**Discovering the physical and functional connections utilizing phylogenetics of drug metabolic enzyme in dissimilar species with the help of computational algorithms.**

**Abstract**

The P450 superfamily enzyme in higher organism is an important enzyme for biotransformation of drugs and are therefore of significance for metabolite during intake food material date to date. The metabolizing using cytochromes P450 enzymes have a crucial part in the oxidative metabolism of a broad array of endogenous and exogenous compounds. At Present, more applications depend on the survival of a support phylogenetic tree for the approval of biological suppositions that are useful for researchers. The main goal of this project is to utilize phylogenetic tool on drug metabolism activity profiles and the functional clinical impact of genetic variation in drug metabolizing using P450 at different organisms.

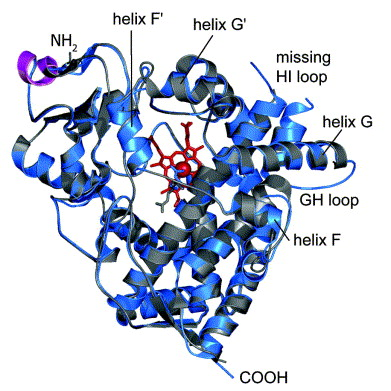
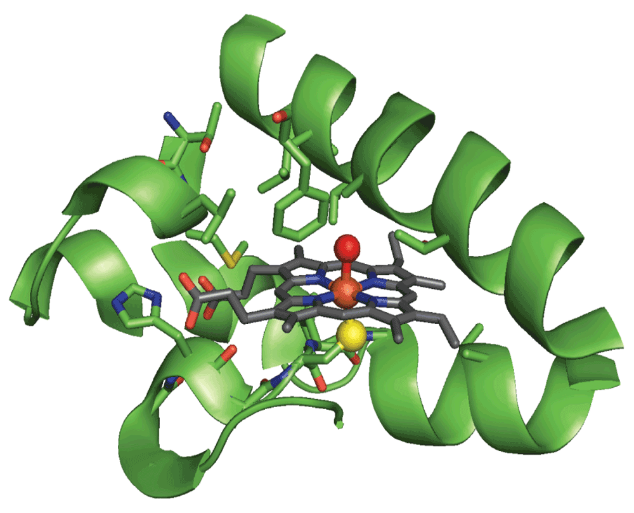
**Key words;** Cytochromes P450, drugs, phylogenetic tool

**Introduction**

Phylogenetics is an area of research which addresses various biological questions such as relationships among organism or genes, the demographic changes and migration patterns of species. To date, this technique is facilitated to extract the information at molecular level. This resources relationship information such as functional or evolutionary can be extracted by gathering phylogenetic relationship using DNA, RNA or Protein sequences at different organisms. The advance tool to compare genomes and exploit datasets obtained by NGS, the best way by performing analysis and reveal some good information.

The cytochromes P450 (CYPs) constitute the major enzyme family capable of catalyzing the oxidative biotransformation of most drugs and are therefore of significance for clinical pharmacology. The cytochrome enzymes have several families and groups, 57 commonly accepted functional human CYPs. Of these, CYP1, 2, and 3 groups are held responsible for the biotransformation of many small chemical and protein drugs in clinical applications. Cytochromes P450 enzymes are principally situated in the liver, there is a considerable proof that individual P450s are also conveyed in other tissues.

Common variation of this gene causes enzyme to function differently. Drug metabolism may be fast or slow depending on gene and polymorphism. If this information is known to the researchers, then can enhance target specific treatment in the medicinal world. Since, it is not practical to implement any study directly to the human being without being tested and approved clinically, functional relationships must be determined cautiously. Here, phylogeny is an asset to enhance this study by helping to determine functional relationship of the enzyme at different species.



**B.**

**Fig: 1 A.** Structure of cytochrome P450, **1B**. classic active site of the cytochrome P450, the aromatic peroxygenase heme-thiolate proteins.

**Materials and Method:**

Different tools such as MegaX, Clustalw, MAFFT and muscle are used. These tools use different algorithm that implement progressive approach for alignment of sequences. Algorithms used by Clustalw: It implements progressive approach for alignment of sequences. Once there is error then it will not be possible to correct while using Clustalw. Algorithm used by muscle: It implements iterative approach for alignment of sequence. It uses progressive and refinement process iteratively.

**Materials**

To know the genetic similarity at amino acid level and genetic distance, six animals sequences has been selected for the comparison and contrast studies.

Homo sapiens: (humans), Pan: (Chimpanzee), Macaca mulatta: (Rhesus Monkey), Oryctolagus cuniculus: (Rabbit),Sus: (Pig),Mus musculus: house mouse.

To know more about cytochrome P450 and its relation to evolution blastp analysis has been performed in 500 species in various levels of organisms.

**Sequences materials**

>Human p450

MLFPISMSATEFLLASVIFCLVFWVIRASRPQVPKGLKNPPGPWGWPLIGHMLTLGKNPHLALSRMSQQY

GDVLQIRIGSTPVVVLSGLDTIRQALVRQGDDFKGRPDLYTFTLISNGQSMSFSPDSGPVWAARRRLAQN

GLKSFSIASDPASSTSCYLEEHVSKEAEVLISTLQELMAGPGHFNPYRYVVVSVTNVICAICFGRRYDHN

HQELLSLVNLNNNFGEVVGSGNPADFIPILRYLPNPSLNAFKDLNEKFYSFMQKMVKEHYKTFEKGHIRD

ITDSLIEHCQEKQLDENANVQLSDEKIINIVLDLFGAGFDTVTTAISWSLMYLVMNPRVQRKIQEELDTV

IGRSRRPRLSDRSHLPYMEAFILETFRHSSFVPFTIPHRKLWVNPSEFLPERFLTPDGAIDKVLSEKVII

FGMGKRKCIGETIARWEVFLFLAILLQRVEFSVPLGVKVDMTPIYGLTMKHACCEHFQMQLRS

>Monkey p450

MLFRISMSATEFLLASLIFCLVFWVIRASRPRVPKGLKNPPGPWGWPLIGHILTLGKNPHLALSRMSQRYGDVLQIRIGSTPVLVLSGLDTIRQALVQQGDDFKGRPNLYSFTLISNGQSMSFGPDSGPVWAARRRLAQNGLKSFSIASDPASSSSCYLEEHVSKEAEVLISKLQEQMAGPGHFNPYRYVVISVANVICAICFGQRYDHNHQELLSLVNLSNNFGEVVGSGNPADFIPILRYLPNRSLNGFKDLNEKFHSFMQKMIKEHYKTFEKGHIRDITDSLIEHCQEKQLDENANIQLSDEKIVNVVLDLFGAGFDTVTTAISWSLMYLVTNPRVQRKIQEELDTVIGRSRRPRLSDRSHLPYMEAFILETFRHSSFVPFTIPHSTTRDTSLKGFYIPKGRCVFVNQWQINHDQKL

WVNPSEFLPERFITPDGAIDKVLSEKVILFGLGKRKCIGETIARWEVFLFLAILLQRVEFSVPPGVKVDMTPIYGLTMKHACCEHFQMQLRS

>Mouse p450

MEPSVLLLLALLVGFLLLLARGHPKSRGNFPPGPRPLPLLGNLLQMDRGGLLKSLIQLREKYGDVFTVHLGPRPVVMLCGTDTIREALVGQAEAFSGRGTVAVVEPTFKEYGVIFANGERWKTLRRFSLATMRDFGMGKRSVEERIQEEAQCLVEELRKSQGAPLDPTFLFQCITANVICSIVFGERFEYTDRQFLRLLELFYQTFSLISSFSSQMFELFSGFLKYFPGAHRQISKNLQELLDYIGHSVERHKATLDPSVPRDFIDIYLLRMEKEKSNQNAEFHHQNLMMSVLSLFFVGTETSSTTLHYGFLLMLKYPHVTEKVQKEIDQVIGSHRLPTLDDRTKMPYSDAVIHEIQRFSDLIPIGVPHRVTKDTLFRGYLLPKNTEVYPILSSALHDPQYFEQPDSFNPDQFLDANGAL

KKSEAFLPFSTGQIFDQKSVGKRICLGESIARSELFLFFTSILQNFSVASHVAPKDIDLTPKESGIGKIPPTYQICFLAR

>Chimpanzee p450

MGLEALVPLAVIVTIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHVDFQNTPYCFDQLRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQILGFGPRSQGVFLARYGPAWREQRRFSVSTLRNLGLGKKSLEQWVTEEAACLCAAFANHSGRPFRPNGLLDKAVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFLREVLNAIPVLLHIPALAGKVLRFQKAFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAEMEKAKGNPESSFNDENLRIVVADLFSAGIVTTSTTLAWGLLLMILHPDVQRRVQQEIDDVIGQVRRPEMGDQARMPYTTAVIHEVQRFGDIVPLGVTHMTSRDIEVQGFRIPKGTTLFTNLSSVLKDKAVWEKPFRFHPEHF

LDAQGHFVKPEAFLPFSAGRRACLGEPLARMELFLFFTSLLQHFSFSVPTGQPRPSHHGVFAFLVTPSPYELCAVPR

>Pig p450

MTALGITVALLVWLVTLLLISIWKHIHSSWKLPPGPFPLPIVGNIFQLDLKNIPKSFTMLAERYGPVFTVYLGSRRIVVLHGYKAVKEVLLHYKNEFSGRGEIPTFQVHKDKGVIFNNGPTWRDTRRFSLTTLRDFGMGKQGNEQRIQREAHFLLEALRKTHGQPFDPTFLIGCAPCNVISDILFRQHFDYNDKTCLRLMSMFNENFYLLSTGWIQLYNNFSGYLRYLPGSHRKLMKNISEIKDYALERVKDHRDSLEPSCPRDFTDTLLMEMEKEKYSAEPIYTLDNIAVTVADMFFAGTETTSTTLRYGLLILMKYPEVEEKLHEEIDRVIGPNRIPAIKDRLVMPYLDAVVHEIQRFIDLIPSNLPHEATRDTDFRDYIIPKGTVVIPTLDSVLYDSQEFPEPEKFKPEHFLNENGK

FKYSDHFKAFSAGKRVCVGEGLARMELFLFMAAILQHFNLKSLVDPKDIDLSPIAIGFAKIPPHYKLCVIPRSQV

>Rabbit p450

MVSDFGLPTFISATELLLASAVFCLVFWVAGASKPRVPKGLKRLPGPWGWPLLGHVLTLGKNPHVALARLSRRYGDVFQIRLGSTPVVVLSGLDTIKQALVRQGDDFKGRPDLYSFSFVTKGQSMIFGSDSGPVWAARRRLAQNALNSFSVASDPASSSSCYLEEHVSQEAENLISKFQELMAAVGHFDPYRYVVMSVANVICAMCFGRRYDHDDQELLSLVNLNDEFGKVAASGSPADFFLILRYLPNPALDTFKDLNERFYSFTQERVKEHCRSFEKGHIRDITDSLIKHYRVDRLDENANVQVSDEKTVGIVLDLFGAGFDTVTTAISWSLMYLVTKPRIQRKIQEELDAVVGRARRPRFSDRPQLPYLEAVIMETFRHTSFLPFTIPHSTTRDTSLGGFYIPKGRCVFVNQWQNNH

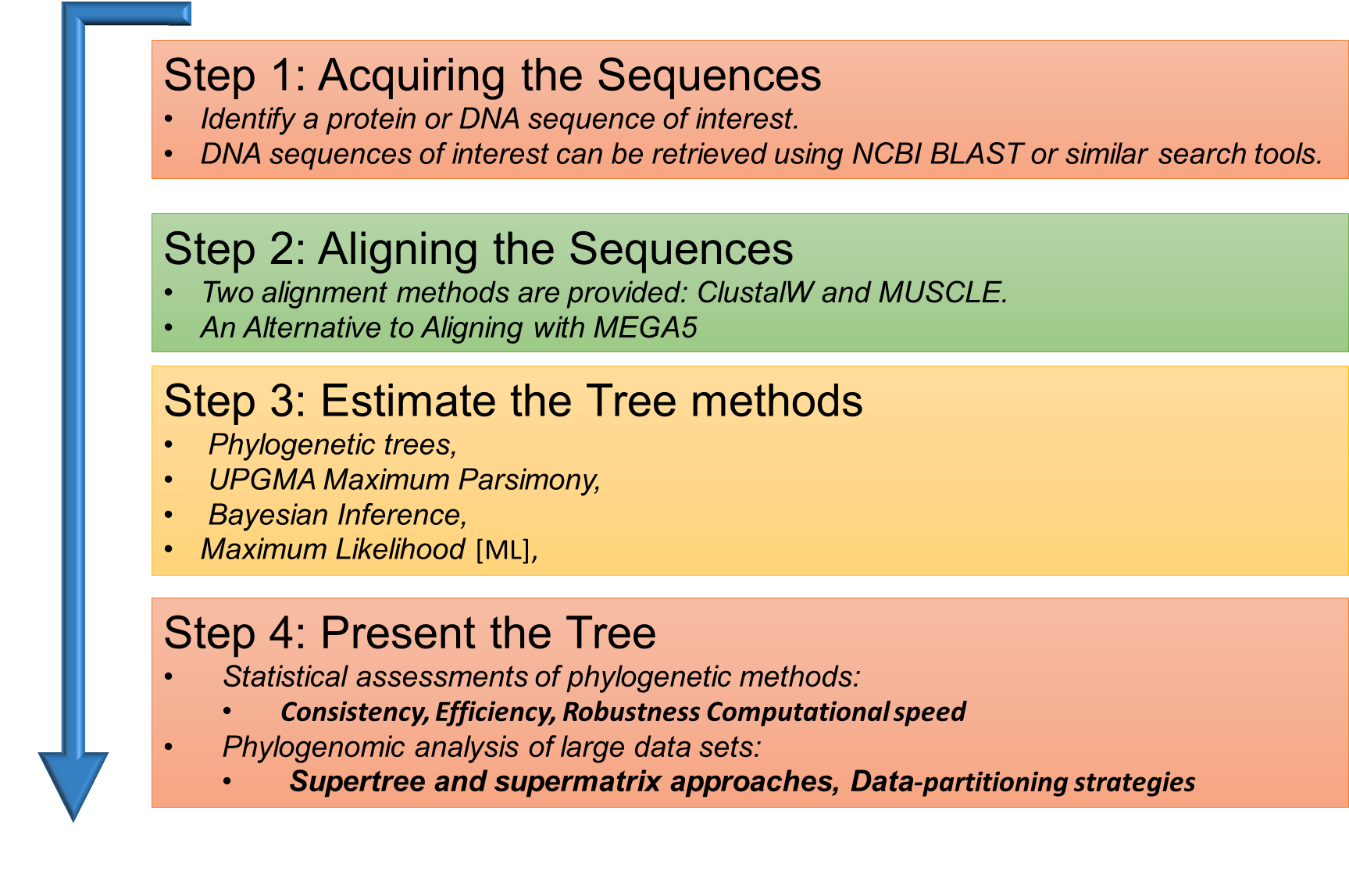
DPELWGDPEAFRPERFLTPSGAVDKALTEKVLLFGLGKRKCIGETIGRLEVFLFLATLLQQVEFSVSPGTTVDMTPIYGLTMKHARCEHFQAKLRFEA

**Operating Systems and Phylogeny analysis tool:**

Any operating system windows, mac or Linux can be used but the best choice for open source tool is Linux and MegaX.

**Building Phylogenetic Trees**

The following is the steps to build phylogenetic tree:



**Fig: 2 Flow charts of producing the phylogenetic tree of a gene of concern (GOI).**

**Results:**

Here, cytochrome P450 enzyme of human, mouse, pig, rabbit, monkey and chimpanzee sequences are analyze this tool. These organisms are primary organisms to test the drug metabolites in clinical studies for evaluation of drugs effectives.

**Multiple sequence alignment**

The desire organisms of cytochrome P450 sequences alignment using ClustalW

**Table 1. Multiple sequence alignment and similarity of cytochrome P450**

Rabbit MVSDFGLPTFISATELLLASAVFCLVFWVA-GASKPRVPKGLKRLPGPWGWPLLGHVLTL 59

Human ----MLFPISMSATEFLLASVIFCLVFWVI-RASRPQVPKGLKNPPGPWGWPLIGHMLTL 55

Monkey ----MLFRISMSATEFLLASLIFCLVFWVI-RASRPRVPKGLKNPPGPWGWPLIGHILTL 55

Chimpanzee ----------MGLE-ALVPLAVIVTIFLLLVDLMHRRQRWAARYPPGPLPLPGLGNLLHV 49

Mouse ------------MEPSVLLLLALLVGFLLLLARGHPKSRG--NFPPGPRPLPLLGNLLQM 46

Pig ----------MTALGITVALLVWLVTLLLISIWKHIHSSW--KLPPGPFPLPIVGNIFQL 48

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Rabbit GK-NPHVALARLSRRYGDVFQIRLGSTPVVVLSGLDTIKQALVRQGDDFKGRPDLYSFSF 118

Human GK-NPHLALSRMSQQYGDVLQIRIGSTPVVVLSGLDTIRQALVRQGDDFKGRPDLYTFTL 114

Monkey GK-NPHLALSRMSQRYGDVLQIRIGSTPVLVLSGLDTIRQALVQQGDDFKGRPNLYSFTL 114

Chimpanzee DFQNTPYCFDQLRRRFGDVFSLQLAWTPVVVLNGLAAVREALVTHGEDTADRPPVPITQI 109

Mouse DRGGLLKSLIQLREKYGDVFTVHLGPRPVVMLCGTDTIREALVGQAEAFSGRGTVAVVEP 106

Pig DLKNIPKSFTMLAERYGPVFTVYLGSRRIVVLHGYKAVKEVLLHYKNEFSGRGEIPTFQ- 107

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Rabbit VTKGQ--SMIFGSDSGPVWAARRRLAQNALNSFSVASDPASSSSCYLEEHVSQEAENLIS 176

Human ISNGQ--SMSFSPDSGPVWAARRRLAQNGLKSFSIASDPASSTSCYLEEHVSKEAEVLIS 172

Monkey ISNGQ--SMSFGPDSGPVWAARRRLAQNGLKSFSIASDPASSSSCYLEEHVSKEAEVLIS 172

Chimpanzee LGFGPRSQGVFLARYGPAWREQRRFSVSTLRNLGLGKK-------SLEQWVTEEAACLCA 162

Mouse T---FKEYGV-IFANGERWKTLRRFSLATMRDFGMGKR-------SVEERIQEEAQCLVE 155

Pig V---HKDKGV-IFNNGPTWRDTRRFSLTTLRDFGMGKQ-------GNEQRIQREAHFLLE 156

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Rabbit KFQELMAAVGHFDPYRYVVMSVANVICAMCFGRRYDHDDQELLSLVNLNDEFGKVAASGS 236

Human TLQELMAGPGHFNPYRYVVVSVTNVICAICFGRRYDHNHQELLSLVNLNNNFGEVVGSGN 232

Monkey KLQEQMAGPGHFNPYRYVVISVANVICAICFGQRYDHNHQELLSLVNLSNNFGEVVGSGN 232

Chimpanzee AFANHSG--RPFRPNGLLDKAVSNVIASLTCGRRFEYDDPRFLRLLDLAQEGLKEESGFL 220

Mouse ELRKSQG--APLDPTFLFQCITANVICSIVFGERFEYTDRQFLRLLELFYQTFSLISSFS 213

Pig ALRKTHG--QPFDPTFLIGCAPCNVISDILFRQHFDYNDKTCLRLMSMFNENFYLLSTGW 214

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Rabbit P---ADFFLILRYLPNPALDTFKDLNERFYSFTQERVKEHCRSFEKG-HIRDITDSLIKH 292

Human P---ADFIPILRYLPNPSLNAFKDLNEKFYSFMQKMVKEHYKTFEKG-HIRDITDSLIEH 288

Monkey P---ADFIPILRYLPNRSLNGFKDLNEKFHSFMQKMIKEHYKTFEKG-HIRDITDSLIEH 288

Chimpanzee REVLNAIPVLL-HIPALAGKVLRFQK-AFLTQLDELLTEHRMTWDPAQPPRDLTEAFLAE 278

Mouse SQMFELFSGFLKYFPGAHRQISKNLQ-ELLDYIGHSVERHKATLDPS-VPRDFIDIYLLR 271

Pig IQLYNNFSGYLRYLPGSHRKLMKNIS-EIKDYALERVKDHRDSLEPS-CPRDFTDTLLME 272

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Rabbit YRVDRLDENANVQVSDEKTVGIVLDLFGAGFDTVTTAISWSLMYLVTKPRIQRKIQEELD 352

Human CQEKQLDENANVQLSDEKIINIVLDLFGAGFDTVTTAISWSLMYLVMNPRVQRKIQEELD 348

Monkey CQEKQLDENANIQLSDEKIVNVVLDLFGAGFDTVTTAISWSLMYLVTNPRVQRKIQEELD 348

Chimpanzee MEK--AKGNPESSFNDENLRIVVADLFSAGIVTTSTTLAWGLLLMILHPDVQRRVQQEID 336

Mouse MEK--EKSNQNAEFHHQNLMMSVLSLFFVGTETSSTTLHYGFLLMLKYPHVTEKVQKEID 329

Pig MEK--EKYSAEPIYTLDNIAVTVADMFFAGTETTSTTLRYGLLILMKYPEVEEKLHEEID 330

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Rabbit AVVGRARRPRFSDRPQLPYLEAVIMETFRHTSFLPFTIPHSTTRDTSLGGFYIPKGRCVF 412

Human TVIGRSRRPRLSDRSHLPYMEAFILETFRHSSFVPFTIPH-------------------- 388

Monkey TVIGRSRRPRLSDRSHLPYMEAFILETFRHSSFVPFTIPHSTTRDTSLKGFYIPKGRCVF 408

Chimpanzee DVIGQVRRPEMGDQARMPYTTAVIHEVQRFGDIVPLGVTHMTSRDIEVQGFRIPKGTTLF 396

Mouse QVIGSHRLPTLDDRTKMPYSDAVIHEIQRFSDLIPIGVPHRVTKDTLFRGYLLPKNTEVY 389

Pig RVIGPNRIPAIKDRLVMPYLDAVVHEIQRFIDLIPSNLPHEATRDTDFRDYIIPKGTVVI 390

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Rabbit VNQWQNNHDPELWGDPEAFRPERFLTPSGAVDKALTEKVLLF---------GLGKRKCIG 463

Human ---------RKLWVNPSEFLPERFLTPDGAIDKVLSEKVIIF---------GMGKRKCIG 430

Monkey VNQWQINHDQKLWVNPSEFLPERFITPDGAIDKVLSEKVILF---------GLGKRKCIG 459

Chimpanzee TNLSSVLKDKAVWEKPFRFHPEHFLDAQGHFVKP--EAFLPF---------SAGRRACLG 445

Mouse PILSSALHDPQYFEQPDSFNPDQFLDANGALKKS--EAFLPFSTGQIFDQKSVGKRICLG 447

Pig PTLDSVLYDSQEFPEPEKFKPEHFLNENGKFKYS--DHFKAF---------SAGKRVCVG 439

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Rabbit ETIGRLEVFLFLATLLQQVEFSVSPGTTV-DMTPIYGLTMKH----ARCEHFQAKLRFEA 518

Human ETIARWEVFLFLAILLQRVEFSVPLGVKV-DMTPIYGLTMKH----ACCEHFQMQLRS-- 483

Monkey ETIARWEVFLFLAILLQRVEFSVPPGVKV-DMTPIYGLTMKH----ACCEHFQMQLRS-- 512

Chimpanzee EPLARMELFLFFTSLLQHFSFSVPTGQPRP-SHHGVFAFLVTPSPYELCAVPR------- 497

Mouse ESIARSELFLFFTSILQNFSVASHVAPKDIDLTPKESGIGKIPPTYQICFLAR------- 500

Pig EGLARMELFLFMAAILQHFNLKSLVDPKDIDLSPIAIGFAKIPPHYKLCVIPRSQV---- 495

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**Table 2. Summary of Percent Identity Matrix - generated by utilizing Clustal2.1**

1: Rabbit **100.00** 75.57 76.37 31.61 29.65 29.61

2: Human 75.57 **100.00** 93.79 29.01 29.56 28.85

3: Monkey 76.37 93.79 **100.00** 29.75 29.44 29.19

4: Chimpanzee 31.61 29.01 29.75 **100.00** 40.16 38.04

5: Mouse 29.65 29.56 29.44 40.16 **100.00** 48.98

6: Pig 29.61 28.85 29.19 38.04 48.98 **100.00**

**Table 3. Multiple sequence alignment by MEGA-X**

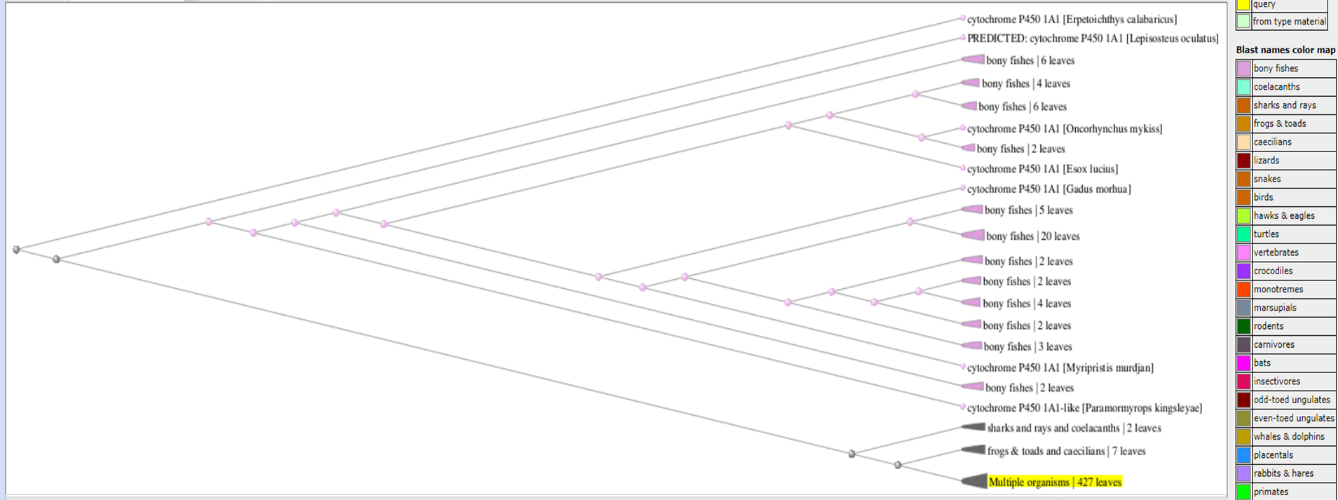
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**Pylogentic tree analysis**

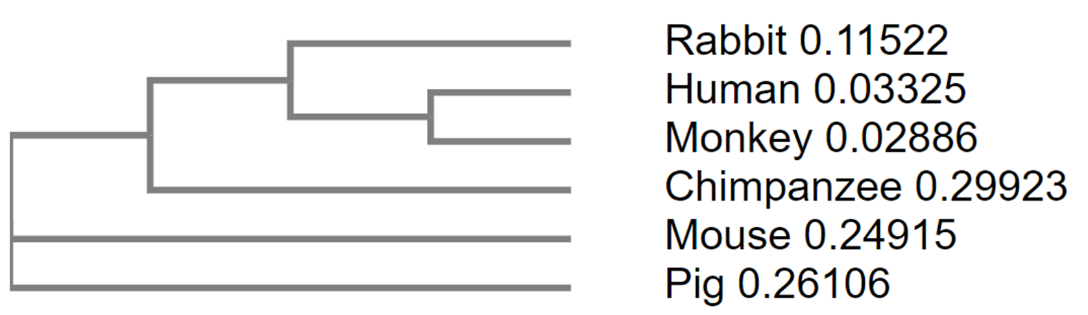
A phylogenetic tree is **a visible** **illustration** of **the connection** **between** **one of a kind** organisms, **showing** the **direction** **through** evolutionary time from a **commonplace** ancestor to **distinctive** descendants. Similarities and divergence **amongst** **related** **biological** sequences **found out** **by** **series** alignment **often** **need to** be rationalized and pictured **within the** context of phylogenetic **timber**. Molecular phylogenetics is the **division** of phylogeny that evaluates genetic, heritable molecular **variations**, predominately in DNA sequences, to **enhance** **data** on an organism’s evolutionary connections. The resemblance of **biological** **abilities** and molecular processes in **dwelling** organisms sharply **indicates** that species originated from a **rare** ancestor. Molecular phylogenetics **creates the use of** **structure** and **function** of molecules **and how** they **vary** **over the years** **to infer** **these** evolutionary connections. From **those** considerations, **it's far** **feasible** to **choose** the **procedures** **through** which **multiplicity** **among** species has been **accomplished**. The **outcome** of a molecular phylogenetic **evaluation** is conveyed in a phylogenetic tree.

**Fig: 3 Phylogrnetic tree by Fast minimum evolution method at Grishin protein distance among various speicies**

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Yellow color indicated all higher specices such as human, mice rabbit, pig and money etc.

**Fig: 4 Neighbour-joining tree: with no space corrections in the branch length at Cladogram.**

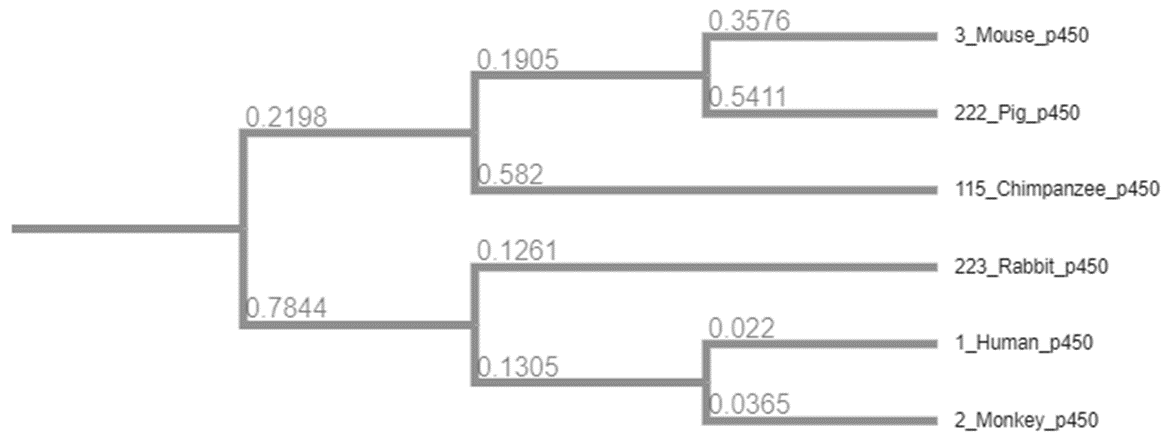
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[https://www.ebi.ac.uk](https://www.ebi.ac.uk/Tools/services/web/toolresult.ebi?jobId=clustalo-I20191110-144700-0750-56020592-p2m&analysis=phylotree)

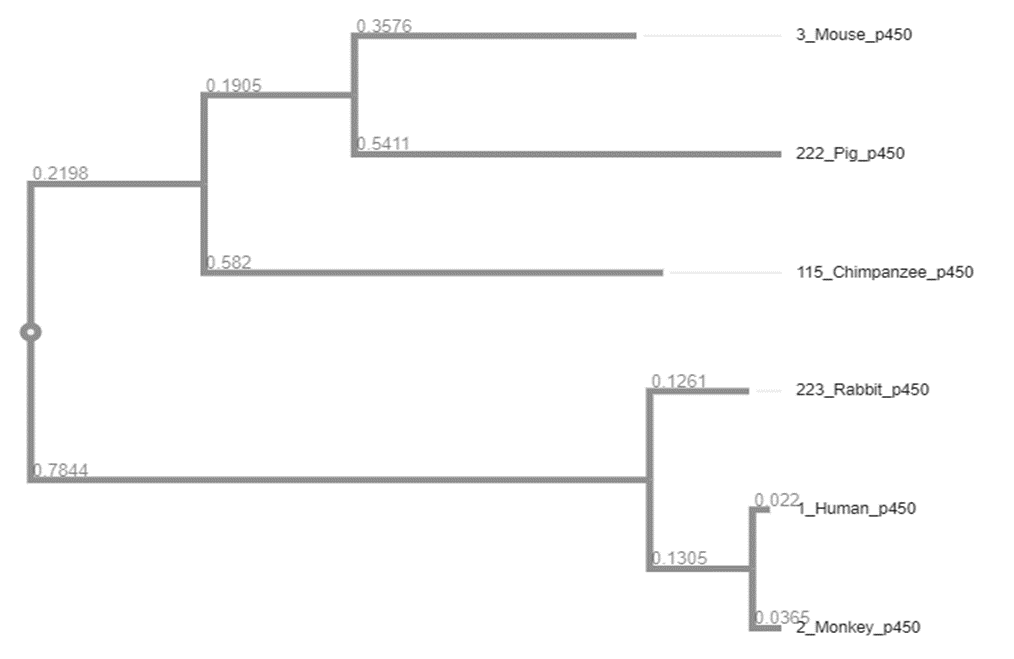
**BlastP analysis**

Blastp analysis: As mentioned above to know the genetic distance between various living organisms Blast analysis has been performed the results are as fallows.

**Fig: 5 Cladogram display ignores length value**

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**Fig: 6 Phylogram display uses branch length values with aligned labels**

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**Fig:7 Phylogram tree cladogram neighbor-join method**

**A screenshot of a cell phone

Description automatically generated**

**A screenshot of a cell phone

Description automatically generatedFig:8 Phylogram tree cladogram at maximum similarity method**

**Conclusion:**

After analysis of sequence and phylogenetic results indicated that cytochrome P450 is very highly conserved sequences and close evolution relation. The amino acid sequence within humans and rhesus monkey stand identical while between humans and chimpanzees there is far difference of amino acid in comparison followed by rabbit. The pig and mouse cytochrome P450 are differ than other specifics. This relationship of this tree indicated that selection of animal model to drug test for clinical application is very critical to translate the drug metabolite profile end goal in human application. This comparison study suggests that rhesus monkey is an ideal model followed by rabbit animal model to evaluate drug metabolite.

**References**

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