

# **Identification and Analysis of Drug-Metabolizing Enzyme Genes in Cynomolgus Monkey**

**- Cytochrome P450s -**

**Shin Nippon Biomedical Laboratories, Ltd.  
Pharmacokinetics and Bioanalysis Center  
Genome Research Group**

**Yasuhiro Uno**

**2010JSSX 2010.10.08**

# Outline

- **Comprehensive isolation of cytochrome P450 (CYP) cDNAs in cynomolgus monkey**
  - **Characterization of CYP2C76 (not orthologous to human CYPs)**
  - **CYP2C76 in pitavastatin metabolism (species difference)**
  - **Discovery of null genotype in cynomolgus *CYP2C76* gene**
  - **Molecular analysis of other cynomolgus CYPs in CYP2-4 family**
  - **Discovery of genetic variants (including null genotypes) in cynomolgus and rhesus *CYP3A4* and *CYP3A5* genes**
  - **Isolation and analysis of glutathione S-transferase cDNAs in cynomolgus monkey**
- ..... etc.

# Outline

- **Comprehensive isolation of cytochrome P450 (CYP) cDNAs in cynomolgus monkey**
- **Characterization of CYP2C76 (not orthologous to human CYPs)**
- **CYP2C76 in pitavastatin metabolism (species difference)**
- **Discovery of null genotype in cynomolgus *CYP2C76* gene**
- **Molecular analysis of other cynomolgus CYPs in CYP2-4 family**
- **Discovery of genetic variants (including null genotypes) in cynomolgus and rhesus *CYP3A4* and *CYP3A5* genes**
- **Isolation and analysis of glutathione S-transferase cDNAs in cynomolgus monkey**
- ..... etc.

## **Species Difference in Drug Metabolism**

- **Species difference is well-known in drug metabolism (i.e. rat and human).**
- **Due to the evolutionarily close relationship to human, monkey is used in drug metabolism.**
- **However, the difference is occasionally seen in monkey, as compared with human.**

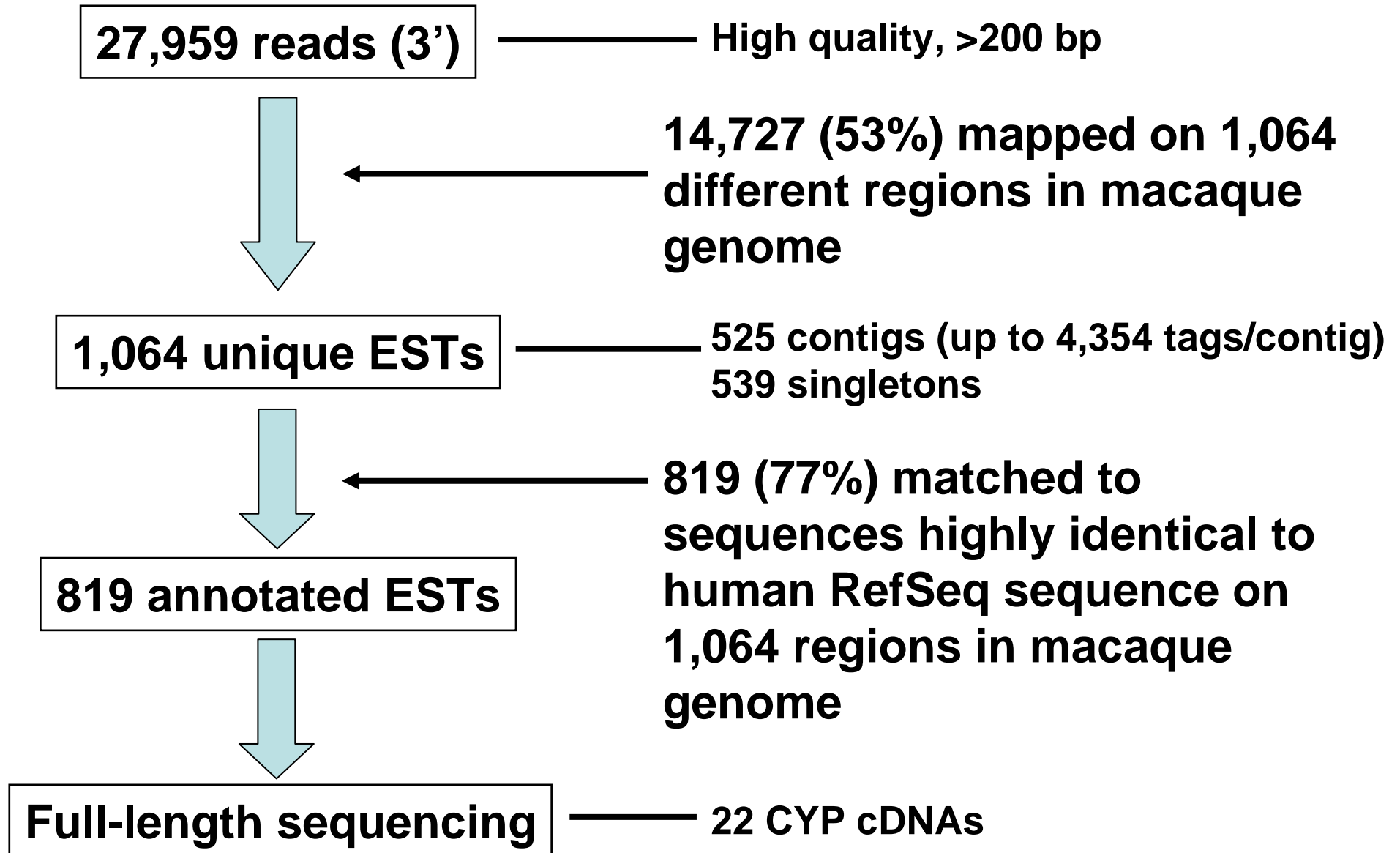
# Cytochrome P450 (CYP)

- **CYP forms gene family comprised of 57 functional genes and 58 pseudogenes in human.**
- ***CYP* genes within subfamily are highly identical to each other and form the gene cluster in the genome.**
- **The number of *CYP* genes within subfamily differs among species.**
  - ➔ **Species difference in drug metabolism**
- **Lack of genetic information hampered elucidating species differences at molecular level in monkey.**
  - ➔ **Identify CYP cDNAs in cynomolgus monkey**

# **EST (Expressed Sequence Tags) Sequencing**

- **Full-length cDNA library was generated from cynomolgus monkey liver.**
- **The end sequence of cDNAs was determined for randomly picked clones by one-pass sequencing.**
- **ESTs (cDNAs) obtained were annotated using human RefSeq mRNAs using BLAST program.**

# Sequence Processing



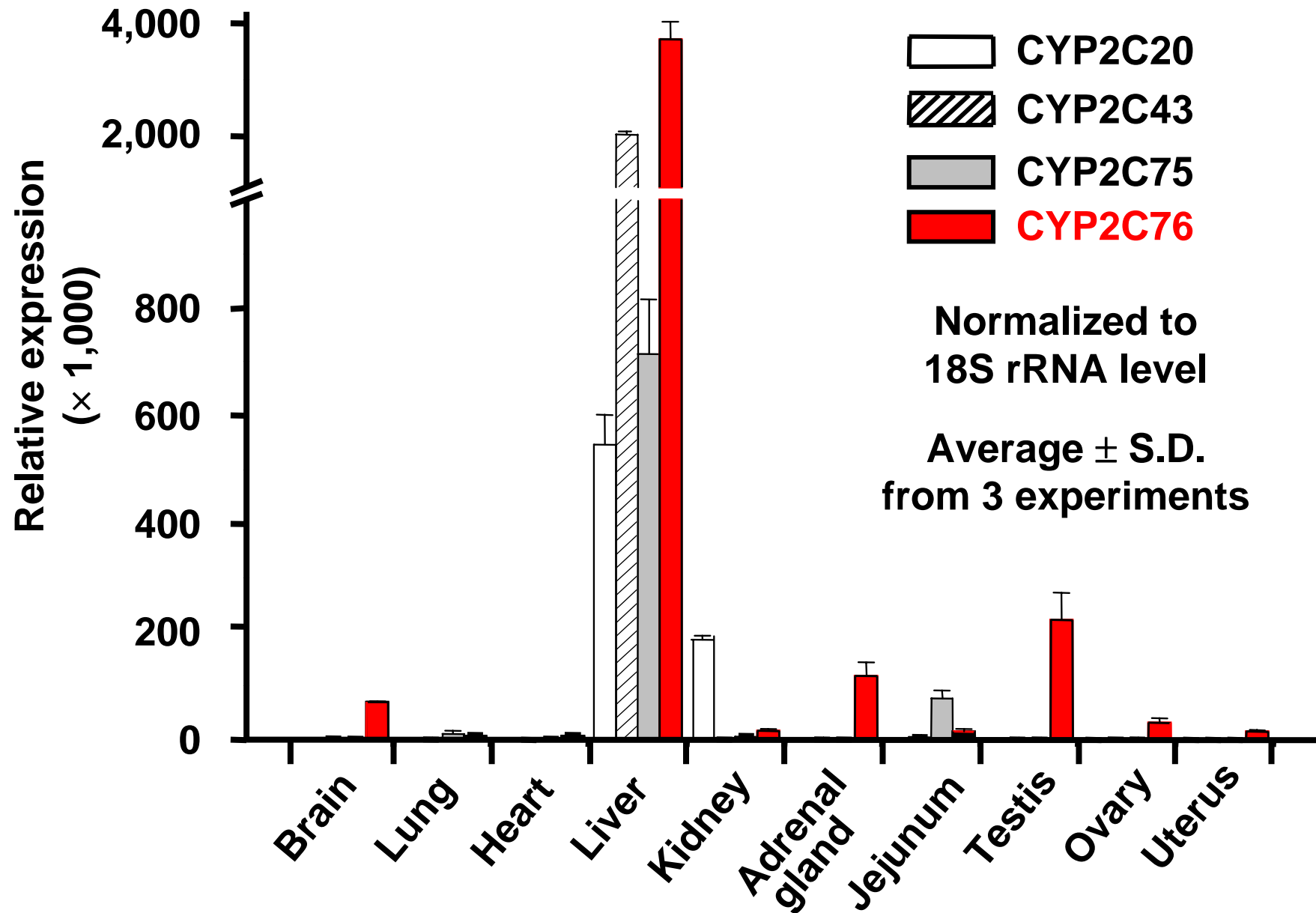
## CYP cDNAs Isolated from Cynomolgus Monkey Liver

Monkey	Human	Sequence identity (%)	
		cDNA	Amino acids
CYP2A23	{ CYP2A13	95	94
CYP2A24		{ CYP2A6	95
CYP2C20(8)*	CYP2C8	95	92
CYP2C43	{ CYP2C9	94	93
CYP2C75		{ CYP2C19	95
CYP2C76	CYP2Cs	81	71
CYP2D17*	CYP2D6	94	93
CYP2E1*	CYP2E1	95	94
CYP2J2	CYP2J2	95	95
CYP3A5	CYP3A5	94	91
CYP3A8(4)*	CYP3A4	95	94
CYP4A11	CYP4A11	96	95
CYP4F3v2	CYP4F3v2	95	96
CYP4F11	CYP4F11	93	91
CYP4F12	CYP4F12	93	92
CYP4F45(2)	CYP4F2	95	96

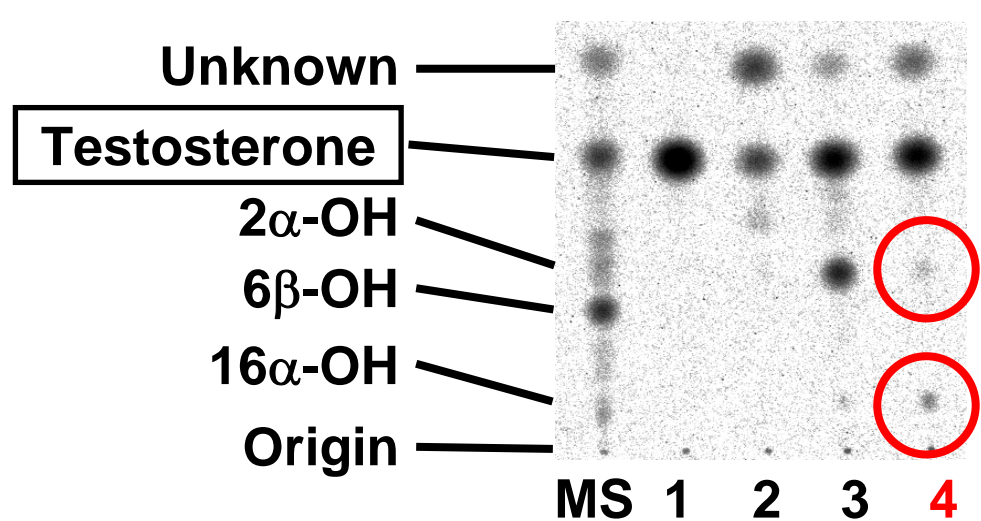
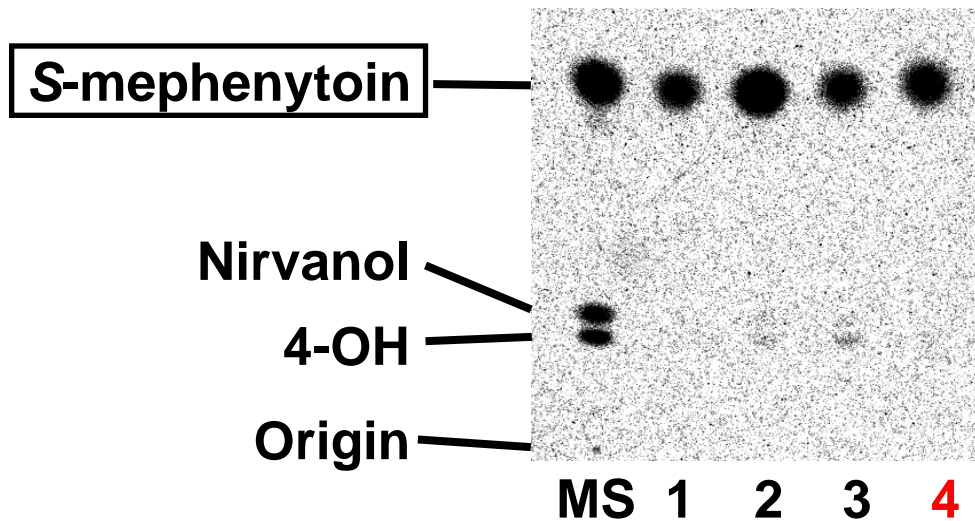
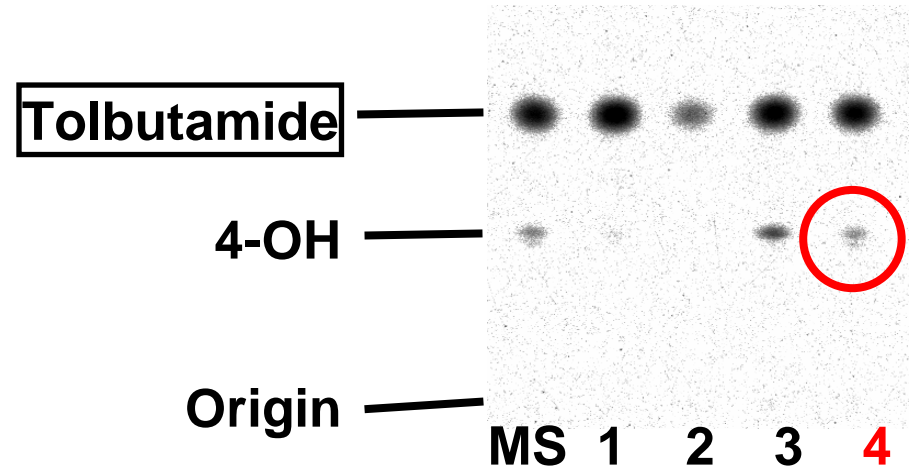
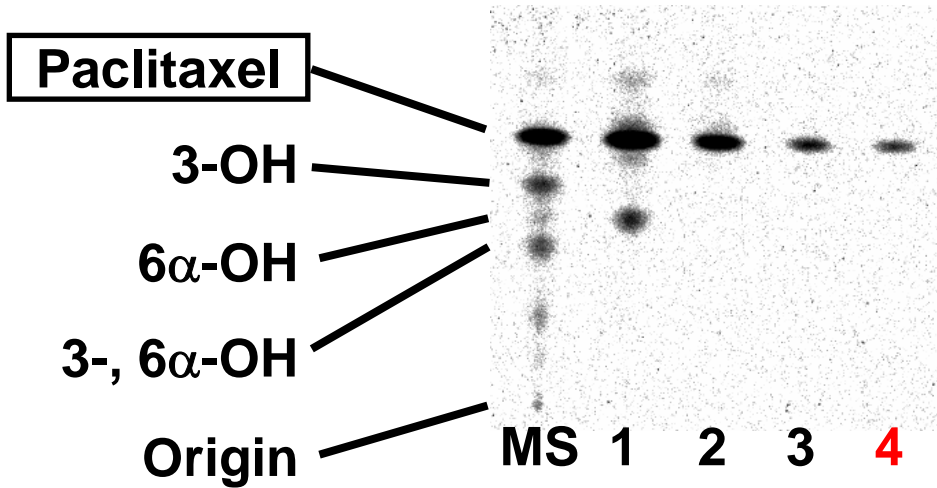
\* Known CYP cDNAs



# Tissue Expression Pattern of Cynomolgus Monkey CYP2C mRNAs



# Drug Metabolism Mediated by CYP2C76

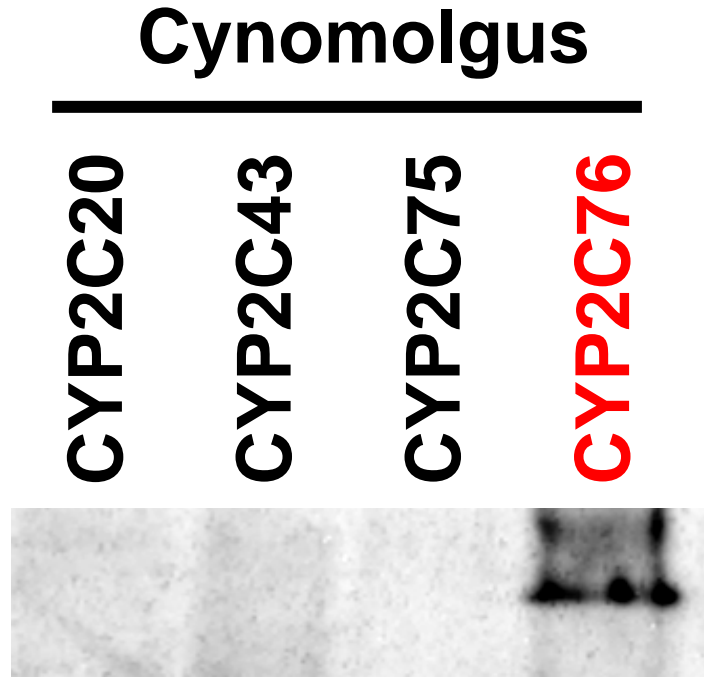


MS, Monkey liver microsomes

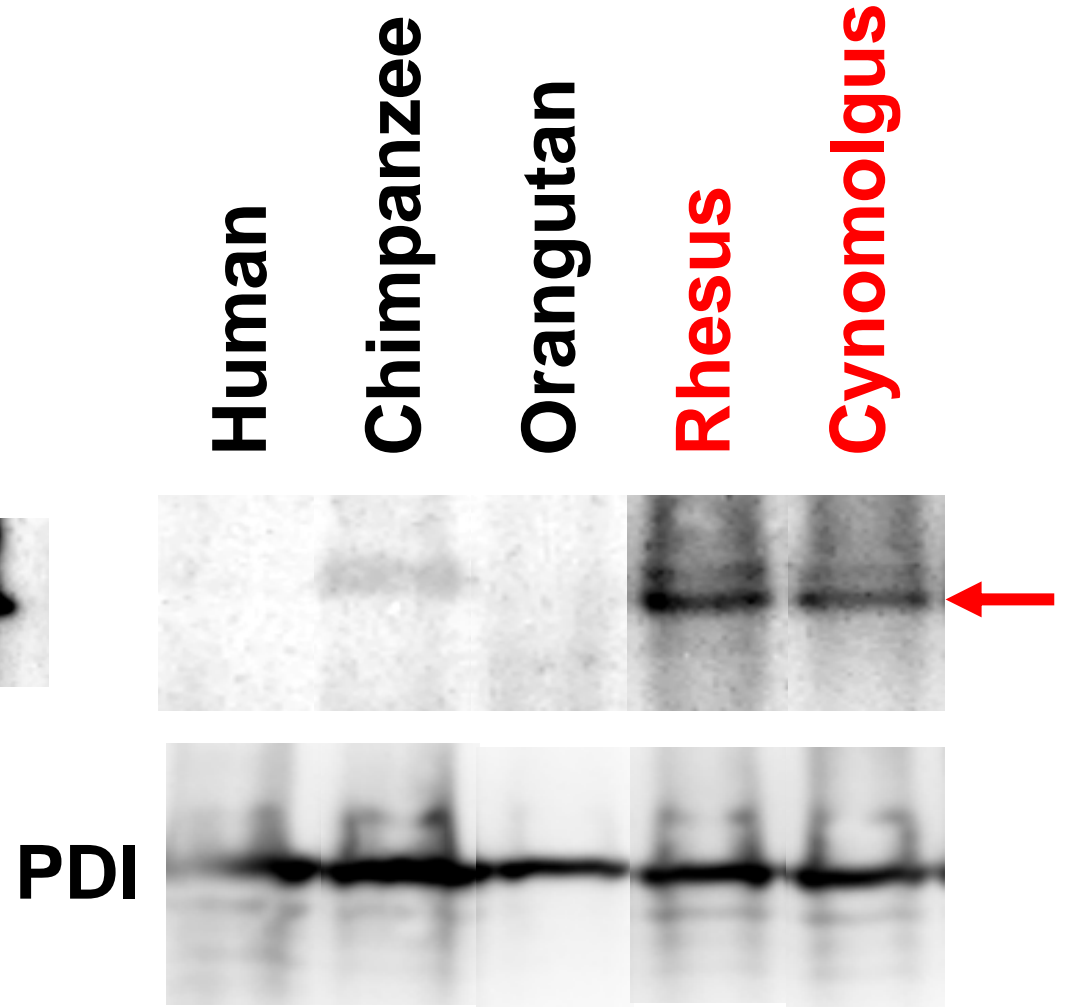
1, CYP2C20; 2, CYP2C43; 3, CYP2C75; 4, CYP2C76

# CYP2C76 Protein Expression in Primate Liver

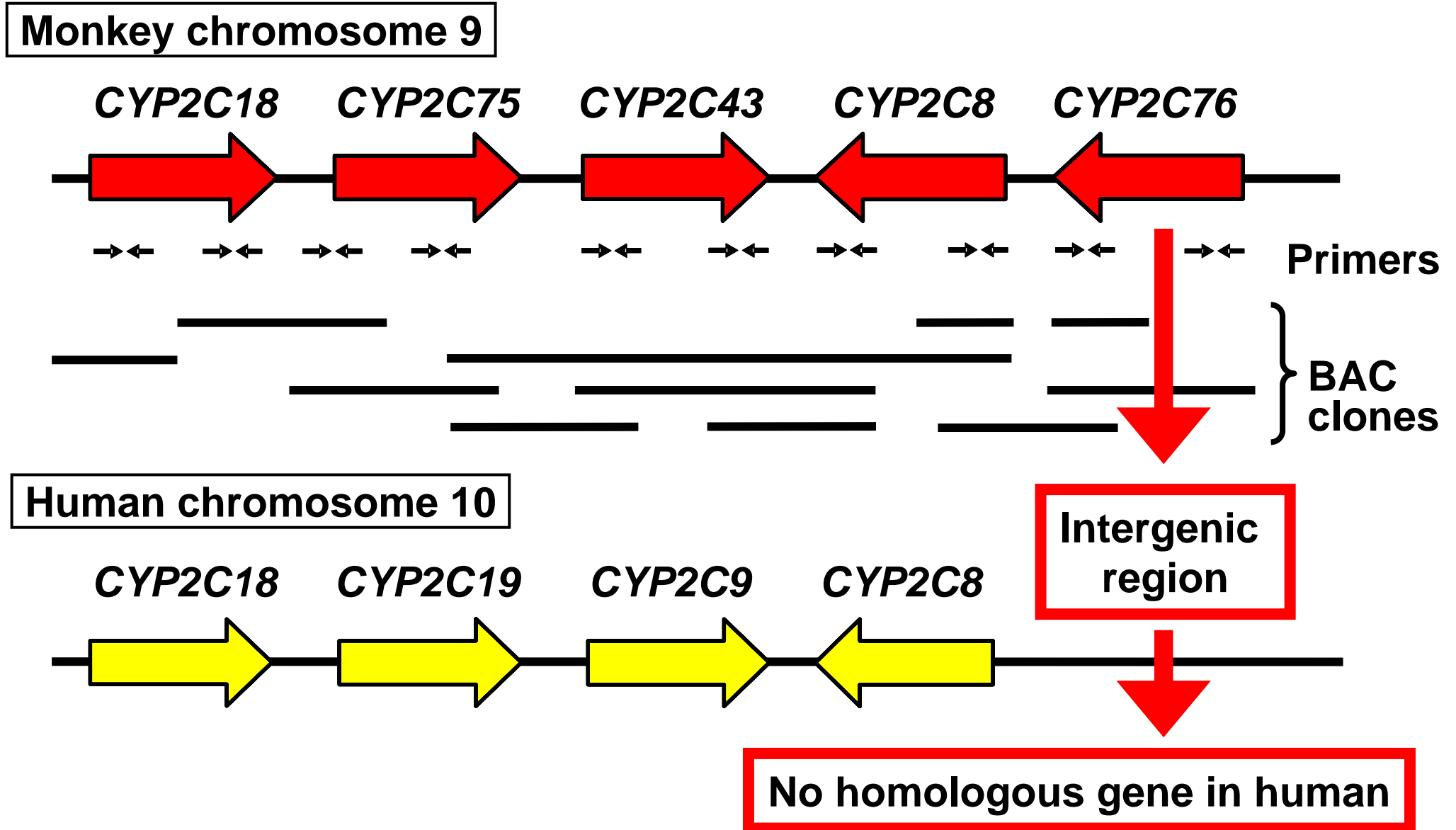
**A**



**B**



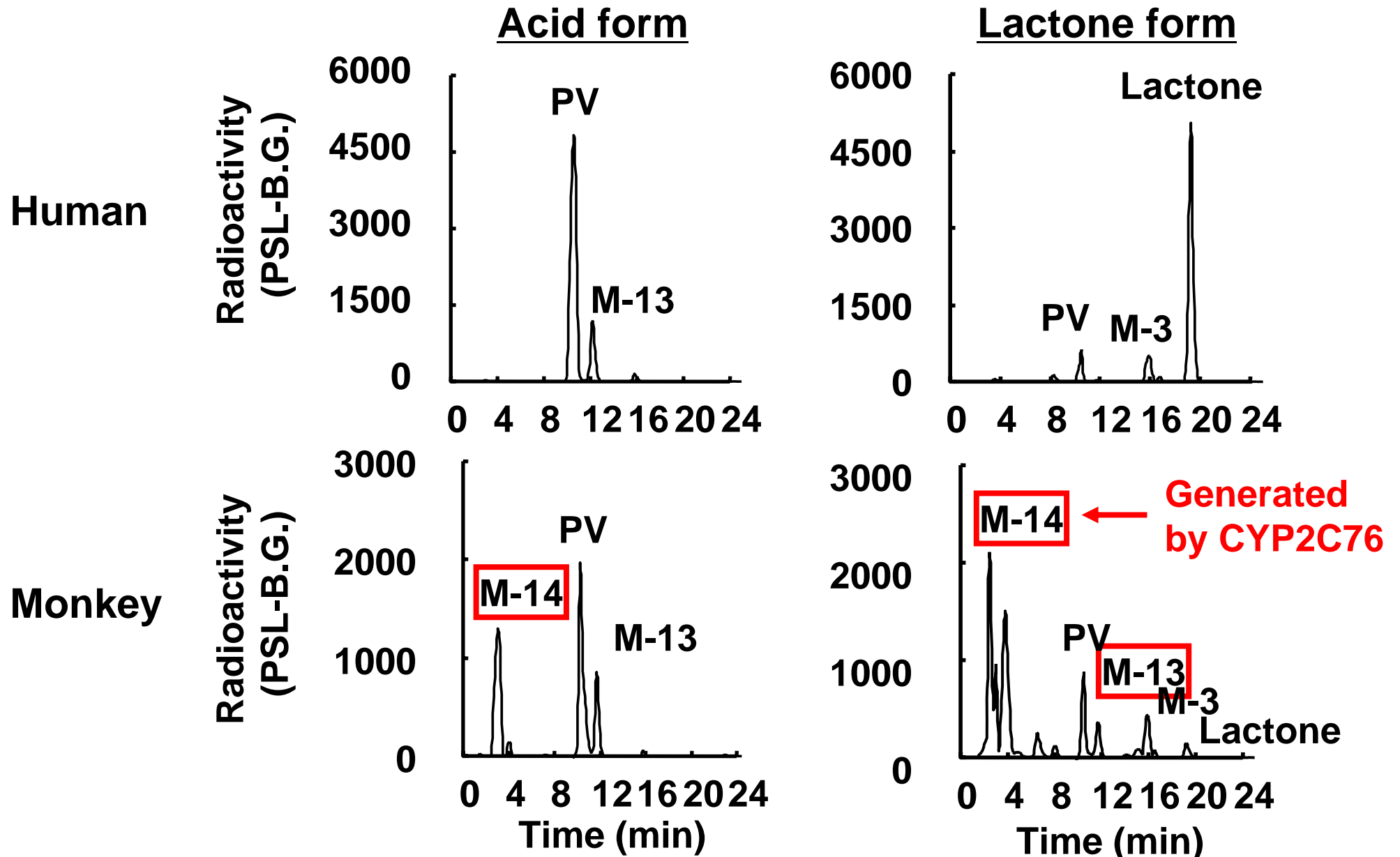
# CYP2C Gene Cluster in Monkey Genome



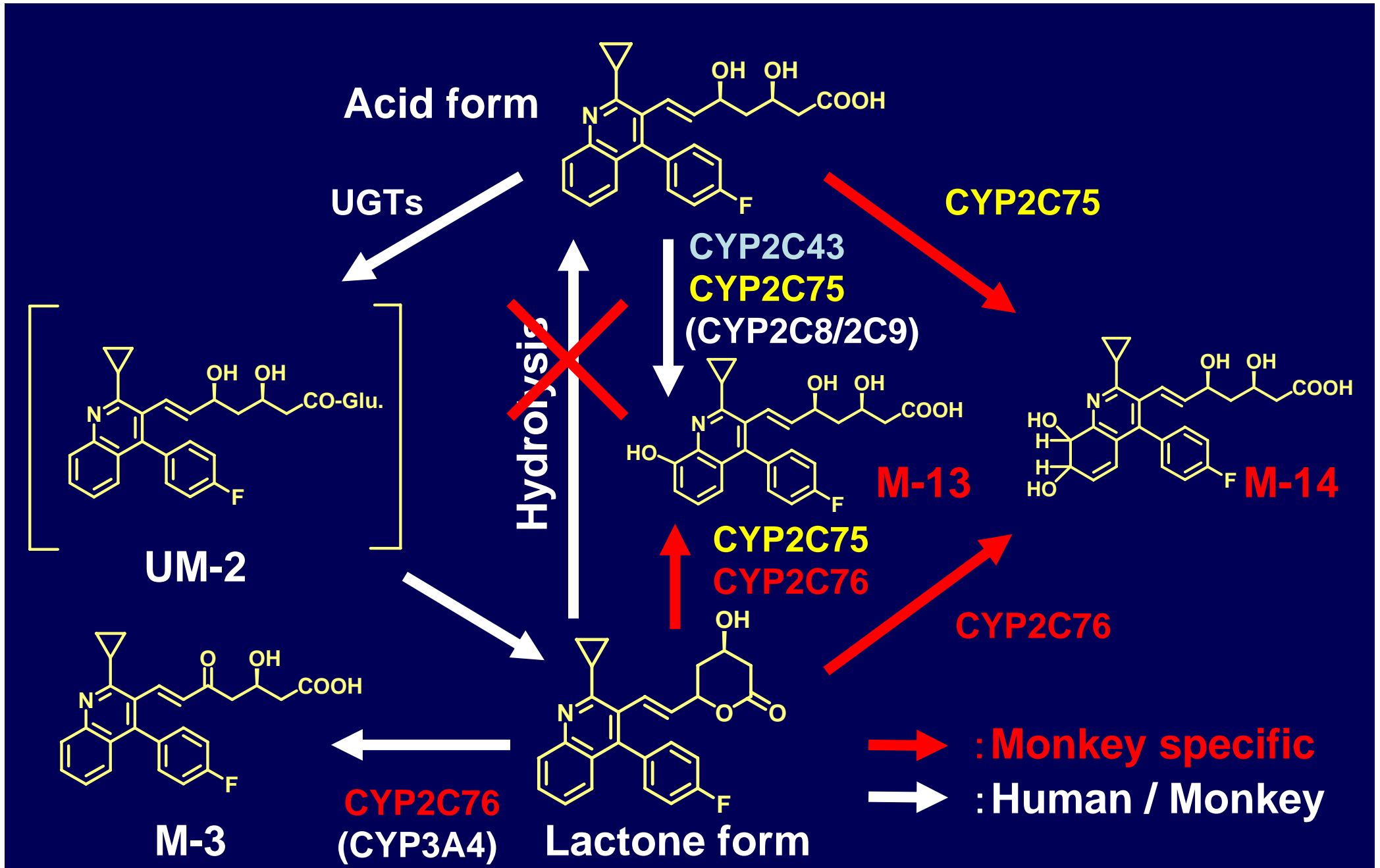
# CYP2C76

- **Maybe a major CYP2C in monkey liver.**
  - **A functional drug-metabolizing enzyme, expressed in liver.**
  - **Ortholog was not present in human.**
- ⇒ CYP2C76 accounts for species difference in drug metabolism between monkey and human?**
- ⇒ Investigate metabolism of pitavastatin (cholesterol-lowering drug )**

# HPLC Chromatograms of Pitavastatin and Its Metabolites in Human and Monkey Hepatic Microsomes



# Difference of Pitavastatin Metabolism between Monkey and Human

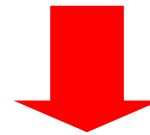


# CYP2C76

- **Not orthologous to human CYPs.**
- **Responsible for species difference between monkey and human in drug metabolism.**



- **Monkeys without CYP2C76 function might show more similar metabolic pattern to human.**

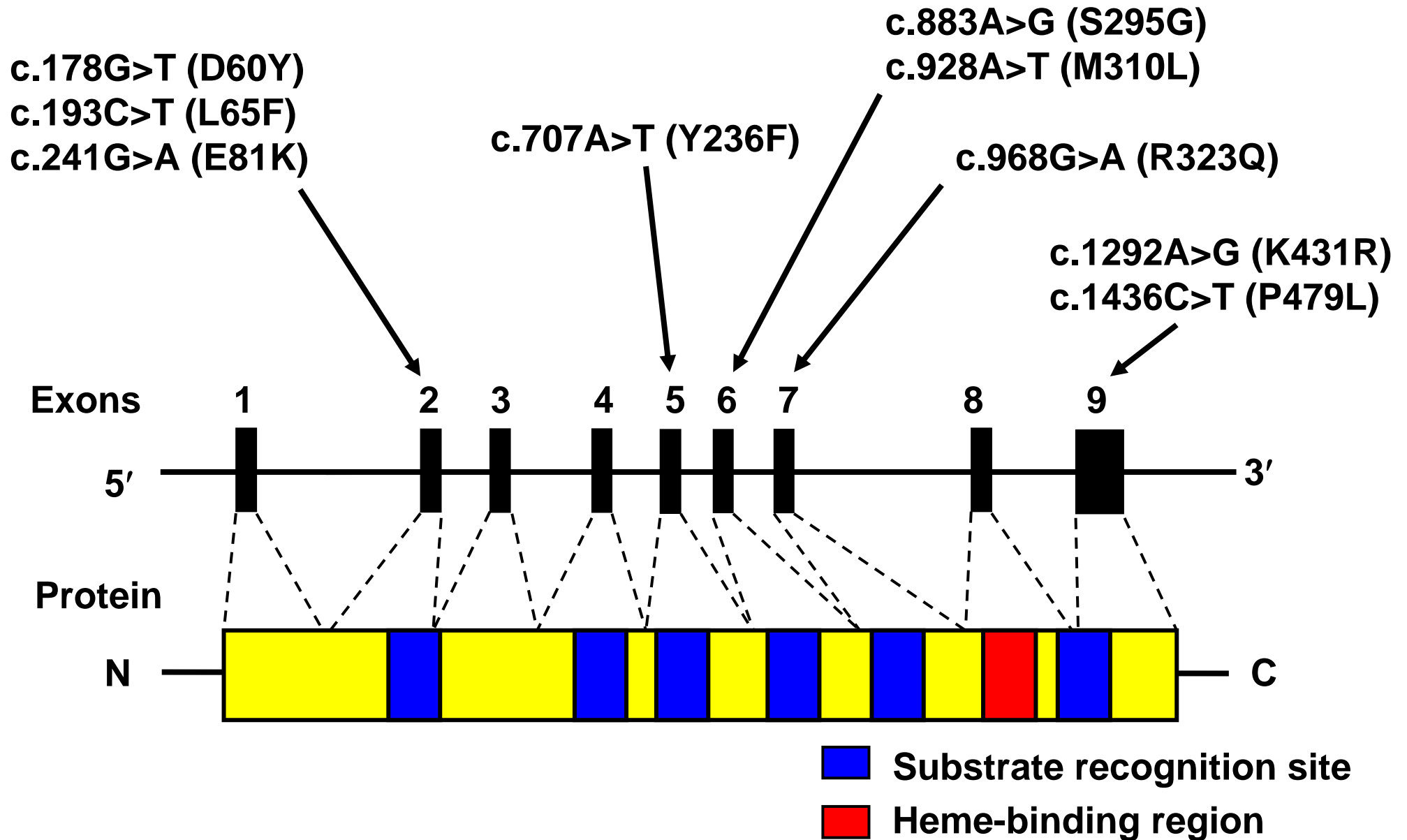


➤ ~~Knockout~~ ~~Knockdown~~

- **Null or defective alleles can be utilized?**

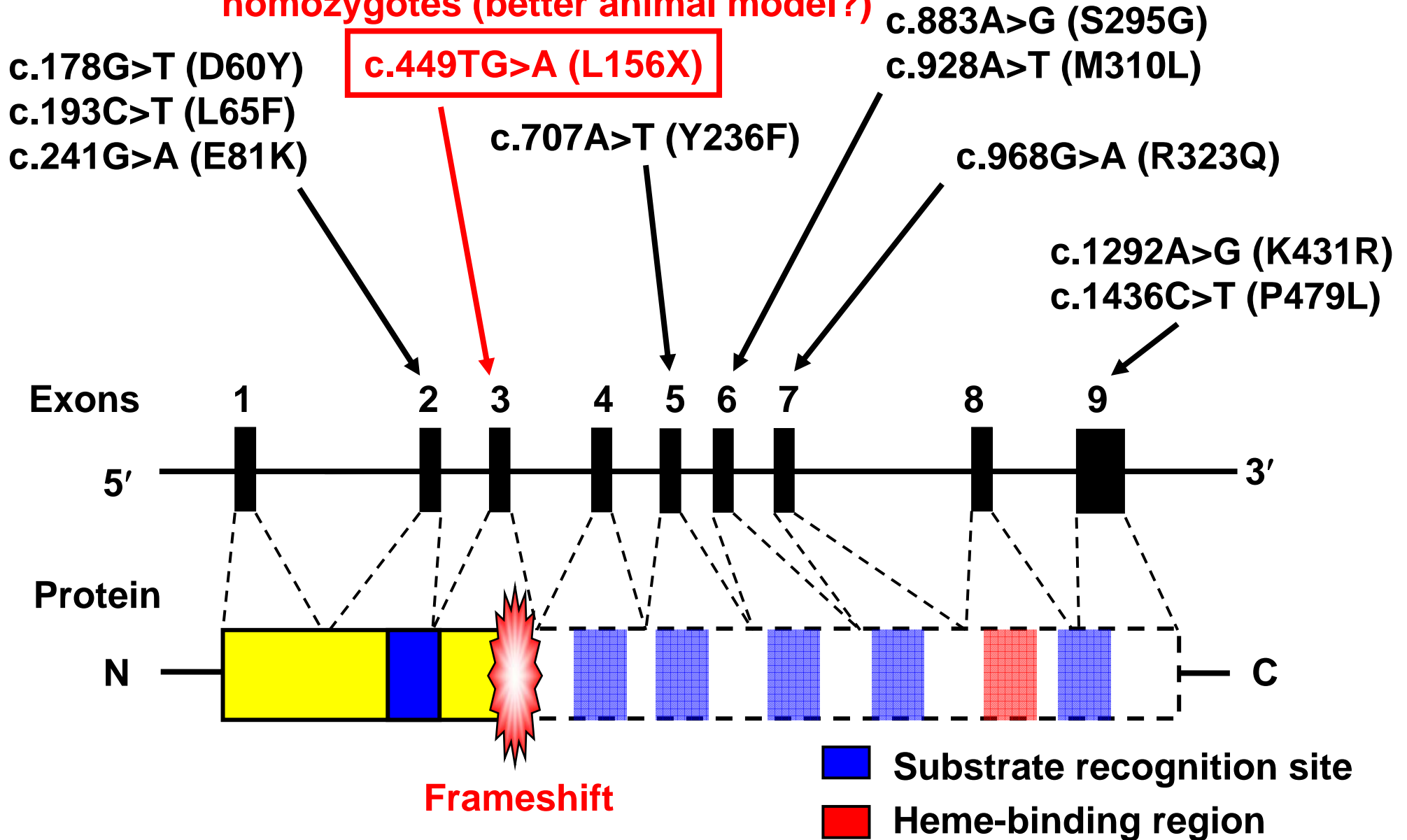


# Genetic Variants Identified in *CYP2C76*



# Genetic Variants Identified in *CYP2C76*

7 heterozygotes: can be used to produce homozygotes (better animal model?)



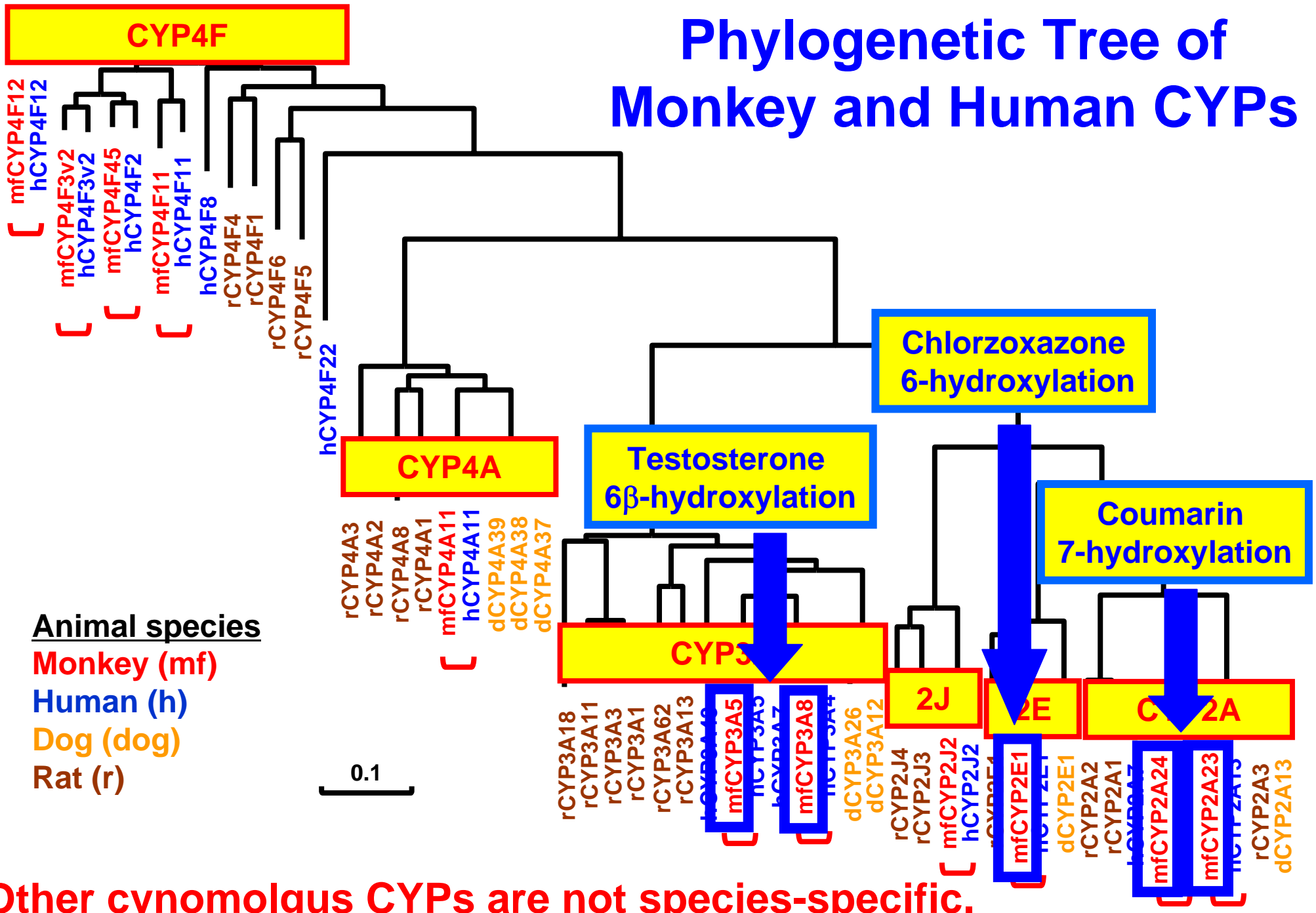
## Other Cynomolgus CYPs Isolated by EST Sequencing are Species-specific?

Monkey	Human	Sequence identity (%)	
		cDNA	Amino acids
CYP2A23 } CYP2A24 }	{ CYP2A13 { CYP2A6	95 95	94 93
CYP2C20(8)*	CYP2C8	95	92
CYP2C43 } CYP2C75 }	{ CYP2C9 { CYP2C19	94 95	93 93
CYP2C76	CYP2Cs	81	71
CYP2D17*	CYP2D6	94	93
CYP2E1* CYP2J2 CYP3A5 CYP3A8(4)* CYP4A11 CYP4F3v2 CYP4F11 CYP4F12 CYP4F45(2)	CYP2E1 CYP2J2 CYP3A5 CYP3A4 CYP4A11 CYP4F3v2 CYP4F11 CYP4F12 CYP4F2	95 95 94 95 96 95 93 93 95	94 95 91 94 95 96 91 92 96

Further  
analyzed

\* Known CYP cDNAs

# Phylogenetic Tree of Monkey and Human CYPs



# Acknowledgments

## Hokkaido University

- Lab. of Translational Research
- Lab. of Drug Metabolism

## Show Pharmaceutical University

- Dr. Hiroshi Yamazaki

## Hyogo University of Health Sciences

- Dr. Hideki Fujino

## University of Tokyo

- Dr. Yutaka Suzuki
- Dr. Sumio Sugano

## Tokai University

- Dr. Ituro Inoue

## SNBL

- Mr. Masahiro Utoh
- Dr. Koichiro Fukuzaki
- Dr. Ryoichi Nagata

**Dr. Tetsuya Kamataki**