Extreme evolutionary conservation and spatio-temporal expression analysis of CNEs and their neighboring genes lead to association of CNE enhancers to their respective genes. (A) Comparative syntenic analysis of human and amphibian depicts the conservation of both the paralogs SP4 (blue) and SP8 (purple) in the nearest vicinity of the CNE (green). Analogy in the expression pattern of the CNE and both of these paralogs suggest the association of this CNE with both SP4 and SP8 gene. (B) When we increased the depth of our syntenic comparison up till the fishes it became evident that only SP8 is conserved along with the CNE in fishes proposing it as a target gene for hs110.
Figure 2. Predicting the target genes of human enhancers by analyzing their genic environment within and across vertebrate genomes (hs189/hs831/hs187).

Careful analysis of genomic context of human enhancers helped in establishing an unambiguous association between them and target genes.  (A) Human enhancer-target gene duplicated early in vertebrate history, before tetrapod-teleost split. Conserved association between duplicated enhancers and SOX paralogs indicate that these duplicated human enhancers (CNE-SOX14 and CNE-SOX21) are specific to SOX14 and SOX21 genes.  (B) Duplication of human enhancer at the root of vertebrate lineage and preservation of linkage of duplicated enhancers with MEIS family members both within and across genomes, unmistakably suggest the specificity of these duplicated cis-regulatory regions (CNE-MEIS1 and CNE-MEIS2) towards MEIS1 and MEIS2 promoters.  (C) Human FOXP1 and FOXP2 paralogs harbor duplicated set of enhancers within their introns. The association of these anciently conserved enhancers with FOXP1 and FOXP2 genes is confirmed by their physical proximity to teleost fish orthologs of human FOXP1 and FOXP2 genes.
Figure 3. Target gene identification of human CNE-enhancers through orthology mapping (hs137/hs376).

Analyzing the genic environment of human CNE-enhancers in teleost fish orthologous loci and expression pattern of reporter gene helps in identifying their target genes. (A) Human CNE-enhancer and target gene duplicated before tetrapod-teleost divergence. Comparative syntenic analysis of human duplicated loci in multiple fish lineages clearly suggest that these duplicated human enhancers are associated with the regulation of human DACH paralogs residing on human chromosome 13 and X. (B) Duplication of human enhancer at the root of vertebrate lineage and preserved linkage of duplicated enhancers with TCF family members both within and across genomes, suggest the specificity of these duplicated CNEs towards TCF4 and TCF12 human paralogs.
Figure 4. Target gene identification of human CNE-enhancers through orthology mapping (hs466 /mm466).

Comparative analysis help in assigning the CNE enhancer to its target genes. (A) The CNE-enhancer duplicated in human lineage. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy on human chromosome suggests the association of this enhancer with *DPY19L1*. (B) The localization of a CNE-enhancer in the intergenic space between *UBE2V2* and *EFCABI* on human chromosome 8 suggest that this enhancer might be associated with one of these genes but not with *SNAI1* that is present ~ 200 kb upstream of *EFCABI*. However the careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy on human chromosome 20 clearly indicates that these duplicated human enhancers (*CNE_SNAI1* and *CNE_SNAI1*) are associated with the regulation of human *SNAI* paralogs.
Human CNE-enhancers by tracing the genic context of their orthologous copies in teleost fish lineage. (A) Human cis-regulatory element positioned in the intergenic space between PKN2 and LMO4, suggesting that this enhancer might be associated with one of these genes. Examining the neighboring genes of this enhancer in teleost fish suggest that PKN2 is a bystander gene because in all teleost fish analyzed this gene is physically uncoupled from CNE enhancer. Another gene HS2ST1 present in the neighborhood of human CNE-enhancer (upstream of PKN2) is similarly linked to this enhancer in all teleost fish analyzed. However the duplication of this locus in stickleback indicated that this gene is also a bystander gene as in one of the duplicated fragment (on Group XIII) HS2ST1 ortholog is lost. The only gene in the human locus that preserved its association with this CNE-enhancer in all teleost fish analyzed (even in duplicated loci of stickleback) is LMO4. Therefore human cis-regulatory element positioned in the intergenic space between PKN2 and LMO4 is unambiguously associated with LMO4 and named CNE-LMO4. (B) Human CNE-enhancer positioned within the intronic interval of HDAC9 gene on chromosome 7. Approximately 2 Mb of human locus encompassing this enhancer (containing at least 6 genes) was analyzed for the maintenance of conserved gene contents in teleost fish. The locus appeared to be duplicated in medaka and zebrafish. The differential gene loss from teleost duplicated loci suggest that CNE-enhancer within intragenic interval of human HDAC9 gene is associated with the regulation of TWIST1. It is noteworthy that in zebrafish in addition to locus duplication event an independent gene duplication event occurred that produced two tandem copies of TWIST1 and associated CNE-TWIST1. (C) Human CNE-enhancer positioned within the intronic interval of EBF1 gene on chromosome 5. Comparative analysis of this locus in teleost fish revealed that genomic interval encompassing this conserved enhancer is duplicated in zebrafish and Fugu. It appeared that after duplication of the locus, one copy of this CNE-enhancer had been lost in both fishes. Tracing the correlation between gene loss-enhancer loss/gene retention-enhancer retention in fish duplicated loci suggest that CNE-enhancer within the intragenic interval of human EBF1 might be associated with the regulation EBF1 gene. (D) Comparative analysis of a genic context around a CNE-enhancer within the intronic interval of human WWOX suggest that this enhancer act at a distance of ~2Mb on MAF gene.
Figure 6. Target gene identification of human CNE-enhancers through orthology mapping (hs234/hs1418/hs249).

Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes. (A) CNE-enhancer within an intron of human ELP4 gene is associated with neighboring PAX6 gene. (B) Human CNE-enhancer positioned on chromosome 7 probably duplicated in the common ancestor of teleost fish. Comparative syntenic analysis of human locus in multiple fish lineages and redundancy in the CNE induced reporter expression pattern and endogenous expression pattern of EN2 clearly suggest that this CNE enhancer is associated with the regulation of human EN2. (C) Conserved positioning of CNE-enhancer within an intergenic space between human CENTG2 and GBX2 and all other fish lineages and expression pattern studies suggest that this CNE is associated with either of flanking genes.
Assigning the target genes to CNE-enhancers through comparative genomic analysis. (A) Human CNE-enhancer is positioned within the intronic interval of ZFPM2 gene on chromosome 8. Comparative analysis of this locus in teleost fish revealed that genomic interval encompassing this conserved enhancer is duplicated in zebrafish. The conserved linkage of CNE with ZFPM2 in all lineages and its endogenous expression unmistakably links this CNE with the regulation of ZFPM2 gene. (B) Human CNE enhancer positioned on chromosome 2 duplicated in the common ancestor of teleost fish. Human-fish conserved positioning of CNE-enhancer within an intergenic space between human FIGN and KCNH7 and redundant expression pattern of these two genes suggest the association of this CNE enhancer with either of neighboring genes.
Assigning the target genes to CNE-enhancers through comparative genomic analysis.  

(A) CNE-enhancer within an intergenic space between human *GALNT5* and *GPD2* is associated with *ACVR1*.  

(B) CNE-enhancer within an intron of human *MEIS2* gene is associated with the promoter of the same gene. Other human-fish conserved genes within this locus appeared to be bystanders as their expression pattern is unrelated to the activity of this cis-acting region.  

(C) CNE-enhancer within an intergenic space between human *ATBF1* and *PMFBP1* is associated with *ATBF1* gene.  

(D) Human-fish conserved positioning of a CNE-enhancer within an intergenic space between human *FANCL* and *BCL11A* and redundant expression pattern of these two genes suggest that a single enhancer could interact with more than one neighboring genes.
Figure 9. Target gene identification of human CNE-enhancers through orthology mapping (hs378/hs169/Hs112/hs23).

Analyzing the genic architecture of human loci in teleost and comparing the reporter expression domains of CNE-enhancers with neighboring genes expression aided in assigning the cis-regulatory elements to human (A) TSHZ1 (B) FOXD3 (C) DMRT3 and (D) IRX6 genes.
Figure 10. Target gene identification of human CNE-enhancers through orthology mapping (hs741/hs488/hs762).

Analysis of human CNE-enhancers loci in teleost fish orthologous genomic intervals helps in identifying their target genes. (A) CNE-enhancer within an intron of human RSRC1 gene is associated with neighboring SHOX2. (B) CNE-enhancer within an intergenic space between human SOX21 and GPR180 is associated with SOX21. (C) CNE-enhancer within an intergenic space between human CDCA1 and PBX1 is associated with PBX1.
Human-fish comparative analysis of human loci and comparing the reporter expression domains induced by CNEs with neighboring genes expression pattern helped in assigning the target genes to CNE-enhancers. (A) An intergenic CNE-enhancer showed conserved positioning with respect to ARX and POLA genes in human and teleost fish lineages. The expression pattern of these genes is highly redundant and in accordance with CNE-enhancer induced reporter expression, and suggest that this enhancer might be influencing the expression of both of its neighboring genes. (B) The physical location of human CNE-enhancer within the intronic region of PBX3 is conserved in its orthologous intervals in fish lineages, along with other bystander genes. However the endogenous expression pattern of PBX3 matches with that of reporter gene expression, clearly inferring the association of enhancer with PBX3. (C) Human-fish comparative syntenic analysis revealed the conservation of CNE between EBF1 and CLINT1 along with these genes, moreover the expression pattern suggest specificity of this CNE enhancer to human EBF1. (D) Human-fish comparative syntenic analysis revealed the conservation of CNE and PRDM16 linkage in both lineages, moreover the expression pattern also suggest specificity of this CNE enhancer to human PRDM16.
Comparing the human fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes. (A) Both synteny analysis and endogenous expression pattern clearly associate to human ZNF312 gene. (B) A human CNE-enhancer positioned in the intergenic space between human INSC and SOX6 gene is similarly associated with the zebrafish SOX6 but not with INSC strongly suggesting that the human SOX6 is under the regulatory control of this intergenic enhancer. (C) Human-fish conserved linkage and expression analysis associate a human intergenic enhancer with HNRNPA3 gene. (D) The localization of CNE-enhancer in the intergenic space between TBX3 and THRAP2 and conservation of this arrangement down to chicken lineage prevents the unambiguous assignment of this CNE-enhancer to one of these genes. However comparing the endogenous expression patterns of both of these flanking genes with the reporter expression pattern induced by this CNE in transgenic mice assay clearly associate this CNE-enhancer to human TBX3 gene. (E) Analyzing the expression pattern and conservation in fish lineage shows that an intragenic CNE-enhancer is regulating the expression of FAF1.
Figure 13. Target gene identification of human CNE-enhancers through orthology mapping (hs1/hs192/hs121/hs242).

Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes. (A) The conserved localization of CNE in the intergenic region near FOXF1 and harmony of their redundant endogenous expression pattern with that of reporter expression induced by this CNE-enhancer in transgenic mice assay suggest the association of this enhancer with FOXF1 gene. (B) A human fish positioning of CNE enhancer between DNAJC19 and SOX2 genes and redundancy in the endogenous expression domains of these two genes and CNE induces reporter expression pattern hinders the unambiguous association of this CNE enhancer with either of flanking human genes. (C) Human fish comparative analysis of CNE containing locus and comparison of expression domains of CNE enhancer induced reporter expression with endogenous expression pattern of genes present in the human locus suggest that this intragenic human CNE enhancer is associated with regulation of POLA genes. (D) The conserved physical location of CNE-enhancer within intronic region of ZAK in fish and human lineages suggest its association with ZAK gene, however the expression studies of neighboring conserved genes infers that this CNE might be regulating the expression of CDCA7, PDK1 and RAPGEF4 rather than ZAK.
Figure 14. Target gene identification of human CNE-enhancers through orthology mapping (hs4/hs244/hs260).

Comparative analysis and expression pattern studies help in associating the enhancer to its target gene. (A) Comparative analysis of CNE enhancer in fish and human lineages show conserved localization of enhancer along with MAF and WWOX genes. Moreover comparing the endogenous expression of these genes with reporter gene expression of CNE also suggest the associates this enhancer with WWOX or MAF. (B) The CNE-enhancer is located within intronic region of PTD004 in human and some fish lineages. The comparative syntenic analysis of human locus with multiple fish lineages suggest its association with SP3 or CIR, moreover expression pattern studies also favors this association. (C) Comparative syntenic analysis of CNE enhancer containing region suggest the association of CNE with either CXXC4 or PPA2.
Figure 15. Target gene identification of human CNE-enhancers through orthology mapping (hs654/hs698/hs669/hs1330).

Comparative analysis and expression pattern studies help in finding the target gene for CNE enhancer. (A) The CNE-enhancer is located in the intergenic space between ZIC1 and AGTR1 and this positioning is conserved in human-fish comparison, however comparing the endogenous expression pattern of flanking genes with reporter expression induces by this CNE enhancer suggest that this CNE enhancer might be associated with regulation of human AGTR1 gene. (B) Human CNE enhancer is located in the intronic region of ST18 on chromosome 8. Comparative analysis of this locus in fish lineage clearly link this CNE enhancer with the regulation of RB1CC1 gene moreover endogenous expression pattern of RB1CC1 also suggest it as a target gene for this enhancer. (C) Human fish comparative analysis of CNE enhancer containing region shows conservation of CNE near OTUD6B suggesting it to be the target gene for this CNE. (D) The conserved localization of CNE-enhancer within intronic region of NBEA in human and all fish lineages, and its redundant expression in accordance with CNE enhancer induced reporter gene expression unmistakably links this CNE enhancer with the regulation of NBEA gene.
Figure 16. Target gene identification of human CNE-enhancers through orthology mapping (hs307/hs578/Hs532).

(A) The conserved positioning of CNE-enhancer within intronic region of BNC2 in human and some fish lineages suggest its association with BCN2 whereas human fish comparative syntenic analysis and expression pattern studies favors its association with CNTLN rather than BNC2. (B) Human CNE enhancer is located in the intergenic space between BUB3 and GPR26. Comparative syntenic analysis of the human locus with orthologous region of this element in teleost fish lineage reveals the association of this CNE with GPR26, also the expression pattern of GPR26 is in accordance with that of reporter gene expression leading to the unambiguous association of GPR26 as the target gene for this CNE. (C) Conserved positioning of the enhancer near GSH1 gene in human and fish lineages along with endogenous expression of GSH1 in accordance with CNE induced reporter gene expression clearly suggests that this enhancer is associated with regulation of GSH1 gene.
Figure 17. Target gene identification of human CNE-enhancers through orthology mapping (hs113/hs411).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) CNE-enhancer is positioned within an intergenic space between human RCNI and WT1 on chromosome 11. Comparative syntenic analysis in fish lineage and expression pattern studies suggest the association of this CNE with PAX6 or WT1. (B) Conserved localization of CNE-enhancer in the intergenic space between human KCNJ3 and NR4A2 and all other fish lineages and expression pattern of these genes in accordance with CNE enhancer suggest that this CNE is associated with either of flanking genes.
Human enhancer target gene duplicated early in vertebrate history before mammal fish divergence. Comparing the genic content of CNE-EBF paralogous loci in human genome and their orthologous loci in multiple fish lineages clearly suggests that duplicated copies of human CNE-EBF enhancer are associated with the regulation of paralogous copies of EBF family members i.e.; EBF1, EBF3 and EBF4. The examination of fish loci also suggest the lineage specific duplication of CNE-EBF enhancer in teleost fish.
Figure 19. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs1859/hs1425).

Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. **(A)** Comparative syntenic analysis of human and teleost fishes and the expression pattern analysis clearly depicts the connection between the CNE and LMO1, because of their conservation throughout the vertebrate genome. **(B)** Analyzing the expression pattern and conservation in tetrapod and fish lineages show that an intragenic CNE-enhancer is regulating the expression of AUTS2.
Figure 20. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs1316/ hs1315).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. (A) Not only the CNE but also its intronic location in CADPS, conserved in both human and teleost lineages, suggests the link exists between CNE and CADPS which is further confirmed by endogenous expression pattern analysis of both the CNE and the gene. (B) Studying the orthologous genomic content in the neighboring region of CNE enhancer in tetrapod-teleost lineages demonstrate the relationship between CNE and its target gene TFAP2A.
Figure 21. Comparing the human-fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes (hs1186/hs1318).

Target genes of CNE enhancers are identified through orthology mapping in Human and teleost fishes. (A) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of SALL3. Redundant expression pattern of the gene and CNE is also in accordance with each other. (B) Conservation of ZFX4 and HNF4G throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
Figure 22. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs906/hs828).

Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) Conserved localization of CNE and HHIP in tetrapod-teleost lineages demonstrate the linkage between them. Redundant expression pattern analysis of CNE and HHIP also emphasizes this idea. (B) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with MEIS2.
Figure 23. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs858/hs935).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Both synteny analysis and endogenous expression pattern clearly associate the CNE to human ZNF536 gene. (B) CNE residing between EMX2 and RAB11FIP2 in humans is also conserved along with EMX2, with differential loss and gain of bystander genes, in teleost fishes. Comparison of reporter gene expression of CNE enhancer and EMX2 also confers EMX2 as target gene of CNE.
Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. **(A)** Comparative syntenic analysis of human and teleost fishes and the expression pattern analysis clearly depicts the connection between the CNE and GSX2, because of their conservation throughout the vertebrate genome. **(B)** Analyses of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of $SP8$. 

Figure 24. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs687/hs701).
Figure 25. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs712/hs720).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod and fish lineages, cis regulatory networks of human genes are defined. (A) Conserved localization of CNE and FAM175A in tetrapod-teleost lineages demonstrate the linkage between them. Redundant expression pattern analysis of CNE and FAM175A also emphasizes this idea. (B) Studying the orthologous genomic content in the neighboring region of CNE enhancer in human and teleost lineages demonstrate the relationship between CNE and its target gene GPR85.
Target genes of CNE enhancers are identified through orthology mapping in Human and teleost fishes. (A) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *EBF3*. Redundant expression pattern of the gene and CNE is also in accordance with each other. (B) Conservation of *ZFHX4* and *HNF4G* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) CNE residing between *ETV1* and *ARL4A* in humans is also conserved along with *ETV1*, with differential loss and gain of bystander genes, in teleost fishes. Comparison of reporter gene expression of CNE enhancer and *ETV1* also confers *ETV1* as target gene of CNE. (B) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with *IRX4*. 

**Figure 27. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs749/hs754).**
Figure 28: Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs755/hs775).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Conservation of *GPR101* and *ZIC3* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Conserved intronic localization of CNE and *ATP9B* in tetrapod-teleost lineages demonstrate the linkage between them. Redundant expression pattern analysis of CNE and *ATP9B* also emphasizes this idea.
Target genes of CNE enhancers are identified through orthology mapping in Human and teleost fishes. (A) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *THSD7A*. Redundant expression pattern of the gene and CNE is also in accordance with each other. (B) Conservation of *DERA* and *MGST1* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
Figure 30. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs595/hs607).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. (A) Not only the CNE but also its intronic location in ADK, conserved in both human and teleost lineages, suggests the link exists between CNE and ADK which is further confirmed by endogenous expression pattern analysis of both the CNE and the gene. (B) Conservation of LMO3 and MGST1 throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
A

Human
Medaka
Stickleback
Zebrfish
Fugu
Tetradon

200kb
100kb
100kb
100kb
50kb
50kb

CNE_BARHL2 / LRR8C
chr1
chr4
grpVIII
chr6
sca67
chr1

B

Human
Zebrfish
Stickleback
Fugu

100kb
100kb
100kb
100kb

CNE_FIGN/KCNH7
chr2
chr9
grpXVI
sca46

GRB14  MGA75B  FIH1  FIGN  GCA  KCNH7  COBLL1
Figure 31. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs612/hs640).

Target genes of CNE enhancers are identified through orthology mapping in Human and teleost fishes. (A) Conservation of *LBARHL2* and *LRRC8C* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Conservation of *FIGN* and *KCNH7* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. (A) Conservation of PAX-1 and NKX2-2 throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Analyses of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of PPP2R2D.
Figure 33. Human CNE-enhancers by tracing the genic context of their orthologous copies in teleost fish lineage (hs540/hs672).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  (A) Analyses of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of *DACH1*.  (B) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with *EMX2*. 
Figure 34. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs679/hs529).

Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. **(A)** CNE residing between *FUSSEL18* and *SMAD2* in humans is also conserved along with *SMAD2*, with differential loss and gain of bystander genes, in teleost fishes. Comparison of reporter gene expression of CNE enhancer and *SMAD2* also confers *SMAD2* as target gene of CNE. **(B)** By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *SH3GL2*. Redundant expression pattern of the gene and CNE is also in accordance with each other.
Figure 35. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs536/hs671).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. **(A)** By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *TSHZ3*. Redundant expression pattern of the gene and CNE is also in accordance with each other. **(B)** Analyses of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of *DPYD*. 
Figure 36. Comparing the human-fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes (hs541/hs543).

Target genes of CNE enhancers are identified through orthology mapping in Human and teleost fishes. (A) Conservation of *SIX2* and *SIX3* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with *ISL1*. 
Figure 37. Human CNE-enhancers by tracing the genic context of their orthologous copies in teleost fish lineage (hs553/hs559).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. **(A)** Conservation of *HAT1* and *DLX* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. **(B)** Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of *PITX2*. 
A

Human

CNE-FOXG1B  500kb  chr14

Zebralfish

50kb  chr17

C14orf23  FOXG1B  PRKD1  FOXG1A

B

Human

1000kb  CNE-ZFH1XB  chr2

Medaka

100kb  chr2

Fugu

100kb  chr2

Tetradon

100kb  chr3

ORC4  ACVR2A  ZFH1XB  MBD5
Figure 38. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs566/hs568).

Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. (A) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *FOXG1B*. Redundant expression pattern of the gene and CNE is also in accordance with each other. (B) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of *ZFHX1B*. 
Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) Conservation of *LMO3* and *DERA* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of *LHX1*. Redundant expression pattern of the gene and CNE is also in accordance with each other.
Figure 40. Comparing the human-fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes (hs407/hs416).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. (A) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of ZFHX1B. (B) Conservation of TBR1 and RBMS1 throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE.
Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Conservation of *DLX1* and *DLX2* throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with *FGF13*.
Figure 42. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs435/hs242).

Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. (A) Conservation of ZNF312 and CADPS throughout the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of SP3.
Figure 43. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs281/hs118).

Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) Genic architecture analysis of the orthologous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with TFEB. (B) By tracing the genomic content around the CNE in tetrapod and fish lineages it becomes evident that the CNE is regulatory element of POLA. Redundant expression pattern of the gene and CNE is also in accordance with each other.
A

- Human
  - 500kb CNE-SALL1/NKD1
  - chr16

- Zebrashish
  - 200kb
  - chr7

- Stickleback
  - 100kb
  - grpI

- Medaka
  - 200kb
  - chr3

- Fugu
  - 200kb
  - sca28

B

- Human
  - 100kb
  - CNE-GLI3
  - chr7

- Zebrashish
  - 100kb
  - chr24

- Stickleback
  - 100kb
  - grpXXI

- Medaka
  - 100kb
  - chr20

- Fugu
  - 10kb
  - sca210

- Tetradon
  - 10kb
  - chr6
Figure 44. Comparing the human-fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes (hs76/hs111).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. (A) Conservation of SALL1 and NKD1 through out the vertebrate genome and endogenous expression pattern analysis of CNE matches with both these genes proposes them as target genes of CNE. (B) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of GLI3.
Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of ZNF503. Conservation of duplicated copy of this gene along with the CNE in fishes verifies this suggestion. (B) Duplication event of the CNE occurred only in humans and the gene entangled with the CNE, in duplicated copy, is TCF. Genic architecture analysis of the orthologous and paralogous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with TCF12.
A

B
Systematic analysis of the surrounding region of CNE enhancers, conserved across the tetrapod-teleost lineages, carved a pathway to associate the CNE enhancer to its target genes. (A) Duplication is occurred in both human and fish lineages. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy in human and fishes suggests the association of this enhancer with ZNF423. (B) Duplication is occurred in both human and fish lineages. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy in human and fishes suggests the association of this enhancer with ZNF521.
Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of ZNF503. Conservation of duplicated copy of this gene along with the CNE in fishes verifies this suggestion. (B) Duplication is occurred in only in teleost fishes, and the gene entangled with the CNE, in duplicated copy, is OTX1. Genic architecture analysis of the orthologous and paralogous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with OTX1.
Figure 48. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs399/hs262).

Through Comparative genomic analysis of functionally defined CNE enhancers, across tetrapod-teleost lineages, cis regulatory networks of human genes are defined. (A) Duplication is occurred in both human and fish lineages. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy in human and fishes suggests the association of this enhancer with BCL11A. (B) Duplication is occurred in fishes. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy in human and fishes suggests the association of this enhancer with CRHBP.
Gene, controlled by specific regulatory elements, are identified through the systematic analysis of orthologous genomic content, which harbors functionally identified CNE enhancers, of tetrapod-teleost lineages. (A) Analysis of expression pattern and conservation in tetrapod and fish lineages shows that CNE-enhancer is regulating the expression of IRX4. Conservation of duplicated copy of this gene along with the CNE in fishes verifies this suggestion. (B) Duplication is occurred in both human and teleost fishes, and the gene entangled with the CNE, in duplicated copy, is LMO3. Genic architecture analysis of the orthologous and paralogous loci holding the CNE enhancers in vertebrate genome recommends the association of CNE with LMO3.
Figure 50. Predicting the target genes of human enhancers by analyzing their genic environment within and across vertebrate genomes (hs921/hs915).

(A) CNE-enhancer within intron of human CENTG2 gene is associated with neighboring GBX2. (B) A human CNE-enhancer positioned in the intergenic space between human EDNRB and POU4F1 is associated with RNF219 and RBM26 according to the endogenous expression pattern analysis of genes and CNE-induced reporter gene expression.
(A) The physical location of human CNE-enhancer within the intronic region of $KIAA1900$ ($KLHL32$) is conserved in its orthologous intervals in fish lineages, along with other bystander genes. However the endogenous expression pattern of $KIAA1900$ and $MMS22L$ matches with that of reporter gene expression, clearly inferring the association of enhancer with $KIAA1900$ and $MMS22L$. (B) Analyzing the expression pattern and conservation in fish lineage shows that an intragenic CNE-enhancer is regulating the expression of $TRPS1$ gene.

Figure 51. Analyzing the genic environment of human CNE-enhancers in teleost fish orthologous loci and expression pattern of reporter gene helps in identifying their target genes (hs676/hs919).
Figure 52. Comparative analysis helps in assigning the CNE-enhancer to its target genes (hs329/hs675).

(A) A human CNE-enhancer positioned in the intergenic space between human ACADSB and HMX3 is associated with HMX2 according to the endogenous expression pattern analysis of genes and CNE-induced reporter gene expression. (B) CNE-enhancer within an intron of human ARHGAP15 gene is associated with neighboring ZEB2 gene. Syntenic and expression pattern analysis supports this association.
(A) Conserved localization of CNE within the intronic region of ZFHX3, across the vertebrate lineage (except Tetraodon) associates this CNE-enhancer with its host gene. Analysis of expression pattern of genes and CNE-induced reporter gene expression along with syntenic analysis also associates this CNE with DHX38 gene. (B) A human CNE, present within the Intergenic region between MMS22L and POU3F2 genes, is associated with POU3F2 genes. Expression pattern of MMS22L is not matched with CNE-induced reporter gene expression.
An intragenic CNE is associated with its harboring gene C9orf28 (FAM125B). Synteny and expression pattern analysis also associate this CNE with PBX3 and LMX1B genes. A human CNE is present between RALGAPA2 and XRN2. Comparative syntenic analysis reveals that XRN2 is lost in fish lineage. Expression pattern analysis and interspecies conservation supports the association of this CNE-enhancer with RALGAPA2 and NKX2-2 genes.
Figure 55. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs271).

(A) CNE-enhancer within an intron of human \textit{FAM172A} gene is associated with neighboring \textit{NR2F1} gene according to the expression pattern analysis.
Figure 56. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs860/hs646).

(A) A human CNE is present within intergenic region of *GTPBP9* and *SP9* genes. According to the expression pattern analysis and interspecies genic conservation, this CNE is associated with *SP3* and *PDK1* genes. (B) An intragenic CNE is associated with its harboring gene *HAT1*. Syntenic and expression pattern analysis also associate this CNE with *CYBRD1* gene.
Figure 57. Analyzing the genic architecture of human (hs841/hs809).

(A) A CNE is present within intron of KIAA1598 gene. This CNE is associated with KIAA1598 and HSPA12A genes. (B) Conserved intragenic position of CNE-enhancer and expression pattern analysis clearly associates this CNE with its harboring gene LMO4.
(A) A human CNE-enhancer is located within the intergenic region between ZFAND2A and UNCX genes. Endogenous expression pattern and CNE-induced reporter gene expression suggest the association between this CNE and UNCX. Expression pattern of ZFAND2A is unrelated to CNE expression pattern. (B) A CNE is positioned between MEIS2 and TMCO5 at human chromosome. Careful syntenic analysis shows that TMCO5 is lost in teleost fish lineage, leaving the MEIS2 best candidate for CNE association. Moreover, analysis of endogenous gene expression of MEIS2 and CNE-induced reporter gene activity pattern clearly associates this CNE with MEIS2.
Conserved localization of human CNE within the intron of MRPS9 gene, in human and teleost fish lineages, proposes the association between CNE and its harboring gene. But careful expression pattern analysis of both, the genes and CNE-enhancer suggests that this CNE is regulating the POU3F3 and NCK2 genes. (B) A human CNE is located within the intergenic region between PARP8 and ISL1 genes. As, expression pattern of ISL1 is not in accordance with CNE-enhancer, this CNE is associated with PARP8 gene.
(A) Conserved positioning of human CNE between *PLSCR5* and *ZIC4* indicates that this CNE is associated with its flanking genes, but expression pattern supports *ZIC1* gene to be the target for this CNE. (B) A human CNE is associated with one of its flanking genes, *POU3F1*, and a neighboring gene *UTP11L*. The expression pattern of other flanking gene *RRAGC* is not in accordance with CNE-induced reporter gene expression.

**Figure 60.** Comparing the human-fish genic content and their reporter gene expression lead to association of CNE enhancers to their respective genes (hs213/hs238).
Conserved allotment of a human CNE between its flanking genes, *SHFM1* and *DLX6*, across human and teleost fish lineages, and endogenous expression pattern analysis clearly associates this CNE with its flanking genes.

(A) Conserved allotment of a human CNE between its flanking genes, *SHFM1* and *DLX6*, across human and teleost fish lineages, and endogenous expression pattern analysis clearly associates this CNE with its flanking genes. (B) A human CNE present within the intron of *SMG6* gene is associated with a neighbor gene *HIC1*. 

Figure 61. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs298/ hs924).
A CNE-enhancer is located within the Intergenic region between *SORL1* and *BLID* genes in human genome. Careful syntenic analysis revealed that *BLID* is lost in fish genome. Analysis of expression pattern of genes and CNE-induced reporter gene activity associates this CNE with a neighboring gene, *UBASH3B*. Although, a human CNE is having conserved localization within intron of *SOX6* gene, across human and teleost fish lineages, expression pattern analysis supports the association between this CNE and *PLEKHA7* gene.

**Figure 62**. Comparative analysis and expression pattern studies help in associating the enhancer to its target gene (hs872/hs236).
Expression pattern analysis of conserved genes with CNE-induced reporter gene expression associates this CNE with SATB1, PLCL2 and RAB5A genes. This association is also supported by conserved synteny across human and teleost fish genomes. (B) One of the flanking genes of a human CNE, PKHD1, is lost in fish genomes. There are two conserved genes near the CNE, TFAP2B and TFAP2D, which are suggested target genes of this CNE-enhancer.
Figure 64. Human CNE-enhancers by tracing the genic context of their orthologous copies in teleost fish lineage (hs258/hs764).

(A) Although, positioning of a human intragenic CNE in conserved across human and teleost fish lineages, expression pattern analysis indicates the SOX2 as the target gene for this CNE. (B) A human CNE is located within the Intergenic region between ATG4C and FOXD3 and this localization is conserved across human and teleost fish lineages. Expression pattern of ATG4C is different from that of CNE-induced reporter gene. Careful syntenic and expression pattern analysis reveals that this CNE is associated with FOXD3 and DOCK7 genes.
(A) Intergenomic conserved synteny and expression pattern profile associates this human CNE with \textit{PRKACB} gene. (B) A human CNE is associated with \textit{PROX1} and \textit{SMYD2} gene. One of the flanking genes, \textit{RPS6KC1}, is lost in fish lineage.
(A) Careful syntenic analysis of CNE in human and fish lineage reveals that CNE is duplicated only in fish genomes. Conserved localization pattern of duplicated CNEs and their corresponding paralogous gene, and expression pattern analysis clearly refers NR2F1 as the target gene for this CNE. (B) A CNE is duplicated only in fish lineage (Except Zebrafish). Conserved localization pattern of duplicated CNEs and their corresponding paralogous gene, and expression pattern analysis clearly refers ZNF503 as the target gene for this CNE.

Figure 66. Predicting the target genes of human enhancers by analyzing their genic environment within and across vertebrate genomes (hs271/hs320).
Figure 67. Analyzing the genic environment of human CNE-enhancers in teleost fish orthologous loci and expression pattern of reporter gene helps in identifying their target genes (hs631/hs297).

(A) Syntenic analysis of a human CNE in teleost fish lineage reveals that this CNE is duplicated only in Medaka. CNE duplication pattern and endogenous expression profile suggests that this CNE is associated with ZNF703 and dCNE is associated with paralog ZNF503. (B) A CNE is duplicated only in Zebrafish. Intergenomic conserved synteny, duplication pattern and expression profile refers NEUROD6 as the target gene for this CNE.
A CNE is duplicated only in Zebrafish. Intergenomic conserved synteny, duplication pattern and expression profile refers $SP8$ as the target gene for this CNE.

**Figure 68.** Comparative analysis helps in assigning the CNE-enhancer to its target genes (hs110).
Duplication of human enhancer at the root of vertebrate lineage and preservation of linkage of duplicated enhancers with \textit{BCL11} family members both within and across genomes, unmistakably suggest the specificity of these duplicated cis-regulatory regions towards \textit{BCL11B} and \textit{BCL11A} promoters.

\textbf{Figure 69 . Target gene identification of human CNE-enhancers through orthology mapping (hs622).}
Human enhancer-target gene duplicated early in vertebrate history, before tetrapod-teleost split. Conserved association between duplicated enhancers and IRX paralogs indicate that these duplicated human enhancers (CNE-IRX2, CNE-IRX1 and CNE-IRX5, CNE-IRX3) are specific to IRX2, IRX1, IRX5 and IRX3 respectively.

Figure 70. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals helps in identifying their target genes (hs261).
Human enhancer-target gene duplicated early in vertebrate history, before tetrapod-teleost split. Conserved association between duplicated enhancers and $EBF$ paralogs indicate that these duplicated human enhancers ($CNE-EBF3$ and $CNE-EBF1$) are specific to $EBF3$ and $EBF1$, respectively.

**Figure 71. Assigning the target genes to CNE-enhancers through comparative genomic analysis (hs232).**

Human enhancer-target gene duplicated early in vertebrate history, before tetrapod-teleost split. Conserved association between duplicated enhancers and $EBF$ paralogs indicate that these duplicated human enhancers ($CNE-EBF3$ and $CNE-EBF1$) are specific to $EBF3$ and $EBF1$, respectively.
Duplication of human enhancer at the root of vertebrate lineage and preservation of linkage of duplicated enhancers with *FOXP* family members both within and across genomes, unmistakably suggest the specificity of these duplicated cis-regulatory regions (*CNE-FOXP2* and *CNE-FOXP1*) towards *FOXP2* and *FOXP1* promoters.
Human enhancer-target gene duplicated early in vertebrate history, before tetrapod-teleost split. Conserved association between duplicated enhancers and IRX paralogs indicate that these duplicated human enhancers (CNE-IRX5, CNE-IRX6 and CNE-IRX2, CNE-IRX4) are specific to IRX5, IRX6, IRX2 and IRX4 respectively.

Figure 73. Analyzing the genic architecture of human (hs26).
Figure 74. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1802/hs1651).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  (A) Both synteny analysis and endogenous expression pattern clearly associate the CNE to human $ZEB2$ gene.  (B) CNE-enhancer within an intergenic space between human $CEP135$ and $KIA1211$ gene is associated with neighboring $EXOC1$ gene.
Figure 75. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1496/hs1450).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Both synteny analysis and endogenous expression pattern clearly associate the CNE to human SOX11 gene. (B) CNE-enhancer within an intergenic space between human NFIA and TM2D1 is associated with NFIA gene.
Figure 76. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1362/hs1434).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) CNE-enhancer within an intron of human \textit{SETBP1} gene is associated with neighboring \textit{SYT4}. (B) CNE-enhancer within an intron of human \textit{C10orf11} gene is associated with neighboring \textit{KCNMA1}. 
Figure 77. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1325/hs1437).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) The localization of a human CNE-enhancer in the intergenic space between NPVF and NFE2L3 genes suggests that this enhancer might be associated with one of these genes but not with SNX10 that is present ~ 104.8 kb downstream of NFE2L3. However the careful analysis of genic context of orthologous regions of this element in teleost fish and human clearly associate the CNE to human SNX10 gene. (B) CNE-enhancer within an intergenic space between human COMTD1 and ZNF503 genes. Careful analysis of genic context of orthologous regions of this element in teleost fish and human clearly associate the CNE to human ZNF503.
Figure 78. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1344/hs1507).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  

(A) CNE-enhancer within an intergenic space between human *OPA1* and *HES1* is associated with *OPA1*.  

(B) Syntenic analysis associate CNE-enhancer to *SLC4A3* gene but its expression pattern is not clearly defined but syntenic analysis provides a strong evidence is favor of its association with *SLC4A3* gene.
Figure 79. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1273/hs1268).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) CNE-enhancer within an intergenic space between human *OSR1* and *TTC32* is associated with *OSR1*. (B) CNE-enhancer within an intron of human *RBM33* gene is associated with neighboring *SHH* gene.
Figure 80. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1257/hs1122).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Both synteny analysis and endogenous expression pattern clearly associate the CNE to human \textit{ESRRG} gene. (B) Both synteny analysis and endogenous expression pattern clearly associate CNE-enhancer within an intron of human \textit{PAH} gene with neighboring \textit{IGF1} gene.
Figure 81. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1791/hs1305).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  

(A) CNE-enhancer is positioned within an intergenic space between human OTX2 and EXOC5 on chromosome 14 suggest its association with either of these genes. however the comparative syntenic analysis in fish lineage and expression studies of neighboring conserved genes infers that this CNE might be regulating the expression of downstream MUDENG and upstream c14orf101 gene.  

(B) CNE-enhancer within an intron of human MRPS28 gene is not associated with the promoter of the same gene, rather comparative syntenic analysis in fish lineage and expression studies of neighboring conserved genes infers that this CNE is acting the at a distance of ~150.8 kb downstream on HEY1 and STMN2 genes.
Figure 82. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1333/hs1304).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) An CNE-enhancer within the intronic region of NBEA is conserved in its orthologous intervals in fish lineages, along with downstream DCLK1 and other bystander genes. However the endogenous expression pattern of NBEA and DCLK1 matches with that of reporter gene expression, clearly inferring the association of enhancer with NBEA and downstream DCLK1 genes. (B) The conserved physical location of CNE-enhancer within intronic region of C10orf34 gene suggest its association with C10orf34 gene, however the expression studies of neighboring conserved genes infers that this CNE might be regulating the expression of USP25 and NRIP1 genes rather than C10orf34.
Figure 83. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1251/hs1235).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) CNE-enhancer is positioned within an intergenic space between human C3orf55 and SHOX2 on chromosome 3. Comparative syntenic analysis in fish lineage and expression pattern studies suggest the association of this CNE with SHOX2 or RSRC1. (B) The CNE-enhancer located within intronic region of PBX1 in human and some fish lineages is conserved along ALDH9A1 and other bystander genes. The comparative syntenic analysis of human locus with multiple fish lineages suggest its association with PBX1 and ALDH9A1, moreover expression pattern studies also favors this association.
Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) The physical location of human CNE enhancer within the intronic region of TEAD1 is conserved in its orthologous intervals in fish lineages, along with PTH and BTBD10 genes. However the endogenous expression pattern is clearly inferring the association of enhancer with TEAD1, PTH and BTBD10. (B) An intergenic CNE-enhancer showed conserved positioning with respect to JAG1 and BTBD3 along with MKKS genes in human and teleost fish lineages. The expression pattern of these genes is highly redundant and in accordance with CNE-enhancer induced reporter expression, and suggests that this enhancer might be influencing the expression of all of these genes.
Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  

(A) Analyzing the expression pattern and conservation in fish lineage shows that CNE-enhancer is regulating the expression of $FZD8, GJD4, CCNY$.  

(B) Analyzing the expression pattern and conservation in fish lineage shows that human CNE-enhancer positioned in the intergenic space between human $SPRY1$ and $ANKRD50$ gene is regulating the expression of flanking $SPRY1$ and downstream $NUDT6$ and $FGF2$ genes.
Figure 86. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1652/hs1301).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  (A) The conserved physical location of CNE-enhancer within intronic region of ATOH8 in fish and human lineages suggest its association with ATOH8 gene, however the expression studies of neighboring conserved genes infers that this CNE might be regulating the expression of ST3GAL5 rather than ATOH8.  (B) Human CNE-enhancer and target gene duplicated before tetrapod-teleost divergence. Comparative syntenic analysis of human duplicated loci in multiple fish lineages clearly suggest that these duplicated human enhancers are associated with the regulation of human SOX paralogs(SOX6,SOX5) residing on human chromosome 11 and 12.
The image illustrates genomic regions A and B with CNE-FOXP2 and CNE-SOX2 respectively. Regions are depicted across different species, including human, Fugu, stickleback, and medaka for region A, and human, zebrafish, and stickleback for region B. The diagram shows gene distributions and proximity with annotations for key genes involved.
Figure 87. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1798/hs1332).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  

(A) Human cis-regulatory element positioned in the intergenic space between \textit{MDFIC} and \textit{TFEC}, suggesting that this enhancer might be associated with one of these genes but not with \textit{FOXP2} that is present $\sim 1.11$ Mb upstream of \textit{MDFIC}. However the careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy on Tetraodon chromosome 2 clearly indicates that the only gene in the human locus that preserved its association with this CNE-enhancer in all teleost fish analyzed (even in duplicated loci of tetraodon) is \textit{FOXP2}. Therefore this human enhancer is unambiguously associated with the regulation of human \textit{FOXP2} gene.

(B) CNE-enhancer within an intergenic space between \textit{SOX2OT} and \textit{ATP11B} genes, suggesting that this enhancer might be associated with one of these genes but careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous (\textit{SOX9,SOX8}) in Stickleback clearly indicates that the only gene in the human locus that preserved its association with this CNE-enhancer in all teleost fish analyzed (even in duplicated loci of Stickleback) is \textit{SOX2} within intron of human \textit{SOX2OT} gene. Therefore this human enhancer is unambiguously associated with the regulation of human \textit{SOX2} gene.
Figure 88. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1309/hs1397).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) Human CNE-enhancer duplicated in human lineage is positioned within the intronic interval of NFIA gene on chromosome 1. Careful analysis of genic context of orthologous regions of this element in teleost fish and its duplicated paralogous copy on human chromosome suggests the association of this enhancer with NFIA gene. (B) Duplicated human CNE enhancer within an intergenic space between MALT1 and ZNF532 is associated with either of these genes where as careful analysis of genic context of orthologous regions of this element in teleost fish reveal that it is associated with ZNF532.
Figure 89. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1390/hs1258).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes.  (A) CNE-enhancer within an intron of human \textit{IGFBPL1} gene is duplicated in Human. Comparative syntenic analysis of this locus revealed that genomic interval encompassing this conserved enhancer in all lineages links this CNE with the regulation of neighboring \textit{ANKRD18A} & \textit{ANKRD29} gene. Although its expression pattern is not clearly defined but syntenic analysis provide a strong evidence in favor of its association with \textit{ANKRD18A} & \textit{ANKRD29} gene.  (B) Human CNE-enhancer is positioned within the intronic interval of \textit{PTCH1} gene. Comparative analysis of this locus in teleost fish revealed that genomic interval encompassing this conserved enhancer is duplicated in zebrafish (\textit{PTCH1},\textit{PTCH2}). The conserved linkage of CNE with \textit{PTCH1} in all lineages and its endogenous expression unmistakably links this CNE with the regulation of \textit{PTCH1} gene.
Figure 90. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1331/hs1322).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. **(A)** CNE-enhancer within an intron of human *POU2F1* gene is associated with the promoter of the same gene. Other human-fish conserved genes within this locus appeared to be bystanders as their expression pattern is unrelated to the activity of this cis-acting region. **(B)** CNE-enhancer within an intron of human *NEK7* gene is associated with the promoter of the same gene. Other human-fish conserved genes within this locus appeared to be bystanders as their expression pattern is unrelated to the activity of this cis-acting region.
Figure 91. Analysis of genic environment of human CNE-enhancers in teleost fish orthologous genomic intervals and observing their expression patterns helps in identifying their target genes (hs1475/hs1857).

Analysis of CNE-enhancers in humans and its orthologous genomic intervals teleost fish lineage helps in associating CNE to their target genes. (A) CNE-enhancer within an intron of human ROBO1 gene is associated with the promoter of same gene. (B) CNE-enhancer within an intergenic space between human SLC6A9 and KLF17gene is associated with neighboring B4GALT2 gene.