sox8, 9* and *10* genes are thought to operate in a concerted fashion to effect digit cartilage [15].

**Figure 44: Expression Profile of *Sox7* in Mouse Foetal Panel**

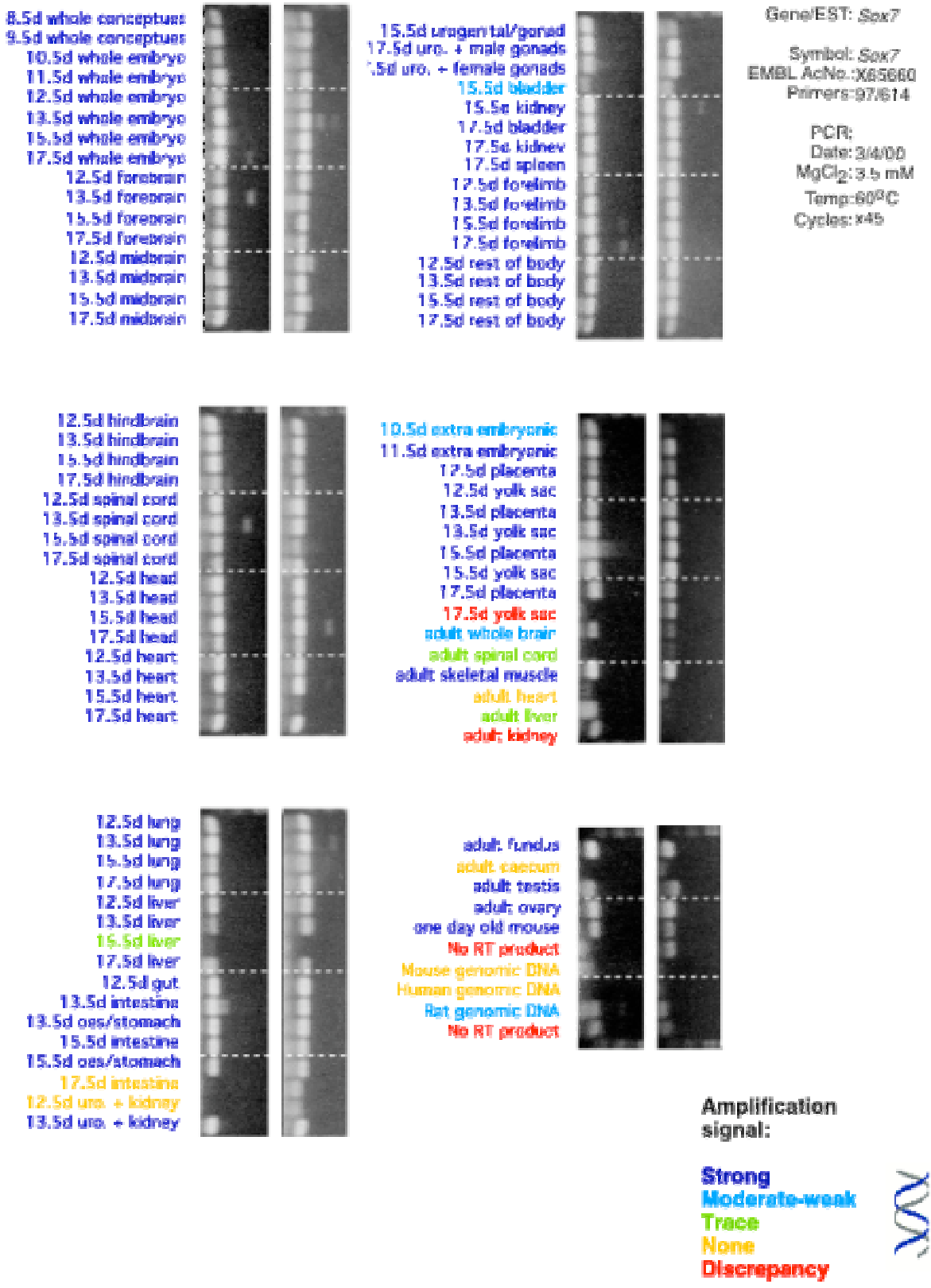


**Figure 45: Expression Profile of *Sox8* in Mouse Foetal Panel**

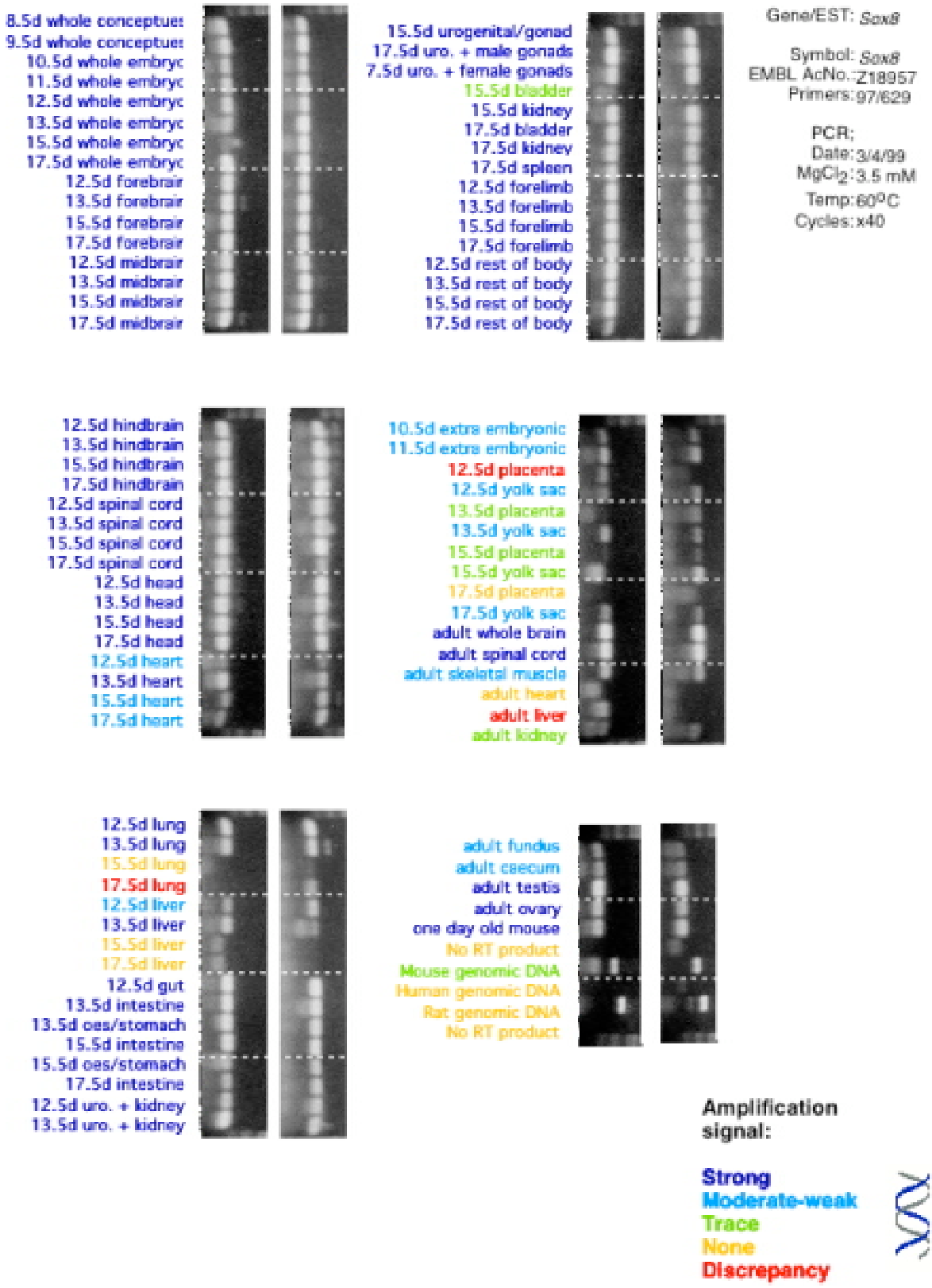


**Figure 46: Expression Profile of *Sox9* in Mouse Foetal Panel**

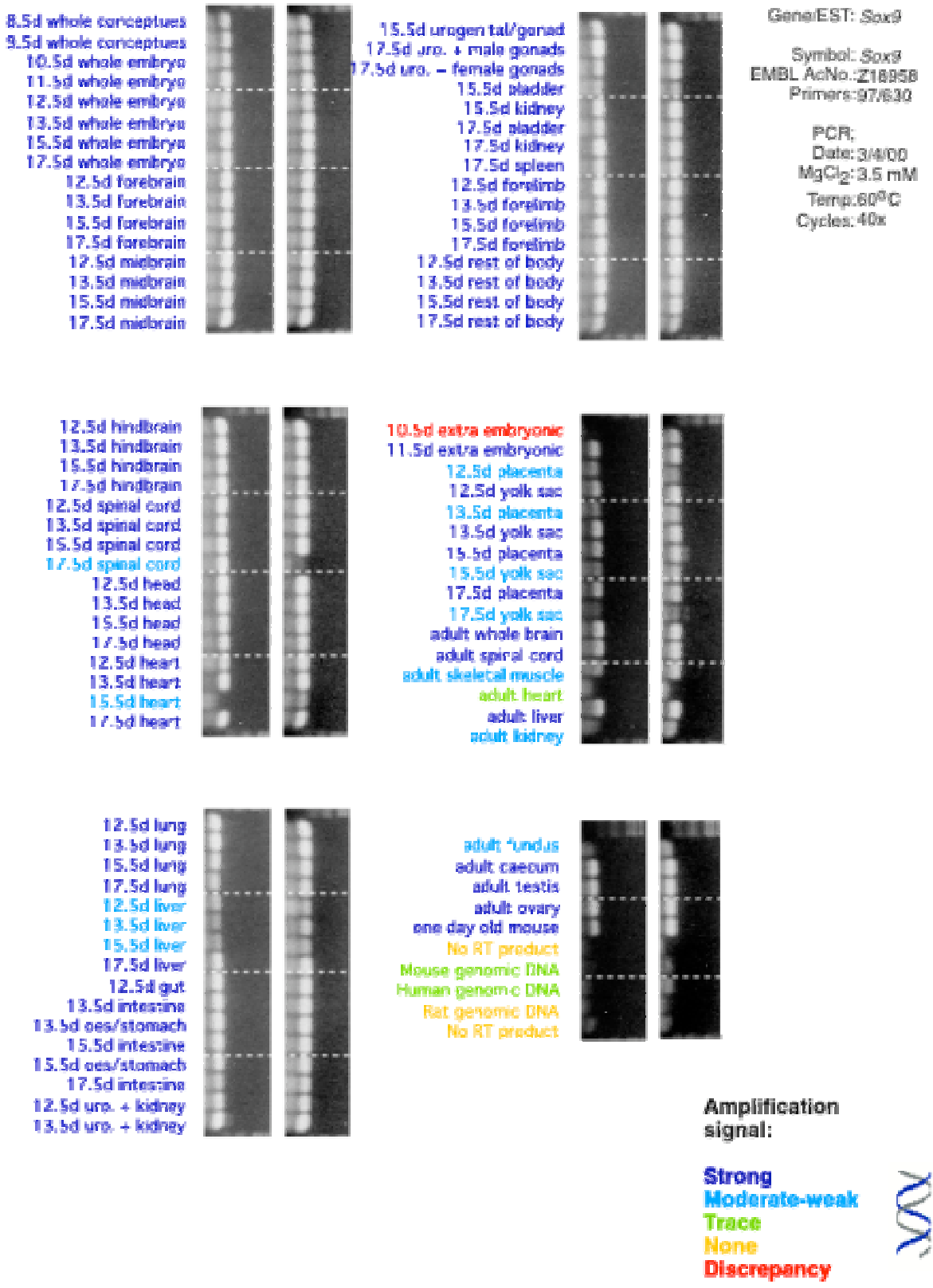
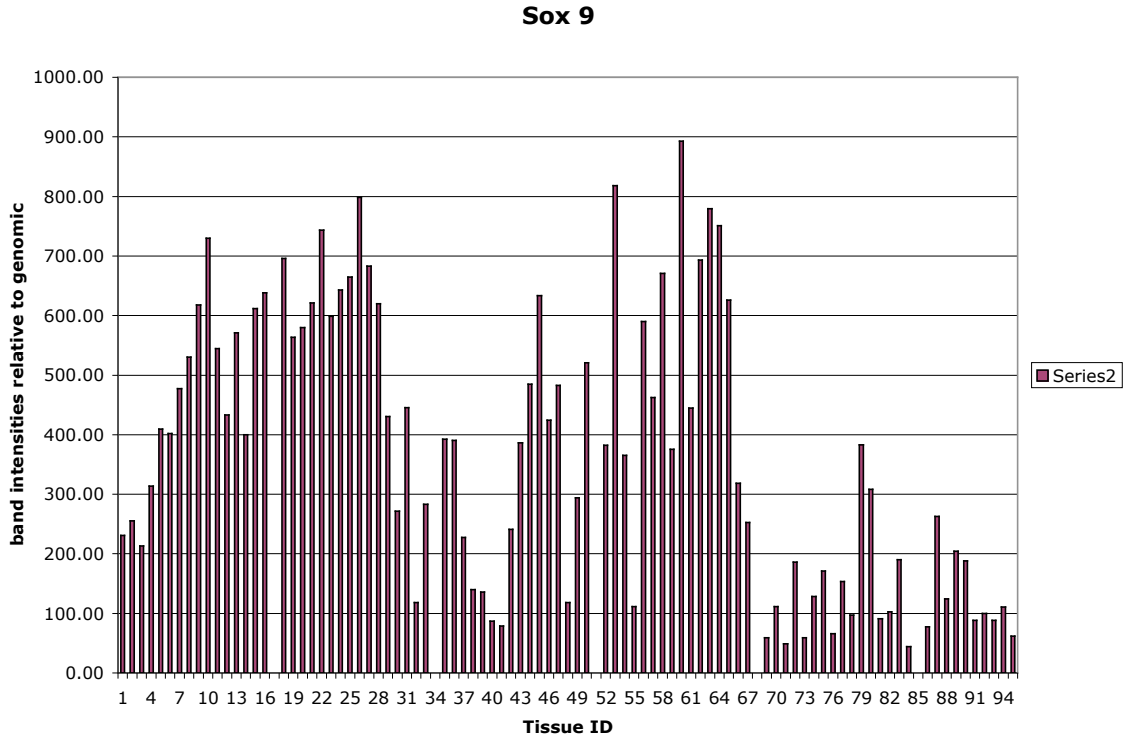


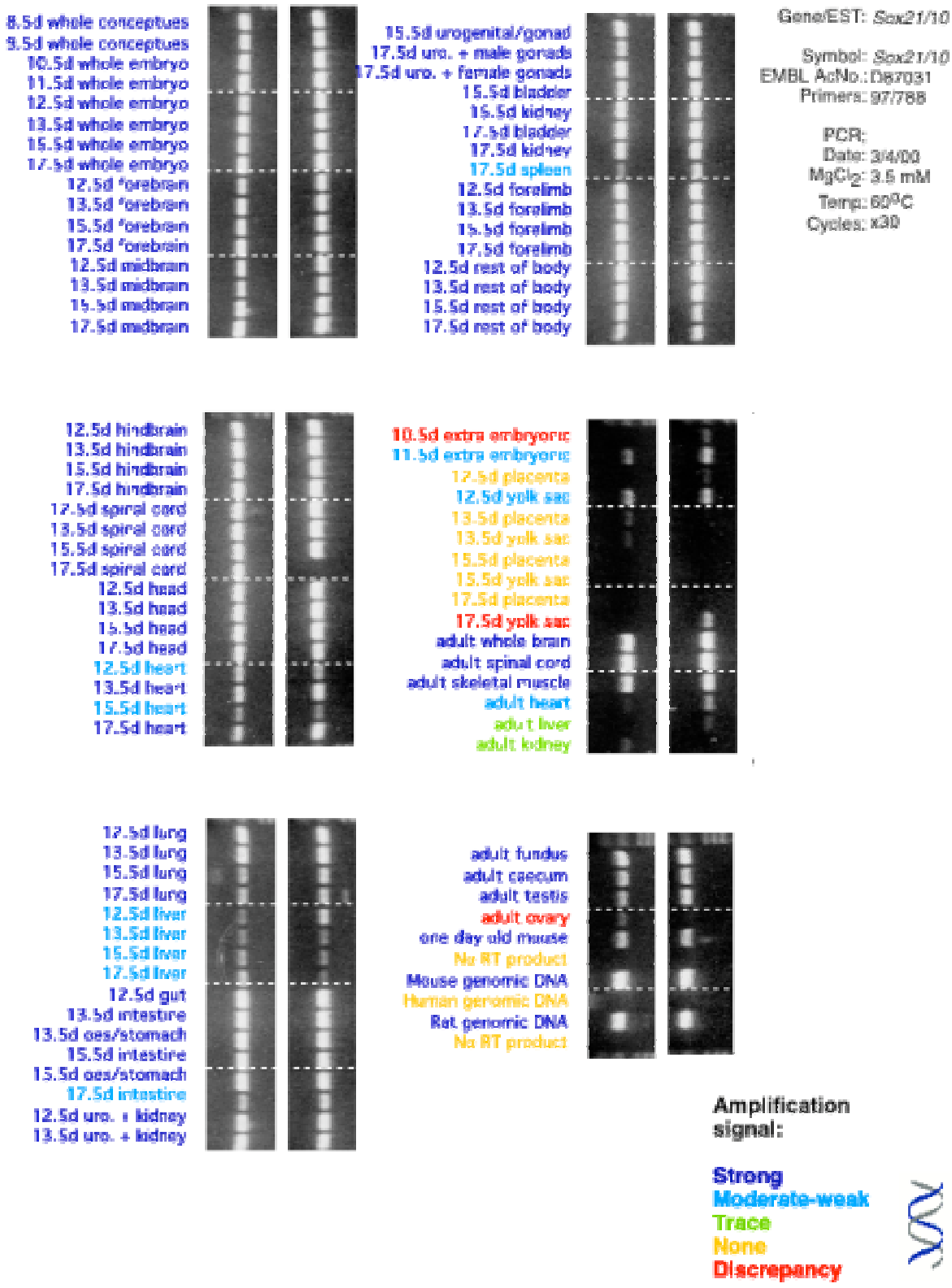
**Figure 47: Graphical representation of gel image in Figure 46.**




***Sox9***

Figure 46: *Sox9* expression is found in all tissues of the developing and adult mouse, in this and earlier studies [2]. The graph, Figure 47, of this gel highlights the regions of highest expression as brain (9-21), spinal cord (22-25), regions of the oesophagus/stomach/intestine (43-49), plus lower expression found in liver samples (39-41) 12.5d, 13.5d and 15.5d. All the urogenital regions except for 15.5d bladder show high expression, as does the placental and yolk sac tissues. From the adult section, brain (79), spinal cord (80) and caecum (87) are showing greatest expression. A lack of this gene leads to the disease campomelic dysplasia, a bone malformation and sex reversal [23]. Sox 9 is important in the maintenance of Sertoli cell development during testis formation [24] and implicated as the main element driving cartilage formation through binding to the type XI collagen gene Col11a2 [25].

**Figure 48: Expression Profile of *Sox10* in Mouse Foetal Panel**



***Sox10***

Figure 48: *Sox10* and *Sox21* were initially thought to be two different sequences, but they have since been assigned the same name of *Sox10* [26]. *Sox10* in this study is found throughout the developing foetus with the exception of placenta and from 13.5d the yolk sac. It is also low in all the liver tissues. Mutations in *Sox10* cause a colon malformation, identified in humans as Waardenburg-Hirschsprung disease [27]. *Sox10* has been found associated with developing and mature glial cells of the CNS, and Northern blot and *in-situ* analysis has shown the *Sox10* transcript in the brain regions of mouse (and rat) [28]. Western analysis identified *Sox10* protein in cultured rat oligodendrocytes and Schwann cells. Whole mount *in-situ* illustrated the presence of *Sox10* in a wide variety of neural tissue, including facial-cranial ganglia, brain regions, spinal cord and outer wall of stomach [28]. It may be of interest to note the absence of expression in maternal tissues for 13.5d – 17.5d, given the widely found expression in foetal and adult tissues.

***Sox11***

Figure 49: *Sox11* in this study is found in the full range of developing tissues, with lower levels found in liver samples. The adult portion of this panel reflects expression patterns found in an earlier study [2], confirming the high levels found in neuronal tissue and testis. From studies in chick, *cSox11* has been shown to be associated with the neural epithelium [29] and the developing CNS and PNS (peripheral nervous system) of mice [13].

**Figure 49: Expression Profile of *Sox11* in Mouse Foetal Panel**

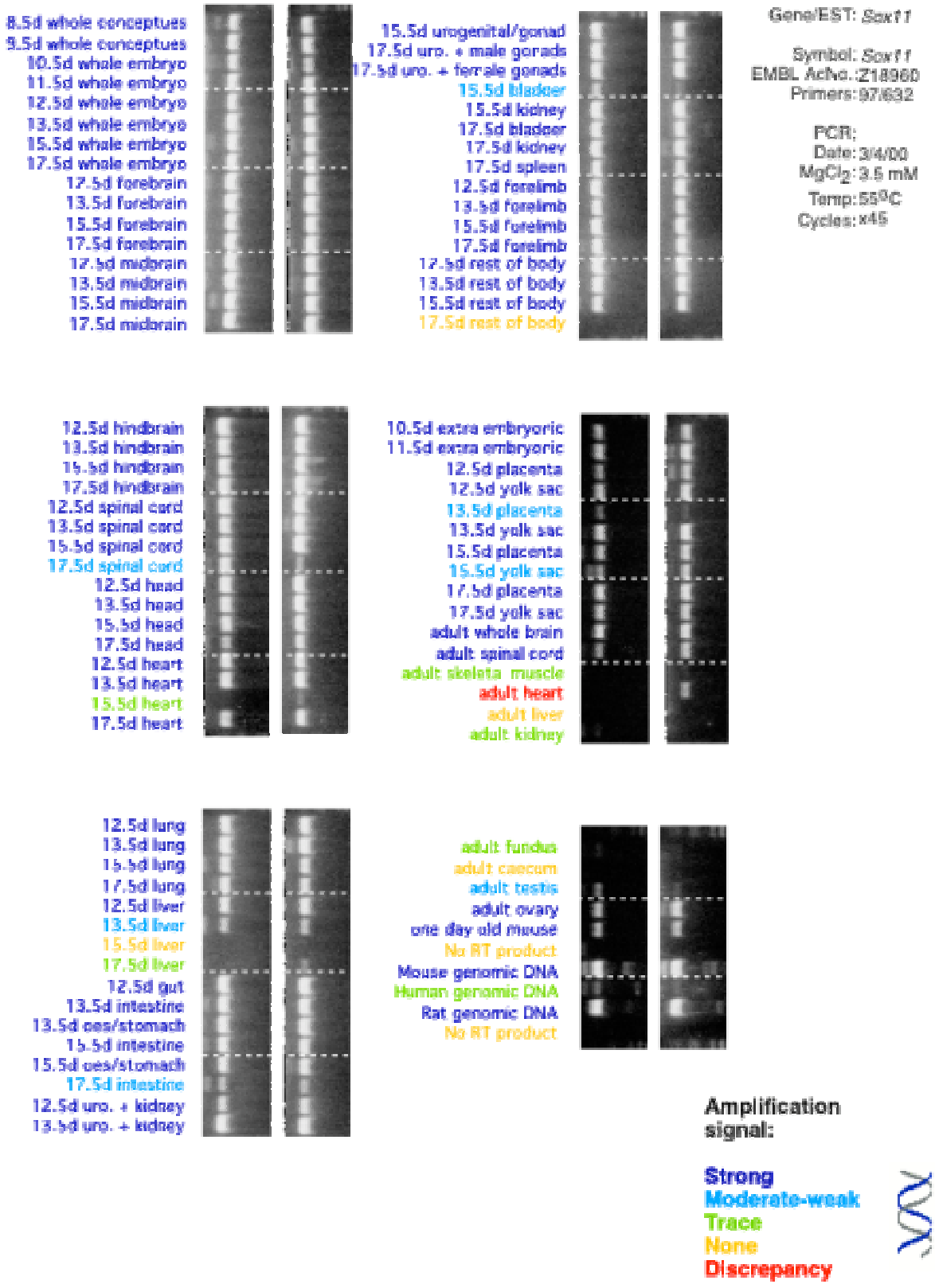
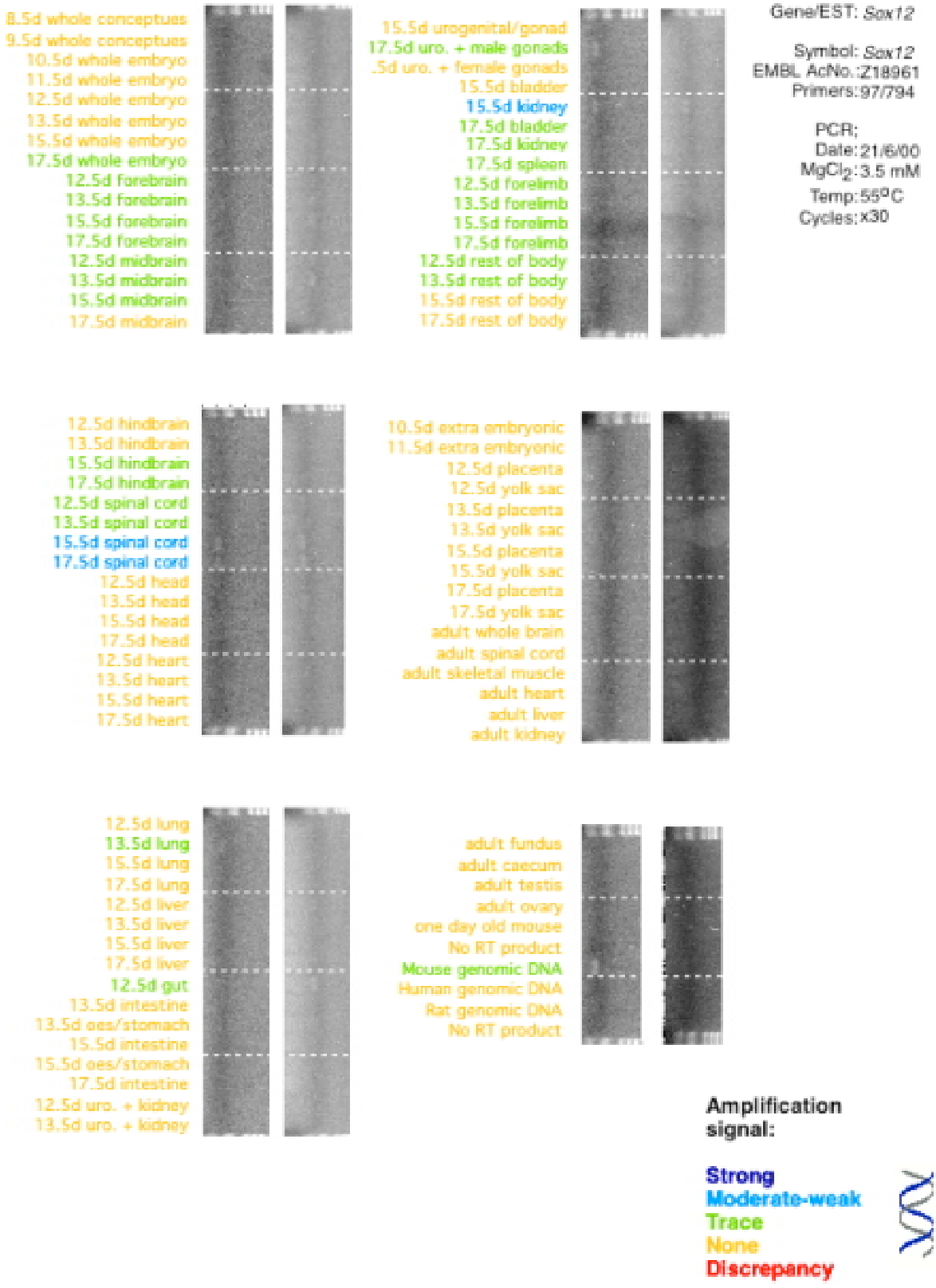


**Figure 50: Expression Profile of *Sox12* in Mouse Foetal Panel**



***Sox12***

Figure 50: *Sox12* in this study showed faint expression in some brain regions, spinal cord, 13.5d lung, 12.5d gut 17.5d urogenital male tissues and 15.5d kidney. There is very little reported on this gene since it was initially discovered [30]. However, *Sox22* has been renamed *Sox12* [31] and studies on the human *SOX22* show expression in a variety of adult and fetal tissues and organs, so it is plausible to expect *Sox12* to be similarly important in the differentiation and maintenance of a range of cell types [32].

***Sox13***

Figure 51: *Sox13* expression in this study shows expression in all tissues throughout the adult and foetus. An earlier report of Northern analysis showed the *Sox13* transcript to be restricted to ovary and kidney [33] (other tissues tested include brain, thymus, spleen and testis). This group D Sox gene expression is reported to be associated with the development of arterial walls [31].

***Sox14***

Figure 52: *Sox14* showed a very distinct pattern of expression in this study of foetal tissues. Undetected in very early stages (8.5d, 9.5d and 10.5d), with high expression in all brain and spinal cord regions through out development to adult. Expression was also found at 15.5d for intestine and oesophagus/stomach, urogenital/gonad and yolk sac; 17.5d urogenital male specific gonad and yolk sac; rest of body for 12.5d and 13.5d, with trace amounts at 15.5d. The adult section of this panel reflects that found in our earlier study [2]. The *Sox14* expression has been found in a highly restricted pattern in neurons in the developing mouse brain and

spinal cord [34], confirming the findings in this study.

**Figure 51: Expression Profile of *Sox13* in Mouse Foetal Panel**

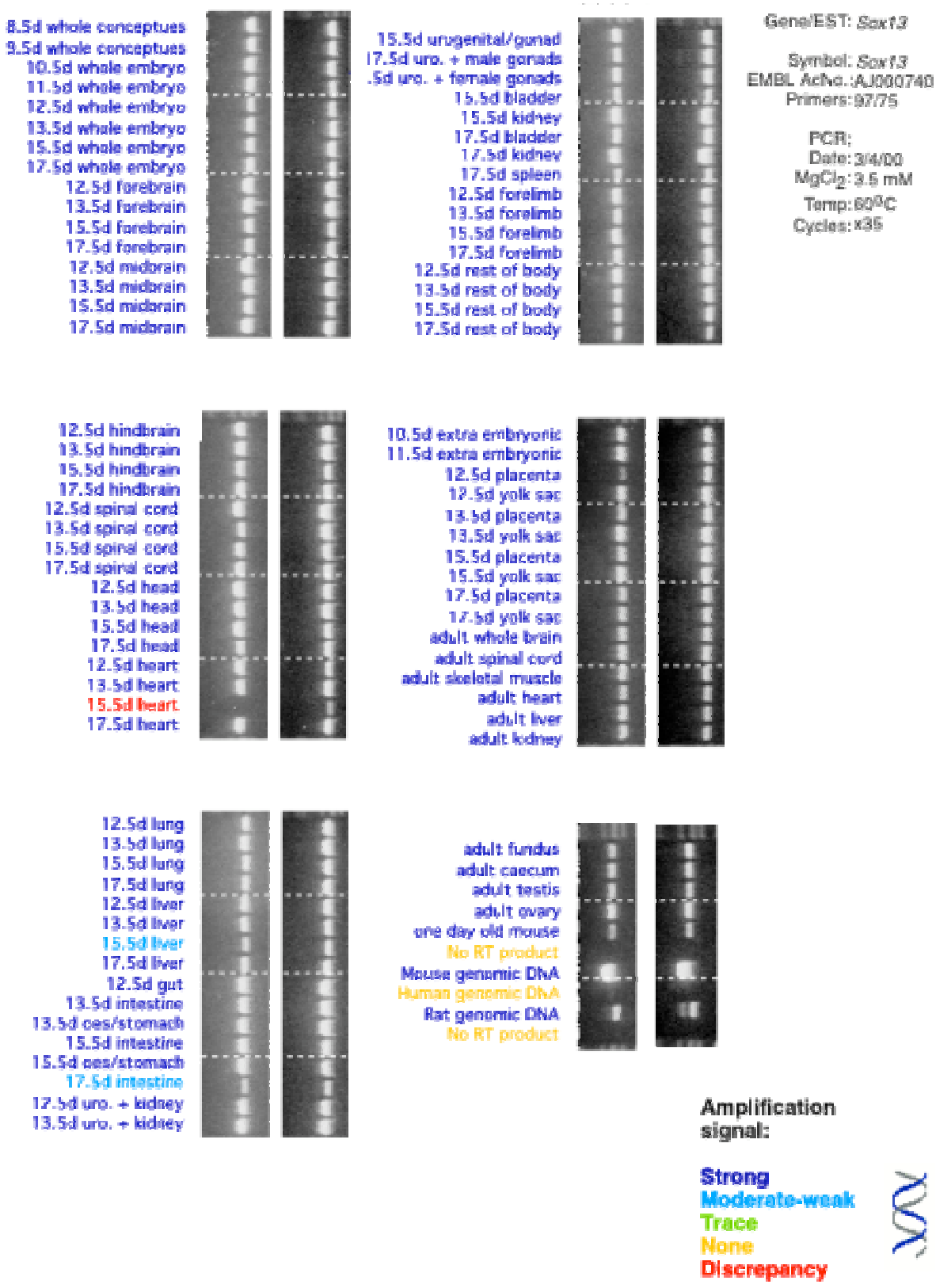


**Figure 52: Expression Profile of *Sox14* in Mouse Foetal Panel**

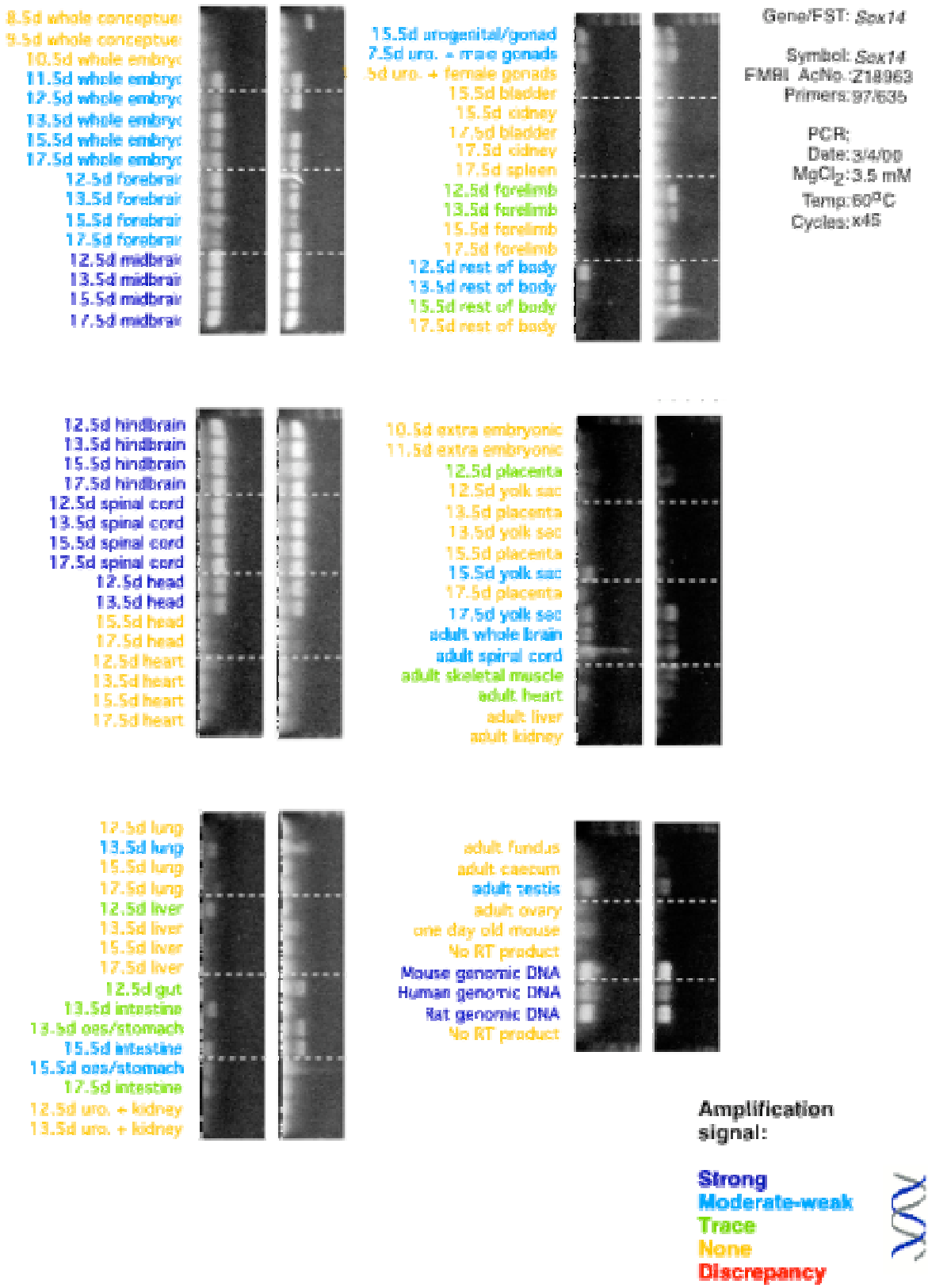


**Figure 53: Expression Profile of *Sox15* in Mouse Foetal Panel**

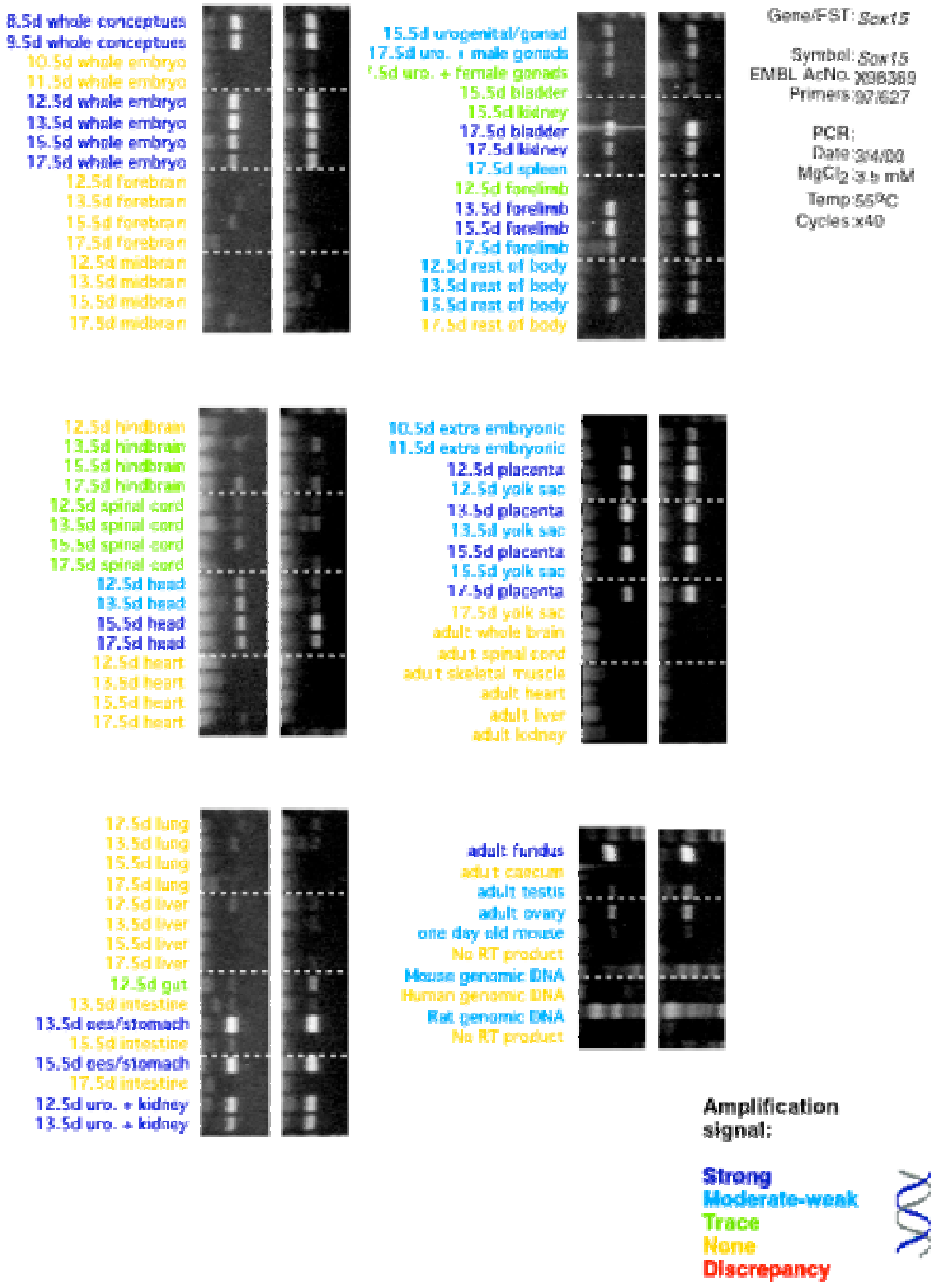
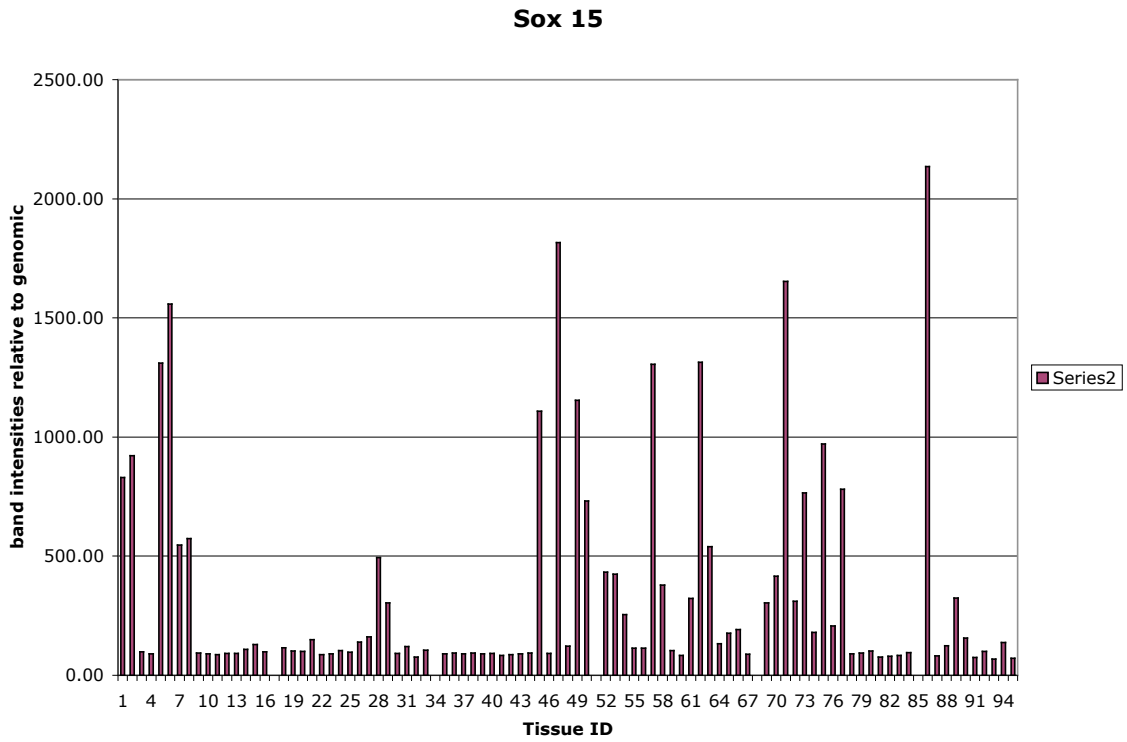


**Figure 54: Graphical representation of gel image in Figure 53.**




Densitometric measurements of gel band fluorescence displayed as a percentage of the mouse genomic fluorescent band intensity in position 92. Tissue identifications are located in Figure 35.

***Sox15***

Figure 53: *Sox15* expression in this study illustrates high levels of *Sox15* in a variety of tissues. The whole organism for 10.5d and 11.5d interestingly gave no expression. Trace amounts of expression were found in the hindbrain and spinal cord, with no expression in forebrain and midbrain, though expression was found in the remainder of the head. No expression was detected in heart, lung, liver or intestine, with high amounts in oesophagus and stomach, urogenital regions of 12.5d, 13.5d, bladder 17.5d, kidney 17.5d. More expression was found in 13.5d and 15.5d forelimb relative to 12.5d and 17.5d, but less in the rest of body at 17.5d relative to earlier stages, and more in placenta relative to yolk sac. Figure 54 is the graphical

representation of the gel, illustrating minimal expression from head regions (28 & 29), significant expression from oesophagus/stomach regions (45, 47) and urogential/kidney (49 & 50) relative to intestine and is mirrored in the adult panel where fundus (85) has much higher expression than caecum (86). The adult section relates to the expression pattern found earlier [2].

It has been reported that the human *SOX15* is expressed in skeletal muscle and is required for myogenesis [35]. However, this report is not confirmed in this study on mouse.

***Sox16***

Figure 55: *Sox16*, like *Sox15,* showed a variety of expression patterns in these tissues. Undetected in the 11.5d whole organism, with expression found in all other stages for whole organism. Brain and spinal cord regions showed very low or no levels of expression, but again expression was detected in the remainder of the head. Heart, lung and liver tissues showed only trace expression in 17.5d lung and 12.5d liver. Expression was found in the gut regions of 12.5d to 15.5d (though not intestine tissues) and urogenital (urogenital/gonad) regions for all stages. It was detected in the 17.5d bladder and kidney, but undetected in these tissues for 15.5d. The pattern of forelimb expression found for *Sox15* was mirrored here, with more found in 13.5d and 15.5d than either 12.5d or 17.5d. Also 17.5d rest of body showed no expression. Again, mirroring the *Sox15,* placental tissues showed a higher expression relative to the yolk sac for all stages. In adult tissues, *Sox16* was detected in skeletal muscle, fundus and ovary, with trace amounts in spinal cord and testis.

Little is known about the *Sox16* gene, though it is reported to have strong homologies with *Sox15* at the sequence level [36].

**Figure 55: Expression Profile of *Sox16* in Mouse Foetal Panel**

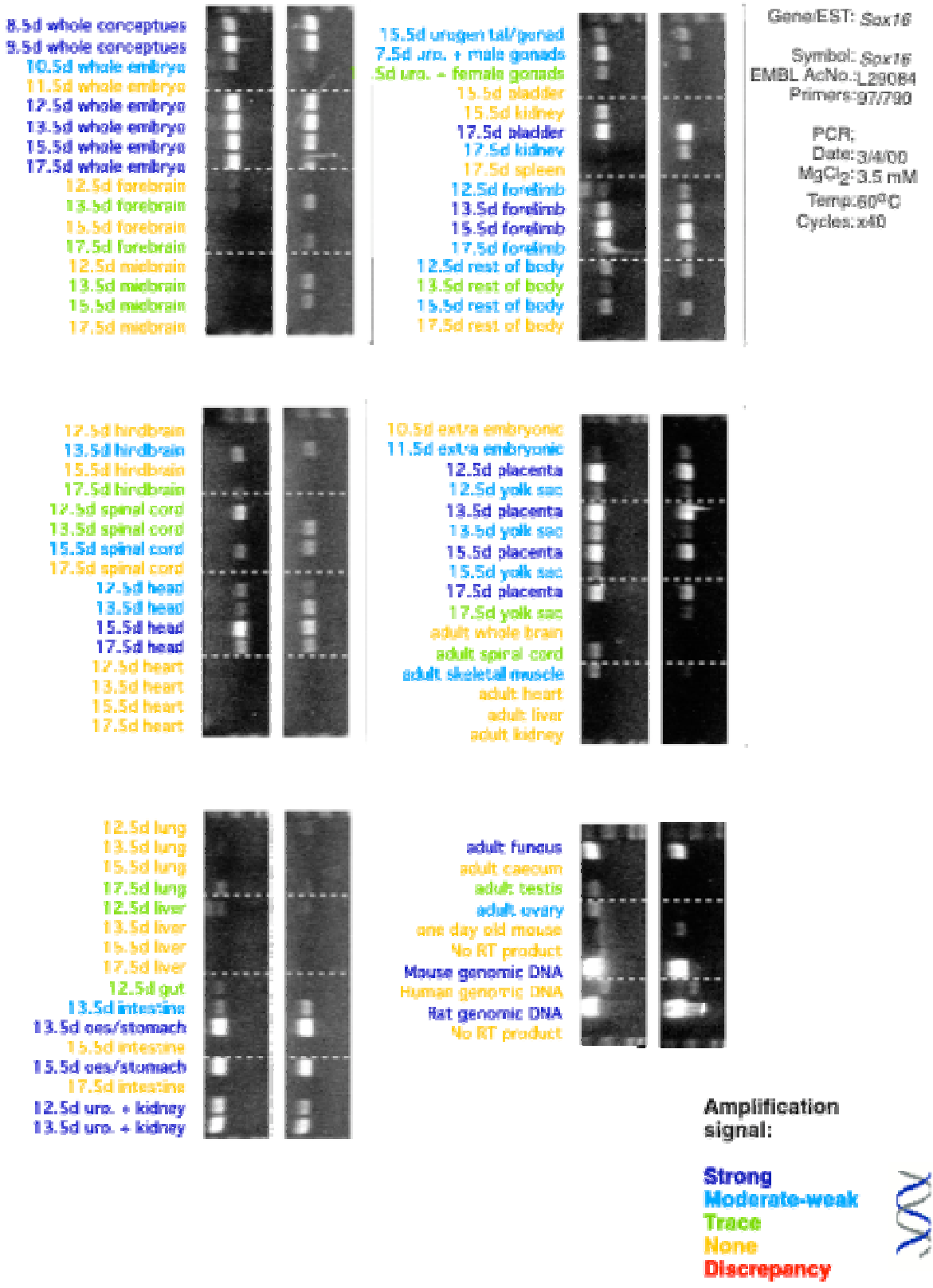
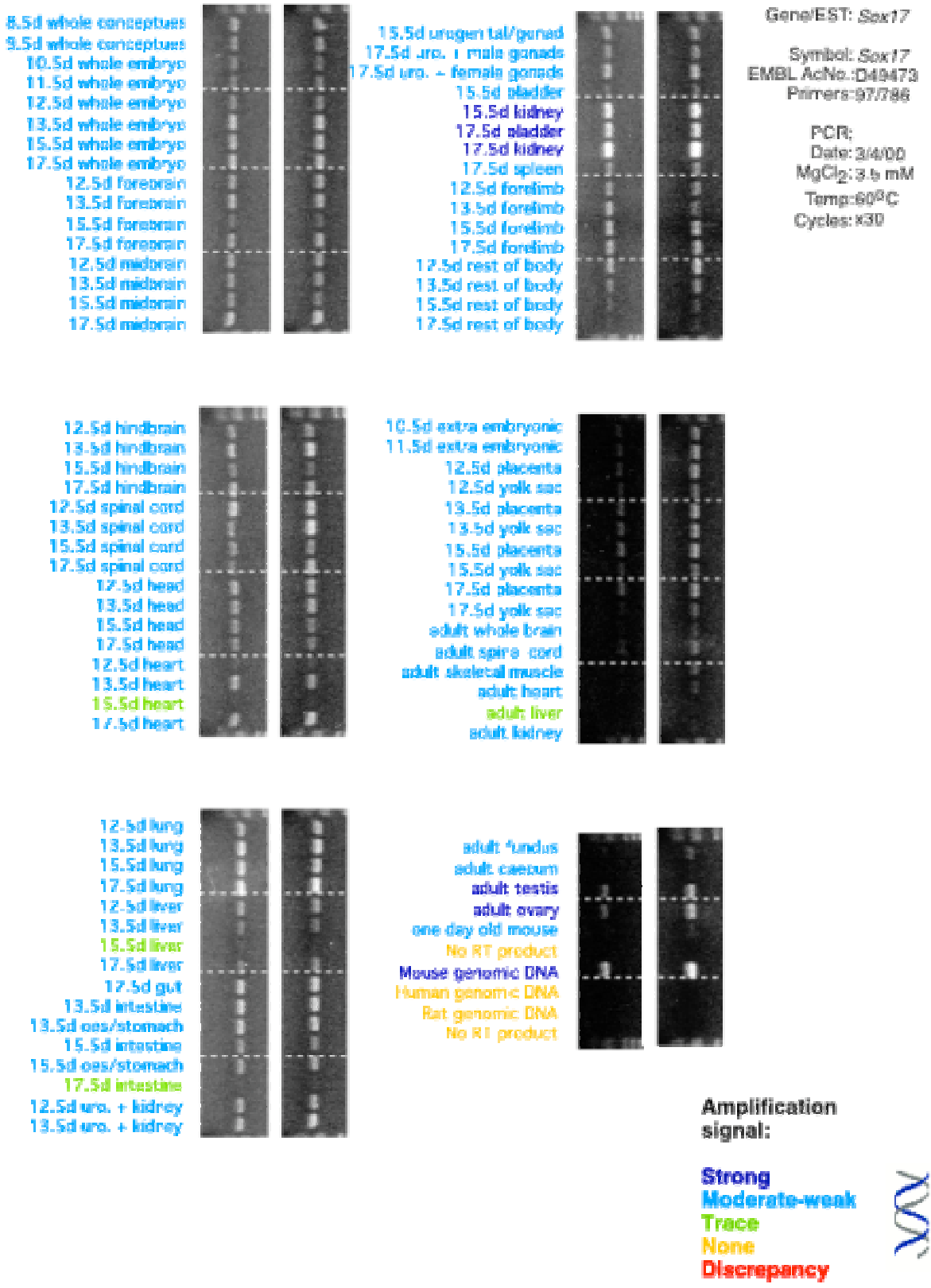


**Figure 56: Expression Profile of *Sox17* in Mouse Foetal Panel**



***Sox17***

Figure 56: *Sox 17* expression is found in all tissues, strong in adult ovary and testis, plus foetal tissues 15.5d kidney, 17.5d kidney and bladder.

The gene *Sox17* has been shown to be implicated in mouse spermatogenesis [37], which fits nicely with the strong expression found in adult testis. Another important role for *Sox17* has been identified in early mouse endoderm development [38], which might explain the general expression found through out foetal development in this study.

***Sox19***

Figure 57: *Sox19* in this study showed a poor expression profile and I have failed to score the results for this reason. It is included in the study to illustrate results, which require a primer redesign.

***SoxLZ***

Figure 58: of *SoxLZ* was found to be strongly expressed in most tissues, from foetal and adult stages. Tissues, which were particularly light included 15.5d heart, 17.5d intestine, 15.5d bladder, 17.5d spleen and the placental samples for all stages.

***Slc17a2***

Figure 59: *Slc17a2,* Solute carrier type 17 2a is a renal sodium phosphate/hydrogen co-transporter previously found to be highly specific for adult kidney [2], found here expressed in the 15.5d and 17.5d kidney and spleen of the 17.5d, with trace amounts in the 15.5d whole embryo and 13.5d hindbrain.

**Figure 57: Expression Profile of *Sox19* in Mouse Foetal Panel**

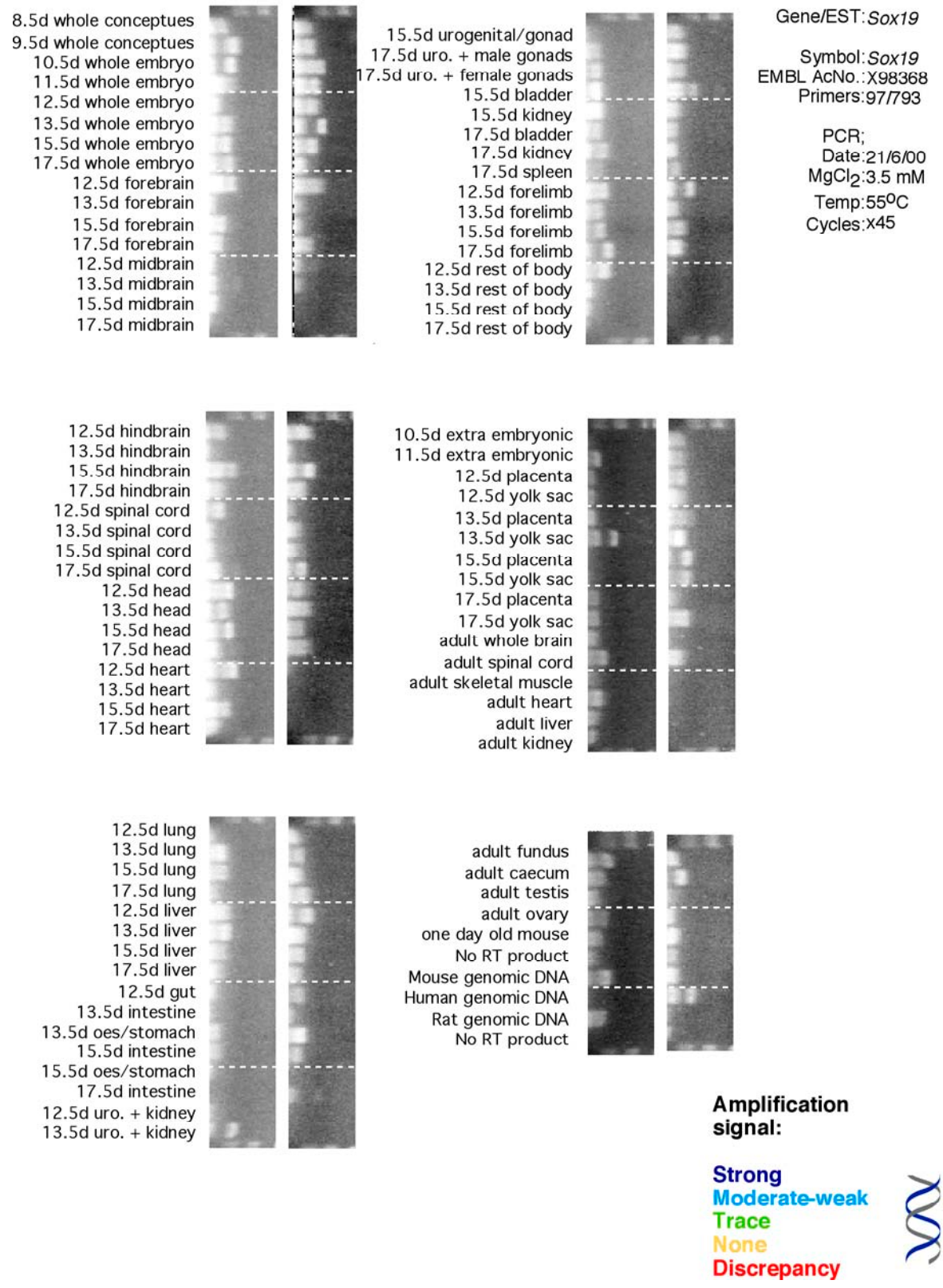


**Figure 58: Expression Profile of *SoxLZ* in Mouse Foetal Panel**

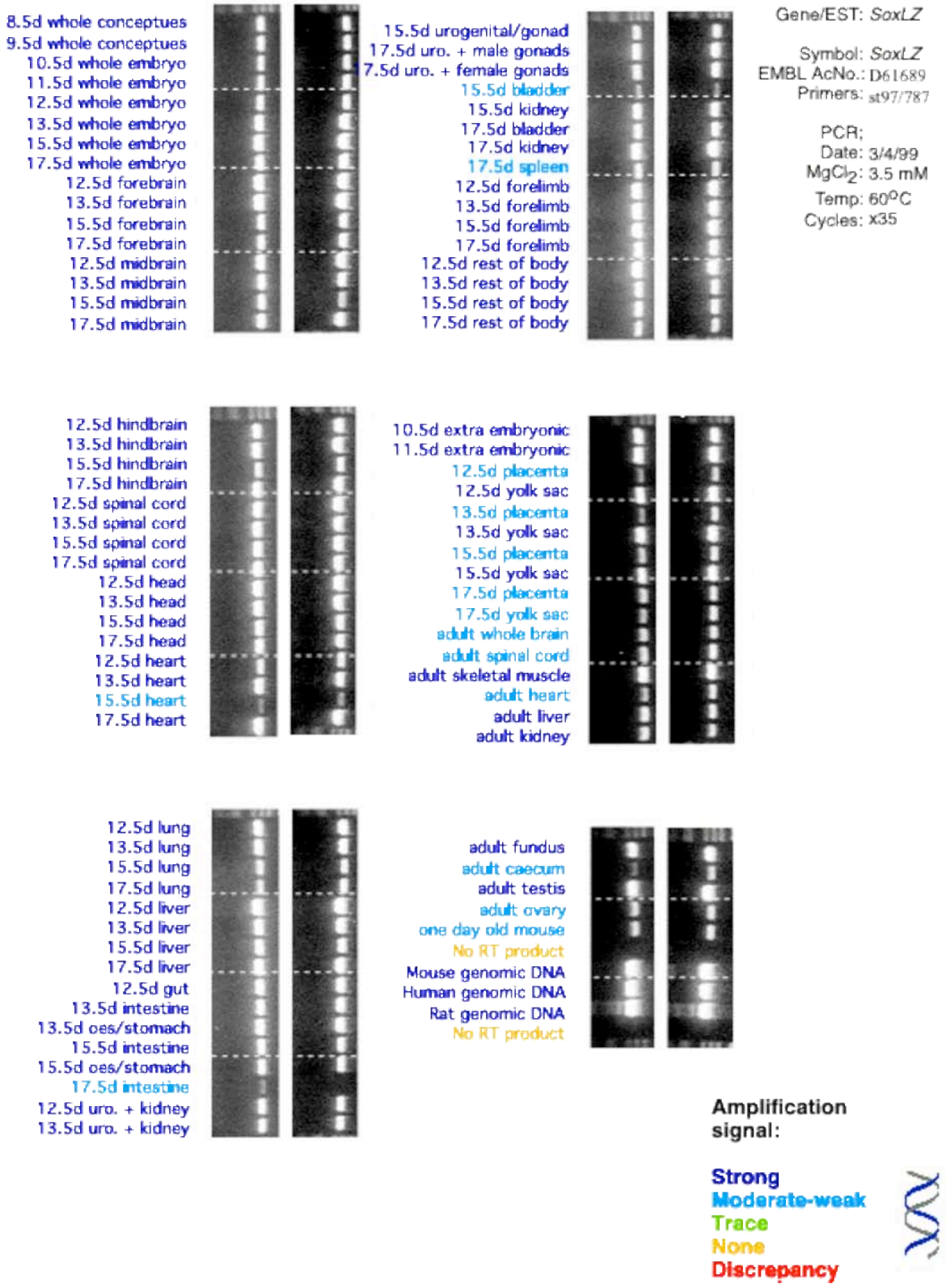
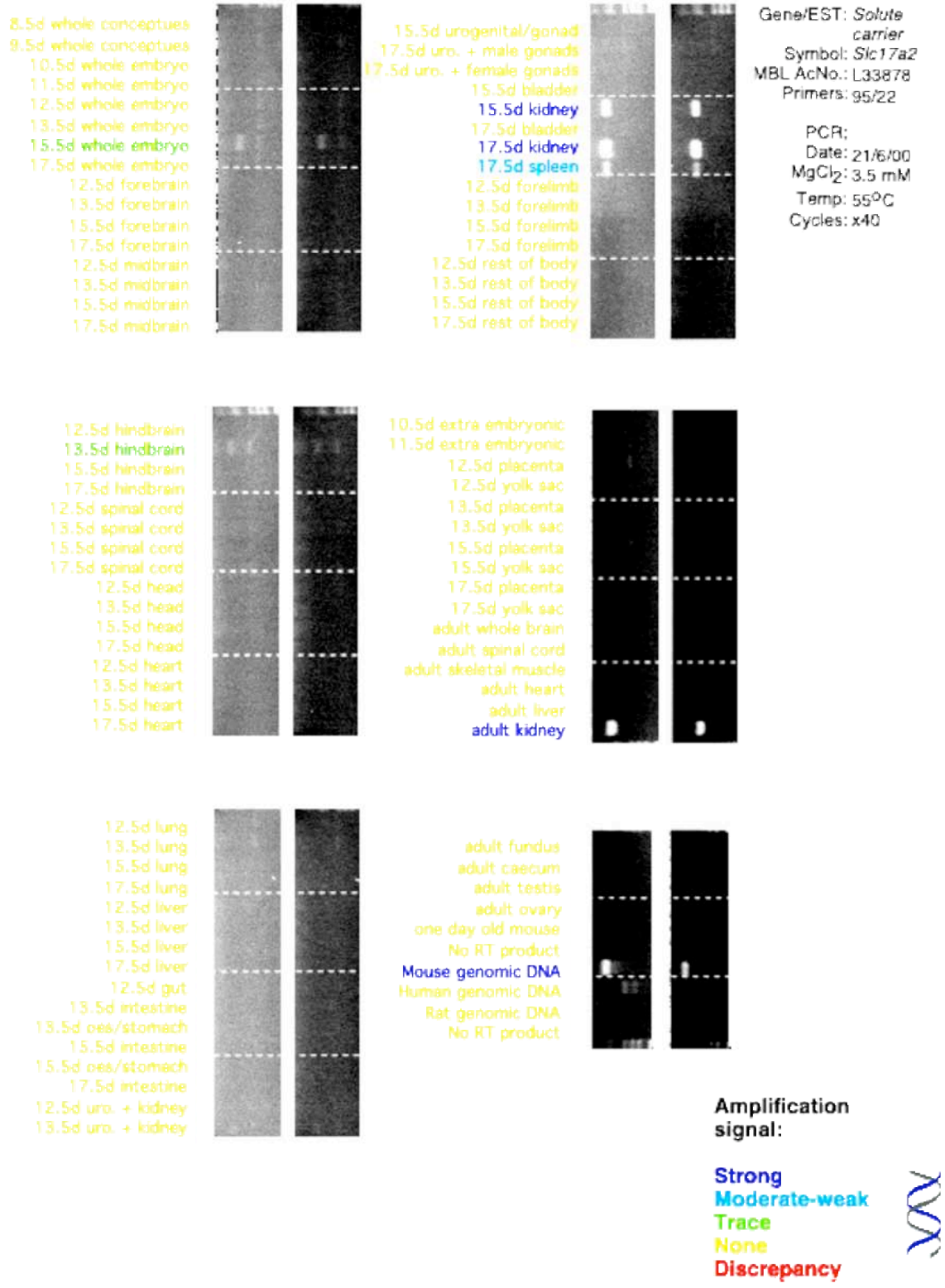


**Figure 59: Expression Profile of *Solute Carrier type 17a2* in Mouse Foetal Panel**



***Csna1***

Figure 60: *Csna1*, Alpha Casein found here only in the liver and midbrain for 17.5d had in an earlier study been found in the pregnant mammary gland and mid term foetus only [2]. This gene is associated with the metabolism of casein found in milk and so was not expected to be found in very early stages.

***FABPi***

Figure 61: *FABPi,* Fatty Acid Binding Protein intestinal in this study was found in trace amounts in the 12.5d and 17.5d whole embryo with higher amounts in the 15.5d whole embryo. High expression was also found in stomach/intestinal regions for all stages, with lower amounts in the extra embryonic tissues and yolk sacs of later stages. In the adult section, this gene was found at high levels in the liver and caecum confirming our earlier study [2].

**Figure 60: Expression Profile of *Alpha Casein* in Mouse Foetal Panel**

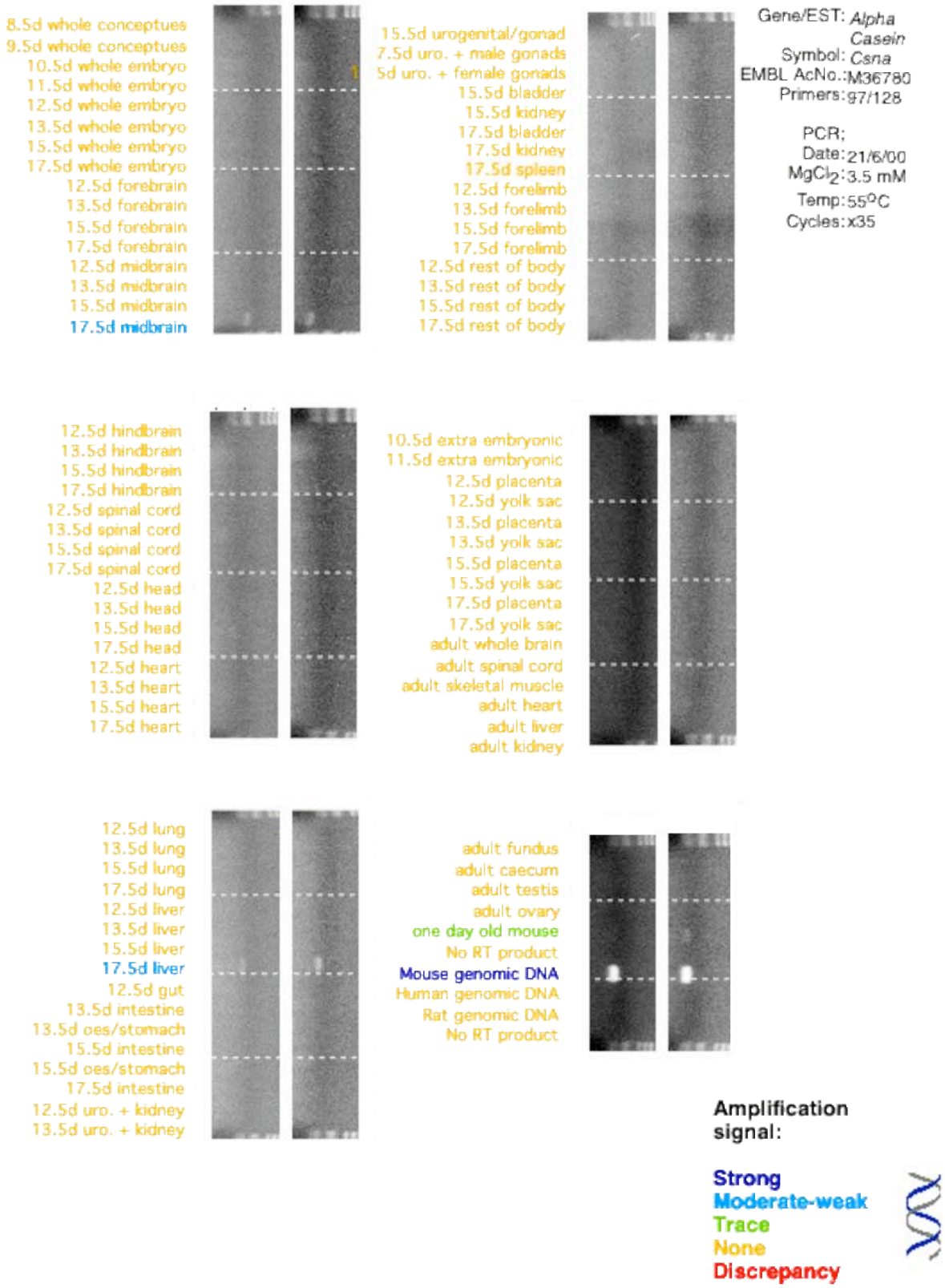
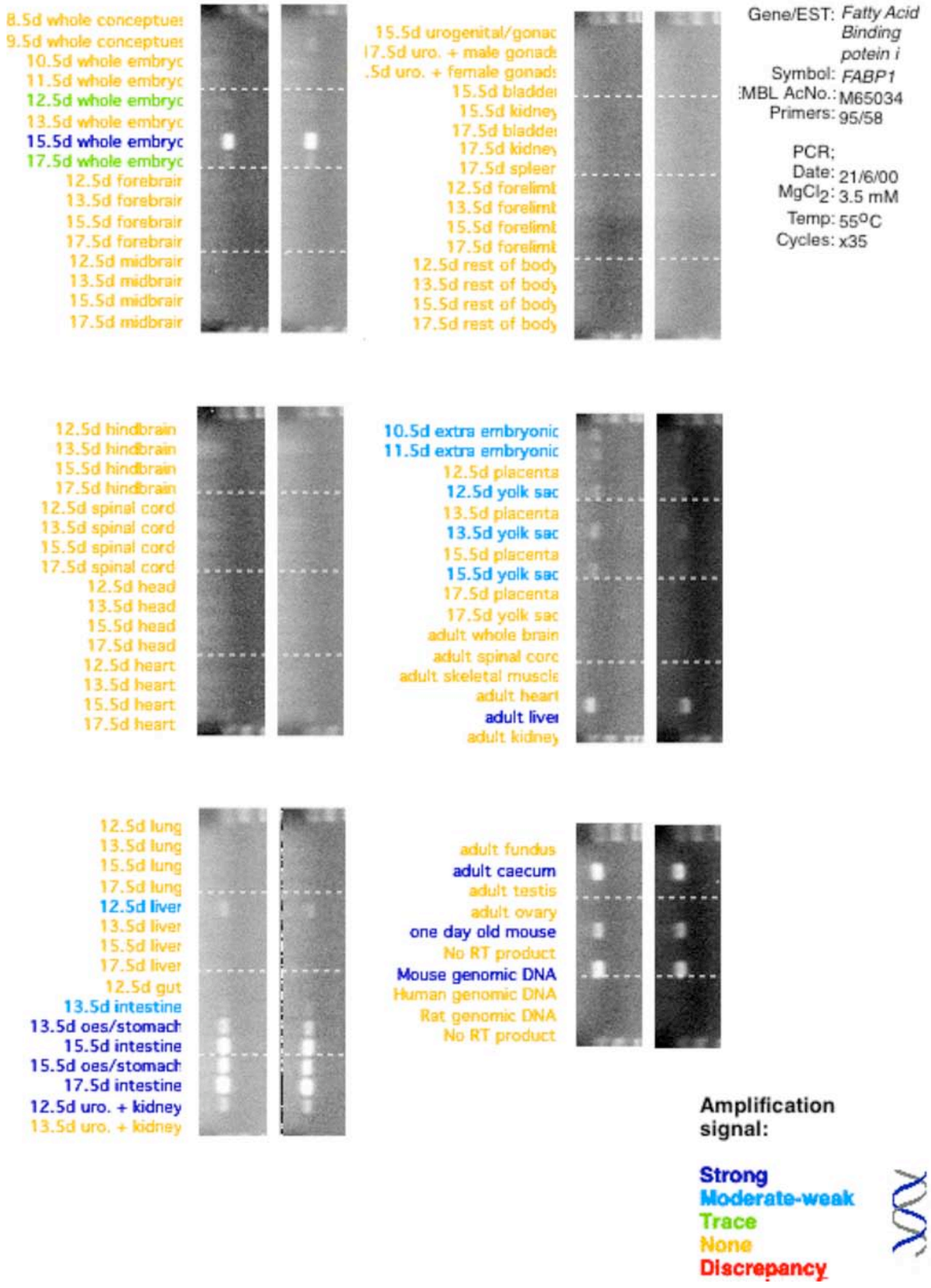


**Figure 61: Expression Profile of *Fatty Acid Binding Protein intestinal* in Mouse Foetal Panel**



### 2.4.2. Concluding Remarks

Profiles of gene expression from these tissues illustrated as agarose gels, provide some information. However, a clearer interpretation of this information is obtained, when these gels are interrogated computationally and presented as graphs, illustrating a broader range of expression intensities across the various tissues. For a complete and thorough analysis, a comparison of the individual tissue expression of the housekeeping genes as a direct relationship to each of the genes profiled would I believe provide a more accurate measure of the genes in this study. Modern methods of gene expression tend to include this form of relationship, providing a more uniform base line from which results are then presented.

The following profiles were chosen, as sufficiently different from each other, for further investigation by *in-situ* hybridization, *Sox 2, 4, 6, 15, 16, 17* and *LZ2*, in order to identify the sub-cellular localisation of the gene expression illustrated in this study.

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