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Classification and nomenclature of all human homeobox genes

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Abstract

Background: The homeobox genes are a large and diverse group of genes, many of which play important roles in the embryonic development of animals. Increasingly, homeobox genes are being compared between genomes in an attempt to understand the evolution of animal development. Despite their importance, the full diversity of human homeobox genes has not previously been described.

Results: We have identified all homeobox genes and pseudogenes in the euchromatic regions of the human genome, finding many unannotated, incorrectly annotated, unnamed, misnamed or misclassified genes and pseudogenes. We describe 300 human homeobox loci, which we divide into 235 probable functional genes and 65 probable pseudogenes. These totals include 3 genes with partial homeoboxes and 13 pseudogenes that lack homeoboxes but are clearly derived from homeobox genes. These figures exclude the repetitive *DUX1* to *DUX5* homeobox sequences of which we identified 35 probable pseudogenes, with many more expected in heterochromatic regions. Nomenclature is established for approximately 40 formerly unnamed loci, reflecting their evolutionary relationships to other loci in human and other species, and nomenclature revisions are proposed for around 30 other loci. We use a classification that recognizes 11 homeobox gene 'classes' subdivided into 102 homeobox gene 'families'.

Conclusion: We have conducted a comprehensive survey of homeobox genes and pseudogenes in the human genome, described many new loci, and revised the classification and nomenclature of homeobox genes. The classification scheme may be widely applicable to homeobox genes in other animal genomes and will facilitate comparative genomics of this important gene superclass.

Background

Homeobox genes are characterized by the possession of a particular DNA sequence, the homeobox, which encodes a recognizable although very variable protein domain, the homeodomain [1,2]. Most homeodomains are 60 amino acids in length, although exceptions are known. Many homeodomain proteins are transcription factors with important roles in embryonic patterning and cell differentiation, and several have been implicated in human diseases and congenital abnormalities [3].

The homeobox genes have been variously subdivided into superclasses, classes, subclasses or groups, although there has been much inconsistency in the use of these terms.

The most commonly recognized groupings are the ANTP, PRD, LIM, POU, HNF, SINE, TALE, CUT, PROS and ZF groups (or variants of these names), although these are not always given equal rank in classification schemes [1,2,4-8]. There is more consensus in classification at a lower level, just above the level of the gene, where very similar genes are grouped into gene families. Widely recognized gene families include Dlx, Evx, Msx, Cdx, En, Otx, Pitx, Otx and Emx (or variants of these names), amongst many others, although there is variation particularly concerning how many gene families are used for the HOX, PAX and NK homeobox genes. Despite the numerous discrepancies, the common principle of classification is the same. The goal of any scheme is to mirror evolutionary diversification, so that 'closely related' genes are placed in the same gene family, and related gene families are placed in the same gene class or other higher grouping. It should be borne in mind, however, that the pathway of evolutionary diversification is never completely known for any large and complex set of genes.

The initial analyses of the draft human genome sequence published in 2001 included estimates of the number of human homeobox genes. Venter et al [9] found 160 homeobox genes, containing 178 homeobox sequences, using large-scale automated classification; while the IHGSC team [10] gave a much higher estimate of 267 homeobox genes. Both were based on draft coverage of the human genome and would be expected to be missing some genes, as well as confusing pseudogenes with genes. In the same year, Banerjee-Basu and Baxevanis [8] presented an analysis of 129 human homeodomain sequences, but this was far from a comprehensive survey. More recently, there have been two more accurate surveys of homeobox genes in the human genome. Nam and Nei [11] found 230 homeobox genes, containing 257 homeobox sequences. Ryan et al [7] found 228 homeodomain sequences in the NCBI RefSeq database of October 2004. Our analyses (described here) revealed many homeobox genes that were incorrectly annotated, named or classified and many homeobox pseudogenes that had previously been missed. We report a complete survey of homeobox loci in the euchromatic regions of the human genome, appropriate gene nomenclature and a consistent classification scheme.

Results and Discussion

How many homeobox genes and pseudogenes?

Using exhaustive database screening, followed by manual examination of sequences, we identified 300 homeobox loci in the human genome. Distinguishing which of these loci are functional genes and which are non-functional pseudogenes was difficult in some cases. Most loci classified as pseudogenes in this study are integrated reverse-transcribed transcripts, readily recognized by their dispersed genomic location, complete lack of intron sequences, and (in some cases) 3' homopolymeric run of adenine residues. A small minority are duplicated copies of genes, recognized by physical linkage to their functional counterparts and the same (or similar) exon-intron arrangement. In general, retrotransposed gene copies are non-functional (and therefore pseudogenes) from the moment of integration because they lack 5' promoter regions necessary for transcription. However, such sequences can occasionally acquire new promoters and become functional as 'retrogenes'. Duplicated gene copies often possess 5'promoter regions (as they are often encompassed by the duplication event); most degenerate to pseudogenes due to redundancy in a process known as non-functionalization, however some can be preserved as functional genes through sub- or neo-functionalization. Thus, in both instances, reliable indicators of non-functionality were sought in order to assign pseudogene status, notably frameshift mutations, premature stop codons and non-synonymous substitutions at otherwise conserved sites in the original coding region.

We currently estimate that the 300 human homeobox loci comprise 235 functional genes and 65 pseudogenes (Table 1). These figures include three functional genes that possess partial homeobox sequences (*PAX2*, *PAX5* and *PAX8*) and retrotransposed pseudogenes that correspond to only part of the original transcript, whether or not it includes the homeobox region or indeed any of the original coding region. Consequently, 13 retrotransposed pseudogenes that lack homeobox sequences are included (*NANOGP11*, *TPRX1P1*, *TPRX1P2*, *POU5F1P7*, *POU5F1P8*, *IRX4P1*, *TGIF2P2*, *TGIF2P3*, *TGIF2P4*, *CUX2P1*, *CUX2P2*, *SATB1P1*, *ZEB2P1*). We do not include *PAX1*, *PAX9* and *CERS1*; these are functional genes without homeobox motifs, albeit closely related to true homeobox genes (the other PAX and CERS genes).

The total number of homeobox sequences in the human genome is higher than 300 for two reasons. First, several genes and pseudogenes possess more than one homeobox sequence, notably members of the Dux (double homeobox), Zfhx and Zhx/Homez gene families. Second, we have excluded a set of sequences related to human *DUX4* (*DUX1* to *DUX5*), which have become part of 3.3 kb repetitive DNA elements present in multiple copies in the genome [12-14]. Few of these tandemly-repeated sequences are likely to be functional as expressed proteins, and all were probably derived by retrotransposition from functional DUX gene transcripts (see below). The fact that they are not included in the total count, therefore, is likely to have limited bearing on understanding the diversity and normal function of human homeobox genes. Hence, our figure of 300 homeobox loci is the most useful current

Table 1: Numbers of human genes, pseudogenes and gene families in each homeobox gene class. The human homeobox gene superclass contains a total of 235 probable functional genes and 65 probable pseudogenes. These are divided between 102 gene families, which are in turn divided between eleven gene classes.

Class	Subclass	Number of gene families	Number of genes	Number of pseudogenes
ANTP	HOXL	14	52	0
	NKL	23	48	19[b]
PRD	PAX	3	7[a]	0
	PAXL	28	43	24[c, d]
LIM		6	12	0
POU		7	16	8[e]
HNF		2	3	0
SINE		3	6	0
TALE		6	20	10[f]
CUT		3	7	3[g]
PROS		1	2	0
ZF		5	14	1[h]
CERS		1	5[i]	0
Totals		***102***	***235[a]***	***65[b-h]***

[a]Includes *PAX2*, *PAX5* and *PAX8* that have a partial homeobox; excludes *PAX1* and *PAX9* that lack a homeobox.
[b]Includes *NANOGP11* that lacks a homeobox.
[c]Excludes intronless and repetitive *DUX1* to *DUX5* sequences.
[d]Includes *TPRX1P1* and *TPRX1P2* that lack a homeobox.
[e]Includes *POU5F1P7* and *POU5F1P8* that lack a homeobox.
[f]Includes *IRX4P1*, *TGIF2P2*, *TGIF2P3* and *TGIF2P4* that lack a homeobox.
[g]Includes *CUX2P1*, *CUX2P2* and *SATB1P1* that lack a homeobox.
[h]Includes *ZEB2P1* that lacks a homeobox.
[i]Excludes *CERS1* that lacks a homeobox.

estimate of the repertoire of human homeobox genes and pseudogenes.

Classification

We propose a simple classification scheme for homeobox genes, based on two principal ranks: gene class and gene family. A gene class contains one or more gene families, which in turn will contain one or more genes. In a few cases, it is useful to erect an intermediate rank between these levels, and for this we use the term subclass. For the entire set of homeobox genes, we use the term superclass.

For the rank of gene family, we use a specific evolutionary-based definition based on common practice in the field of comparative genomics and developmental biology. We define a gene family as a set of genes derived from a single gene in the most recent common ancestor of bilaterian animals (here defined as the latest common ancestor of *Drosophila* and human). This definition has been made explicitly in previous work [2,6] but is actually a principle that has been in widespread, but rather inconsistent, use for over a decade [15]. For example, amongst the homeobox genes, the En (engrailed) gene family was originally defined to include human *EN1* and *EN2*, plus *Drosophila en* and *inv* [16]; these four genes arose by independent duplication from a single gene in the most recent common ancestor of insects and vertebrates. Moving outside the homeobox genes, this principle is also widespread; for example, the Hh (hedgehog) gene family was defined to include mouse *Shh*, *Dhh* and *Ihh*, plus *Drosophila hh* [17]. To clarify boundaries between gene families, we conducted molecular phylogenetic analyses of human homeodomain sequences, using a range of protostome and occasionally cnidarian homeodomain sequences as outgroups (Additional files 1 and 2).

While the gene family definition described above is generally workable for homeobox genes, by necessity there are some exceptions. One type of exception relates to genes with an unknown ancestral number. For example, there is uncertainty as to whether there were one or two Dlx (distal-less) genes in the most recent common ancestor of bilaterians; however it is common practice to refer to a single Dlx gene family [18]. Thus, we stick with convention for this set of genes. There is similar uncertainty over the ancestral number of Irx (iroquois) genes [19], and again we treat these as a single gene family. The HOX genes are an interesting case as their precise number in the most recent common ancestor of bilaterians is unknown due to lack of phylogenetic resolution between 'central' genes [20]. Here we divide the HOX genes into seven gene families: the 'anterior' Hox1 and Hox2 gene families, the 'group 3' Hox3 gene family, the 'central' Hox4, Hox5 and Hox6-8 gene families, and the 'posterior' Hox9-13 gene family. Another type of exception relates to 'orphan' genes. These are genes that have been found in one species (for example human) but not in other species, or at least not in a wide diversity of Metazoa. Some of these will be ancient genes that have been secondarily lost from the genomes of some species, in which case these comply with our evolutionary definition of a gene family made above. Others, however, will be rapidly evolving genes that originated from another homeobox gene and then diverged to such an extent that their origins are unclear [21]. Whenever origins are unclear, we must define a new gene family to encompass those genes, even though they may not date back to the latest common ancestor of bilaterians. In these cases, the gene family is erected to recognize a set of distinct genes on the basis of DNA and protein sequence, rather than on evolutionary origins.

Using the aforementioned criteria, we recognize 102 homeobox gene families in the human genome (Table 1). We are aware that other homeobox gene families exist in bilaterians but have been lost from humans (for example, Nk7, Ro, Hbn, Repo and Cmp; [7]), and we recognize that some gene family boundaries will alter as new information is obtained. Nonetheless, at the present time the 102 gene families provide a sound framework for the study of human homeobox genes.

It is much more difficult to propose a rigorous evolutionary definition for the rank of gene class. Every attempt to classify genes above the level of gene family involves a degree of arbitrariness. We define gene classes by taking two principal criteria into account. First, gene classes should ideally be monophyletic assemblages of gene families. To identify probable monophyletic groups of gene families, we conducted molecular phylogenetic analyses of homeodomain sequences, and looked for sets of gene families that group together stably, regardless of the precise composition of the dataset used (Figures 1, 2, 3; Additional files 3, 4, 5). Some gene families were difficult to place from sequence data alone, and were found in different gene classes (or subclasses) depending on the precise dataset analyzed or the phylogenetic method employed. This is perhaps not surprising as trees that encompass many homeobox genes can only be built with a short sequence alignment (the homeodomain); under these conditions, phylogenetic trees can only be used as a guide to possible classification, not the absolute truth. In ambiguous cases, we used the chromosomal location of genes to guide possible resolution between alternative hypotheses. Second, some homeobox gene classes can be characterized by the presence of additional protein domains outside of the homeodomain [2]. Recognized protein domains associated with homeodomains include the PRD domain, LIM domain, POU-specific domain, POU-like domain, SIX domain, various MEINOX-related domains, the CUT domain, PROS domain, and various ZF domains [2].

Using the aforementioned criteria, we recognize eleven homeobox gene classes in the human genome: ANTP, PRD, LIM, POU, HNF, SINE, TALE, CUT, PROS, ZF and CERS (Table 1). There is no expectation that the eleven gene classes will be of similar size, simply because some classes will have undergone more expansion by gene duplication than others. In the human genome, the ANTP and PRD classes are much larger than the other classes. Although gene classes should ideally be monophyletic, it is possible that the ZF homeobox gene class, characterized by the presence of zinc finger motifs in most of its members, is polyphyletic (Figure 3; Additional file 5). In other words, domain shuffling may have brought together a homeobox sequence and a zinc finger sequence on more than one occasion. The same may also be true for the LIM class; alternatively the apparent polyphyly of LIM-class homeodomains could be a consequence of LIM domain loss or artefactual placement of some ZF-class homeodomains in phylogenetic analyses (Figure 3; Additional file 5).

In theory, it is possible to recognize higher level associations above the level of the gene class, because the diversification of homeobox genes will have taken place by a continual series of gene duplication events. We do not propose names for hierarchical levels above the rank of class, and consider that gene name, gene family and gene class (and occasionally subclass) convey sufficient information for most purposes.

We use a consistent convention for writing gene classes and gene families. We present the names of all gene classes in abbreviated non-italicized upper case – for example, the ANTP and PRD classes – to avoid confusion with gene symbols (*Antp* and *prd*) or indeed gene names (*Antennapedia* and *paired*). In contrast, we present the names of all gene families in non-italicized title case; for example, the Cdx, En and Gsc gene families. We have used this style consistently in recent work [6,21-23] and note that several other authors have done likewise [4,7,24]. We suggest that this style, and most of these gene family names, can be used in other bilaterian genomes. Extending the scheme to non-bilaterians is more difficult, however, and awaits clarification of the relationship between the homeobox genes of sponges, placozoans, cnidarians and bilaterians [7,25].

The ANTP homeobox class

The ANTP class derives its name from the *Antennapedia* (*Antp*) gene, one of the HOX genes within the ANT-C homeotic complex of *Drosophila melanogaster*. The human genome has 39 HOX genes, arranged into four Hox clusters. Here we divide the HOX genes into seven gene families: Hox1, Hox2, Hox3, Hox4, Hox5, Hox6-8 and Hox9-13. The HOX genes are not the only ANTP-class genes, and we recognize a total of 37 gene families in this class (Table 1). We divide these 37 gene families between two subclasses that are relatively well-supported in phylogenetic analyses: the HOXL and the NKL subclasses (Figure 1; Additional file 3). As previously discussed, the subclasses are largely consistent with the chromosomal positions of genes [26,27]. The HOXL (HOX-Like or HOX-Linked) genes primarily map to two fourfold paralogous regions: the Hox paralogon (2q, 7p/q, 12q and 17q) and the ParaHox paralogon (4q, 5q, 13q and Xq) (Figure 4). The NKL (NK-Like or NK-Linked) genes are more dispersed, but there is a concentration on the NKL or MetaHox paralogon (2p/8p, 4p, 5q and 10q) (Figure 4). Somewhat aberrantly, the Dlx and En gene families group with the NKL subclass in phylogenetic analyses (Figure 1; Additional file 3), but with the HOXL subclass on the basis of chromosomal positions (Figure 4).

Most of the 37 gene families in the ANTP class have been clearly defined before. We draw attention here to several

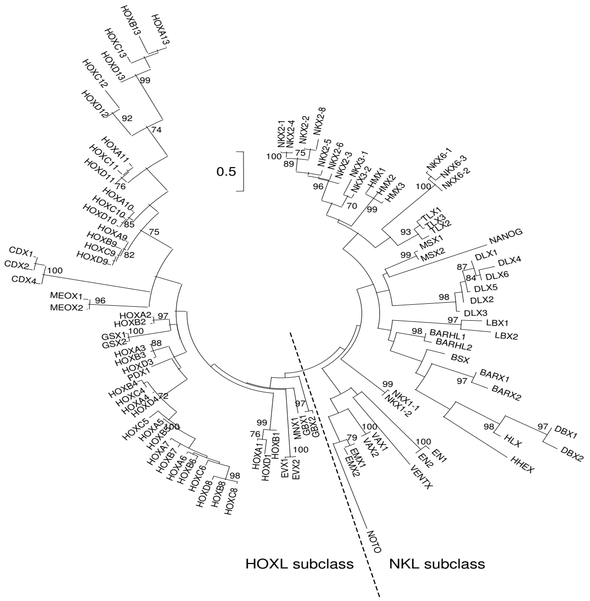

Figure 1

Maximum likelihood phylogenetic tree of human ANTP-class homeodomains. Arbitrarily rooted phylogenetic tree of human ANTP-class homeodomains constructed using the maximum likelihood method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded. The proposed division between the HOXL and NKL subclasses is indicated. The position of *EN1* and *EN2* is unstable; this tree places them in the NKL subclass, whereas neighbor-joining analysis of the same dataset places them at the base of the two subclasses (Additional file 3). Interrelationships of genes in the Nk4 and Nk2.2 families are also unstable (in this tree and Additional file 3 respectively); in these cases synteny within and between genomes clearly resolves gene families. Detailed relationships between different gene families should not be inferred from this tree.

cases that could cause confusion. Other details can be found in Table 2.

◦ Cdx, Gsx and Pdx gene families. Some authors refer to the Pdx gene family as the Xlox gene family [28]. One gene from each of these families (*CDX2*, *GSX1* and *PDX1*) forms the ParaHox cluster at 13q12.2 (Figure 4), and clustering of Cdx, Gsx and Pdx genes is ancestral for chordates [28].

◦ Mnx gene family. This gene family name derives from a previous study [29]. The family includes one gene in the human genome: *MNX1* (formerly *HLXB9*), and two genes in the chicken genome: *Mnx1* (formerly *HB9*) and *Mnx2* (formerly *MNR2*). Some authors refer to the Mnx gene family as the Exex gene family due to the *Drosophila* ortholog *exex* [7].

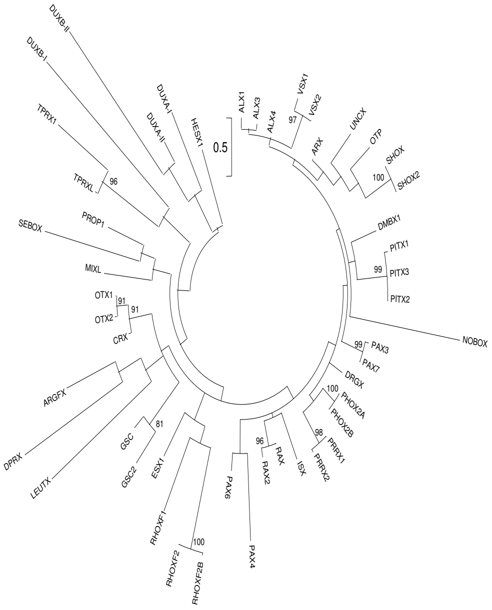

Figure 2

Maximum likelihood phylogenetic tree of human PRD-class homeodomains. Arbitrarily rooted phylogenetic tree of human PRD-class homeodomains constructed using the maximum likelihood method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded, as are the partial homeodomains of PAX2, PAX5 and PAX8, and the HOPX homeodomain because its extremely divergent sequence destabilizes the overall tree topology. Roman numeral suffixes are used to distinguish multiple homeodomains encoded by a single Dux-family gene. In this tree Dux-family homeodomains are not monophyletic, even within the same gene; however, monophyly is recovered by neighbor-joining analysis (Additional file 4). Detailed relationships between different gene families should not be inferred from this tree.

◦ Dlx gene family. It is currently unclear if this gene family is derived from one or more genes in the common ancestor of bilaterians [18]. Phylogenetic analyses place this gene family firmly within the NKL subclass (Figure 1; Additional file 3), but chromosomal positions (on the Hox chromosomes 2, 7 and 17) place it within the HOXL subclass (Figure 4). Here we favor placement of the Dlx gene family within the NKL subclass due to strong phylogenetic support.

◦ En gene family. Phylogenetic analyses place this gene family either within the NKL subclass (maximum likelihood; Figure 1) or close to the division between the NKL and HOXL subclasses (neighbor-joining; Additional file 3). Here we place the En gene family within the NKL subclass, although we note that human *EN2* maps close to the clear HOXL-subclass genes *GBX1* and *MNX1* on chromosome 7 (Figure 4).

◦ Nk2.1 and Nk2.2 gene families. The genes *NKX2-1* (formerly *TITF1*), *NKX2-4*, *NKX2-2* and *NKX2-8* divide into two distinct gene families each with an invertebrate ortholog, not a single Nk2 gene family. *NKX2-1* and *NKX2-4* are collectively orthologous to *Drosophila scro* and amphioxus *AmphiNk2-1* [30,31]; these comprise one gene family: Nk2.1. *NKX2-2* and *NKX2-8* are collectively orthologous to *Drosophila vnd* and amphioxus *AmphiNk2-2* [31,32]; these comprise a second gene family: Nk2.2.

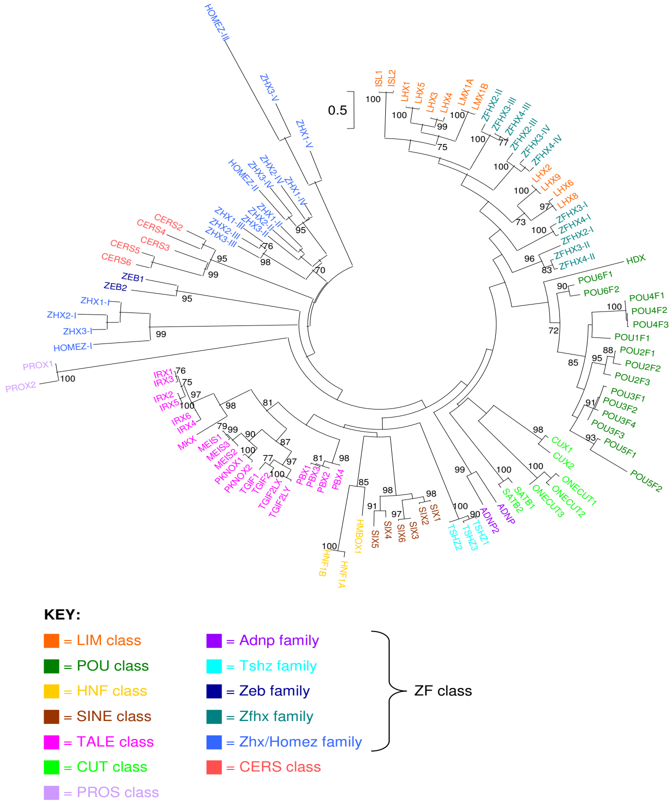

Figure 3

Maximum likelihood phylogenetic tree of human homeodomains excluding ANTP and PRD classes. Arbitrarily rooted phylogenetic tree of human homeodomains excluding the ANTP and PRD classes constructed using the maximum likelihood method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded. Roman numeral suffixes are used to distinguish multiple homeodomains encoded by a single gene. Classes and/or families are color coded as shown in the key. The LIM and ZF classes are not recovered as two distinct monophyletic groups, a result also found by neighbor-joining analysis (Additional file 5). The multiple homeodomains of Zfhx-family proteins and Zhx/Homez-family proteins are also dispersed in the tree, presumably artefactually. Detailed relationships between different gene families should not be inferred from this tree.

◦ Nk4 gene family. The genes *NKX2-3*, *NKX2-5* and *NKX2-6* form a gene family, quite distinct from other human genes that confusingly share the prefix *NKX2*. These three genes are actually orthologs of *Drosophila tin* (formerly *NK4*); they are not orthologs of *Drosophila vnd* (formerly *NK2*) or *scro* [33]. Therefore, they do not belong to the Nk2.1 or Nk2.2 gene families, but belong to a separate Nk4 gene family. As the three gene names have very extensive current usage, it may be difficult for revised names to be used consistently. In this situation, we don't alter the current names, but raise for discussion the possibility of these genes being renamed to the more logical *NKX4-1*

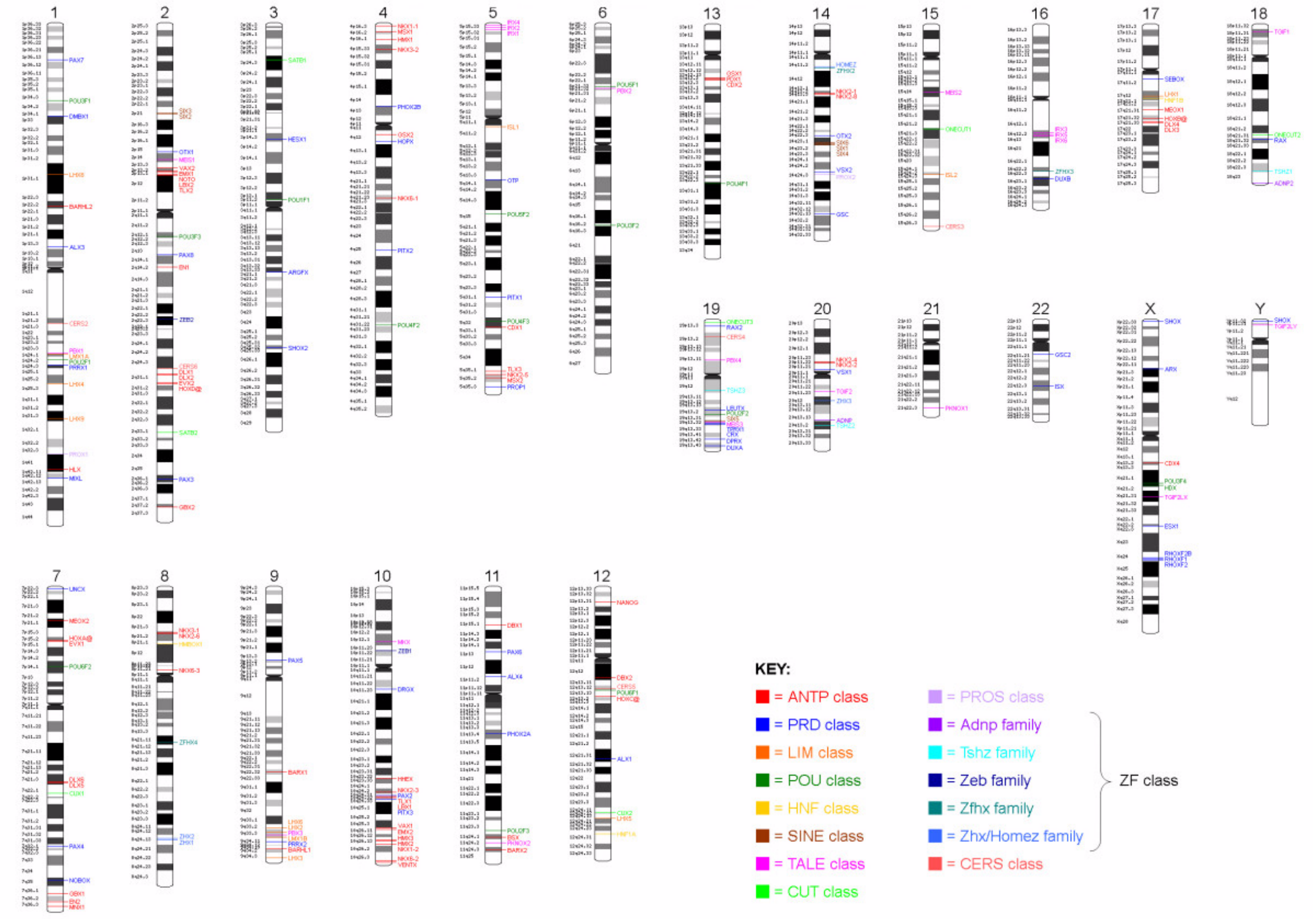

Figure 4

Chromosomal distribution of human homeobox genes. Ideograms of human chromosomes showing the locations of human homeobox genes. Hox clusters are each shown as a single line for simplicity. Probable pseudogenes are not shown. Genes are color coded according to their class or family (see key). Map positions were obtained through the Ensembl Genome Browser.

(*NKX2-5*), *NKX4-2* (*NKX2-6*) and *NKX4-3* (*NKX2-3*), or to *CSX1* (*NKX2-5*), *CSX2* (*NKX2-6*) and *CSX3* (*NKX2-3*), based on the alternative name *CSX1* for *NKX2-5* [34].

◦ Noto gene family. This gene family falls close to the division between the ANTP and PRD classes in phylogenetic analyses (Additional files 1 and 2). We favor placement within the ANTP class as the human *NOTO* gene is chromosomally linked to the clear ANTP-class (NKL-subclass) genes *EMX1*, *LBX2*, *TLX2* and *VAX2* on chromosome 2 (Figure 4), suggesting ancestry by ancient tandem duplication.

Most of the 100 genes in the ANTP class have been adequately named previously. However, several genes were unnamed or misnamed prior to this study. We have updated these as follows.

◦ *GSX2* [Entrez Gene ID: 170825] is the second of two human members of the Gsx gene family. This previously unnamed gene has clear orthology to mouse *Gsh2*, inferred from sequence identity and synteny. We designate the gene *GSX2* and revise the nomenclature of the other human member of the family from *GSH1* to *GSX1* [Entrez Gene ID: 219409], in accordance with homeobox gene nomenclature convention.

◦ *MNX1* [Entrez Gene ID: 3110] is the only member of the Mnx gene family in the human genome. This gene was previously known as *HLXB9*; we rename it *MNX1* because it is not part of a series of at least nine related genes.

◦ *PDX1* [Entrez Gene ID: 3651] is the only member of the Pdx gene family in the human genome. This gene was previously known as *IPF1*; we rename it *PDX1* because the majority of published studies use this as the gene symbol.

Table 2: Human ANTP class homeobox genes and pseudogenes

Human ANTP-class homeobox genes and pseudogenes
HOXL subclass

Family	Gene symbol	Gene name	Location	Entrez gene ID	Previous symbols
Cdx	*CDX1*	caudal type homeobox 1	5q32	1044	
	CDX2	caudal type homeobox 2	13q12.2	1045	CDX3
	CDX4	caudal type homeobox 4	Xq13.2	1046	
Evx	*EVX1*	even-skipped homeobox 1	7p15.2	2128	
	EVX2	even-skipped homeobox 2	2q31.1	344191	
Gbx	*GBX1*	gastrulation brain homeobox 1	7q36.1	2636	
	GBX2	gastrulation brain homeobox 2	2q37.2	2637	
Gsx	*GSX1*	GS homeobox 1	13q12.2	219409	GSH1
	GSX2	GS homeobox 2	4q12	170825	GSH2
Hox1	*HOXA1*	homeobox A1	7p15.2	3198	HOX1F
	HOXB1	homeobox B1	17q21.32	3211	HOX2I
	HOXD1	homeobox D1	2q31.1	3231	HOX4G
Hox2	*HOXA2*	homeobox A2	7p15.2	3199	HOX1K
	HOXB2	homeobox B2	17q21.32	3212	HOX2H
Hox3	*HOXA3*	homeobox A3	7p15.2	3200	HOX1E
	HOXB3	homeobox B3	17q21.32	3213	HOX2G
	HOXD3	homeobox D3	2q31.1	3232	HOX4A
Hox4	*HOXA4*	homeobox A4	7p15.2	3201	HOX1D
	HOXB4	homeobox B4	17q21.32	3214	HOX2F
	HOXC4	homeobox C4	12q13.13	3221	HOX3E
	HOXD4	homeobox D4	2q31.1	3233	HOX4B
Hox5	*HOXA5*	homeobox A5	7p15.2	3202	HOX1C
	HOXB5	homeobox B5	17q21.32	3215	HOX2A
	HOXC5	homeobox C5	12q13.13	3222	HOX3D
Hox6-8	*HOXA6*	homeobox A6	7p15.2	3203	HOX1B
	HOXB6	homeobox B6	17q21.32	3216	HOX2B
	HOXC6	homeobox C6	12q13.13	3223	HOX3C
	HOXA7	homeobox A7	7p15.2	3204	HOX1A
	HOXB7	homeobox B7	17q21.32	3217	HOX2C
	HOXB8	homeobox B8	17q21.32	3218	HOX2D
	HOXC8	homeobox C8	12q13.13	3224	HOX3A
	HOXD8	homeobox D8	2q31.1	3234	HOX4E
Hox9-13	*HOXA9*	homeobox A9	7p15.2	3205	HOX1G
	HOXB9	homeobox B9	17q21.32	3219	HOX2E
	HOXC9	homeobox C9	12q13.13	3225	HOX3B
	HOXD9	homeobox D9	2q31.1	3235	HOX4C
	HOXA10	homeobox A10	7p15.2	3206	HOX1H
	HOXC10	homeobox C10	12q13.13	3226	HOX3I
	HOXD10	homeobox D10	2q31.1	3236	HOX4D, HOX4E
	HOXA11	homeobox A11	7p15.2	3207	HOX1I
	HOXC11	homeobox C11	12q13.13	3227	HOX3H
	HOXD11	homeobox D11	2q31.1	3237	HOX4F
	HOXC12	homeobox C12	12q13.13	3228	HOX3F
	HOXA13	homeobox A13	7p15.2	3209	HOX1J
	HOXB13	homeobox B13	17q21.32	10481	
	HOXC13	homeobox C13	12q13.13	3229	HOX3G
	HOXD13	homeobox D13	2q31.1	3239	HOX4I
Mnx	*MNX1*	motor neuron and pancreas homeobox 1	7q36.3	3110	HLXB9, HB9, HOXHB9
Meox	*MEOX1*	mesenchyme homeobox 1	17q21.31	4222	MOX1
	MEOX2	mesenchyme homeobox 2	7p21.1	4223	MOX2, GAX
Pdx	*PDX1*	pancreatic and duodenal homeobox 1	13q12.2	3651	IPF1, IUF1, IDX1, STF1
NKL subclass					
Barhl	*BARHL1*	BarH-like homeobox 1	9q34.13	56751	
	BARHL2	BarH-like homeobox 2	1p22.2	343472	
Barx	*BARX1*	BARX homeobox 1	9q22.32	56033	
	BARX2	BARX homeobox 2	11q24.3	8538	
Bsx	*BSX*	brain specific homeobox	11q24.1	390259	
Dbx	*DBX1*	developing brain homeobox 1	11p15.1	120237	

Table 2: Human ANTP class homeobox genes and pseudogenes *(Continued)*

	DBX2	developing brain homeobox 2	12q12	440097	
Dlx	*DLX1*	distal-less homeobox 1	2q31.1	1745	
	DLX2	distal-less homeobox 2	2q31.1	1746	TES1
	DLX3	distal-less homeobox 3	17q21.33	1747	
	DLX4	distal-less homeobox 4	17q21.33	1748	DLX7, DLX8, DLX9, BP1
	DLX5	distal-less homeobox 5	7q21.3	1749	
	DLX6	distal-less homeobox 6	7q21.3	1750	
Emx	*EMX1*	empty spiracles homeobox 1	2p13.2	2016	
	EMX2	empty spiracles homeobox 2	10q26.11	2018	
En	*EN1*	engrailed homeobox 1	2q14.2	2019	
	EN2	engrailed homeobox 2	7q36.3	2020	
Hhex	*HHEX*	hematopoietically expressed homeobox	10q23.33	3087	HEX, PRH, PRHX
Hlx	*HLX*	H2.0-like homeobox	1q41	3142	HLX1, HB24
Lbx	*LBX1*	ladybird homeobox 1	10q24.32	10660	LBX1H, HPX6
	LBX2	ladybird homeobox 2	2p13.1	85474	
Msx	*MSX1*	msh homeobox 1	4p16.2	4487	HOX7
	MSX2	msh homeobox 2	5q35.2	4488	HOX8, MSH
	MSX2P1	msh homeobox 2 pseudogene	17q23.2	55545	HPX5, MSX2P
Nanog	*NANOG*	Nanog homeobox	12p13.31	79923	
	NANOGP1	Nanog homeobox pseudogene 1	12p13.31	404635	NANOG2
	NANOGP2	Nanog homeobox pseudogene 2	2q36.1	414131	NANOGP4
	NANOGP3	Nanog homeobox pseudogene 3	6p12.1	340217	
	NANOGP4	Nanog homeobox pseudogene 4	7p15.1	414132	NANOGP2
	NANOGP5	Nanog homeobox pseudogene 5	9q31.1	414133	
	NANOGP6	Nanog homeobox pseudogene 6	10q24.2	414134	
	NANOGP7	Nanog homeobox pseudogene 7	14q32.12	414130	NANOGP3
	NANOGP8	Nanog homeobox pseudogene 8	15q14	388112	NANOGP1
	NANOGP9	Nanog homeobox pseudogene 9	Xq12	349386	NANOGP6
	NANOGP10	Nanog homeobox pseudogene 10	Xp11.3	349372	NANOGP5
	NANOGP11	Nanog homeobox pseudogene 11	6q25.2	414135	
Nk1	*NKX1-1*	NK1 homeobox 1	4p16.3	54279	NKX1.1, HSPX153, HPX153
	NKX1-2	NK1 homeobox 2	10q26.13	390010	NKX1.2, C10orf121
Nk2.1	*NKX2-1*	NK2 homeobox 1	14q13.3	7080	NKX2.1, NKX2A, TTF1, TITF1
	NKX2-4	NK2 homeobox 4	20p11.22	4823	NKX2.4, NKX2D
Nk2.2	*NKX2-2*	NK2 homeobox 2	20p11.22	4821	NKX2.2, NKX2B
	NKX2-8	NK2 homeobox 8	14q13.3	26257	NKX2.8, NKX2H
Nk3	*NKX3-1*	NK3 homeobox 1	8p21.2	4824	NKX3.1, NKX3A
	NKX3-2	NK3 homeobox 2	4p15.33	579	NKX3.2, NKX3B, BAPX1
Nk4	*NKX2-3*	NK2 homeobox 3	10q24.2	159296	NKX2.3, NKX2C, NKX4-3, CSX3
	NKX2-5	NK2 homeobox 5	5q35.1	1482	NKX2.5, NKX2E, NKX4-1, CSX, CSX1
	NKX2-6	NK2 homeobox 6	8p21.2	137814	NKX2.6, NKX4-2, CSX2
Nk5/Hmx	*HMX1*	H6 family homeobox 1	4p16.1	3166	NKX5-3, H6
	HMX2	H6 family homeobox 2	10q26.13	3167	NKX5-2, H6L
	HMX3	H6 family homeobox 3	10q26.13	340784	NKX5-1
Nk6	*NKX6-1*	NK6 homeobox 1	4q21.23	4825	NKX6.1, NKX6A
	NKX6-2	NK6 homeobox 2	10q26.3	84504	NKX6.2, NKX6B, GTX
	NKX6-3	NK6 homeobox 3	8p11.21	157848	NKX6.3
Noto	*NOTO*	notochord homeobox	2p13.2	344022	
Tlx	*TLX1*	T-cell leukemia homeobox 1	10q24.32	3195	HOX11, TCL3
	TLX2	T-cell leukemia homeobox 2	2p13.1	3196	HOX11L1, NCX
	TLX3	T-cell leukemia homeobox 3	5q35.1	30012	HOX11L2, RNX
Vax	*VAX1*	ventral anterior homeobox 1	10q26.11	11023	
	VAX2	ventral anterior homeobox 2	2p13.3	25806	
Ventx	*VENTX*	VENT homeobox	10q26.3	27287	VENTX2, HPX42B
	VENTXP1	VENT homeobox pseudogene 1	Xp21.3	139538	VENTX2P1, NA88A
	VENTXP2	VENT homeobox pseudogene 2	13q31.1	347975	VENTX2P2
	VENTXP3	VENT homeobox pseudogene 3	12q21.1	349814	VENTX2P3
	VENTXP4	VENT homeobox pseudogene 4	3p24.2	152101	VENTX2P4
	VENTXP5	VENT homeobox pseudogene 5	8p12	442384	
	VENTXP6	VENT homeobox pseudogene 6	8q21.11	552879	
	VENTXP7	VENT homeobox pseudogene 7	3p24.3	391518	VENTX1, HPX42

Human ANTP class homeobox genes and pseudogenes including full names, chromosomal locations, Entrez Gene IDs and previous symbols. *NANOGP1* is a duplicate of *NANOG*.

◦ *BSX* [Entrez Gene ID: 390259] is the only member of the Bsx gene family in the human genome. We designate this previously unnamed gene *BSX* on the basis of clear orthology to the mouse *Bsx* gene, inferred from sequence identity and synteny.

◦ *DBX1* [Entrez Gene ID: 120237] and *DBX2* [Entrez Gene ID: 440097] are the only two members of the Dbx gene family in the human genome. We designate these previously unnamed genes *DBX1* and *DBX2* on the basis of clear orthology to mouse *Dbx1* and *Dbx2*, inferred from sequence identity and synteny.

◦ *NKX1-1* [Entrez Gene ID: 54729] and *NKX1-2* [Entrez Gene ID: 390010] are the only two members of the Nk1 gene family in the human genome. These genes were previously known as *HSPX153* and *C10orf121* respectively; we rename them *NKX1-1* and *NKX1-2* on the basis of clear orthology to mouse *Nkx1-1* and *Nkx1-2*, inferred from sequence identity and synteny.

◦ *NKX2-1* [Entrez Gene ID: 7080] is the first of two human members of the Nk2.1 gene family. This gene was previously known as *TITF1*; we rename it *NKX2-1* to show that it is a member of the Nk2.1 gene family.

◦ *NKX2-6* [Entrez Gene ID: 137814] is the third of three human members of the Nk4 gene family. We designate this previously unnamed gene *NKX2-6* on the basis of clear orthology to mouse *Nkx2-6*, inferred from sequence identity and synteny, although nomenclature revision for the entire Nk4 gene family should be discussed (see above).

◦ *NKX3-2* [Entrez Gene ID: 579] is the second of two human members of the Nk3 gene family. This gene was previously known as *BAPX1*; we rename it *NKX3-2* to show that it is a member of the Nk3 gene family.

◦ *NKX6-3* [Entrez Gene ID: 157848] is the third of three human members of the Nk6 gene family. We designate this previously unnamed gene *NKX6-3* on the basis of clear orthology to mouse *Nkx6-3*, inferred from sequence identity and synteny.

◦ *VENTX* [Entrez Gene ID: 27287] is the only functional member of the Ventx gene family in the human genome. This gene was previously known as *VENTX2*. We remove the numerical suffix from this gene symbol because we discovered that the sequence formerly known as *VENTX1* is actually a retrotransposed pseudogene derived from this gene. Accordingly, we also replace the *VENTX1* symbol with *VENTXP7* (see below).

In contrast to the previous descriptions of probable functional genes, there has been much less research on pseudogenes within the ANTP class. Eleven pseudogenes derived from the human *NANOG* gene have been described previously [22], while four pseudogenes in the Ventx gene family have been reported following routine annotation of the human genome. We have identified two additional Ventx-family pseudogenes (*VENTXP5* and *VENTXP6*), and also found two cases of pseudogenes that were originally mistaken for functional genes (*MSX2P1* and *VENTXP7*). In all cases, we have clarified the origins and organization of these pseudogenes. This research brings the total number of ANTP-class pseudogenes in the human genome to 19.

◦ *MSX2P1* [Entrez Gene ID: 55545]. A short cDNA sequence [EMBL: X74862] related to the Msx gene family was reported previously [35]; the former Entrez Gene record labeled *HSHPX5* was based on this sequence. This locus was later provisionally called *MSX4*, as it was distinct from human *MSX1* and *MSX2*, and by synteny it was clearly not the ortholog of mouse *Msx3* [27]. It is now clear that this locus was formed by retrotransposition of mRNA from *MSX2* and hence we name it *MSX2P1*. The genomic sequence of *MSX2P1* can now be accessed via the Reference Sequence collection [RefSeq: NR_002307]. The pseudogene shares 91% sequence identity with *MSX2* mRNA, lacks intronic sequence, and has remnants of a 3' poly(A) tail. It is intriguing, but probably coincidental, that the *MSX2P1* pseudogene has integrated at 17q23.2, close to several ANTP-class genes (HOXB cluster, *MEOX1*, *DLX3* and *DLX4*).

◦ *NANOGP1* [Entrez Gene ID: 404635]. We follow Booth and Holland [22] and classify *NANOGP1* as a pseudogene that arose by tandem duplication of *NANOG*. The alternative view, argued by Hart et al [36], is that this locus is a functional gene, and should be named *NANOG2*. There is evidence for transcription of this locus in human embryonic stem cells [36], and for selection-driven conservation of the open reading frame [37], but as yet no clear evidence for function.

◦ *NANOGP8* [Entrez Gene ID: 388112]. We follow Booth and Holland [22] and classify *NANOGP8* as a retrotransposed pseudogene. The alternative view, argued by Zhang et al [38], is that this locus is a functional retrogene. There is evidence for transcription and translation of this locus in cancer cell lines and tumors [38], but no evidence yet for a role in normal tissues.

◦ *VENTXP1* [Entrez Gene ID: 139538], *VENTXP2* [Entrez Gene ID: 347975], *VENTXP3* [Entrez Gene ID: 349814] and *VENTXP4* [Entrez Gene ID: 152101]. These four *VENTX* retrotransposed pseudogenes have been reported previously, and were originally known as *VENTX2P1* to *VENTX2P4*. The correction of the *VENTX2* gene symbol to simply *VENTX* (see above) means that each of the pseudogene names should also change; we rename them *VENTXP1* to *VENTXP4*. *VENTXP1* is transcribed but due to mutations it can no longer encode a homeodomain protein; it can however encode an antigenic peptide (NA88A) responsible for T-cell stimulation in response to melanoma [39].

◦ *VENTXP5* [Entrez Gene ID: 442384]. We designate this previously unnamed sequence *VENTXP5* because it is clearly a retrotransposed pseudogene of *VENTX*. The genomic sequence of *VENTXP5* can now be accessed via the Reference Sequence collection [RefSeq: NG_005091]. The pseudogene shares 83% identity with *VENTX* mRNA (after masking of an Alu element in the parental mRNA sequence), lacks intronic sequence, and has remnants of a 3' poly(A) tail.

◦ *VENTXP6* [Entrez Gene ID: 552879]. We designate this previously unannotated sequence *VENTXP6* because it is clearly a retrotransposed pseudogene of *VENTX*. Its lack of annotation may reflect the fact that it is located within an intron of an unrelated and well characterized gene, *STAU2*. The genomic sequence of *VENTXP6* can now be accessed via the Reference Sequence collection [RefSeq: NG_005090]. The pseudogene shares 87% identity with *VENTX* mRNA (after masking of an Alu element in the parental mRNA sequence) and lacks intronic sequence.

◦ *VENTXP7* [Entrez Gene ID: 391518]. A short cDNA sequence [EMBL: X74864] was reported previously and named *HPX42* [35]. This was later renamed the *VENTX1* gene, after it was found to be related to *Xenopus* Ventx-family genes. Our analysis of the genomic sequence at this locus reveals that it is actually a retrotransposed pseudogene of the *VENTX* gene (formerly *VENTX2*); thus we designate it *VENTXP7*. The genomic sequence of *VENTXP7* can now be accessed via the Reference Sequence collection [RefSeq: NR_002311]. The pseudogene shares 86% identity with *VENTX* mRNA (after masking of an Alu element in the parental mRNA sequence), lacks intronic sequence, and has remnants of a 3' poly(A) tail.

One other gene could conceivably be included in the ANTP class, but is excluded from our survey. This gene [Entrez Gene ID: 360030; GenBank: AY151139], has been annotated as a homeobox gene and is located just 20 kb from *NANOG*. However, no homeodomain was detected when the deduced protein was analyzed for conserved domains. Also, secondary structure prediction did not predict the expected organisation of alpha helices. Alignment with the NANOG homeodomain reveals identity of the KQ and WF motifs, either side of the same intron position (44/45), but few other shared residues. It is possible, but unproven, that the locus arose by tandem duplication of part, or all, of the *NANOG* homeobox gene. This gene has generated two retrotransposed pseudogenes: one at 2q11.2 and another at 12q24.33.

The PRD homeobox class

The PRD class derives its name from the *paired* (*prd*) gene of *Drosophila melanogaster*. In previous studies, the PRD class has been subdivided in several different ways, often based on identify of the amino acid at residue 50 in the homeodomain, for example S50, K50 and Q50. These categories are not monophyletic groupings of genes and so can be misleading if we aim for a classification scheme that reflects evolution [5]. Here we divide the PRD class into two subclasses of unequal size: the PAX subclass (containing seven PAX genes, excluding *PAX1* and *PAX9*), and the PAXL subclass (containing 43 non-PAX genes and many pseudogenes) (Table 1). PAX genes are defined by possession of a conserved paired-box motif, distinct from the homeobox, coding for the 128-amino-acid PRD domain. Of the nine human genes possessing a paired-box (*PAX1* to *PAX9*), only four also contain a complete homeobox (*PAX3*, *PAX7*, *PAX4* and *PAX6*). Three genes have a partial homeobox (*PAX2*, *PAX5* and *PAX8*), while two lack a homeobox entirely (*PAX1* and *PAX9*). Phylogenetic analyses using PAX genes from a range of species suggest that these are secondary conditions, and that the ancestral PAX gene probably possessed both motifs [40]. The PAX genes do not constitute a single gene family, because it is clear that the latest common ancestor of the Bilateria contained four PAX genes. Three of these are ancestors of the PRD-class homeobox gene families Pax2/5/8, Pax3/7 and Pax4/6; the fourth is the ancestor of *PAX1* and *PAX9*. Thus the PAX subclass contains three gene families. We divide the PAXL subclass into 28 gene families, although as explained below not all of these date to the base of the Bilateria. Thus, we recognize a total of 31 gene families in the PRD class (Table 1).

Many of the 31 gene families in the PRD class have been clearly defined before. We draw attention here to newly defined gene families and cases that could cause confusion. Other details can be found in Table 3.

◦ Argfx, Dprx and Tprx gene families. There are no known invertebrate members of these three gene families. Therefore, these are exceptions to the rule defining gene families as dating to the base of the Bilateria. The Dprx and Tprx gene families may have arisen by duplication and very extensive divergence from *CRX*, a member of the Otx gene family, during mammalian evolution; origins of *ARGFX* are obscure [21].

Table 3: Human PRD class homeobox genes and pseudogenes

Human PRD-class homeobox genes and pseudogenes					
Family	**Gene symbol**	**Gene name**	**Location**	**Entrez gene IDc**	**Previous symbols**
Alx	*ALX1*	ALX homeobox 1	12q21.31	8092	CART1
	ALX3	ALX homeobox 3	1p13.3	257	
	ALX4	ALX homeobox 4	11p11.2	6059	
Argfx	*ARGFX*	arginine-fifty homeobox	3q13.33	503582	
	ARGFXP1	arginine-fifty homeobox pseudogene 1	5q23.2	503583	
	ARGFXP2	arginine-fifty homeobox pseudogene 2	17q11.2	503640	
Arx	*ARX*	aristaless related homeobox	Xp21.3	170302	ISSX
Dmbx	*DMBX1*	diencephalon/mesencephalon brain homeobox 1	1p34.1	127343	MBX, OTX3, PAXB
Dprx	*DPRX*	divergent paired-related homeobox	19q13.42	503834	
	DPRXP1	divergent paired-related homeobox pseudogene 1	2q32.1	503641	
	DPRXP2	divergent paired-related homeobox pseudogene 2	6p21.31	503643	
	DPRXP3	divergent paired-related homeobox pseudogene 3	14q13.2	503644	
	DPRXP4	divergent paired-related homeobox pseudogene 4	17q11.2	503645	
	DPRXP5	divergent paired-related homeobox pseudogene 5	21q22.13	503646	
	DPRXP6	divergent paired-related homeobox pseudogene 6	Xp11.4	503647	
	DPRXP7	divergent paired-related homeobox pseudogene 7	Xq23	503648	
Drgx	*DRGX*	dorsal root ganglia homeobox	10q11.23	644168	DRG11, PRRXL1
Dux	*DUXA*	double homeobox A	19q13.43	503835	
	DUXAP1	double homeobox A pseudogene 1	2p11.2	503630	
	DUXAP2	double homeobox A pseudogene 2	8q22.3	503631	
	DUXAP3	double homeobox A pseudogene 3	10q11.21	503632	
	DUXAP4	double homeobox A pseudogene 4	10q11.21	503633	
	DUXAP5	double homeobox A pseudogene 5	11q23.3	503634	
	DUXAP6	double homeobox A pseudogene 6	15q26.1	503635	
	DUXAP7	double homeobox A pseudogene 7	20p11.23	503636	
	DUXAP8	double homeobox A pseudogene 8	22q11.21	503637	
	DUXAP9	double homeobox A pseudogene 9	14qcen	503638	
	DUXAP10	double homeobox A pseudogene 10	14q11.2	503639	
	DUXB	double homeobox B	16q23.1	100033411	
Esx	*ESX1*	ESX homeobox 1	Xq22.2	80712	ESX1L, ESXR1
Gsc	*GSC*	goosecoid homeobox	14q32.13	145258	GSC1
	GSC2	goosecoid homeobox 2	22q11.21	2928	GSCL
Hesx	*HESX1*	HESX homeobox 1	3p14.3	8820	RPX, ANF
Hopx	*HOPX*	HOP homeobox	4q12	84525	HOP, OB1, LAGY, NECC1, SMAP31
Isx	*ISX*	intestine specific homeobox	22q12.3	91464	RAXLX
Leutx	*LEUTX*	Leucine twenty homeobox	19q13.2	342900	
Mix	*MIXL*	Mix paired-like homeobox	1q42.12	83881	MIX, MIXL1, MILD1
Nobox	*NOBOX*	NOBOX oogenesis homeobox	7q35	135935	OG2, OG2X
Otp	*OTP*	orthopedia homeobox	5q14.1	23440	
Otx	*OTX1*	orthodenticle homeobox 1	2p15	5013	
	OTX2	orthodenticle homeobox 2	14q22.3	5015	
	OTX2P1	orthodenticle homeobox 2 pseudogene	9q21.2	100033409	OTX2P
	CRX	cone-rod homeobox	19q13.32	1406	OTX3
Pax2/5/8	*PAX2*	paired box 2	10q24.31	5076	
	PAX5	paired box 5	9p13.2	5079	BSAP
	PAX8	paired box 8	2q13	7849	
Pax3/7	*PAX3*	paired box 3	2q36.1	5077	HUP2
	PAX7	paired box 7	1p36.13	5081	HUP1, PAX7B
Pax4/6	*PAX4*	paired box 4	7q32.1	5078	
	PAX6	paired box 6	11p13	5080	
Phox	*PHOX2A*	paired-like homeobox 2a	11q13.4	401	PMX2A, ARIX
	PHOX2B	paired-like homeobox 2b	4p13	8929	PMX2B, NBPhox
Pitx	*PITX1*	pituitary homeobox 1	5q31.1	5307	PTX1, POTX, BFT
	PITX2	pituitary homeobox 2	4q25	5308	PTX2, ARP1, RGS, RIEG, RIEG1
	PITX3	pituitary homeobox 3	10q24.32	5309	PTX3
Prop	*PROP1*	PROP paired-like homeobox 1	5q35.3	5626	
Prrx	*PRRX1*	paired related homeobox 1	1q24.3	5396	PRX1, PMX1, PHOX1
	PRRX2	paired related homeobox 2	9q34.11	51450	PRX2, PMX2
Rax	*RAX*	retina and anterior neural fold homeobox	18q21.31	30062	RX
	RAX2	retina and anterior neural fold homeobox 2	19p13.3	84839	QRX, RAXL1
Rhox	*RHOXF1*	Rhox homeobox family, member 1	Xq24	158800	PEPP1, OTEX
	RHOXF2	Rhox homeobox family, member 2	Xq24	84528	PEPP2
	RHOXF2B	Rhox homeobox family, member 2B	Xq24	727940	PEPP2L

Table 3: Human PRD class homeobox genes and pseudogenes *(Continued)*

Sebox	*SEBOX*	SEBOX homeobox	17q11.2	645832	OG9, OG9X
Shox	*SHOX*	short stature homeobox	Xp22.33/ Yp11.32	6473	SHOXY, GCFX, PHOG
	SHOX2	short stature homeobox 2	3q25.32	6474	SHOT, OG12, OG12X
Tprx	*TPRX1*	tetra-peptide repeat homeobox 1	19q13.32	284355	
	TPRX2P	tetra-peptide repeat homeobox 2 pseudogene	19q13.32	503627	
	TPRX1P1	tetra-peptide repeat homeobox 1 pseudogene 1	10q22.3	503628	
	TPRX1P2	tetra-peptide repeat homeobox 1 pseudogene 2	10q22.3	503629	
	TPRXL	tetra-peptide repeat homeobox-like	3p25.1	348825	
Uncx	*UNCX*	UNC homeobox	7p22.3	340260	PHD1, UNCX4.1
Vsx	*VSX1*	visual system homeobox 1	20p11.21	30813	KTCN, RINX
	VSX2	visual system homeobox 2	14q24.3	338917	RET1, HOX10, CHX10

Human PRD class homeobox genes and pseudogenes including full names, chromosomal locations, Entrez Gene IDs and previous symbols. Pax2/5/8-family genes contain a partial homeobox. *RHOXF2B* is a duplicate of *RHOXF2*. *TPRX2P* is a duplicate of *TPRX1*.

◦ Dux gene family. Members of this gene family are characterized by the presence of two closely-linked homeobox motifs. Most members are intronless sequences present in multiple polymorphic copies within the 3.3 kb family of tandemly repeated elements associated with heterochromatin. These comprise the sequences known as *DUX1* to *DUX5* reported in previous studies [12-14] and numerous *DUX4* copies detected in this study (see below). The absence of introns suggests that these sequences may have originated by retrotransposition from an mRNA transcript, thus they are probably non-functional. There are two noticeable exceptions; these members known as *DUXA* and *DUXB* possess introns, thus either one could be the progenitor for the large number of intronless Dux-family sequences found in the human genome. *DUXA* has spawned 10 retrotransposed pseudogenes and has been described previously [21]. *DUXB* is described here (see below).

◦ Hopx gene family. Phylogenetic analyses places this gene family, containing a single very divergent homeobox gene *HOPX* (formerly *HOP*), either within the PRD class (maximum likelihood; Additional file 1) or close to Zhx/Homez-family genes (neighbor-joining; Additional file 2). We favor placement in the PRD class for three reasons. First, the HOPX homeodomain has highest sequence identity with PRD-class homeodomains (GSC: 38% and PAX6: 36%). Second, the HOPX homeodomain possesses the same combination of residues that are invariably conserved across human PRD-class homeodomains (Additional file 6). Third, the HOPX homeodomain shares the 46/47 intron position seen in many PRD-class homeodomains. *HOPX* does not map particularly near any other homeobox genes, although the closest is *GSX2* in the ANTP class at 4q12 (Figure 4). *HOPX* is not a typical PRD-class homeobox gene; the homeodomain has a single amino acid insertion between helix I and helix II (Additional file 6), and lacks the ability to bind DNA [41,42].

◦ Leutx gene family. This gene family contains a single gene in the human genome, *LEUTX*, and no known invertebrate members. We place *LEUTX* in the PRD class for four reasons. First, there is weak phylogenetic support for this placement (Additional files 1 and 2). Second, the LEUTX homeodomain possesses the same combination of residues that are invariably conserved across human PRD-class homeodomains (except for a leucine at position 20; Additional file 6). Third, the LEUTX homeodomain shares the 46/47 intron position seen in many PRD-class homeodomains. Fourth, the *LEUTX* gene is located close to the PRD-class genes *TPRX1*, *CRX*, *DPRX* and *DUXA* on the distal end of the long arm of chromosome 19 (Figure 4). This fourth observation leads us to hypothesize that this gene family arose by tandem duplication and extensive divergence during mammalian evolution.

◦ Nobox gene family. This gene family falls close to the division between the ANTP and PRD classes in both maximum likelihood and neighbor-joining phylogenetic analyses (Additional files 1 and 2). We favor placement within the PRD class because the NOBOX homeodomain has higher sequence identity with PRD-class homeodomains (up to 55%) than with ANTP-class homeodomains (up to 46%). Chromosomal position does not shed light on the issue, as its location at 7q35 is close to both ANTP- and PRD-class genes (Figure 4).

◦ Otx gene family. This very well known gene family was originally considered to contain human *OTX1* and *OTX2* (and their mouse orthologs) and the *Drosophila otd* gene [43]. Later, it was shown that the *CRX* gene is a member of the same gene family, deriving from the same ancestral gene. Thus, *CRX* could be considered the true *OTX3* gene [44]. Unfortunately, the *OTX3* symbol was formerly used erroneously for a gene in a different family, now called *DMBX1*, thus complicating its future use. The gene family name Otx is derived by majority rule from the constituent genes.

◦ Pax2/5/8 gene family. This gene family is also known as Pax group II; it contains *PAX2*, *PAX5* and *PAX8*, clearly derived from a single ancestral gene [45]. These genes have partial homeoboxes.

◦ Pax3/7 gene family. This gene family is also known as Pax group III; it contains *PAX3* and *PAX7*, clearly derived from a single ancestral gene [46].

◦ Pax4/6 gene family. This gene family is also known as Pax group IV; it contains *PAX4* and *PAX6*. There is confusion as to whether this should be split into two gene families, because invertebrate homologs generally group with *PAX6* in phylogenetic analyses and not as an outgroup to the two genes as might be expected. We follow the generally accepted view and group *PAX4* and *PAX6* into a single gene family, proposing that *PAX4* is a divergent member, not an ancient gene [40].

◦ Rhox gene family. The mouse Rhox cluster was first described as comprising twelve X-linked homeobox genes, all selectively expressed in reproductive tissues [47]. Subsequent studies reported a total of 32 genes in the cluster, with the additional genes attributed to recent tandem duplications [48-51]. The human genome contains three homeobox genes at Xq24 that are clearly members of the Rhox gene family based on sequence identity, molecular phylogenetics, intron positions and chromosomal location. These are *RHOXF1* (formerly *OTEX*/*PEPP1*), *RHOXF2* (formerly *PEPP2*) and *RHOXF2B* (formerly *PEPP2b*/*PEPP3*).

Most of the 50 genes in the PRD class have been adequately named previously. However, several genes were unnamed or misnamed prior to this study. We have updated these as follows.

◦ *ALX1* [Entrez Gene ID: 8092] is the first of three human members of the Alx gene family. This gene was previously known as *CART1*; we rename it *ALX1* because it is related to *ALX3* and *ALX4*; all three genes were formed by duplication from a single ancestral invertebrate gene [52].

◦ *DRGX* [Entrez Gene ID: 117065] is the only member of the newly defined Drgx gene family in the human genome. This gene was previously known as *PRRXL1* and *DRG11*, and there is a clear mouse ortholog (*Prrxl1*). The symbol *PRRXL1* is misleading because it infers membership of the Prrx gene family, containing *PRRX1* and *PRRX2* in the human genome. Several lines of evidence suggest it belongs to a different gene family. First, this gene (at 10q11.23) is not located in the same paralogon as *PRRX1* (1q24.3) and *PRRX2* (9q34.11) so they are not three paralogs generated during genome duplication in early vertebrate evolution. Second, it has a completely different exon-intron structure from the Prrx-family genes, and it does not contain a Prrx domain or an OAR domain (present in *PRRX1* and *PRRX2*; [53]). Third, the homeodomain is only 73% identical to PRRX1 and PRRX2 homeodomains, much lower than the 80-100% usually encountered for members of the same gene family in humans. Finally, we have identified the *Drosophila* ortholog, *IP09201*. The homeodomains of *Drosophila* IP09201 and human DRGX form a highly supported monophyletic group in our maximum likelihood (90%; Additional file 1) and neighbor-joining (97%; Additional file 2) phylogenetic analyses. The new symbol *DRGX* (*dorsal root ganglia homeobox*) incorporates the root of the former symbol *DRG11*, referring to expression of the rodent ortholog in dorsal root ganglia neurons [54].

◦ *DUXB* [Entrez Gene ID: 100033411] is a human member of the Dux (double homeobox) gene family. As previously discussed, most members of this gene family are intronless and are probably derived by retrotransposition of an mRNA transcript from a functional intron-containing Dux gene (or duplication of such an integrant). Booth and Holland [21] described the *DUXA* gene containing five introns (including one within each homeobox), and noted the existence of a second intron-containing human Dux-family gene provisionally designated *DUXB*. The *DUXB* nomenclature is endorsed here. No cDNA or EST sequences have been reported for *DUXB*.

◦ *GSC2* [Entrez Gene ID: 2928] is the second of two human members of the Gsc gene family. This gene was previously known as *GSCL*; we rename it *GSC2* to remove the inadvertent implication that it is not a true gene, and also to reflect the clear orthology to chick *Gsc2* as inferred by phylogenetic analysis and synteny.

◦ *HOPX* [Entrez Gene ID: 84525] is the only member of the newly defined Hopx gene family in the human genome. The mouse version of the gene was first identified first and named *Hop* (*homeodomain only protein*) because the encoded protein is just 73 amino acids long, with 61 of these making up the homeodomain [41,42]. The *HOP* gene symbol is not ideal as it is also used for unrelated genes, including *hopscotch* in *Drosophila* and *hop-sterile* in mouse. Therefore, we revise the gene symbol from *HOP* to *HOPX* (*HOP homeobox*) in accordance with homeobox gene nomenclature convention.

◦ *LEUTX* [Entrez Gene ID: 342900] is the only member of the newly defined Leutx gene family in the human genome. We designate this previously unnamed gene *LEUTX* (leucine twenty homeobox) to reflect the presence of a leucine residue at the otherwise highly conserved homeodomain position 20; other PRD-class homeodomains have a phenylalanine at this position (Additional file 6). Studies of mutations in other homeobox genes suggest that mutation to leucine alters transcriptional activity of a homeodomain protein [55].

◦ *RAX2* [Entrez Gene ID: 84839] is the second of two human members of the Rax gene family. This gene was previously known as *RAXL1*; we rename it *RAX2* to standardize nomenclature.

◦ *RHOXF1* [Entrez Gene ID: 158800] and *RHOXF2* [Entrez Gene ID: 84528] are two of three human members of the Rhox gene family. These genes were previously known as *OTEX/PEPP1* and *PEPP2* respectively. The prefix *PEPP* is not suitable as it is used for numerous aminopeptidase P-encoding genes. Thus, we replace the gene symbols *OTEX/PEPP1* and *PEPP2* with *RHOXF1* and *RHOXF2* respectively, to reflect their orthologous relationship with the mouse Rhox cluster (containing 32 genes, see above) whilst avoiding inadvertent equivalence to specific genes within the cluster.

◦ *RHOXF2B* [Entrez Gene ID: 727940] is the third human member of the Rhox gene family. This locus was referred to in previous studies as *PEPP2b* [56] and *PEPP3* [51]. The prefix *PEPP* cannot be approved for reasons noted above. *RHOXF2B* is located very close to *RHOXF1* and *RHOXF2* at Xq24 and is clearly a very recent duplicate of *RHOXF2*. The genomic sequences at these two loci share 99% identity over exonic, intronic and approximately 20 kb flanking regions. Over the coding region, there are just two nucleotide substitutions (both nonsynonymous); one of these results in an unusual change within the homeodomain (arginine to cysteine at position 18). We currently list *RHOXF2B* as a functional gene, although it is possible that it is a duplicated pseudogene.

◦ *SEBOX* [Entrez Gene ID: 645832] is the only member of the Sebox gene family in the human genome. The human gene is the ortholog of mouse *Sebox* based on their locations in syntenic chromosomal regions (17q11.2 and 11B5 respectively) and presence of the same intron positions. However, sequence identity is lower than normal for orthologous genes in mouse and human (78% amino acid identity over the homeodomain) and there is evidence that the human gene has undergone divergence. Most surprisingly, the human sequence has two unusual substitutions in the homeodomain [57]. At homeodomain position 51, the human sequence codes for lysine whereas mouse has asparagine; an earlier analysis of 346 homeodomain sequences found asparagine to be invariant at this position [1,2]. Similarly, at homeodomain position 53, human has tryptophan whereas mouse has arginine; this position is almost invariably arginine [1,2]. These sequence changes in the important third helix raise the possibility that human *SEBOX* could have accumulated mutations as a non-functional pseudogene. Until this is shown more clearly we consider it to be a functional, but divergent, gene. This gene was previously known as *OG9X* with *SEBOX* as the alternative symbol; we favor *SEBOX* because the *OG* prefix was originally used for several unrelated homeobox genes.

◦ *UNCX* [Entrez Gene ID: 340260] is the only member of the Uncx gene family in the human genome. This gene was previously known as *UNCX4.1*; we remove the numerals to give *UNCX* as these do not denote a series within a gene family.

◦ *VSX2* [Entrez Gene ID: 338917] is the second of two human members of the Vsx gene family. This gene was previously known as *CHX10*; we rename it *VSX2* to better reflect its paralogous relationship to *VSX1*. *VSX2* has been used as an alias for this gene in other vertebrate species and the gene symbol *CHX10* has the disadvantage of implicitly suggesting presence of at least nine paralogs in human (*CHX1* to *CHX9*), which do not exist.

Unlike the situation with the ANTP class, many of the pseudogenes within the PRD class have been well characterized. A previous study has described and named two pseudogenes in the Argfx gene family, seven pseudogenes in the Dprx gene family, four pseudogenes in the Tprx gene family, and 10 pseudogenes derived from the *DUXA* gene [21]. There is also a possibility that the *SEBOX* and *RHOXF2B* loci are non-functional pseudogenes, as described above. We have identified a previously undescribed pseudogene from the Otx gene family (*OTX2P1*), and argue that the majority of Dux-family sequences are pseudogenes.

◦ *OTX2P1* [Entrez Gene ID: 100033409]. We designate this previously undescribed sequence *OTX2P1* because it is clearly a retrotransposed pseudogene of *OTX2*. The genomic DNA sequence of *OTX2P1* shares significant homology with *OTX2* transcript variant 2 [RefSeq: NM_172337]. There is an Alu element (AluSx subfamily) insertion, a Made1 (Mariner derived element 1) insertion, and a 1182-nucleotide deletion in *OTX2P1* compared to *OTX2*. The *OTX2P1* sequence lacks introns, ends with a poly(A) tail, and harbors critical sequence alterations (including a three-nucleotide insertion introducing a stop codon into the deduced homeodomain).

◦ *DUX1* [EMBL: AJ001481], *DUX2* [GenBank: AF068744], *DUX3* [GenBank: AF133130] and *DUX5* [GenBank: AF133131]. These sequences have been cloned in previous studies [12,13]. We detected no matches with 100% identity to *DUX1*, *DUX2*, *DUX3* or *DUX5* in build 35.1 of the human genome sequence, which covers the euchromatic regions of each chromosome. This concurs with previous studies indicating that *DUX1*, *DUX2*, *DUX3* and

DUX5 are found in heterochromatin on human acrocentric chromosomes; each is apparently present in multiple copies within members of the 3.3 kb family of tandemly repeated DNA elements [12,13]. Because the majority of human heterochromatin has not been sequenced, and may be variable between individuals, the exact number of copies of *DUX1*, *DUX2*, *DUX3* and *DUX5* is unknown. It is also debatable whether these loci encode functional proteins. These sequences lack introns and, as discussed above, are most likely derived from intron-containing genes in the Dux family, such as *DUXA* or *DUXB*.

◦ *DUX4* [GenBank: AF117653]. This sequence has been extensively studied as some of its multiple copies exist within the 3.3 kb repetitive elements of the D4Z4 locus at 4q35 [14]. The polymorphic D4Z4 locus is linked to facioscapulohumeral muscular dystrophy (FSHD); between 12 and 96 tandem copies of 3.3 kb elements are present in unaffected individuals and deletions leaving a maximum of eight such elements have been associated with FSHD [58]. In build 35.1 of the human genome sequence, we identified 35 loci at 10 chromosomal locations containing a total of 58 *DUX4* (and highly similar) homeobox sequences. This should not be taken as a precise figure due to copy number polymorphism and the possibility of additional copies existing in currently unsequenced heterochromatic regions. Some of the copies are 100% identical to the previously reported *DUX4* sequence over the homeobox regions, others have single nucleotide polymorphisms, some have critical sequence mutations, and others have just a single homeobox. Most of the copies are located in tandemly repeated arrays (for example, on chromosomes 4, 10 and 16) and others are alone in the genome (for example, a single copy resides at 3p12.3). The majority of *DUX4* copies are unlikely to encode functional proteins as suggested by their intronless, mutated and tandemly repeated nature. The lack of introns indicates they are most likely derived from intron-containing genes in the Dux family, such as *DUXA* or *DUXB*.

The LIM homeobox class

The LIM class encodes proteins with two LIM domains (named from the nematode *lin-11*, mammalian *Isl1* and nematode *mec-3* genes) N-terminal to a typical (i.e. 60-amino-acid) homeodomain. The LIM domain is a protein-protein interaction domain of approximately 55 amino acids comprising two specialised cysteine-rich zinc fingers in tandem [59]. Importantly, human genes also exist that encode LIM domains but not homeodomains. These LIM domains are divergent from the LIM domains encoded by LIM homeobox genes, and hence these genes are unlikely to be derived by loss of the homeobox. There is one exception: the human Lmo gene family encodes LIM domains that have been grouped by sequence similarity and domain arrangement with the LIM domains of the LIM homeobox gene class [59]. Thus, this gene family may have secondarily lost the homeobox, although this remains untested. Only genes encoding both LIM domains and homeodomains are included in our LIM homeobox gene count.

We have identified a total of twelve LIM-class homeobox genes in the human genome (Tables 1 and 4), consistent with previous work [60]. Phylogenetic analyses of homeodomains do not always recover the LIM class as a monophyletic group, depending on the dataset and method used (Figure 3; Additional files 1, 2 and 5), but it is likely that the class evolved from a single fusion event that brought together LIM domains and a homeodomain. Phylogenetic analyses of homeodomains divide the LIM class into six gene families (Figure 3; Additional files 1, 2 and 5), consistent with previous studies [60]. Each gene family has two human members and dates to a single ancestral gene in the most recent common ancestor of bilaterians [60]. We have not found any human LIM-class pseudogenes.

The POU homeobox class

The POU class generally encodes proteins with a POU-specific domain (named from the mammalian genes *Pit1* (now *Pou1f1*), *OCT1* and *OCT2* (now *POU2F1* and *POU2F2*), andnematode *unc-86*) N-terminal to a typical homeodomain. The POU-specific domain is a DNA-binding domain of approximately 75 amino acids; the POU-specific domain and the homeodomain are collectively known as the bipartite POU domain [61].

We have identified a total of 16 POU-class homeobox genes in the human genome (Tables 1 and 4). The genes form a distinct grouping even if the POU-specific domain is disregarded – phylogenetic analyses of homeodomains recover the POU class as a monophyletic group (Figure 3; Additional files 1, 2 and 5). There are six widely recognized gene families within the POU class (Pou1 to Pou6), and nomenclature revisions approximately 10 years ago clarified which genes belong to which gene family [62]. We have placed two additional genes (*HDX* and *POU5F2*) in the POU class on the basis of their deduced homeodomain sequences, even though one of these genes (*HDX*) does not encode a POU-specific domain. We have erected a new gene family for this gene, bringing the total number of gene families in the POU class to seven. We have also identified a total of eight POU-class pseudogenes in the human genome (Tables 1 and 4); we have named six of these (*POU5F1P2*, *POU5F1P4* to *POU5F1P8*), and revised the nomenclature of one other (*POU5F1P3*).

◦ *HDX* [Entrez Gene ID: 139324]. This gene was previously known as *CXorf43*. The gene encodes a highly divergent atypical (68-amino-acid) homeodomain but not a

Table 4: Human LIM, POU, HNF, SINE, TALE, CUT, PROS, ZF AND CERS class homeobox genes and pseudogenes

Human LIM-class homeobox genes					
Family	**Gene symbol**	**Gene name**	**Location**	**Entrez gene ID**	**Previous symbols**
Isl	*ISL1*	ISL LIM homeobox 1	5q11.2	3670	
	ISL2	ISL LIM homeobox 2	15q24.3	64843	
Lhx1/5	*LHX1*	LIM homeobox 1	17q12	3975	LIM1
	LHX5	LIM homeobox 5	12q24.13	64211	
Lhx2/9	*LHX2*	LIM homeobox 2	9q33.3	9355	LH2
	LHX9	LIM homeobox 9	1q31.3	56956	
Lhx3/4	*LHX3*	LIM homeobox 3	9q34.3	8022	M2-LHX3
	LHX4	LIM homeobox 4	1q25.3	89884	GSH4
Lhx6/8	*LHX6*	LIM homeobox 6	9q33.2	26468	LHX6.1
	LHX8	LIM homeobox 8	1p31.1	431707	LHX7
Lmx	*LMX1A*	LMX LIM homeobox 1A	1q24.1	4009	LMX1, LMX1.1
	LMX1B	LMX LIM homeobox 1B	9q33.3	4010	LMX2, LMX1.2
Human POU-class homeobox genes and pseudogenes					
Hdx	*HDX*	highly divergent homeobox	Xq21.1	139324	CXorf43
Pou1	*POU1F1*	POU class 1 homeobox 1	3p11.2	5449	PIT1, GHF1
Pou2	*POU2F1*	POU class 2 homeobox 1	1q24.2	5451	OCT1, OTF1
	POU2F2	POU class 2 homeobox 2	19q13.2	5452	OCT2, OTF2
	POU2F3	POU class 2 homeobox 3	11q23.3	25833	OCT11, PLA1, EPOC1, SKN1A
Pou3	*POU3F1*	POU class 3 homeobox 1	1p34.3	5453	OCT6, OTF6, SCIP
	POU3F2	POU class 3 homeobox 2	6q16.2	5454	OCT7, OTF7, BRN2, POUF3
	POU3F3	POU class 3 homeobox 3	2q12.1	5455	OTF8, BRN1
	POU3F4	POU class 3 homeobox 4	Xq21.1	5456	OTF9, BRN4
Pou4	*POU4F1*	POU class 4 homeobox 1	13q31.1	5457	BRN3A, RDC1, Oct-T1
	POU4F2	POU class 4 homeobox 2	4q31.22	5458	BRN3B, BRN3.2
	POU4F3	POU class 4 homeobox 3	5q32	5459	BRN3C
Pou5	*POU5F1*	POU class 5 homeobox 1	6p21.33	5460	OCT3, OTF3, OCT4, OTF4
	POU5F1P1	POU class 5 homeobox 1 pseudogene 1	8q24.21	5462	OTF3C, OTF3P1, POU5FLC8
	POU5F1P2	POU class 5 homeobox 1 pseudogene 2	8q22.3	100009665	
	POU5F1P3	POU class 5 homeobox 1 pseudogene 3	12p13.31	642559	OTF3L, POU5F1L, POU5FLC12
	POU5F1P4	POU class 5 homeobox 1 pseudogene 4	1q22	645682	POU5FLC1
	POU5F1P5	POU class 5 homeobox 1 pseudogene 5	10q21.3	100009667	
	POU5F1P6	POU class 5 homeobox 1 pseudogene 6	3q21.3	100009668	
	POU5F1P7	POU class 5 homeobox 1 pseudogene 7	3q12.1	100009669	
	POU5F1P8	POU class 5 homeobox 1 pseudogene 8	17q25.3	100009670	
	POU5F2	POU class 5 homeobox 2	5q15	134187	SPRM1
Pou6	*POU6F1*	POU class 6 homeobox 1	12q13.13	5463	BRN5, MPOU, TCFB1
	POU6F2	POU class 6 homeobox 2	7p14.1	11281	WT5, WTSL, RPF1
Human HNF-class homeobox genes					
Hmbox	*HMBOX1*	homeobox containing 1	8p12	79618	HNF1LA, PBHNF
Hnf1	*HNF1A*	HNF1 homeobox A	12q24.31	6927	TCF1, HNF1, LFB1
	HNF1B	HNF1 homeobox B	17q12	6928	TCF2, LFB3, VHNF1
Human SINE-class homeobox genes					
Six1/2	*SIX1*	SIX homeobox 1	14q23.1	6495	
	SIX2	SIX homeobox 2	2p21	10736	
Six3/6	*SIX3*	SIX homeobox 3	2p21	6496	
	SIX6	SIX homeobox 6	14q23.1	4990	OPTX2, Six9
Six4/5	*SIX4*	SIX homeobox 4	14q23.1	51804	AREC3
	SIX5	SIX homeobox 5	19q13.32	147912	DMAHP
Human TALE-class homeobox genes and pseudogenes					

Table 4: Human LIM, POU, HNF, SINE, TALE, CUT, PROS, ZF AND CERS class homeobox genes and pseudogenes *(Continued)*

Irx	*IRX1*	iroquois homeobox 1	5p15.33		IRX-5
	IRX1P1	iroquois homeobox 1 pseudogene 1	13q12.12	79192	IRXA1
	IRX2	iroquois homeobox 2	5p15.33	646390	
	IRX3	iroquois homeobox 3	16q12.2	153572	IRX-1
	IRX4	iroquois homeobox 4	5p15.33	50805	
	IRX4P1	iroquois homeobox 4 pseudogene 1	18p11.22	100009671	
	IRX5	iroquois homeobox 5	16q12.2	79190	IRX2A
	IRX6	iroquois homeobox 6	16q12.2		IRX-3, IRX7
Meis	*MEIS1*	Meis homeobox 1	2p14	4211	
	MEIS2	Meis homeobox 2	15q14	4212	MRG1
	MEIS3	Meis homeobox 3	19q13.32	56917	MRG2
	MEIS3P1	Meis homeobox 3 pseudogene 1	17p12	4213	MRG2, MEIS3, MEIS4
	MEIS3P2	Meis homeobox 3 pseudogene 2	17p11.2	257468	
Mkx	*MKX*	mohawk homeobox	10p12.1	283078	IRXL1, IFRX, C10orf48
Pbx	*PBX1*	pre-B-cell leukemia homeobox 1	1q23.3	5087	
	PBX2	pre-B-cell leukemia homeobox 2	6p21.32	5089	G17, HOX12, PBX2MHC
	PBX2P1	pre-B-cell leukemia homeobox 2 pseudogene 1	3q24	5088	PBXP1, PBX2
	PBX3	pre-B-cell leukemia homeobox 3	9q33.3	5090	
	PBX4	pre-B-cell leukemia homeobox 4	19p13.11	80714	
Pknox	*PKNOX1*	PBX/knotted homeobox 1	21q22.3	5316	PREP1, PKNOX1C
	PKNOX2	PBX/knotted homeobox 2	11q24.2	63876	PREP2
Tgif	*TGIF1*	TGFB-induced factor homeobox 1	18p11.31	7050	TGIF, HPE4
	TGIF1P1	TGFB-induced factor homeobox1 pseudogene 1	19q13.32	126052	
	TGIF2	TGFB-induced factor homeobox 2	20q11.23	60436	
	TGIF2P1	TGFB-induced factor homeobox 2 pseudogene 1	1q44	126826	
	TGIF2P2	TGFB-induced factor homeobox 2 pseudogene 2	15q21.1	100009674	
	TGIF2P3	TGFB-induced factor homeobox 2 pseudogene 3	15q21.1	100009672	
	TGIF2P4	TGFB-induced factor homeobox 2 pseudogene 4	14q24.2	100009673	
	TGIF2LX	TGFB-induced factor homeobox 2-like, X-linked	Xq21.31	90316	TGIFLX (retrogene)
	TGIF2LY	TGFB-induced factor homeobox 2-like, Y-linked	Yp11.2	90655	TGIFLY (retrogene)
Human CUT-class homeobox genes and pseudogenes					
Onecut	*ONECUT1*	one cut homeobox 1	15q21.3	3175	HNF6, HNF6A
	ONECUT2	one cut homeobox 2	18q21.31	9480	OC2
	ONECUT3	one cut homeobox 3	19p13.3	390874	
Cux	*CUX1*	cut-like homeobox 1	7q22.1	1523	CUTL1, CUX, CDP, COY1
	CUX2	cut-like homeobox 2	12q24.12	23316	CUTL2
	CUX2P1	cut-like homeobox 2 pseudogene 1	10p14	-	
	CUX2P2	cut-like homeobox 2 pseudogene 2	4q32.1	-	
Satb	*SATB1*	SATB homeobox 1	3p24.3	6304	
	SATB2	SATB homeobox 2	2q33.1	23314	
Human PROS-class homeobox genes					
Prox	*PROX1*	prospero homeobox 1	1q41	5629	
	PROX2	prospero homeobox 2	14q24.3	283571	
Human ZF-class homeobox genes and pseudogenes					
Adnp	*ADNP*	activity-dependent neuroprotector homeobox	20q13.13	23394	ADNP1
	ADNP2	ADNP homeobox 2	18q23	22850	ZNF508
Tshz	*TSHZ1*	teashirt zinc finger homeobox 1	18q22.3	10194	TSH1
	TSHZ2	teashirt zinc finger homeobox 2	20q13.2	128553	TSH2, ZNF218, ZABC2, OVC10-2

Table 4: Human LIM, POU, HNF, SINE, TALE, CUT, PROS, ZF AND CERS class homeobox genes and pseudogenes *(Continued)*

	TSHZ3	teashirt zinc finger homeobox 3	19q12	57616	TSH3, ZNF537
Zeb	*ZEB1*	zinc finger E-box binding homeobox 1	10p11.22	6935	ZFHX1A, deltaEF1, TCF8, ZEB
	ZEB2	zinc finger E-box binding homeobox 2	2q22.3	9839	ZFHX1B, SIP1, SMADIP1
	ZEB2P1	zinc finger E-box binding homeobox 2 pseudogene 1	4p15.32	100033412	
Zfhx	*ZFHX2*	zinc finger homeobox 2	14q11.2	85446	
	ZFHX3	zinc finger homeobox 3	16q22.3	463	ATBT, ATBF1
	ZFHX4	zinc finger homeobox 4	8q21.11	79776	ZFH4
Zhx/ Homez	*ZHX1*	zinc fingers and homeoboxes 1	8q24.13	11244	
	ZHX2	zinc fingers and homeoboxes 2	8q24.13	22882	
	ZHX3	zinc fingers and homeoboxes 3	20q12	23051	TIX1
	HOMEZ	homeobox and leucine zipper encoding	14q11.2	57594	
Human CERS-class homeobox genes					
Cers	*CERS2*	ceramide synthase 2	1p36.13-q24.1	29956	LASS2, TRH3, TMSG1
	CERS3	ceramide synthase 3	15q26.3	204219	LASS3
	CERS4	ceramide synthase 4	19p13.3	79603	LASS4, TRH1
	CERS5	ceramide synthase 5	12q13.12	91012	LASS5, TRH4
	CERS6	ceramide synthase 6	2q31	253782	LASS6

Human homeobox genes and pseudogenes, excepting the ANTP and PRD classes, including full names, chromosomal locations, Entrez Gene IDs and previous symbols. The *HOMEZ* gene is in the ZF class but encodes a protein with leucine zippers instead of zinc fingers.

POU-specific domain, and thus it is debatable whether it should be placed within the POU class. Phylogenetic analyses of homeodomains place it basally in a clade with the POU class (Figure 3; Additional files 1 and 5), or within the POU class (Additional file 2), suggesting that the HDX protein either diverged before the POU-specific domain became associated with the homeodomain or lost the POU-specific domain during evolution. Further information on this gene may allow this tentative classification to be revisited.

◦ *POU5F2* [Entrez Gene ID: 134187]. We designate this previously unnamed gene *POU5F2* on the basis of clear orthology to the mouse *Sprm1* gene, which has been assigned the second member of the Pou5 gene family [63]. The symbol *POU5F2* ensures the gene conforms with standardized nomenclature for the POU class.

◦ *POU5F1P2* [GeneID: 100009665], *POU5F1P3* (formerly *POUF51L*) [GeneID: 5461], *POU5F1P4* [GeneID: 100009666], *POU5F1P5* [GeneID: 100009667], *POU5F1P6* [GeneID: 100009668], *POU5F1P7* [GeneID: 100009669] and *POU5F1P8* [GeneID: 100009670]. Prior to this study, a single retrotransposed pseudogene of the *POU5F1* gene had been annotated and designated *POU5F1P1* [Entrez Gene ID: 5462]. Another *POU5F1*-related sequence of unknown status had been annotated and designated *POUF5F1L* [GeneID: 5461]. We replace the gene symbol *POUF5F1L* with *POU5F1P3* as this sequence is a retrotransposed pseudogene of *POUF51*. Our analyses of the human genome sequence identified a further six pseudogenes of *POU5F1*, which we name sequentially *POU5F1P2*, *POU5F1P4* through to *POU5F1P8*. Each clearly aligns to the mRNA sequence of *POU5F1* but with sequence alterations, indicating origin by retrotransposition. *POU5F1P2* and *POU5F1P6* have frameshift mutations in the homeobox. *POU5F1P5* and *POU5F1P6* have stop codons in the homeobox. *POU5F1P7* and *POU5F1P8* are partial integrants of *POU5F1* mRNA excluding the homeobox – *POU5F1P7* covers part of the 3' untranslated region and *POU5F1P8* a short region around the start codon.

The HNF homeobox class

The HNF class (named after the rat gene *Hnf1*) encodes proteins with a POU-like domain N-terminal to a highly atypical homeodomain. The POU-like domain, as its name indicates, is weakly similar in sequence to the POU-specific domain [64]; more importantly, it has nearly the same three-dimensional structure and mode of DNA binding as the POU-specific domain [65].

We have identified a total of three HNF-class homeobox genes in the human genome (Tables 1 and 4), consistent with previous work [66,67]. The homeodomains encoded by the human *HNF1A* and *HNF1B* genes are atypical in possessing 21 extra amino acid residues between the second and third alpha helices (Additional file 6). We place these two genes in a single gene family (Hnf1) within the HNF class, implying derivation from a single invertebrate gene. Examination of their chromosomal locations concurs with this view. *HNF1A* and *HNF1B* map to parts of the genome known to have duplicated in early vertebrate evolution, namely 12q24.31 (*HNF1A*, near *LHX5* and on the same arm as the HOXC cluster) and 17q12 (*HNF1B*, between *LHX1* and the HOXB cluster) (Figure 4). The use of the A and B suffixes is unfortunate, as numerals are generally used to distinguish paralogs of this age, but is retained at present due to widespread and stable use. The homeodomain encoded by the human *HMBOX1* gene is atypical in possessing 15 extra amino acid residues between the second and third alpha helices (Additional file 6). Phylogenetic analyses confirm previous suggestions [67] that *HMBOX1* is more distantly related to *HNF1A* and *HNF1B* (Figure 3; Additional files 1, 2 and 5). We place this gene in a separate gene family (Hmbox) within the same class. We have not found any human HNF-class pseudogenes.

The SINE homeobox class

The SINE class (named after the *Drosophila* gene *so*: *sine oculis*) encodes proteins with a SIX domain N-terminal to a typical homeodomain. The SIX domain is a DNA-binding domain of approximately 115 amino acids; both the SIX domain and the homeodomain are required for DNA binding [68].

We have identified a total of six SINE-class homeobox genes in the human genome (Tables 1 and 4), consistent with previous work [68,69]. The genes form a distinct grouping even if the SIX domain is disregarded – phylogenetic analyses of homeodomains recover the SIX class as a monophyletic group (Figure 3; Additional files 1, 2 and 5). Phylogenetic analyses of homeodomains divide the SIX class into three gene families (Figure 3; Additional files 1, 2 and 5), consistent with previous studies [68,69]. Each gene family has two human members and dates to a single ancestral gene in the most recent common ancestor of bilaterians [68,69]. We have not found any human SINE-class pseudogenes.

The TALE homeobox class

TALE (three amino acid loop extension) class genes are distinguished by the presence of three extra amino acids between the first and second alpha helices of the encoded homeodomain [1,2,70]. Genes belonging to the TALE class encode proteins with various domains outside of the atypical homeodomain.

We have identified a total of 20 TALE-class homeobox genes in the human genome (Tables 1 and 4). The genes form a distinct grouping in phylogenetic analyses even when the three extra homeodomain residues are excluded from the sequence alignment (Figure 3; Additional file 5). Bürglin [2] has given the TALE group the rank of 'superclass' and distinguished between several 'classes' by the presence of distinct domains outside of the homeodomain. These are the IRX domain, MKX domains, the MEIS domain, the PBC domain and TGIF domains [2,71-73]. Along with some others [4,7,24], we have given the TALE group the rank of 'class' containing several 'gene families'; this maintains consistent terminology throughout the present paper. Phylogenetic analyses of homeodomains divide the TALE class into six gene families (Figure 3; Additional files 1, 2 and 5), including an Mkx family containing the recently described *MKX* gene, which is distinguished from Irx-family genes phylogenetically and by absence of an IRX domain [73,74]. It should be noted that the established name of the Pknox gene family does not indicate orthology with Knox-family genes of plants. We have also identified a total of 10 TALE-class pseudogenes in the human genome (Tables 1 and 4); we have named six of these (*IRX4P1*, *TGIF1P1* and *TGIF2P1* to *TGIF2P4*), and revised the nomenclature of two others (*IRX1P1* and *PBX2P1*).

◦ *IRX1P1* [Entrez Gene ID: 646390]. This sequence was previously known as *IRXA1*; we rename it *IRX1P1* because it is clearly a retrotransposed pseudogene of *IRX1* and not a functional gene. The *IRX1P1* sequence aligns to the mRNA of *IRX1* but has a frameshift mutation and two stop codons in the homeobox.

◦ *IRX4P1* [Entrez Gene ID: 100009671]. We designate this previously unannotated sequence *IRX4P1* because it is clearly a retrotransposed pseudogene of *IRX4*. The *IRX4P1* sequence is a partial integrant derived from a region of the *IRX4* mRNA around the stop codon; it lacks the homeobox.

◦ *PBX2P1* [Entrez Gene ID: 5088]. This sequence was previously known as *PBXP1*; we rename it *PBX2P1* because it is clearly a retrotransposed pseudogene of *PBX2*. The former name of *PBXP1* did not indicate its transcript of origin. The *PBX2P1* sequence aligns to the mRNA of *PBX2* but has a frameshift mutation in the coding region.

◦ *TGIF1P1* [Entrez Gene ID: 126052]. We designate this previously unannotated sequence *TGIF1P1* because it is clearly a retrotransposed pseudogene of *TGIF1*. The locus has many sequence alterations when compared to *TGIF1* mRNA, including a 48 nucleotide insertion within the homeobox.

◦ *TGIF2P1* [GeneID: 126826], *TGIF2P2* [GeneID: 100009674], *TGIF2P3* [GeneID: 100009672] and *TGIF2P4* [GeneID: 100009673]. These four sequences were unannotated prior to this study. We designate them *TGIF2P1* to *TGIF2P4* because they are clearly pseudogenes of *TGIF2*. Each aligns to the mRNA sequence of *TGIF2* but with sequence alterations, indicating origin by retrotransposition. *TGIF2P1* has many sequence alterations, including a frameshift mutation in the homeobox. *TGIF2P2* and *TGIF2P3* are very similar neighboring loci that must have originated by tandem duplication of a retrotransposed *TGIF2* mRNA; neither includes the homeobox. *TGIF2P4* is a short partial integrant derived from part of the 3' untranslated region of *TGIF2* mRNA.

The CUT homeobox class

The CUT class (named after the *Drosophila* gene *cut*) generally encodes proteins with one or more CUT domains N-terminal to a typical homeodomain. The CUT domain is a DNA-binding domain of approximately 75 amino acids [75]. There are three widely recognized gene families within the CUT class in humans (Onecut, Cux, Satb; [76]). A fourth gene family (Cmp), lacking a CUT domain but sharing a CMP domain with the Satb gene family, is absent from vertebrates. Bürglin and Cassata [76] have proposed that the vertebrate Satb gene family evolved from the invertebrate Cmp gene family.

We have identified a total of seven CUT-class homeobox genes in the human genome (Tables 1 and 4). Although grouped together by presence of CUT domains, the homeodomains of the Onecut, Cux and Satb gene families are quite divergent and do not always form a monophyletic group in phylogenetic analyses (Additional files 2 and 5). Topologies that separate the gene families are also only weakly supported, so it is most parsimonious to assume that the class is actually monophyletic but the constituent genes underwent rapid sequence divergence following their initial duplications. We have revised the nomenclature of two CUT-class genes (*CUX1* and *CUX2*). We have also identified a total of three CUT-class pseudogenes in the human genome (Tables 1 and 4); we have named all of these (*CUX2P1*, *CUX2P2* and *SATB1P1*).

◦ *CUX1* [Entrez Gene ID: 1523] and *CUX2* [Entrez Gene ID: 23316]. These genes were previously known as *CUTL1* and *CUTL2* respectively. We rename them *CUX1* and *CUX2* in accordance with homeobox gene nomenclature convention.

◦ *CUX2P1* and *CUX2P2*. These sequences were unannotated prior to this study. We designate them *CUX2P1* and *CUX2P2* because they are clearly retrotransposed pseudogenes of *CUX2*. Both are short partial integrants derived from *CUX2* mRNA, excluding the homeobox – *CUX2P1* covers part of the coding region at the 5' end and *CUX2P2* part of the 3' untranslated region.

◦ *SATB1P1* [Entrez Gene ID: 100033410]. We designate this previously unannotated sequence *SATB1P1* because it is clearly a retrotransposed pseudogene of *SATB1*. *SATB1P1* is a short partial integrant derived from part of the 3' untranslated region of *SATB1* mRNA; it does not encompass the homeobox.

The PROS homeobox class

The PROS class (named after the *Drosophila* gene *pros*) encodes proteins with a PROS domain C-terminal to an atypical homeodomain. The PROS domain is a DNA-binding domain of approximately 100 amino acids [77]. PROS-class genes encode a highly divergent homeodomain with three extra amino acids. These additional residues are inserted at a different position compared to the TALE class, being between the second and third alpha helices (Additional file 6).

We have identified a total of two PROS-class homeobox genes in the human genome (Tables 1 and 4), which we have placed in a single gene family (Prox). The highly divergent homeodomain sequence and unusual structural features provide justification for PROS being a separate gene class, despite the small number of genes. In phylogenetic analyses, PROS-class homeodomains are situated on a long branch, very distant from other classes (Figure 3; Additional files 1, 2 and 5). The human *PROX1* gene is well characterized; we have identified and named its paralog, *PROX2*. We have not found any human PROS-class pseudogenes.

◦ *PROX2* [Entrez Gene ID: 283571]. We designate this previously unannotated gene *PROX2* on the basis of clear orthology to the mouse *Prox2* gene, inferred from sequence identity and synteny. The homeobox of human *PROX2* has two introns and unusually the splice sites of the first (5') intron (AT-AA) do not follow the GT-AG donor-acceptor rule. This has also been noted for mouse *Prox2* [78].

The ZF homeobox class

The ZF (zinc finger) class generally encodes proteins with zinc finger motifs, in addition to one or more homeodomains. As noted earlier, phylogenetic analyses of homeodomains does not recover the ZF class as a monophyletic group (Figure 3; Additional files 1, 2 and 5). We recognize that this suggests that zinc finger motifs and homeodomains may have been brought together on three separate occasions in evolution; nonetheless, it is convenient and informative to group these into a single class. Inclusion of the *HOMEZ* gene in the ZF class may be surprising, as this gene does not encode zinc fingers. However, as previously noted [79] and reproduced in our phylogenetic analyses (Figure 3; Additional files 1, 2 and 5), the multiple homeodomain sequences of this gene are clearly related to those encoded by the *ZHX1*, *ZHX2* and *ZHX3* genes.

We have identified a total of 14 ZF-class homeobox genes in the human genome (Tables 1 and 4), which we have placed in five gene families (Adnp, Tshz, Zeb, Zfhx and Zhx/Homez). We have also identified one ZF-class pseudogenes in the human genome (Tables 1 and 4). We have revised the nomenclature of five of these loci (*ADNP2*, *ZEB1*, *ZEB2*, *ZEB2P1* and *ZFHX3*).

◦ *ADNP2* [Entrez Gene ID 22850]. This gene was previously known as *ZNF508*; we rename it *ADNP2* to reflect its paralogous relationship to *ADNP*.

◦ *ZEB1* [Entrez Gene ID: 6935] and *ZEB2* [Entrez Gene ID: 9839]. These genes were previously known as *ZFHX1A* and *ZFHX1B* respectively. We rename them *ZEB1* and *ZEB2* to distinguish them from genes belonging to the distantly related Zfhx gene family.

◦ *ZEB2P1* [Entrez Gene ID: 100033412]. This retrotransposed pseudogene of *ZEB2* has been described previously [80]. Our new nomenclature (*ZEB2P1*) reflects the origin of this locus.

◦ *ZFHX3* [Entrez Gene ID: 463]. This gene was previously known as *ATBF1*; we rename it *ZFHX3* to reflect its close relationship to *ZFHX2* and *ZFHX4*; indeed *ZFHX3* was a synonym for this gene.

The CERS homeobox class

The highly unusual CERS (ceramide synthase) class, also known as the LASS (longevity assurance) class, comprises a single gene family that is highly conserved amongst eukaryotes and includes the yeast gene and original member *LAG1*. There are six CERS-class genes in the human genome (*CERS1* to *CERS6*) and five of these (*CERS2* to *CERS6*) encode proteins with a homeodomain sequence [81,82]. These are, however, extremely divergent from the homeodomains of other gene classes. Secondary structure prediction analyses suggest these sequences have the potential to encode three alpha helices in the appropriate positions (data not shown). The most surprising characteristic of these genes is that biochemical studies predict them to encode transmembrane proteins, with the homeodomain on the cytosolic side of the endoplasmic reticulum membrane, and hence they could not act as DNA-binding proteins or transcription factors [81,82]. It is possible that an ancestor of these genes gained a homeobox through exon shuffling, or alternatively this could represent convergent evolution. We include only *CERS2* to *CERS6* in our comprehensive compilation of human homeobox genes, as *CERS1* lacks a homeobox motif.

Chromosomal distribution of human homeobox genes

The chromosomal locations of genes can give clues to evolutionary ancestry, including patterns of gene duplication, and the possible existence of gene clusters. In Figure 4, we show the chromosomal locations of all human homeobox genes. We do not include probable pseudogenes on these ideograms, because most of these have originated by reverse transcription of mRNA and secondary integration into the genome, and hence give no insight into ancestral locations of genes. The highly repetitive *DUX1* to *DUX5* sequences are also not shown, as these have undergone secondary amplification and are also most likely non-functional (see above).

The first observation is that there are homeobox genes on every human chromosome. Even the two sex chromosomes harbor homeobox genes, with *SHOX* (*short stature homeobox*) in the PAR1 pseudoautosomal region at the tip of the short arms of X and Y being the best known. Haploinsufficiency of *SHOX* is implicated in the short stature phenotype of Turner syndrome patients who lack one copy of the X chromosome [83]. There are also nine other homeobox genes in non-pseudoautosomal regions of the X chromosome, including three tandemly-arranged members of the Rhox gene family, collectively homologous to the multiple Rhox (reproductive homeobox) genes of mouse. Only one of the homeobox genes on the X chromosome, the TALE-class gene *TGIF2LX*, has a distinct homolog on the Y chromosome, called *TGIF2LY*. These genes map to the largest homology block shared by the unique regions of the X and Y chromosomes, spanning 3.5 Mb. It has been proposed that the ancestor of these two genes arose by retrotransposition of *TGIF2* mRNA [84].

The autosomes with the lowest number of homeobox genes are chromosomes 21 (with just *PKNOX1*) and 22 (with *GSC2* and *ISX*). Examination of the remaining autosomes reveals that homeobox genes are quite dispersed with some interesting regional accumulations. The best known examples of close linkage between homeobox genes are the four Hox clusters on human chromosomes 2, 7, 12 and 17, comprising 9, 11, 9 and 10 genes respectively; each of these is shown as just a single line on each ideogram for simplicity (Figure 4). These should not be considered in isolation, however, because many other ANTP-class genes map in the vicinity of the Hox clusters [26,27]. These include genes very tightly linked to the Hox clusters, notably the Evx-family genes (on chromosomes 2 and 7), Dlx-family genes (on chromosomes 2 and 17), and Meox-family genes (on chromosomes 2 and 17).

There are other concentrations of ANTP-class genes away from the Hox clusters. These are the ParaHox cluster (*GSX1*, *PDX1*, *CDX2*) on chromosome 13, and four sets of NKL-subclass genes on 2p/8p (split), 4p, 5q and 10q, hypothesized to be derived from an ancestral array by duplication [26,33]. The accumulation on the distal half of the long arm of chromosome 10 is particularly striking, comprising eleven ANTP-class genes from 10 gene families. This is not a tight gene cluster, but it is compatible with ancestry by extensive tandem gene duplication followed by dispersal. Discounting the rather aberrant case of the Hox clusters, this region of the long arm of chromosome 10 is the most homeobox-rich region of the human genome.

There are additional groupings of homeobox genes outside the ANTP class. These include two TALE-class Irx clusters on chromosomes 5 and 16 homologous to the described mouse Irx clusters [19], and a set of PRD-class genes on chromosome 19 proposed to be derived from the *CRX* homeobox gene by duplication and rapid divergence [21]. Perhaps the most interesting case, however, is found on the tip of the long arm of chromosome 9, where there is a concentration of homeobox genes from disparate gene classes. Four LIM-class genes, one ANTP-class gene, one PRD-class gene and one TALE-class gene are found in this location. Although dispersed over a large region, and not forming a tight gene cluster, the linkages are nonetheless intriguing. It is possible that these linkages reflect ancestry from the very ancient gene duplications that must have generated the distinctive homeobox gene classes found within animal genomes.

Conclusion

We identified 300 homeobox loci in the euchromatic regions of the human genome, and divide these into 235 probable functional genes and 65 probable pseudogenes. Not all of these loci possess a homeobox because for completeness we include all sequences derived from homeobox-containing genes. The number of homeobox sequences is also different from the number of loci because several genes contain multiple homeobox motifs. The figures exclude the repetitive *DUX1* to *DUX5* homeobox sequences of which we identified 35 probable pseudogenes, with many more expected in heterochromatic regions.

New or revised nomenclature is proposed for approximately 70 of the 300 homeobox loci in order to clarify orthologous relationships between human and mouse, to indicate evolutionary relationships within a gene family, to distinguish genes from pseudogenes, and to indicate pseudogene origins. The loci are also classified into a simple hierarchical scheme, comprising 102 gene families within eleven gene classes. The classification scheme proposed may be widely applicable to homeobox genes from other animals.

The 235 probable functional homeobox genes map to every human chromosome with some interesting regional concentrations of genes. These include a large number of ANTP-class genes on the distal end of the long arm of chromosome 10, and a combination of LIM-, ANTP-, PRD- and TALE-class genes on the distal end of the long arm of chromosome 9. These associations may be remnants of common ancestry early in animal evolution.

Methods

The finished human genome sequence (build 35.1) was subjected to a series of tBLASTn searches [85,86] using known homeodomain sequences from the ANTP, PRD, LIM, POU, HNF, SINE, TALE, CUT, PROS and ZF classes. No arbitrary E-value cut-off was selected, but instead each list of hits was analyzed manually until true homeodomain sequences ceased to be detected. Definition of a homeodomain used a combination of CD-search for conserved protein domains implemented through BLASTp [85,86] and secondary structure prediction by JPred implemented through the Barton Group, University of Dundee [87]. Each time a new or divergent homeodomain match was found, the tBLASTn process was repeated. Six very divergent gene families were undetected by this method but found by text searching: Hopx, Adnp, Tshz, Zeb, Zhx/Homez and Cers. To ensure that every pseudogene was detected, including truncated or decayed versions lacking the homeobox, the full mRNA sequence of each gene was deduced and used in a BLASTn search of the human genome sequence [85,86]. Pseudogenes were recognized as those genomic regions with similarity to non-repetitive DNA sequences of the parent gene, even if aligning to only part of the locus. Pseudogenes undergo mutational decay and would eventually become unrecognizable, but in practice ambiguous cases were not encountered. Exon-intron structures of novel loci were deduced by comparison between genomic sequence and cDNA, EST or retrotransposed pseudogene sequences, as previously described [21]. Several unnamed human loci were identified as probable orthologs of known mouse genes; orthology was deduced by a combination of homeodomain sequence similarity and synteny, examined through the mouse genome sequence (build 34.1) and the Ensembl Genome Browser [88].

Phylogenetic analyses were performed with homeodomain sequences, after each had been edited to an alignment of 60 amino acids (Additional file 7), using the maximum likelihood [89] and neighbor-joining [90] methods. Maximum likelihood trees were constructed using PhyML [91], with a JTT model of amino acid substitution, four categories of between-site rate heterogeneity, a gamma distribution parameter estimated from the data and 500 bootstrap resamplings. Neighbor-joining trees were constructed using PHYLIP (.)[92], with a JTT model of amino acid substitution and 1000 bootstrap resamplings. For defining human gene families, all *Drosophila* homeodomains were first combined with all human homeodomains in maximum likelihood and neighbor-joining analyses to enable divergent *Drosophila* genes to be identified and removed. These include genes lost from human, as well genes known to have undergone unusually rapid evolution in *Drosophila*. For the Hox3 family the rapidly evolving *Drosophila* genes *bcd*, *zen* and *zen2* were then replaced by an ortholog from centipede (*Sm Hox3b*), and for the Nk4 family the rapid evolving *Drosophila* gene *tin* was replaced by an ortholog from annelid (*Pd NK4*). In addition, six genes from other protostome or cnidarian genomes were added to represent gene families known to be missing from *Drosophila* (Pdx family: *Ps Xlox*; Alx family: *Nv CART1*; Dmbx family: *Hv manacle*; Pou1 family: *Nv POU1*; Hnf1 family: *Nv HNF*; Pknox family: *Am Prep*). Only 100 bootstrap resamplings were performed on this dataset because of its large size (354 homeodomains). Trees were displayed using TreeExplorer [93]. Genes encoding partial homeodomains, and probable pseudogenes, were not included in the phylogenetic analyses. With short alignments, phylogenetic trees can only be used as guides to relationships, not absolute indicators of evolutionary history, and the trees presented in this paper should be interpreted in this light.

Authors' contributions

PWHH designed the study and contributed to gene identification and to gene nomenclature revisions. HAFB carried out database searches, annotations and phylogenetic analyses and contributed to gene nomenclature revisions. PWHH and HAFB drafted the manuscript. EB contributed to gene nomenclature revisions, discussed these with the research community and databases, and implemented the agreed changes.

All authors edited and approved the final manuscript.

Additional material

Additional file 1

Maximum likelihood phylogenetic tree of all human plus selected protostome and cnidarian homeodomains for identification of gene families*. Arbitrarily rooted phylogenetic tree of all human plus selected protostome and cnidarian homeodomains constructed using the maximum likelihood (ML) method. Bootstrap values supporting gene family designations are shown. Homeodomain sequences derived from pseudogenes are excluded. This ML tree should be compared with the neighbor-joining (NJ) tree shown in Additional file 2.*

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[http://www.biomedcentral.com/content/supplementary/1741-7007-5-47-S1.pdf]

Additional file 2

Neighbor-joining phylogenetic tree of all human plus selected protostome and cnidarian homeodomains for identification of gene families*. Arbitrarily rooted phylogenetic tree of all human plus selected protostome and cnidarian homeodomains constructed using the neighbor-joining (NJ) method. Bootstrap values supporting gene family designations are shown. Homeodomain sequences derived from pseudogenes are excluded. Comparison of NJ and ML trees, and description of the dataset used, is given in the legend to Additional file 1. Several artefactual mixing of classes occurs in this NJ tree, notably splitting of the CUT class, mixing of the TALE and SINE classes and aberrant placement of* HOPX.

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Additional file 3

Neighbor-joining phylogenetic tree of human ANTP-class homeodomains, for comparison to maximum likelihood tree*. Arbitrarily rooted phylogenetic tree of human ANTP-class homeodomains constructed using the neighbor-joining method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded. The proposed division between the HOXL and NKL subclasses is indicated. The position of* EN1 *and* EN2 *is unstable; this tree places them close to the base of the HOXL/NKL divergence, whereas maximum likelihood analysis of the same dataset places them firmly in the NKL subclass (Figure 1). Interrelationships of genes in the Nk2.2 and Nk4 families are also unstable (in this tree and Figure 1 respectively); in these cases synteny within and between genomes clearly resolves gene families. Detailed relationships between different gene families should not be inferred from this tree.*

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Additional file 4

Neighbor-joining phylogenetic tree of human PRD-class homeodomains, for comparison to maximum likelihood tree*. Arbitrarily rooted phylogenetic tree of human PRD-class homeodomains constructed using the neighbor-joining method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded, as are the partial homeodomains of PAX2, PAX5 and PAX8, and the HOPX homeodomain because its extremely divergent sequence destabilizes the overall tree topology. Roman numeral suffixes are used to distinguish multiple homeodomains encoded by a single Dux-family gene. Detailed relationships between different gene families should not be inferred from this tree.*

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Additional file 5

***Neighbor-joining phylogenetic tree of human homeodomains excluding ANTP and PRD classes, for comparison to maximum likelihood tree**. Arbitrarily rooted phylogenetic tree of human homeodomains excluding the ANTP and PRD classes constructed using the neighbor-joining method. Bootstrap values supporting internal nodes with over 70% are shown. Homeodomain sequences derived from pseudogenes are excluded. Roman numeral suffixes are used to distinguish multiple homeodomains encoded by a single gene. Classes and/or families are color coded as shown in the key. The LIM and ZF classes are not recovered as two distinct monophyletic groups, a result also found by maximum likelihood analysis (Figure 3). The multiple homeodomains of Zfhx-family proteins and Zhx/Homez-family proteins are also dispersed in the tree, presumably artefactually. Monophyly of the CUT class is not recovered in this tree, but is by maximum likelihood analysis (Figure 3). Detailed relationships between different gene families should not be inferred from this tree.*

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Additional file 6

***Multiple sequence alignment of all human plus selected protostome and cnidarian homeodomains**. The consensus homeodomain sequence (shown several times for reference) was derived from a compilation of 247 human homeodomain sequences. The three horizontal lines indicate the positions of the three alpha-helices. The numbering scheme refers to amino acid position in the canonical 60-amino-acid homeodomain; insertions relative to this sequence are shown when present. Black shaded resides are invariant between all human homeodomains within each class (or family in the case of the ZF homeodomains). Sequence accession numbers are shown. For each gene family designation, maximum likelihood and neighbor-joining bootstrap support values are indicated (see Additional files 1 and 2). These values are not shown if the gene family does not form a monophyletic group in phylogenetic analyses (in which case n/a is written) or if an invertebrate homolog could not be found.*

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Additional file 7

***Phylogenetic input file**. All human and invertebrate homeodomains used in phylogenetic analyses are shown, after alignment and removal of insertions to give a uniform 60-amino-acid alignment.*

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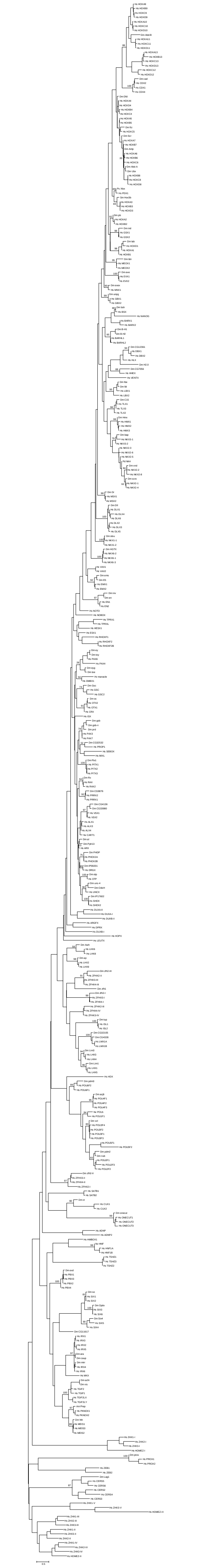
Additional file 1

The dataset used for both ML and NJ analyses includes all human homeodomains, most Drosophila melanogaster homeodomains, plus selected additional homeodomains from other protostomes or cnidarians when the gene family is divergent or absent in Drosophila. Divergent Drosophila genes that do not group with human genes were identified by construction of a preliminary, nonbootstrapped ML and NJ trees, and subsequently removed from the dataset. These include genes lost from human, as well genes known to have undergone unusually rapid evolution in Drosophila. For the Hox3 family the rapidly evolving Drosophila genes bcd, zen and zen2 were replaced with Sm Hox3b, and for the Nk4 family the rapid evolving Drosophila gene tin was replaced with Pd NK4. In addition, six genes from other protostome or cnidarian genomes were added to represent gene families known to be missing from Drosophila (Pdx family: Ps Xlox; Alx family: Nv CART1; Dmbx family: Hv manacle; Pou1 family: Nv POU1; Hnf1 family: Nv HNF; Pknox family: Am Prep). Species abbreviations: Am, Apis mellifera (honeybee); Dm, Drosophila melanogaster (fruitfly); Hv, Hydra vulgaris (hydrozoan); Nv, Nematostella vectensis (starlet sea anemone); Pd, Platynereis dumerilii (annelid worm); Ps, Phascolion strombus (sipunculan worm); Sm, Strigamia maritima (centipede).

ML performed more poorly than NJ in recovering several well known gene families, notably Hox4, Hox5, Nk4 and Alx. In contrast, ML did recover PROP1 and CG32532 as a true gene family; NJ did not. The invertebrate gene does not always lie as a strict outgroup to all human genes in a family; this effect is expected when using a short alignment. Instead, distinct grouping of invertebrate and human genes is taken as evidence of ancestry from a single gene. A few ambiguous cases were encountered, notably divergence of Drosophila H2.0 in the proposed Hlx gene family, and resolution within the Pax4/6 gene family, which is recovered as two families in NJ but one in ML. As explained in the text, several human gene families contain 'orphan' genes without invertebrate orthologs; these are Barx, Nanog, Noto, Vax, Ventx, Argfx, Dprx, Dux, Esx, Hesx, Hopx, Isx, Leutx, Mix, Nobox, Rhox, Sebox, Tprx, Hdx, Pou5, Hmbox, Satb, Adnp and Zhx/Homez. Zeb and Mkx would be placed in this category based on our ML and NJ trees, although other data suggest that Drosophila zfh1 and CG11617 respectively may be the protostome orthologs [73,94]. Tshz is only an apparent orphan family; the clear Drosophila ortholog simply lacks the homeobox [95,96]. Phylogenetic analysis is just one source of evidence for allocation of genes to gene families and identification of boundaries between gene families; complementary criteria used are synteny between species and paralogy within the human genome. Our ML and NJ trees should not be used to allocate gene families to gene classes, because other diagnostic characters such as insertions within the homeodomain, key amino acid residues, and several motifs outside of the homeodomain are excluded from the analysis. Indeed, artefactual mixing of the TALE and SINE classes occurs in both ML and NJ trees.


Hs HOXA9
Hs HOXB9
Hs HOXC9
Hs HOXD9
Hs HOXA10
Hs HOXC10
Hs HOXD10
Dm Abd-B
Hs HOXA11
Hs HOXC11
Hs HOXD11
66
Hs HOXA13
Hs HOXB13
Hs HOXC13
Hs HOXD13
Hs HOXC12
Hs HOXD12
Dm cad
Hs CDX2
100
Hs CDX1
Hs CDX4
Dm Dfd
Hs HOXA4
Hs HOXD4
Hs HOXB4
Hs HOXC4
Hs HOXA5
Hs HOXB5
Dm ftz
Hs HOXC5
Dm Scr
Hs HOXA7
Hs HOXB7
Dm Antp
Hs HOXA6
20
Hs HOXB6
Hs HOXC6
Dm Abd-A
Dm Ubx
Hs HOXB8
Hs HOXC8
Hs HOXD8
89
Ps Xlox
Hs PDX1
Sm Hox3b
Hs HOXA3
69
Hs HOXB3
Hs HOXD3
92
Dm pb
Hs HOXA2
Hs HOXB2
Dm ind
98
Hs GSX1
Hs GSX2
Dm lab
Hs HOXD1
98
Hs HOXA1
Hs HOXB1
Dm btn
95
Hs MEOX1
Hs MEOX2
Dm eve
100
Hs EVX1
Hs EVX2
90
Dm exex
Hs MNX1
Dm unpg
99
Hs GBX1
Hs GBX2
89
Dm bsh
Hs BSX
Hs NANOG
Hs BARX1
Hs BARX2
Dm B-H1
Dm B-H2
95
Hs BARHL1
Hs BARHL2
94
Dm CG12361
Hs DBX1
Hs DBX2
Hs HLX
Dm H2.0
88
Dm CG7056
Hs HHEX
Hs VENTX
Dm lbe
Dm lbl
95
Hs LBX1
Hs LBX2
Dm C15
Hs TLX1
98
Hs TLX3
Hs TLX2
99
Dm Hmx
Hs HMX1
Hs HMX2
Hs HMX3
Dm bap
79
Hs NKX3-1
Hs NKX3-2
Hs NKX2-3
Hs NKX2-6
Hs NKX2-5
Pd NK4
Dm vnd
84
Hs NKX2-2
Hs NKX2-8
Dm scro
62
Hs NKX2-1
Hs NKX2-4
98
Dm Dr
Hs MSX1
Hs MSX2
Dm Dll
Hs DLX1
Hs DLX4
100
Hs DLX6
Hs DLX2
Hs DLX3
Hs DLX5
Dm slou
100
Hs NKX1-1
Hs NKX1-2
Dm HGTX
Hs NKX6-2
100
Hs NKX6-1
Hs NKX6-3
Hs VAX1
Hs VAX2
Dm ems
92
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Dm inv
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Hs ESX1
Hs RHOXF1
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Hs RHOXF2B
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Dm toe
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Dm oc
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Hs ISX
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Dm unc-4
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Hs POU2F2
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Hs ZFHX2-I
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Hs ONECUT2
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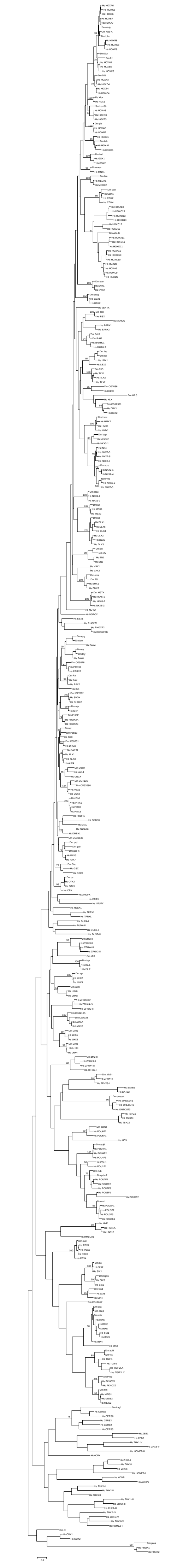
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Hs SIX4
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Dm caup
Dm mirr
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Hs PKNOX2
Dm hth
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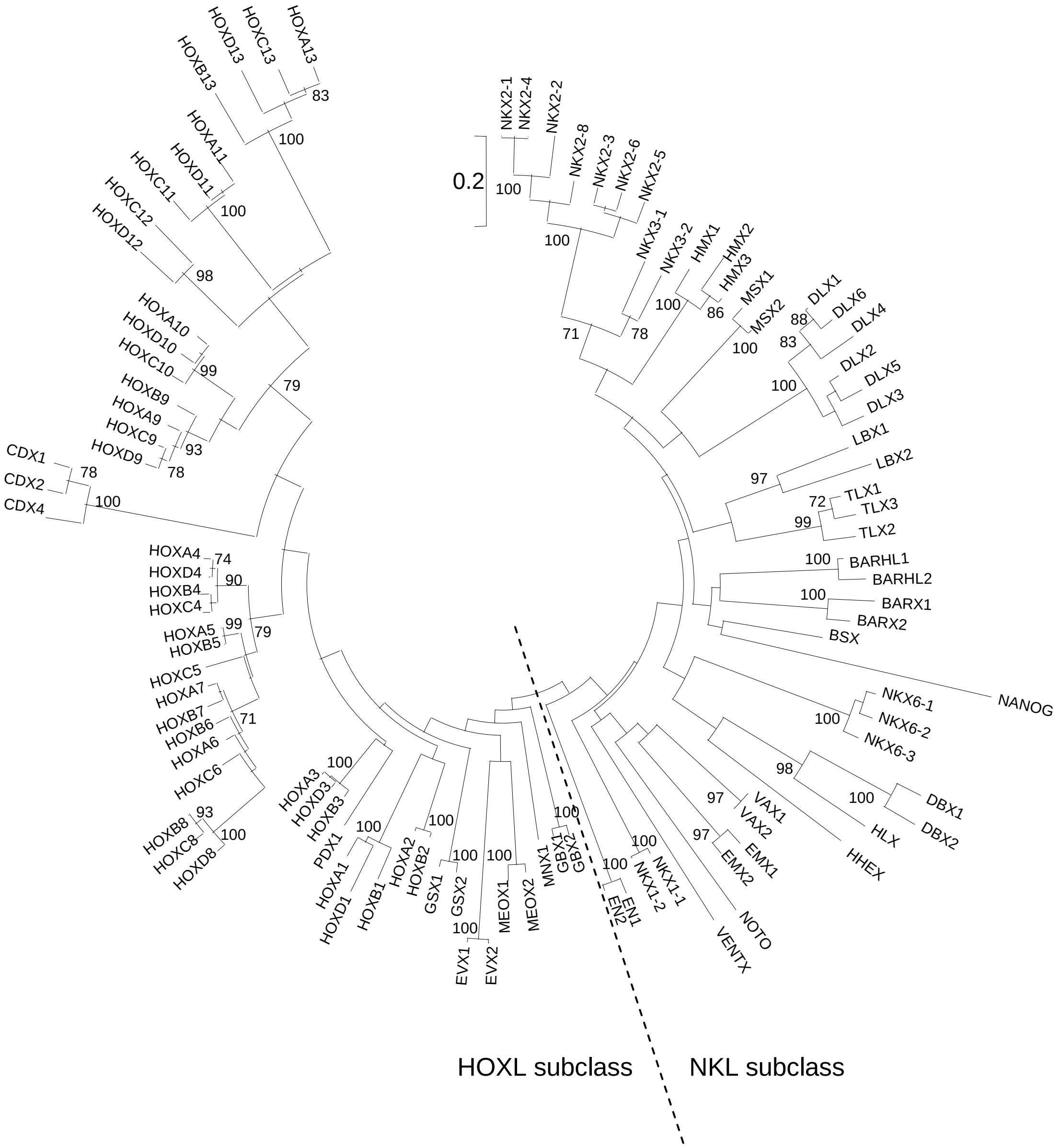
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VAX2
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HLX
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BARX2
BARX1
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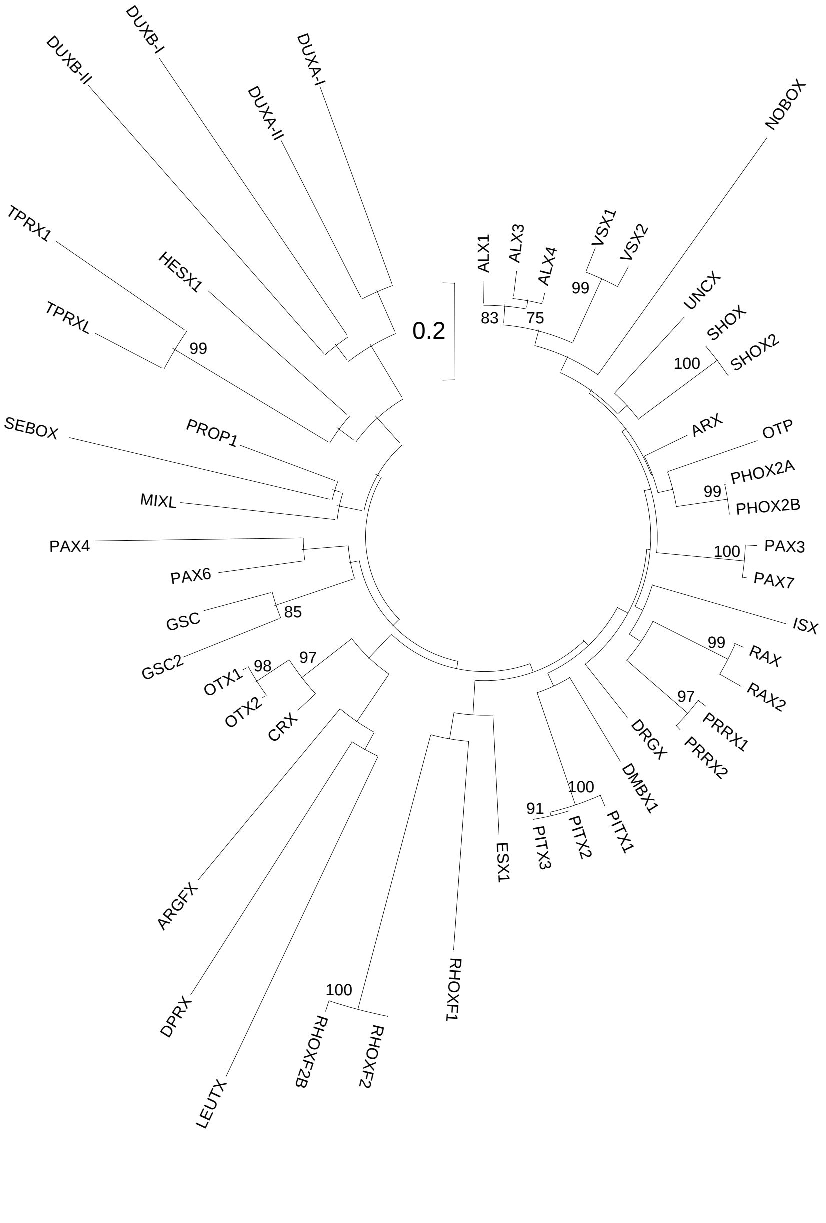
NKL subclass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ALX3
ALX4
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UNCX
SHOX
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ARX
OTP
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PHOX2A
PHOX2B
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PAX7
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RAX
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CRX
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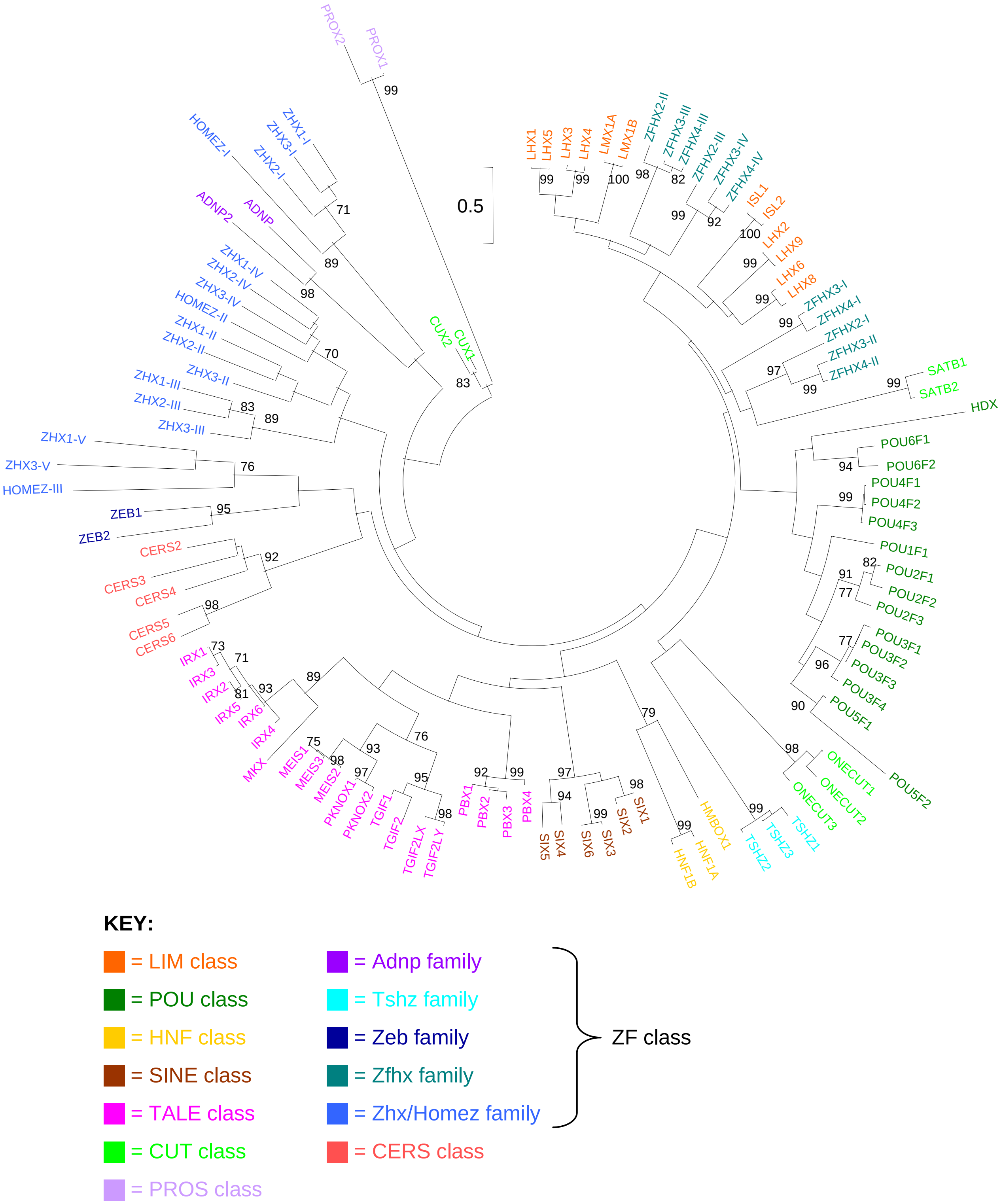
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CUX1
83
KEY:
🟧 = LIM class
🟩 = POU class
🟨 = HNF class
🟫 = SINE class
🟪 = TALE class
🟩 = CUT class
🟪 = PROS class
🟪 = Adnp family
🟦 = Tshz family
🟦 = Zeb family
🟩 = Zfhx family
🟦 = Zhx/Homez family
🟥 = CERS class
ZF class

ANTP class

```
                                  ______I______     ____II____    _______III_______
                                10        20        30        40        50        60
                        ....|....|....|....|....|....|....|....|....|....|....|....|
          Consensus     KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE      Accession       ML bs   NJ bs


HOXL subclass

Cdx       Hs CDX1       KDKYRVVYTDHQRLELEKEFHYSRYITIRRKSELAANLGLTERQVKIWFQNRRAKERKVN      NP_001795       100     80
          Hs CDX2       KDKYRVVYTDHQRLELEKEFHYSRYITIRRKAELAATLGLSERQVKIWFQNRRAKERKIN      NP_001256
          Hs CDX4       KEKYRVVYTDHQRLELEKEFHCNRYITIQRKSELAVNLGLSERQVKIWFQNRRAKERKMI      NP_005184
          Dm cad        KDKYRVVYTDFQRLELEKEYCTSRYITIRRKSELAQTLSLSERQVKIWFQNRRAKERKQN      NP_476954

Evx       Hs EVX1       MRRYRTAFTREQIARLEKEFYRENYVSRPRRCELAAALNLPETTIKVWFQNRRMKDKRQR      NP_001980       100     100
          Hs EVX2       VRRYRTAFTREQIARLEKEFYRENYVSRPRRCELAAALNLPETTIKVWFQNRRMKDKRQR      NP_001073927
          Dm eve        VRRYRTAFTRDQLGRLEKEFYKENYVSRPRRCELAAQLNLPESTIKVWFQNRRMKDKRQR      NP_523670

Gbx       Hs GBX1       SRRRRTAFTSEQLLELEKEFHCKKYLSLTERSQIAHALKLSEVQVKIWFQNRRAKWKRIK      NP_001092304    99      100
          Hs GBX2       NRRRRTAFTSEQLLELEKEFHCKKYLSLTERSQIAHALKLSEVQVKIWFQNRRAKWKRVK      NP_001476
          Dm unpg       SRRRRTAFTSEQLLELEREFHAKKYLSLTERSQIATSLKLSEVQVKIWFQNRRAKWKRVK      NP_477146

Gsx       Hs GSX1       SKRMRTAFTSTQLLELEREFASNMYLSRLRRIEIATYLNLSEKQVKIWFQNRRVKHKKEG      NP_663632       98      100
          Hs GSX2       GKRMRTAFTSTQLLELEREFSSNMYLSRLRRIEIATYLNLSEKQVKIWFQNRRVKHKKEG      NP_573574
          Dm ind        SKRIRTAFTSTQLLELEREFSHNAYLSRLRRIEIANRLRLSEKQVKIWFQNRRVKQKKGG      NP_996087

Hox1      Hs HOXA1      PNAVRTNFTTKQLTELEKEFHFNKYLTRARRVEIAASLQLNETQVKIWFQNRRMKQKKRE      NP_005513       98      99
          Hs HOXB1      PSGLRTNFTTRQLTELEKEFHFNKYLSRARRVEIAATLELNETQVKIWFQNRRMKQKKRE      NP_002135
          Hs HOXD1      SSAIRTNFSTKQLTELEKEFHFNKYLTRARRIEIANCLHLNDTQVKIWFQNRRMKQKKRE      NP_078777
          Dm lab        NNSGRTNFTNKQLTELEKEFHFNRYLTRARRIEIANTLQLNETQVKIWFQNRRMKQKKRV      NP_476613

Hox2      Hs HOXA2      SRRLRTAYTNTQLLELEKEFHFNKYLCRPRRVEIAALLDLTERQVKVWFQNRRMKHKRQT      NP_006726       92      100
          Hs HOXB2      ARRLRTAYTNTQLLELEKEFHFNKYLCRPRRVEIAALLDLTERQVKVWFQNRRMKHKRQT      NP_002136
          Dm pb         PRRLRTAYTNTQLLELEKEFHFNKYLCRPRRIEIAASLDLTERQVKVWFQNRRMKHKRQT      NP_476669

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          Hs HOXB3      SKRARTAYTSAQLVELEKEFHFNRYLCRPRRVEMANLLNLSERQIKIWFQNRRMKYKKDQ      NP_002137
          Hs HOXD3      SKRVRTAYTSAQLVELEKEFHFNRYLCRPRRVEMANLLNLTERQIKIWFQNRRMKYKKDQ      NP_008829
          Sm Hox3b      NKRSRTAYTQSQLVELEKEFHFNRYLCRPRRVELASMLNLTERQIKIWFQNRRMKNKKIK     ABN42908

Hox4      Hs HOXA4      PKRSRTAYTRQQVLELEKEFHFNRYLTRRRRIEIAHTLCLSERQVKIWFQNRRMKWKKDH      NP_002132       n/a     39
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          Hs HOXC4      PKRSRTAYTRQQVLELEKEFHYNRYLTRRRRIEIAHSLCLSERQIKIWFQNRRMKWKKDH      NP_055435
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          Dm Dfd        PKRQRTAYTRHQILELEKEFHYNRYLTRRRRIEIAHTLVLSERQIKIWFQNRRMKWKKDN      NP_477201

Hox5      Hs HOXA5      GKRARTAYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLSERQIKIWFQNRRMKWKKDN      NP_061975       n/a     28
          Hs HOXB5      GKRARTAYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLSERQIKIWFQNRRMKWKKDN      NP_002138
          Hs HOXC5      GKRSRTSYTRYQTLELEKEFHFNRYLTRRRRIEIANNLCLNERQIKIWFQNRRMKWKKDS      NP_061826
          Dm Scr        TKRQRTSYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEH      NP_524248
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Hox6-8	**Hs HOXA6**	GRRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIANALCLTERQIKIWFQNRRMKWKKEN	NP_076919	20	39
	Hs HOXB6	GRRGRQTYTRYQTLELEKEFHYNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKES	NP_061825		
	Hs HOXC6	RRRGRQIYSRYQTLELEKEFHFNRYLTRRRRIEIANALCLTERQIKIWFQNRRMKWKKES	NP_004494		
	Hs HOXA7	RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEH	NP_008827		
	Hs HOXB7	RKRGRQTYTRYQTLELEKEFHYNRYLTRRRRIEIAHTLCLTERQIKIWFQNRRMKWKKEN	NP_004493		
	Hs HOXB8	RRRGRQTYSRYQTLELEKEFLFNPYLTRKRRIEVSHALGLTERQVKIWFQNRRMKWKKEN	NP_076921		
	Hs HOXC8	RRSGRQTYSRYQTLELEKEFLFNPYLTRKRRIEVSHALGLTERQVKIWFQNRRMKWKKEN	NP_073149		
	Hs HOXD8	RRRGRQTYSRFQTLELEKEFLFNPYLTRKRRIEVSHALALTERQVKIWFQNRRMKWKKEN	NP_062458		
	Dm Antp	RKRGRQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN	NP_996172		
	Dm Ubx	RRRGRQTYTRYQTLELEKEFHTNHYLTRRRRIEMAHALCLTERQIKIWFQNRRMKLKKEI	NP_536752		
	Dm Abd-A	RRRGRQTYTRFQTLELEKEFHFNHYLTRRRRIEIAHALCLTERQIKIWFQNRRMKLKKEL	NP_476693		
Hox9-13	**Hs HOXA9**	TRKKRCPYTKHQTLELEKEFLFNMYLTRDRRYEVARLLNLTERQVKIWFQNRRMKMKKIN	NP_689952	66	61
	Hs HOXB9	SRKKRCPYTKYQTLELEKEFLFNMYLTRDRRHEVARLLNLSERQVKIWFQNRRMKMKKMN	NP_076922		
	Hs HOXC9	TRKKRCPYTKYQTLELEKEFLFNMYLTRDRRYEVARVLNLTERQVKIWFQNRRMKMKKMN	NP_008828		
	Hs HOXD9	TRKKRCPYTKYQTLELEKEFLFNMYLTRDRRYEVARILNLTERQVKIWFQNRRMKMKKMS	NP_055028		
	Hs HOXA10	GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISRSVHLTDRQVKIWFQNRRMKLKKMN	NP_061824		
	Hs HOXC10	GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISKTINLTDRQVKIWFQNRRMKLKKMN	NP_059105		
	Hs HOXD10	GRKKRCPYTKHQTLELEKEFLFNMYLTRERRLEISKSVNLTDRQVKIWFQNRRMKLKKMS	NP_002139		
	Hs HOXA11	TRKKRCPYTKYQIRELEREFFFSVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKIN	NP_005514		
	Hs HOXC11	TRKKRCPYSKFQIRELEREFFFNVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKLS	NP_055027		
	Hs HOXD11	SRKKRCPYTKYQIRELEREFFFNVYINKEKRLQLSRMLNLTDRQVKIWFQNRRMKEKKLN	NP_067015		
	Hs HOXC12	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL	NP_776272		
	Hs HOXD12	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV	NP_067016		
	Hs HOXA13	GRKKRVPYTKVQLKELEREYATNKFITKDKRRRISATTNLSERQVTIWFQNRRVKEKKVI	NP_000513		
	Hs HOXB13	GRKKRIPYSKGQLRELEREYAANKFITKDKRRKISAATSLSERQITIWFQNRRVKEKKVL	NP_006352		
	Hs HOXC13	GRKKRVPYTKVQLKELEKEYAASKFITKEKRRRISATTNLSERQVTIWFQNRRVKEKKVV	NP_059106		
	Hs HOXD13	GRKKRVPYTKLQLKELENEYAINKFINKDKRRRISAATNLSERQVTIWFQNRRVKDKKIV	NP_000514		
	Dm Abd-B	VRKKRKPYSKFQTLELEKEFLFNAYVSKQKRWELARNLQLTERQVKIWFQNRRMKNKKNS	NP_650577		
Mnx	**Hs MNX1**	CRRPRTAFTSQQLLELEHQFKFNKYLSRPKRFEVATSLMLTETQVKIWFQNRRMKWKRSK	NP_005506	90	99
	Dm exex	TRRPRTAFTSQQLLELEKQFKQNKYLSRPKRFEVASGLMLSETQVKIWFQNRRMKWKRSK	NP_648164		
Meox	**Hs MEOX1**	ARKERTAFTKEQLRELEAEFAHHNYLTRLRRYEIAVNLDLSERQVKVWFQNRRMKWKRVK	NP_004518	95	100
	Hs MEOX2	PRKERTAFTKEQIRELEAEFAHHNYLTRLRRYEIAVNLDLTERQVKVWFQNRRMKWKRVK	NP_005915		
	Dm btn	NRKERTAFSKTQLKQLEAEFCYSNYLTRLRRYEIAVALELTERQVKVWFQNRRMKCKRIK	NP_732768		
Pdx	**Hs PDX1**	NKRTRTAYTRAQLLELEKEFLFNKYISRPRRVELAVMLNLTERHIKIWFQNRRMKWKKEE	NP_000200	89	100
	Ps Xlox	NKRTRTAYTRAQLLELEKEFHFNKYISRPRRIELAAMLNLTERHIKIWFQNRRMKWKKDE	AAK77134		
NKL subclass					
Barhl	**Hs BARHL1**	PRKARTAFTDHQLAQLERSFERQKYLSVQDRMELAASLNLTDTQVKTWYQNRRTKWKRQT	NP_064448	95	94
	Hs BARHL2	PRKARTAFSDHQLNQLERSFERQKYLSVQDRMDLAAALNLTDTQVKTWYQNRRTKWKRQT	NP_064447		
	Dm B-H1	QRKARTAFTDHQLQTLEKSFERQKYLSVQERQELAHKLDLSDCQVKTWYQNRRTKWKRQT	NP_523387		
	Dm B-H2	QRKARTAFTDHQLQTLEKSFERQKYLSVQDRMELANKLELSDCQVKTWYQNRRTKWKRQT	NP_523386		
Barx	**Hs BARX1**	GRRSRTVFTELQLMGLEKRFEKQKYLSTPDRIDLAESLGLSQLQVKTWYQNRRMKWKKIV	NP_067545	prot/cnid	homolog not found
	Hs BARX2	PRRSRTIFTELQLMGLEKKFQKQKYLSTPDRLDLAQSLGLTQLQVKTWYQNRRMKWKKMV	NP_003649		
Bsx	**Hs BSX**	RRKARTVFSDSQLSGLEKRFEIQRYLSTPERVELATALSLSETQVKTWFQNRRMKHKKQL	NP_001091639	89	100
	Dm bsh	RRKARTVFSDPQLSGLEKRFEGQRYLSTPERVELATALGLSETQVKTWFQNRRMKHKKQL	NP_477350		

Dbx	**Hs DBX1**	GMLRRAVFSDVQRKALEKMFQKQKYISKPDRKKLAAKLGLKDSQVKIWFQNRRMKWRNSK	EAW68342	94	96
	Hs DBX2	GILRRAVFSEDQRKALEKMFQKQKYISKTDRKKLAINLGLKESQVKIWFQNRRMKWRNSK	NP_001004329		
	Dm CG12361	GMMRRAVFSDSQRKGLEKRFQQQKYISKPDRKKLAERLGLKDSQVKIWFQNRRMKWRNSK	NP_647677		
Dlx	**Hs DLX1**	IRKPRTIYSSLQLQALNRRFQQTQYLALPERAELAASLGLTQTQVKIWFQNKRSKFKKLM	NP_835221	100	100
	Hs DLX2	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAASLGLTQTQVKIWFQNRRSKFKKMW	NP_004396		
	Hs DLX3	VRKPRTIYSSYQLAALQRRFQKAQYLALPERAELAAQLGLTQTQVKIWFQNRRSKFKKLY	NP_005211		
	Hs DLX4	LRKPRTIYSSLQLQHLNQRFQHTQYLALPERAQLAAQLGLTQTQVKIWFQNKRSKYKKLL	NP_612138		
	Hs DLX5	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAASLGLTQTQVKIWFQNKRSKIKKIM	NP_005212		
	Hs DLX6	IRKPRTIYSSLQLQALNHRFQQTQYLALPERAELAASLGLTQTQVKIWFQNKRSKFKKLL	NP_005213		
	Dm Dll	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAASLGLTQTQVKIWFQNRRSKYKKMM	NP_523857		
Emx	**Hs EMX1**	PKRIRTAFSPSQLLRLERAFEKNHYVVGAERKQLAGSLSLSETQVKVWFQNRRTKYKRQK	NP_004088	92	100
	Hs EMX2	PKRIRTAFSPSQLLRLEHAFEKNHYVVGAERKQLAHSLSLTETQVKVWFQNRRTKFKRQK	NP_004089		
	Dm ems	PKRIRTAFSPSQLLKLEHAFESNQYVVGAERKALAQNLNLSETQVKVWFQNRRTKHKRMQ	NP_731868		
	Dm E5	PKRVRTAFSPTQLLKLEHAFEGNHYVVGAERKQLAQGLSLTETQVKVWFQNRRTKHKRMQ	NP_524825		
En	**Hs EN1**	DKRPRTAFTAEQLQRLKAEFQANRYITEQRRQTLAQELSLNESQIKIWFQNKRAKIKKAT	NP_001417	97	99
	Hs EN2	DKRPRTAFTAEQLQRLKAEFQTNRYLTEQRRQSLAQELSLNESQIKIWFQNKRAKIKKAT	NP_001418		
	Dm en	EKRPRTAFSSEQLARLKREFNENRYLTERRRQQLSSELGLNEAQIKIWFQNKRAKIKKST	NP_523700		
	Dm inv	DKRPRTAFSGTQLARLKHEFNENRYLTEKRRQQLSGELGLNEAQIKIWFQNKRAKLKKSS	NP_725055		
Hhex	**Hs HHEX**	RKGGQVRFSNDQTIELEKKFETQKYLSPPERKRLAKMLQLSERQVKTWFQNRRAKWRRLK	NP_002720	88	85
	Dm CG7056	RKGGQIRFTSQQTKNLEARFASSKYLSPEERRHLALQLKLTDRQVKTWFQNRRAKWRRAN	NP_650938		
Hlx	**Hs HLX**	RSWSRAVFSNLQRKGLEKRFEIQKYVTKPDRKQLAAMLGLTDAQVKVWFQNRRMKWRHSK	NP_068777	n/a	n/a
	Dm H2.0	KRKRSWSRAVFSNLQRKGLEIQQKYITKPDRRKLAARLNLTDAQVKVWFQNRRMKWRHTR	NP_523488		
Lbx	**Hs LBX1**	RRKSRTAFTNHQIYELEKRFLYQKYLSPADRDQIAQQLGLTNAQVITWFQNRRAKLKREL	NP_006553	95	94
	Hs LBX2	RRKSRTAFTAQQVLELERRFVFQKYLAPSERDGLATRLGLANAQVVTWFQNRRAKLKRDV	NP_001009812		
	Dm lbe	KRKSRTAFTNHQIFELEKRFLYQKYLSPADRDEIAASLGLSNAQVITWFQNRRAKQKRDI	NP_524435		
	Dm lbl	KRKSRTAFTNQQIFELEKRFLYQKYLSPADRDEIAGGLGLSNAQVITWFQNRRAKLKRDM	NP_524434		
Msx	**Hs MSX1**	NRKPRTPFTTAQLLALERKFRQKQYLSIAERAEFSSSLSLTETQVKIWFQNRRAKAKRLQ	NP_002439	98	100
	Hs MSX2	NRKPRTPFTTSQLLALERKFRQKQYLSIAERAEFSSSLNLTETQVKIWFQNRRAKAKRLQ	NP_002440		
	Dm Dr	NRKPRTPFTTQQLLSLEKKFREKQYLSIAERAEFSSSLRLTETQVKIWFQNRRAKAKRLQ	NP_477324		
Nanog	**Hs NANOG**	KQKTRTVFSSTQLCVLNDRFQRQKYLSLQQMQELSNILNLSYKQVKTWFQNQRMKSKRWQ	NP_079141	prot/cnid	homolog not found
Nk1	**Hs NKX1-1**	PRRARTAFTYEQLVALENKFKATRYLSVCERLNLALSLSLTETQVKIWFQNRRTKWKKQN	XP_931434	100	100
	Hs NKX1-2	PRRARTAFTYEQLVALENKFRATRYLSVCERLNLALSLSLTETQVKIWFQNRRTKWKKQN	XP_372331		
	Dm slou	PRRARTAFTYEQLVSLENKFKTTRYLSVCERLNLALSLSLTETQVKIWFQNRRTKWKKQN	NP_476657		
Nk2-1	**Hs NKX2-1**	RRKRRVLFSQAQVYELERRFKQQKYLSAPEREHLASMIHLTPTQVKIWFQNHRYKMKRQA	NP_001073136	62	79
	Hs NKX2-4	RRKRRVLFSQAQVYELERRFKQQKYLSAPEREHLASMIHLTPTQVKIWFQNHRYKMKRQA	CAH71494		
	Dm scro	RRKRRVLFTQAQVYELERRFKQQRYLSAPEREHLASLIHLTPTQVKIWFQNHRYKCKRQA	NP_001015473		
Nk2-2	**Hs NKX2-2**	KRKRRVLFSKAQTYELERRFRQQRYLSAPEREHLASLIRLTPTQVKIWFQNHRYKMKRAR	NP_002500	84	62
	Hs NKX2-8	RKKRRVLFSKAQTLELERRFRQQRYLSAPEREQLASLLRLTPTQVKIWFQNHRYKLKRAR	NP_055175		
	Dm vnd	KRKRRVLFTKAQTYELERRFRQQRYLSAPEREHLASLIRLTPTQVKIWFQNHRYKTKRAQ	NP_476786		
Nk3	**Hs NKX3-1**	QKRSRAAFSHTQVIELERKFSHQKYLSAPERAHLAKNLKLTETQVKIWFQNRRYKTKRKQ	NP_006158	79	88
	Hs NKX3-2	KKRSRAAFSHAQVFELERRFNHQRYLSGPERADLAASLKLTETQVKIWFQNRRYKTKRRQ	NP_001180		
	Dm bap	KKRSRAAFSHAQVFELERRFAQQRYLSGPERSEMAKSLRLTETQVKIWFQNRRYKTKRKQ	NP_732637		

Nk4	Hs NKX2-3	RRKPRVLFSQAQVFELERRFKQQRYLSAPEREHLASSLKLTSTQVKIWFQNRRYKCKRQR	NP_660328	n/a	60
	Hs NKX2-5	RRKPRVLFSQAQVYELERRFKQQRYLSAPERDQLASVLKLTSTQVKIWFQNRRYKCKRQR	NP_004378		
	Hs NKX2-6	RRKPRVLFSQAQVLALERRFKQQRYLSAPEREHLASALQLTSTQVKIWFQNRRYKCKRQR	XP_070619		
	Pd NK4	KRKPRVLFSQAQVYELERRFKQQRYLSAPEREQLASMLKLTSTQVKIWFQNRRYKMKRQR	ABQ10640		
Nk5/Hmx	Hs HMX1	KKKTRTVFSRSQVFQLESTFDLKRYLSTAERAGLAASLQLTETQVKIWFQNRRNKWKRHV	NP_061815	99	100
	Hs HMX2	KKKTRTVFSRSQVYQLESTFDMKRYLSSSERACLASSLQLTETQVKTWFQNRRNKWKRQL	NP_005510		
	Hs HMX3	KKKTRTVFSRSQVFQLESTFDMKRYLSSSERAGLAASLHLTETQVKIWFQNRRNKWKRQL	XP_291716		
	Dm Hmx	KKKTRTVFSRAQVFQLESTFDLKRYLSSSERAGLAASLRLTETQVKIWFQNRRNKWKRQL	NP_524951		
Nk6	Hs NKX6-1	RKHTRPTFSGQQIFALEKTFEQTKYLAGPERARLAYSLGMTESQVKVWFQNRRTKWRKKH	NP_006159	100	100
	Hs NKX6-2	KKHSRPTFSGQQIFALEKTFEQTKYLAGPERARLAYSLGMTESQVKVWFQNRRTKWRKRH	NP_796374		
	Hs NKX6-3	KKHTRPTFTGHQIFALEKTFEQTKYLAGPERARLAYSLGMTESQVKVWFQNRRTKWRKKS	EAW63251		
	Dm HGTX	KKHTRPTFSGQQIFALEKTFEQTKYLAGPERAKLAYALGMSESQVKVWFQNRRTKWRKRH	NP_652614		
Noto	Hs NOTO	QKRVRTMFNLEQLEELEKVFAKQHNLVGKKRAQLAARLKLTENQVRVWFQNRRVKYQKQQ	XP_292889	prot/cnid homolog not found	
Tlx	Hs TLX1	KKKPRTSFTRLQICELEKRFHRQKYLASAERAALAKALKMTDAQVKTWFQNRRTKWRRQT	NP_005512	98	100
	Hs TLX2	RKKPRTSFSRSQVLELERRFLRQKYLASAERAALAKALRMTDAQVKTWFQNRRTKWRRQT	NP_057254		
	Hs TLX3	RKKPRTSFSRVQICELEKRFHRQKYLASAERAALAKSLKMTDAQVKTWFQNRRTKWRRQT	NP_066305		
	Dm C15	RKKPRTSFTRIQVAELEKRFHKQKYLASAERAALARGLKMTDAQVKTWFQNRRTKWRRQT	NP_476873		
Vax	Hs VAX1	PKRTRTSFTAEQLYRLEMEFQRCQYVVGRERTELARQLNLSETQVKVWFQNRRTKQKKDQ	CAI14825	prot/cnid homolog not found	
	Hs VAX2	PKRTRTSFTAEQLYRLEMEFQRCQYVVGRERTELARQLNLSETQVKVWFQNRRTKQKKDQ	NP_036608		
Ventx	Hs VENTX	APRVRTAFTMEQVRTLEGVFQHHQYLSPLERKRLAREMQLSEVQIKTWFQNRRMKHKRQM	NP_055283	prot/cnid homolog not found	

PRD class

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                   ______I______      ____II____    _______III_______
                   10        20         30        40        50        60
....|....|....|....|... .|....|....|....|....|....|....|....|
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		Consensus	Accession	ML bs	NJ bs
		KRRKRTTFTKEQLLELEKEFAKN-PYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE			
Alx	Hs ALX1	KRRHRTTFTSLQLEELEKVFQKT-HYPDVYVREQLALRTELTEARVQVWFQNRRAKWRKRE	NP_008913	n/a	79
	Hs ALX3	KRRNRTTFSTFQLEELEKVFQKT-HYPDVYAREQLALRTDLTEARVQVWFQNRRAKWRKRE	NP_006483		
	Hs ALX4	KRRNRTTFTSYQLEELEKVFQKT-HYPDVYAREQLAMRTDLTEARVQVWFQNRRAKWRKRE	NP_068745		
	Nv CART1	KRRNRTTFTAYQLEEMERVFQKT-HYPDVYTREQLALRCALTEARVQVWFQNRRAKWRKRE	ABB83749		
Argfx	Hs ARGFX	RHKERTSFTHQQYEELEALFSQT-MFPDRNLQEKLALRLDLPESTVKVWFRNRRFKLKKQQ	NP_001012677	prot/cnid homolog not found	
Arx	Hs ARX	QRRYRTTFTSYQLEELERAFQKT-HYPDVFTREELAMRLDLTEARVQVWFQNRRAKWRKRE	NP_620689	23	28
	Dm al	QRRYRTTFTSFQLEELEKAFSRT-HYPDVFTREELAMKIGLTEARIQVWFQNRRAKWRKQE	NP_722629		
	Dm Pph13	QRRYRTTFNTLQLQELERAFQRT-HYPDVFFREELAVRIDLTEARVQVWFQNRRAKWRKQE	NP_477330		
Dmbx	Hs DMBX1	QRRSRTAFTAQQLEALEKTFQKT-HYPDVVMRERLAMCTNLPEARVQVWFKNRRAKFRKKQ	NP_671725	50	49
	Hv manacle	HRRVRTAFTHHQLTTLERTFETS-HYPDVVLRERLASFTGLAESRIQVWFKNRRAKYRKHQ	AAD30998		
Dprx	Hs DPRX	SHRKRTMFTKKQLEDLNILFNEN-PYPNPSLQKEMASKIDIHPTVLQVWFKNHRAKLKKAK	NP_001012746	prot/cnid homolog not found	

Drgx	**Hs DRGX**	QRRNRTTFTLQQLEALEAVFAQT-HYPDVFTREELAMKINLTEARVQVWFQNRRAKWRKTE	NP_001073989	90	97
	Dm IP09201	QRRNRTTFTLQQLEELETAFAQT-HYPDVFTREDLAMKINLTEARVQVWFQNRRAKWRKAE	ABK30908		
Dux	**Hs DUXA-I**	HRRCRTKFTEEQLKILINTFNQK-PYPGYATKQKLALEINTEESRIQIWFQNRRARHGFQK	NP_001012747	prot/cnid homolog not found	
	Hs DUXA-II	ARRCRTTYSASQLHTLIKAFMKN-PYPGIDSREELAKEIGVPESRVQIWFQNRRSRLLLQR	NP_001012747		
	Hs DUXB-I	FWRNRIQYNQSQKDILQSWFQHD-PFPDKAAREQLAKEIGVPESNIQVWFKNYRVKQRKLD	unassigned		
	Hs DUXB-II	ARQKQTFITWTQKNRLVQAFERN-PFPDIATRKKLAEQTGLQESRIQMWFQKQRSLYLKKS	unassigned		
Esx	**Hs ESX1**	KRRRRTAFTQFQLQELENFFDES-QYPDVVARERLAARLNLTEDRVQVWFQNRRAKWKRNQ	NP_703149	prot/cnid homolog not found	
Gsc	**Hs GSC**	KRRHRTIFTDEQLEALENLFQET-KYPDVGTREQLARKVHLREEKVEVWFKNRRAKWRRQK	NP_776248	74	77
	Hs GSC2	TRRHRTIFSEEQLQALEALFVQN-QYPDVSTRERLAGRIRLREERVEVWFKNRRAKWRHQK	NP_005306		
	Dm Gsc	KRRHRTIFTEEQLEQLEATFDKT-HYPDVVLREQLALKVDLKEERVEVWFKNRRAKWRKQK	NP_476949		
Hesx	**Hs HESX1**	GRRPRTAFTQNQIEVLENVFRVN-CYPGIDIREDLAQKLNLEEDRIQIWFQNRRAKLKRSH	NP_003856	prot/cnid homolog not found	
Hopx	**Hs HOPX**	SAETASGPTEDQVEILEYNFNKVDKHPDSTTLCLIAAEAGLSEEETQKWFKQRLAKWRRSE	NP_115884	prot/cnid homolog not found	
Isx	**Hs ISX**	KRRVRTTFTTEQLHELEKIFHFT-HYPDVHIRSQLAARINLPEARVQIWFQNQRAKWRKQE	NP_001008494	prot/cnid homolog not found	
Leutx	**Hs LEUTX**	YRRPRTRFLSKQLTALRELLEKT-MHPSLATMGKLASKLQLDLSVVKIWFKNQRAKWKRQQ	XP_001129035	prot/cnid homolog not found	
Mix	**Hs MIXL**	QRRKRTSFSAEQLQLLELVFRRT-RYPDIHLRERLAALTLLPESRIQVWFQNRRAKSRRQS	NP_114150	prot/cnid homolog not found	
Nobox	**Hs NOBOX**	RKKTRTLYRSDQLEELEKIFQED-HYPDSDKRREIAQTVGVTPQRIMVWFQNRRAKWRKME	O60393	prot/cnid homolog not found	
Otp	**Hs OTP**	QKRHRTRFTPAQLNELERSFAKT-HYPDIFMREELALRIGLTESRVQVWFQNRRAKWKKRK	NP_115485	100	99
	Dm otp	QKRHRTRFTPAQLNELERCFSKT-HYPDIFMREEIAMRIGLTESRVQVWFQNRRAKWKKRK	NP_523799		
Otx	**Hs OTX1**	QRRERTTFTRSQLDVLEALFAKT-RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQ	NP_055377	91	98
	Hs OTX2	QRRERTTFTRAQLDVLEALFAKT-RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQ	NP_068374		
	Hs CRX	QRRERTTFTRSQLEELEALFAKT-QYPDVYAREEVALKINLPESRVQVWFKNRRAKCRQQR	NP_000545		
	Dm oc	QRRERTTFTRAQLDVLEALFGKT-RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQL	NP_511091		
Pax2/5/8	**Hs PAX2**	KHLRADTFTQQQLEALDRVFERP-SYPDVFQASE---------------------------	NP_003981	partial homeodomain	
	Hs PAX5	KQMRGDLFTQQQLEVLDRVFERQ-HYSDIFTTTE---------------------------	NP_057953		
	Hs PAX8	KHLRTDAFSQHHLEPLECPF RQ-HYPEAYASPS---------------------------	NP_003457		
Pax3/7	**Hs PAX3**	QRRSRTTFTAEQLEELERAFERT-HYPDIYTREELAQRAKLTEARVQVWFSNRRARWRKQA	NP_852122	78	79
	Hs PAX7	QRRSRTTFTAEQLEELEKAFERT-HYPDIYTREELAQRTKLTEARVQVWFSNRRARWRKQA	NP_002575		
	Dm prd	QRRCRTTFSASQLDELERAFERT-QYPDIYTREELAQRTNLTEARIQVWFSNRRARLRKQH	NP_523556		
	Dm gsb	QRRSRTTFSNDQIDALERIFART-QYPDVYTREELAQSTGLTEARVQVWFSNRRARLRKQL	NP_523863		
	Dm gsb-n	QRRSRTTFTAEQLEALERAFSRT-QYPDVYTREELAQTTALTEARIQVWFSNRRARLRKHS	NP_523862		
Pax4/6	**Hs PAX4**	GHRNRTIFSPSQAEALEKEFQRG-QYPDSVARGKLATATSLPEDTVRVWFSNRRAKWRRQE	NP_006184	28	29
	Hs PAX6	LQRNRTSFTQEQIEALEKEFERT-HYPDVFARERLAAKIDLPEARIQVWFSNRRAKWRREE	NP_001595		
	Dm ey	LQRNRTSFTNDQIDSLEKEFERT-HYPDVFARERLAGKIGLPEARIQVWFSNRRAKWRREE	NP_524628		
	Dm eyg	FRRNRTTFSPEQLEELEKEFDKS-HYPCVSTRERLSSRTSLSEARVQVWFSNRRAKWRRHQ	NP_524042		
	Dm toy	LQRNRTSFSNEQIDSLEKEFERT-HYPDVFARERLADKIGLPEARIQVWFSNRRAKWRREE	NP_524638		
	Dm toe	FRRNRTTFSPEQLDELEKEFDKS-HYPCVNTREKLAARTALSEARVQVWFSNRRAKWRRHQ	NP_524041		
Phox	**Hs PHOX2A**	QRRIRTTFTSAQLKELERVFAET-HYPDIYTREELALKIDLTEARVQVWFQNRRAKFRKQE	NP_005160	75	71
	Hs PHOX2B	QRRIRTTFTSAQLKELERVFAET-HYPDIYTREELALKIDLTEARVQVWFQNRRAKFRKQE	NP_003915		
	Dm PHDP	QRRIRTTFTSNQLNELEKIFLET-HYPDIYTREEIASKLHLTEARVQVWFQNRRAKFRKQE	NP_523834		

Pitx	**Hs PITX1**	QRRQRTHFTSQQLQELEATFQRN-RYPDMSMREEIAVWTNLTEPRVRVWFKNRRAKWRKRE	NP_002644	100	100
	Hs PITX2	QRRQRTHFTSQQLQELEATFQRN-RYPDMSTREEIAVWTNLTEARVRVWFKNRRAKWRKRE	NP_000316		
	Hs PITX3	QRRQRTHFTSQQLQELEATFQRN-RYPDMSTREEIAVWTNLTEARVRVWFKNRRAKWRKRE	NP_005020		
	Dm Ptx1	QRRQRTHFTSQQLQELEHTFSRN-RYPDMSTREEIAMWTNLTEARVRVWFKNRRAKWRKRE	NP_733410		
Prop	**Hs PROP1**	RRRHRTTFSPVQLEQLESAFGRN-QYPDIWARESLARDTGLSEARIQVWFQNRRAKQRKQE	NP_006252	52	n/a
	Dm CG32532	RRRHRTTFTQEQLAELEAAFAKS-HYPDIYCREELARTTKLNEARIQVWFQNRRAKYRKQE	NP_608318		
Prrx	**Hs PRRX1**	QRRNRTTFNSSQLQALERVFERT-HYPDAFVREDLARRVNLTEARVQVWFQNRRAKFRRNE	NP_073207	92	81
	Hs PRRX2	QRRNRTTFNSSQLQALERVFERT-HYPDAFVREELARRVNLSEARVQVWFQNRRAKFRRNE	NP_057391		
	Dm CG9876	PRRNRTTFSSAQLTALEKVFERT-HYPDAFVREELATKVHLSEARVQVWFQNRRAKFRRNE	NP_611756		
Rax	**Hs RAX**	HRRNRTTFTTYQLHELERAFEKS-HYPDVYSREELAGKVNLPEVRVQVWFQNRRAKWRRQE	NP_038463	94	99
	Hs RAX2	HRRNRTTFTTYQLHQLERAFEAS-HYPDVYSREELAAKVHLPEVRVQVWFQNRRAKWRRQE	NP_116142		
	Dm Rx	HRRNRTTFTTYQLHELERAFEKS-HYPDVYSREELAMKVNLPEVRVQVWFQNRRAKWRRQE	NP_726006		
Rhox	**Hs RHOXF1**	PRTRRTKFTLLQVEELESVFRHT-QYPDVPTRRELAENLGVTEDKVRVWFKNKRARCRRHQ	NP_644811	prot/cnid homolog not found	
	Hs RHOXF2	QQPNVHAFTPLQLQELERIFQRE-QFPSEFLRRRLARSMNVTELAVQIWFENRRAKWRRHQ	NP_115887		
	Hs RHOXF2B	QQPNVHAFTPLQLQELECIFQRE-QFPSEFLRRRLARSMNVTELAVQIWFENRRAKWRRHQ	NP_001093155		
Sebox	**Hs SEBOX**	HRRKRTTFSKGQLLELERAFAAW-PYPNISTHEHLAWVTCLPEAKVQVWFQKRWAKIIKNR	NP_001074306	prot/cnid homolog not found	
Shox	**Hs SHOX**	QRRSRTNFTLEQLNELERLFDET-HYPDAFMREELSQRLGLSEARVQVWFQNRRAKCRKQE	NP_000442	100	100
	Hs SHOX2	QRRSRTNFTLEQLNELERLFDET-HYPDAFMREELSQRLGLSEARVQVWFQNRRAKCRKQE	NP_006875		
	Dm IP17602	QRRSRTNFTLDQLNELERLFEET-HYPDAFMREELSQRLGLSEARVQVWFQNRRAKCRKHE	ABM92832		
Tprx	**Hs TPRX1**	QRQERTVYTESQQKVLEFYFQKD-QYPNYDQRLNLAEMLSLREQQLQVWFKNRRAKLARER	EAW57514	prot/cnid homolog not found	
	Hs TPRXL	QRQDRTIYNWKQQEVLENHFKEE-QYPDYDTRQELAEMLNLREYQVQVWFKNRRAKRSRER	unassigned		
Uncx	**Hs UNCX**	RRRTRTNFTGWQLEELEKAFNES-HYPDVFMREALALRLDLVESRVQVWFQNRRAKWRKKE	NP_001073930	90	85
	Dm unc-4	RRRSRTNFNSWQLEELERAFSAS-HYPDIFMREALAMRLDLKESRVAVWFQNRRAKVRKRE	NP_573242		
	Dm OdsH	KRRGRTNFNSWQLRELERVFQGS-HYPDIFMREALATKLDLMEGRIAVWFQNRRAKWRKQE	NP_523389		
Vsx	**Hs VSX1**	KRRHRTVFTAHQLEELEKAFSEA-HYPDVYAREMLAVKTELPEDRIQVWFQNRRAKWRKRE	NP_055403	97	100
	Hs VSX2	KRRHRTIFTSYQLEELEKAFNEA-HYPDVYAREMLAMKTELPEDRIQVWFQNRRAKWRKRE	NP_878314		
	Dm CG33980	RRHSRTIFTSYQLEKLEEAFKEA-HYPDVYAREMLSLKTELPEDRIQVWFQNRRAKWRKTE	NP_001033832		
	Dm CG4136	RRHGRTIFTSSQLEELEKAFKEA-HYPDVSARELLSMKTGLAEDRIQVWYQNRRAKWRKTE	NP_572232		

LIM class

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           ______I______     ____II____    ________III________
           10        20        30        40        50        60
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	Consensus	KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE	**Accession**	**ML bs**	**NJ bs**
Isl	**Hs ISL1**	TTRVRTVLNEKQLHTLRTCYAANPRPDALMKEQLVEMTGLSPRVIRVWFQNKRCKDKKRS	NP_002193	100	100
	Hs ISL2	TTRVRTVLNEKQLHTLRTCYAANPRPDALMKEQLVEMTGLSPRVIRVWFQNKRCKDKKKS	NP_665804		
	Dm tup	PTRVRTVLNEKQLHTLRTCYNANPRPDALMKEQLVEMTSLSPRVIRVWFQNKRCKDKKKT	NP_476775		

Lhx1/5	**Hs LHX1**	RRGPRTTIKAKQLETLKAAFAATPKPTRHIREQLAQETGLNMRVIQVWFQNRRSKERRMK	NP_005559	96	89
	Hs LHX5	RRGPRTTIKAKQLETLKAAFAATPKPTRHIREQLAQETGLNMRVIQVWFQNRRSKERRMK	NP_071758		
	Dm Lim1	RRGPRTTIKAKQLEVLKTAFNQTPKPTRHIREQLAKETGLPMRVIQVWFQNKRSKERRMK	NP_572505		
Lhx2/9	**Hs LHX2**	TKRMRTSFKHHQLRTMKSYFAINHNPDAKDLKQLAQKTGLTKRVLQVWFQNARAKFRRNL	NP_004780	98	100
	Hs LHX9	TKRMRTSFKHHQLRTMKSYFAINHNPDAKDLKQLAQKTGLTKRVLQVWFQNARAKFRRNL	NP_064589		
	Dm ap	TKRMRTSFKHHQLRTMKSYFAINHNPDAKDLKQLSQKTGLPKRVLQVWFQNARAKWRRMM	NP_724428		
Lhx3/4	**Hs LHX3**	AKRPRTTITAKQLETLKSAYNTSPKPARHVREQLSSETGLDMRVVQVWFQNRRAKEKRLK	NP_055379	94	99
	Hs LHX4	AKRPRTTITAKQLETLKNAYKNSPKPARHVREQLSSETGLDMRVVQVWFQNRRAKEKRLK	NP_203129		
	Dm Lim3	NKRPRTTITAKQLETLKTAYNNSPKPARHVREQLSQDTGLDMRVVQVWFQNRRAKEKRLK	NP_476606		
Lhx6/8	**Hs LHX6**	AKRARTSFTAEQLQVMQAQFAQDNNPDAQTLQKLADMTGLSRRVIQVWFQNCRARHKKHT	NP_055183	58	54
	Hs LHX8	AKRARTSFTADQLQVMQAQFAQDNNPDAQTLQKLAERTGLSRRVIQVWFQNCRARHKKHV	NP_001001933		
	Dm Awh	TKRVRTTFTEEQLQVLQANFQIDSNPDGQDLERIASVTGLSKRVTQVWFQNSRARQKKHI	NP_523907		
Lmx	**Hs LMX1A**	PKRPRTILTTQQRRAFKASFEVSSKPCRKVRETLAAETGLSVRVVQVWFQNQRAKMKKLA	NP_796372	100	100
	Hs LMX1B	PKRPRTILTTQQRRAFKASFEVSSKPCRKVRETLAAETGLSVRVVQVWFQNQRAKMKKLA	NP_002307		
	Dm CG32105	PKRPRTILTSQQRKQFKASFDQSPKPCRKVREALAKDTGLSVRVVQVWFQNQRAKMKKIQ	NP_729801		
	Dm CG4328	PKRPRTILNTQQRRAFKASFEVSPKPCRKVRENLAKDTGLSLRIVQVWFQNQRAKVKKIQ	NP_648567		

POU class

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                ______I______         ____II____     _______III_______
                10        20          30        40        50        60
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	Consensus	KRRK----RTTFTKEQLLELEKEFAKNPY----PSREEREELAASLGLTERQVKVWFQNRRAKWKKQE	**Accession**	**ML bs**	**NJ bs**
Hdx	**Hs HDX**	SRKRALQDRTQFSDRDLATLKKYWDNGMTSLGSVCREKIEAVATELNVDCEIVRTWIGNRRRKYRLMG	NP_653258	prot/cnid	homolog not found
Pou1	**Hs POU1F1**	KRKR----RTTISIAAKDALERHFGEQNK----PSSQEIMRMAEELNLEKEVVRVWFCNRRQREKRVK	NP_000297	94	90
	Nv POU1	RRKR----RTTIGLAAKEALENHFMKQTK----PSSPEIVRIADGLRLDKEVVRVWFCNRRQREKRVK	ABB86471		
Pou2	**Hs POU2F1**	RRKK----RTSIETNIRVALEKSFLENQK----PTSEEITMIADQLNMEKEVIRVWFCNRRQKEKRIN	NP_002688	83	79
	Hs POU2F2	RRKK----RTSIETNVRFALEKSFLANQK----PTSEEILLIAEQLHMEKEVIRVWFCNRRQKEKRIN	NP_002689		
	Hs POU2F3	KRKK----RTSIETNIRLTLEKRFQDNPK----PSSEEISMIAEQLSMEKEVVRVWFCNRRQKEKRIN	NP_055167		
	Dm nub	RRKK----RTSIETTIRGALEKAFLANQK----PTSEEITQLADRLSMEKEVVRVWFCNRRQKEKRIN	NP_476659		
	Dm pdm2	RRKK----RTSIETTVRTTLEKAFLMNCK----PTSEEISQLSERLNMDKEVIRVWFCNRRQKEKRIN	NP_523558		
Pou3	**Hs POU3F1**	KRKK----RTSIEVGVKGALESHFLKCPK----PSAHEITGLADSLQLEKEVVRVWFCNRRQKEKRMT	NP_002690	83	93
	Hs POU3F2	KRKK----RTSIEVSVKGALESHFLKCPK----PSAQEITSLADSLQLEKEVVRVWFCNRRQKEKRMT	NP_005595		
	Hs POU3F3	KRKK----RTSIEVSVKGALESHFLKCPK----PSAQEITNLADSLQLEKEVVRVWFCNRRQKEKRMT	NP_006227		
	Hs POU3F4	KRKK----RTSIEVSVKGVLETHFLKCPK----PAAQEISSLADSLQLEKEVVRVWFCNRRQKEKRMT	NP_000298		
	Dm vvl	KRKK----RTSIEVSVKGALEQHFHKQPK----PSAQEITSLADSLQLEKEVVRVWFCNRRQKEKRMT	NP_523948		
Pou4	**Hs POU4F1**	KKRK----RTSIAAPEKRSLEAYFAVQPR----PSSEKIAAIAEKLDLKKNVVRVWFCNQRQKQKRMK	NP_006228	95	100
	Hs POU4F2	KKRK----RTSIAAPEKRSLEAYFAIQPR----PSSEKIAAIAEKLDLKKNVVRVWFCNQRQKQKRMK	NP_004566		
	Hs POU4F3	RKRK----RTSIAAPEKRSLEAYFAIQPR----PSSEKIAAIAEKLDLKKNVVRVWFCNQRQKQKRMK	NP_002691		
	Dm acj6	KKRK----RTSIAAPEKRSLEAYFAVQPR----PSGEKIAAIAEKLDLKKNVVRVWFCNQRQKQKRIV	NP_524876		

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Pou5       Hs POU5F1    RKRK----RTSIENRVRGNLENLFLQCPK----PTLQQISHIAQQLGLEKDVVRVWFCNRRQKGKRSS                  NP_002692    prot/cnid homolog not found
           Hs POU5F2    GKWR----RASRERRIGNSLEKFFQRCPK----PTPQQISHIAGCLQLQKDVVRVWFYNRSKMGSRPT                  NP_694948

Pou6       Hs POU6F1    KRKR----RTSFTPQAIEALNAYFEKNPL----PTGQEITEIAKELNYDREVVRVWFCNRRQTLKNTS                  NP_002693    89        92
           Hs POU6F2    KRKR----RTSFTPQALEILNAHFEKNTH----PSGQEMTEIAEKLNYDREVVRVWFCNKRQALKNTI                  NP_009183
           Dm pdm3      KRKR----RTSFTPQALELLNAHFERNTH----PSGTEITGLAHQLGYEREVIRIWFCNKRQALKNTV                  NP_610377
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HNF class

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                                 ______I______     ____II____                                  ________III________
                                 10        20        30                                  40        50        60
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           Consensus    KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLG---------------------LTERQVKVWFQNRRAKWKKQE  Accession    ML bs     NJ bs

Hmbox      Hs HMBOX1    RRGSRFTWRKECLAVMESYFNENQYPDEAKREEIANACN--AVIQK---PGKKLS-DLERVTSLKVYNWFANRRKEIKRRA  NP_078843    prot/cnid homolog not found

Hnf1       Hs HNF1A     GRRNRFKWGPASQQILFQAYERQKNPSKEERETLVEECNRAECIQRGVSPSQAQGLGSNLVTEVRVYNWFANRRKEEAFRH  NP_000536    99        98
           Hs HNF1B     MRRNRFKWGPASQQILYQAYDRQKNPSKEEREALVEECNRAECLQRGVSPSKAHGLGSNLVTEVRVYNWFANRRKEEAFRQ  NP_000449
           Nv HNF       SRRNRFKWGPASTNILYQSYEQQRNPSKEEREALVEACNRAECEQRGVSYNNVEGLGFNLVTESRVYNWFANRRKEETFRM  ABB86496
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SINE class

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                                 ______I______     ____II____    ________III________
                                 10        20        30        40        50        60
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           Consensus    KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                       Accession    ML bs     NJ bs

Six1/2     Hs SIX1      GEETSYCFKEKSRGVLREWYAHNPYPSPREKRELAEATGLTTTQVSNWFKNRRQRDRAAE                       NP_005973    81        100
           Hs SIX2      GEETSYCFKEKSRSVLREWYAHNPYPSPREKRELTEATGLTTTQVSNWFKNRRQRDRAAE                       NP_058628
           Dm so        GEETSYCFKEKSRSVLRDWYSHNPYPSPREKRDLAEATGLTTTQVSNWFKNRRQRDRAAE                       NP_476733

Six3/6     Hs SIX3      GEQKTHCFKERTRSLLREWYLQDPYPNPSKKRELAQATGLTPTQVGNWFKNRRQRDRAAA                       NP_005404    100       99
           Hs SIX6      GEQKTHCFKERTRNLLREWYLQDPYPNPSKKRELAQATGLTPTQVGNWFKNRRQRDRAAA                       NP_031400
           Dm Optix     GEQKTHCFKERTRSLLREWYLQDPYPNPTKKRELAKATGLNPTQVGNWFKNRRQRDRAAA                       NP_524695

Six4/5     Hs SIX4      GEETVYCFKEKSRNALKELYKQNRYPSPAEKRHLAKITGLSLTQVSNWFKNRRQRDRNPS                       NP_059116    90        91
           Hs SIX5      GEETVYCFKERSRAALKACYRGNRYPTPDEKRRLATLTGLSLTQVSNWFKNRRQRDRTGA                       NP_787071
           Dm Six4      GEETVYCFKEKSRNALKDCYLTNRYPTPDEKKTLAKKTGLTLTQVSNWFKNRRQRDRTPQ                       NP_649256
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TALE class

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                                 ______I______        ____II____    _______III_______
                                10        20           30        40        50        60
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          Consensus     KRRKRTTFTKEQLLELEKEFAKN---PYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE      Accession      ML bs   NJ bs

Irx       Hs IRX1       DPGRPKNATRESTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_077313      97      38
          Hs IRX2       DPAYRKNATRDATATLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_150366
          Hs IRX3       DPSRPKNATRESTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_077312
          Hs IRX4       SGTRRKNATRETTSTLKAWLQEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_057442
          Hs IRX5       DPAYRKNATRDATATLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_005844
          Hs IRX6       GAGRRKNATRETTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_077311
          Dm ara        LAARRKNATRESTATLKAWLNEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_524045
          Dm caup       LAARRKNATRESTATLKAWLSEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_524046
          Dm mirr       NGARRKNATRETTSTLKAWLNEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN      NP_524047

Meis      Hs MEIS1      RHKKRGIFPKVATNIMRAWLFQHLTHPYPSEEQKKQLAQDTGLTILQVNNWFINARRRIVQPM      NP_002389      69      93
          Hs MEIS2      RQKKRGIFPKVATNIMRAWLFQHLTHPYPSEEQKKQLAQDTGLTILQVNNWFINARRRIVQPM      NP_733777
          Hs MEIS3      RNKKRGIFPKVATNIMRAWLFQHLSHPYPSEEQKKQLAQDTGLTILQVNNWFINARRRIVQPM      NP_001009813
          Dm hth        NQKKRGIFPKVATNILRAWLFQHLTHPYPSEDQKKQLAQDTGLTILQVNNWFINARRRIVQPM      NP_476578

Mkx       Hs MKX        KVRHKRQALQDMARPLKQWLYKHRDNPYPTKTEKILLALGSQMTLVQVSNWFANARRRLKNTV      NP_775847      n/a     n/a
          Dm CG11617    SRATKRLFTPDIKRMLKDWLIRRRENPYPSREEKKQLAAETGLTYTQICNWFANWRRKLKNSE      NP_608502

Pbx       Hs PBX1       ARRKRRNFNKQATEILNEYFYSHLSNPYPSEEAKEELAKKCGITVSQVSNWFGNKRIRYKKNI      NP_002576      100     100
          Hs PBX2       ARRKRRNFSKQATEVLNEYFYSHLSNPYPSEEAKEELAKKCGITVSQVSNWFGNKRIRYKKNI      NP_002577
          Hs PBX3       ARRKRRNFSKQATEILNEYFYSHLSNPYPSEEAKEELAKKCSITVSQVSNWFGNKRIRYKKNI      NP_006186
          Hs PBX4       ARRKRRNFSKQATEVLNEYFYSHLNNPYPSEEAKEELARKGGLTISQVSNWFGNKRIRYKKNM      NP_079521
          Dm exd        ARRKRRNFSKQASEILNEYFYSHLSNPYPSEEAKEELARKCGITVSQVSNWFGNKRIRYKKNI      NP_523360

Pknox     Hs PKNOX1     SKNKRGVLPKHATNVMRSWLFQHIGHPYPTEDEKKQIAAQTNLTLLQVNNWFINARRRILQPM      NP_004562      97      94
          Hs PKNOX2     SKNKRGVLPKHATNIMRSWLFQHLMHPYPTEDEKRQIAAQTNLTLLQVNNWFINARRRILQPM      NP_071345
          Am Prep       GRQKRGVLPKQATSIMRTWLFEHLVHPYPTEDEKRQIASQTNLTLLQVNNWFINARRRILQPM      XP_001120618

Tgif      Hs TGIF1      KRRRRGNLPKESVQILRDWLYEHRYNAYPSEQEKALLSQQTHLSTLQVCNWFINARRRLLPDM      NP_733796      100     88
          Hs TGIF2      KRKRRGNLPKESVKILRDWLYLHRYNAYPSEQEKLSLSGQTNLSVLQICNWFINARRRLLPDM      NP_068581
          Hs TGIF2LX    KKKRKGNLPAESVKILRDWMYKHRFKAYPSEEEKQMLSEKTNLSLLQISNWFINARRRILPDM      NP_620410
          Hs TGIF2LY    KKKRKGNLPAESVKILRDWMYKHRFKAYPSEEEKQMLSEKTNLSLLRISNWFINARRRILPDM      NP_631960
          Dm achi       LRKRRGNLPKTSVKILKRWLYEHRYNAYPSDAEKFTLSQEANLTVLQVCNWFINARRRILPEM      NP_725182
          Dm vis        LRKRRGNLPKSSVKILKRWLYEHRYNAYPSDAEKFTLSQEANLTVLQVCNWFINARRRILPEM      NP_523714
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CUT class

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                               _______I_______     _____II_____   _________III_________
                               10        20        30        40        50        60
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           Consensus       KRRKRTTFTKEQLLELEKEFAK-NPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                  Accession       ML bs     NJ bs

Cux        Hs CUX1         LKKPRVVLAPEEKEALKRAYQQ-KPYPSPKTIEDLATQLNLKTSTVINWFHNYRSRIRREL                  NP_853530       90        n/a
           Hs CUX2         IKKPRVVLAPEEKEALRKAYQL-EPYPSQQTIELLSFQLNLKTNTVINWFHNYRSRMRREM                  NP_056082
           Dm ct           SKKQRVLFSEEQKEALRLAFAL-DPYPNVGTIEFLANELGLATRTITNWFHNHRMRLKQQV                  NP_524764

Onecut     Hs ONECUT1      PKKPRLVFTDVQRRTLHAIFKE-NKRPSKELQITISQQLGLELSTVSNFFMNARRRSLDKW                  NP_004489       99        96
           Hs ONECUT2      QKKSRLVFTDLQRRTLFAIFKE-NKRPSKEMQITISQQLGLELTTVSNFFMNARRRSLEKW                  NP_004843
           Hs ONECUT3      PKKQRLVFTDLQRRTLIAIFKE-NKRPSKEMQVTISQQLGLELNTVSNFFMNARRRCMNRW                  NP_001073957
           Dm onecut       PKKPRLVFTDLQRRTLQAIFKE-TKRPSKEMQVTIARQLGLEPTTVGNFFMNARRRSMDKW                  NP_524842

Satb       Hs SATB1        KTRPRTKISVEALGILQSFIQDVGLYPDEEAIQTLSAQLDLPKYTIIKFFQNQRYYLKHHG                  NP_002962       prot/cnid homolog not found
           Hs SATB2        KPRSRTKISLEALGILQSFIHDVGLYPDQEAIHTLSAQLDLPKHTIIKFFQNQRYHVKHHG                  NP_056080
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PROS class

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                               _______I_______    _____II_____    _________III_________
                               10        20        30        40          50        60
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           Consensus       KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLT---ERQVKVWFQNRRAKWKKQE                Accession       ML bs     NJ bs

Prox       Hs PROX1        GSAMQEGLSPNHLKKAKLMFFYTRYPSSNMLKTYFSDVKFNRCITSQLIKWFSNFREFYYIQM                NP_002754       100       99
           Hs PROX2        LVHIQEGLNPGHLKKAKLMFFFTRYPSSNLLKVYFPDVQFNRCITSQMIKWFSNFREFYYIQM                NP_001073877
           Dm pros         MAPTSSTLTPMHLRKAKLMFFWVRYPSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQM                NP_731565
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ZF class

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                               _______I_______    _____II_____   _________III_________
                               10        20        30        40        50        60
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           Consensus       KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                   Accession       ML bs     NJ bs

Adnp       Hs ADNP         PKGHEDDSYEARKSFLTKYFNKQPYPTRREIEKLAASLWLWKSDIASHFSNKRKKCVRDC                   NP_056154       prot/cnid homolog not found
           Hs ADNP2        PKKYEGRSYEEKKQFLKDYFHKKPYPSKKEIELLSSLFWVWKIDVASFFGKRRYICMKAI                   NP_055728
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                     ______I______           ____II____   _______III_______
                     10        20                      30        40        50        60
                 ....|....|....|....|....|..           ..|....|....|....|....|....|....|
       Consensus KRRKRTTFTKEQLLELEKEFAKNPYPS-----------REEREELAASLGLTERQVKVWFQNRRAKWKKQE       Accession    ML bs    NJ bs

Tshz   Hs TSHZ1  RKGRQSNWNPQHLLILQAQFASSLRETTEGKYIMSDLGPQERVHISKFTGLSMTTISHWLANVKYQLRRTG       NP_005777    no homeodomain in Dm tsh
       Hs TSHZ2  RKGRQSNWNPQHLLILQAQFASSLFQTSEGKYLLSDLGPQERMQISKFTGLSMTTISHWLANVKYQLRKTG       NP_775756
       Hs TSHZ3  RKGRQSNWNPQHLLILQAQFAASLRQTSEGKYIMSDLSPQERMHISRFTGLSMTTISHWLANVKYQLRRTG       NP_065907

                      ______I______   ____II____    _______III_______
                      10        20        30        40        50        60
                 ....|.. ..|....|....|....|....|....|....|....|....|....|....|
       Consensus KRRKRTT-FTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                    Accession    ML bs    NJ bs

Zeb    Hs ZEB1   GNLSPSQPPLKNLLSLLKAYYALNAQPSAEELSKIADSVNLPLDVVKKWFEKMQAGQISVQ                    NP_110378    n/a      n/a
       Hs ZEB2   GMTSPIN-PYKDHMSVLKAYYAMNMEPNSDELLKISIAVGLPQEFVKEWFEQRKVYQYSNS                    NP_055610
       Dm zfh1   KVRVRTA-INEEQQQLKQHYSLNARPSRDEFRMIAARLQLDPRVVQVWFQNNRSRERKMQ                     NP_733402

                     ______I______   ____II____    _______III_______
                     10        20        30        40        50        60
                 ....|....|....|....|....|....|....|....|....|....|....|....|
       Consensus KRRKRTTFTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                     Accession    ML bs    NJ bs

Zfhx   Hs ZFHX3-I    NKRPRTRITDDQLRVLRQYFDINNSPSEEQIKEMADKSGLPQKVIKHWFRNTLFKERQRN                 NP_008816    99       98
       Hs ZFHX4-I    FKRPRTRITDDQLKILRAYFDINNSPSEEQIQEMAEKSGLSQKVIKHWFRNTLFKERQRN                 NP_078997
       Dm zfh2-I     QKRARTRITDDQLKILRAHFDINNSPSEESIMEMSQKANLPMKVVKHWFRNTLFKERQRN                 NP_524623

       Hs ZFHX2-I    RRFSRTKFTEFQTQALQSFFETSAYPKDGEVERLASLLGLASRVVVVWFQNARQKARKNA                 NP_207646    97       92
       Hs ZFHX3-II   KRSSRTRFTDYQLRVLQDFFDANAYPKDDEFEQLSNLLNLPTRVIVVWFQNARQKARKNY                 NP_008816
       Hs ZFHX4-II   KRSSRTRFTDYQLRVLQDFFDTNAYPKDDEIEQLSTVLNLPTRVIVVWFQNARQKARKSY                 NP_078997
       Dm zfh2-II    KRANRTRFTDYQIKVLQEFFENNSYPKDSDLEYLSKLLLLSPRVIVVWFQNARQKQRKIY                 NP_524623

       Hs ZFHX2-II   DKRLRTTILPEQLEILYRWYMQDSNPTRKMLDCISEEVGLKKRVVQVWFQNTRARERKGQ                 NP_207646    91       95
       Hs ZFHX3-III  DKRLRTTITPEQLEILYQKYLLDSNPTRKMLDHIAHEVGLKKRVVQVWFQNTRARERKGQ                 NP_008816
       Hs ZFHX4-III  DKRLRTTITPEQLEILYEKYLLDSNPTRKMLDHIAREVGLKKRVVQVWFQNTRARERKGQ                 NP_078997
       Dm zfh2-III   NKRLRTTILPEQLNFLYECYQSESNPSRKMLEEISKKVNLKKRVVQVWFQNSRAKDKKSR                 NP_524623

       Hs ZFHX2-III  QRRYRTQMSSLQLKIMKACYEAYRTPTMQECEVLGEEIGLPKRVIQVWFQNARAKEKKAK                 NP_207646
       Hs ZFHX3-IV   QKRFRTQMTNLQLKVLKSCFNDYRTPTMLECEVLGNDIGLPKRVVQVWFQNARAKEKKSK                 NP_008816
       Hs ZFHX4-IV   HKRFRTQMSNLQLKVLKACFSDYRTPTMQECEMLGNEIGLPKRVVQVWFQNARAKEKKFK                 NP_078997
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                                 ______I______     ____II____    _______III_______
                                 10        20        30        40        50        60
                       ....|.. ..|....|....|....|....|....|....|....|....|....|....|
          Consensus    KRRKRTT-FTKEQLLELEKEFAKNPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                      Accession        ML bs     NJ bs

Zhx/      Hs ZHX1-I    NSIPTYN-AALDNNPLLLNTYNKFPYPTMSEITVLSAQAKYTEEQIKIWFSAQRLKHGVSW                      NP_001017926     prot/cnid homolog not found
Homez     Hs ZHX2-I    LNTTKYN-SALDTNATMINSFNKFPYPTQAELSWLTAASKHPEEHIRIWFATQRLKHGISW                      NP_055758
          Hs ZHX3-I    SSIPTYN-AAMDSNSFLKNSFHKFPYPTKAELCYLTVVTKYPEEQLKIWFTAQRLKQGISW                      NP_055850
          Hs HOMEZ-I   WTQAAQT-SELDSNEHLLKTFSYFPYPSLADIALLCLRYGLQMEKVKTWFMAQRLRCGISW                      NP_065885

          Hs ZHX1-II   SFGIRAK-KTKEQLAELKVSYLKNQFPHDSEIIRLMKITGLTKGEIKKWFSDTRYNQRNSK                      NP_001017926
          Hs ZHX2-II   TPASDRK-KTKEQIAHLKASFLQSQFPDDAEVYRLIEVTGLARSEIKKWFSDHRYRCQRGI                      NP_055758
          Hs ZHX3-II   ASIYKNK-KSHEQLSALKGSFCRNQFPGQSEVEHLTKVTGLSTREVRKWFSDRRYHCRNLK                      NP_055850

          Hs ZHX1-III  TPQKFKE-KTAEQLRVLQASFLNSSVLTDEELNRLRAQTKLTRREIDAWFTEKKKSKALKE                      NP_001017926
          Hs ZHX2-III  APQKFKE-KTQGQVKILEDSFLKSSFPTQAELDRLRVETKLSRREIDSWFSERRKLRDSME                      NP_055758
          Hs ZHX3-III  TPTKYKE-RAPEQLRALESSFAQNPLPLDEELDRLRSETKMTRREIDSWFSERRKKVNAEE                      NP_055850

          Hs ZHX1-IV   STGKICK-KTPEQLHMLKSAFVRTQWPSPEEYDKLAKESGLARTDIVSWFGDTRYAWKNGN                      NP_001017926
          Hs ZHX2-IV   SPSPAIA-KSQEQVHLLRSTFARTQWPTPQEYDQLAAKTGLVRTEIVRWFKENRCLLKTGT                      NP_055758
          Hs ZHX3-IV   PGKVSCK-KTAQQRHLLRQLFVQTQWPSNQDYDSIMAQTGLPRPEVVRWFGDSRYALKNGQ                      NP_055850
          Hs HOMEZ-II  RQRKTKR-KTKEQLAILKSFFLQCQWARREDYQKLEQITGLPRPEIIQWFGDTRYALKHGQ                      NP_065885

          Hs ZHX1-V    DRGPSLI-KFKTGTAILKDYYLKHKFLNEQDLDELVNKSHMGYEQVREWFAERQRRSELGI                      NP_001017926
          Hs ZHX3-V    FPPGLLV-IAPGNRELLQDYYMTHKMLYEEDLQNLCDKTQMSSQQVKQWFAEKMGEETRAV                      NP_055850
          Hs HOMEZ-III TPPLPIP-PPPPDIQPLERYWAAHQQLRETDIPQLSQASRLSTQQVLDWFDSRLPQPAEVV                      NP_065885
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CERS class

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                                ______I______      ____II____    _______III_______
                                10        20         30        40        50        60
                       ....|....|....|....|.. ..|....|....|....|....|....|....|....|
          Consensus    KRRKRTTFTKEQLLELEKEFAK-NPYPSREEREELAASLGLTERQVKVWFQNRRAKWKKQE                      Accession        ML bs     NJ bs

Cers      Hs CERS2     NIKEKTRLRAPPNATLEHFYLTSGKQPKQVEVELLSRQSGLSGRQVERWFRRRRNQDRPSL                      NP_859530        87        78
          Hs CERS3     GIKETV-RKVTPNTVLENFFKHSTRQPLQTDIYGLAKKCNLTERQVERWFRSRRNQERPSR                      NP_849164
          Hs CERS4     GVRDQTRRQVKPNATLEKHFLTEGHRPKEPQLSLLAAQCGLTLQQTQRWFRRRRNQDRPQL                      NP_078828
          Hs CERS5     GIEDSGPYQAQPNAILEKVFISITKYPDKKRLEGLSKQLDWNVRKIQCWFRHRRNQDKPPT                      NP_671723
          Hs CERS6     NIQANGPQIAPPNAILEKVFTAITKHPDEKRLEGLSKQLDWDVRSIQRWFRQRRNQEKPST                      NP_982288
          Dm Lag1      GIRSSRPKKAANVPILEKTYAKSTRLDKKK-LVPLSKQTDMSEREIERWWRLRRAQDKPST                      NP_727075
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