

The role of CYP3A4 mRNA transcript with shortened 3'-UTR in hepatocyte differentiation, liver development, and response to drug induction

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**Supplemental Table 1.** Slope and R<sup>2</sup> of standard curves and PCR efficiency.

Primer set	Slope	R <sup>2</sup>	PCR Efficiency
CYP3A4 transcript with canonical 3'-UTR	-3.33 ±0.20	0.9996 ±0.0006	1.00 ±0.08
CYP3A4 transcripts with canonical and shorter 3'-UTR	-3.27 ±0.15	0.9982 ±0.0016	1.03 ±0.07
CYP3A4 transcript with intron-6 retention	-3.25 ±0.15	0.9959 ±0.0001	1.03 ±0.07
GAPDH	-3.42 ±0.26	0.9965 ±0.0010	0.97 ±0.10
ACTB	-3.22 ±0.16	0.9972 ±0.0012	0.99 ±0.09

Mean ± S.D. (n=3)

$$\text{PCR efficiency} = 10^{(-1/\text{slope})} - 1$$

GAPDH: glyceraldehyde 3-phosphate dehydrogenase (reference gene 1)

ACTB: actin, beta (reference gene 2)

**Supplemental Table 2.** Fold changes of expression of the CYP3A4 mRNA transcripts in HepaRG cells during hepatocyte differentiation

**2A.** CYP3A4 transcript with canonical 3'-UTR

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Undif HepaRG)	Fold of Change (Power)
Undif HepaRG1	21.58	2.18	-0.47	1.39
Undif HepaRG2	22.81	2.95	0.30	0.81
Undif HepaRG3	22.65	2.90	0.25	0.84
			Mean $\pm$ S.D.	1.0 $\pm$ 0.3
Diff HepaRG1	21.96	1.20	-1.45	2.73
Diff HepaRG2	23.33	2.34	-0.31	1.24
Diff HepaRG3	21.88	0.66	-1.99	3.97
			Mean $\pm$ S.D.	2.6 $\pm$ 1.4

**2B. CYP3A4 transcripts with canonical and shorter 3'-UTR**

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Undif HepaRG)	Fold of Change (Power)
Undif HepaRG1	23.39	2.88	-0.65	1.57
Undif HepaRG2	25.12	4.00	0.47	0.72
Undif HepaRG3	24.68	4.02	0.49	0.71
			Mean $\pm$ S.D.	1.0 $\pm$ 0.5
Diff HepaRG1	22.42	0.51	-3.02	8.14
Diff HepaRG2	21.62	-0.07	-3.60	12.14
Diff HepaRG3	21.87	0.25	-3.28	9.75
			Mean $\pm$ S.D.	10.0 $\pm$ 2.0

## 2C. CYP3A4 transcript with intron-6 retention

Sample#	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Undif HepaRG)	Fold of Change (Power)
Undif HepaRG1	30.61	6.90	0.65	0.64
Undif HepaRG2	28.72	5.86	-0.39	1.31
Undif HepaRG3	28.94	6.17	-0.08	1.06
			Mean $\pm$ S.D.	1.0 $\pm$ 0.3
Diff HepaRG1	27.47	3.18	-3.07	8.38
Diff HepaRG2	26.05	2.43	-3.82	14.11
Diff HepaRG3	25.65	2.19	-4.06	16.73
			Mean $\pm$ S.D.	13.1 $\pm$ 4.3

# 1, 2, 3 represents Biological triplicates.

\* Cq values of technical triplicates were averaged.

^ Minus the mean of two reference genes (GAPDH and ACTB).

Undif HepaRG, undifferentiated HepaRG; Diff HepaRG, differentiated HepaRG.

**Supplemental Table 3.** Fold changes of expression of the CYP3A4 mRNA transcripts in HepaRG cells in response to drugs

**3A.** CYP3A4 transcript with canonical 3'-UTR

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	20.60	2.38	0.48	0.72
DMSO2	21.61	1.54	-0.36	1.29
DMSO3	21.37	1.90	-0.00	1.00
			Mean $\pm$ S.D.	1.0 $\pm$ 0.3
RIF1	18.90	1.28	-0.62	1.53
RIF2	19.70	0.88	-1.02	2.02
RIF3	21.08	-0.57	-2.47	5.55
			Mean $\pm$ S.D.	3.0 $\pm$ 2.2
PB1	19.41	1.38	-0.52	1.43
PB2	20.87	1.50	-0.40	1.32
PB3	20.01	1.33	-0.57	1.48
			Mean $\pm$ S.D.	1.4 $\pm$ 0.1

**3B. CYP3A4 transcripts with canonical and shorter 3'-UTR**

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	23.88	4.07	-0.95	1.94
DMSO2	26.69	6.55	1.53	0.35
DMSO3	25.48	5.53	0.51	0.70
			Mean $\pm$ S.D.	1.0 $\pm$ 0.8
RIF1	19.12	-0.23	-5.25	38.07
RIF2	21.86	0.86	-4.16	17.86
RIF3	19.73	0.18	-4.84	28.66
			Mean $\pm$ S.D.	28.2 $\pm$ 10.1
PB1	19.36	-0.05	-5.07	33.70
PB2	20.81	0.41	-4.61	24.39
PB3	20.18	0.82	-4.20	18.34
			Mean $\pm$ S.D.	25.5 $\pm$ 7.7

### 3C. CYP3A4 transcript with intron-6 retention

Sample#	Cq*	$\Delta Cq^{\wedge}$	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	28.85	8.23	-0.40	1.32
DMSO2	31.28	8.33	-0.30	1.23
DMSO3	30.62	9.70	1.07	0.47
			Mean $\pm$ S.D.	1.0 $\pm$ 0.5
RIF1	24.22	4.02	-4.61	24.34
RIF2	27.67	4.01	-4.62	24.60
RIF3	25.62	4.90	-3.73	13.31
			Mean $\pm$ S.D.	20.8 $\pm$ 6.4
PB1	24.71	4.43	-4.20	18.36
PB2	26.59	3.73	-4.90	29.84
PB3	26.91	6.55	-2.08	4.23
			Mean $\pm$ S.D.	17.5 $\pm$ 12.8

# 1, 2, 3 represents Biological triplicates.

\* Cq values of technical triplicates were averaged.

$\wedge$  Minus the mean of two reference genes (GAPDH and ACTB).

DMSO, 0.1% DMSO solvent control; RIF, rifampicin (10  $\mu$ M); PB, phenobarbital (750  $\mu$ M).

**Supplemental Table 4.** Fold changes of expression of the CYP3A4 mRNA transcripts in primary human hepatocytes in response to drugs

**4A.** CYP3A4 transcript with canonical 3'-UTR

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	19.45	2.58	0.03	0.98
DMSO2	22.22	3.32	0.77	0.59
DMSO3	20.96	2.02	-0.53	1.44
			Mean $\pm$ S.D.	1.0 $\pm$ 0.4
RIF1	18.78	2.01	-0.54	1.45
RIF2	20.92	2.33	-0.22	1.16
RIF3	19.10	0.39	-2.16	4.45
			Mean $\pm$ S.D.	2.4 $\pm$ 1.8
PB1	18.36	1.70	-0.85	1.80
PB2	20.88	2.26	-0.29	1.23
PB3	19.40	0.86	-1.69	3.22
			Mean $\pm$ S.D.	2.1 $\pm$ 1.0



**4B. CYP3A4 transcripts with canonical and shorter 3'-UTR**

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	20.77	3.90	1.57	0.34
DMSO2	25.18	6.27	3.94	0.06
DMSO3	19.89	0.95	-1.38	2.61
			Mean $\pm$ S.D.	1.0 $\pm$ 1.4
RIF1	17.58	0.92	-1.41	2.66
RIF2	18.79	0.20	-2.13	4.37
RIF3	16.81	-1.90	-4.23	18.76
			Mean $\pm$ S.D.	8.6 $\pm$ 8.8
PB1	17.44	0.67	-1.66	3.16
PB2	18.86	0.23	-2.10	4.29
PB3	16.93	-1.60	-3.93	15.24
			Mean $\pm$ S.D.	7.6 $\pm$ 6.7

#### 4C. CYP3A4 transcript with intron-6 retention

Sample Name #	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus DMSO)	Fold of Change (Power)
DMSO1	25.71	8.84	0.96	0.51
DMSO2	27.36	8.46	0.58	0.67
DMSO3	25.95	7.01	-0.87	1.83
			Mean $\pm$ S.D.	1.0 $\pm$ 0.7
RIF1	22.47	5.71	-2.17	4.51
RIF2	25.43	6.84	-1.04	2.05
RIF3	24.29	5.58	-2.30	4.92
			Mean $\pm$ S.D.	3.8 $\pm$ 1.6
PB1	22.29	5.63	-2.25	4.76
PB2	23.58	4.95	-2.93	7.62
PB3	23.98	5.45	-2.43	5.39
			Mean $\pm$ S.D.	5.9 $\pm$ 1.5

# 1, 2, 3 represents Biological triplicates.

\* Cq values of technical triplicates were averaged.

^ Minus the mean of two reference genes (GAPDH and ACTB).

DMSO, 0.1% DMSO solvent control; RIF, rifampicin (10  $\mu$ M); PB, phenobarbital (750  $\mu$ M).

**Supplemental Table 5.** Fold changes of expression of the CYP3A4 mRNA transcripts for human liver samples at different ages.

**5A.** CYP3A4 transcript with canonical 3'-UTR

Fetal	Sample No.	EGA # (days)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	170	20.81	2.33	-0.87	1.83
	2	191	22.00	3.68	0.48	0.72
	3	204	21.11	3.03	-0.17	1.13
	4	227	22.12	4.32	1.12	0.46
					Mean $\pm$ S.D.	1.0 $\pm$ 0.6
Neonatal /pediatric	Sample No.	PNA # (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	4 days	22.35	4.81	1.61	0.33
	2	3	21.00	1.31	-1.89	3.72
	3	4	23.84	4.55	1.35	0.39
	4	7	22.40	3.84	0.64	0.64
					Mean $\pm$ S.D.	1.3 $\pm$ 1.6
Adult	Sample No.	Age (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	17	23.27	3.78	0.58	0.67
	2	23	19.49	-1.73	-4.93	30.55
	3	23	22.21	1.43	-1.77	3.41
	4	36	21.36	0.13	-3.07	8.42
					Mean $\pm$ S.D.	10.8 $\pm$ 13.6

**5B. CYP3A4 transcripts with canonical and shorter 3'-UTR**

Fetal	Sample No.	EGA # (days)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	170	24.18	5.70	-0.90	1.87
	2	191	25.02	6.70	0.10	0.94
	3	204	24.49	6.41	-0.19	1.14
	4	227	27.25	9.44	2.84	0.14
					Mean $\pm$ S.D.	1.0 $\pm$ 0.7

  

Neonatal /pediatric	Sample No.	PNA # (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	4 days	26.77	9.23	2.63	0.16
	2	3	18.93	-0.77	-7.37	165.43
	3	4	23.59	4.29	-2.31	4.94
	4	7	19.99	1.43	-5.17	35.94
					Mean $\pm$ S.D.	51.6 $\pm$ 77.5

  

Adult	Sample No.	Age (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	17	22.11	2.61	-3.99	15.93
	2	23	19.99	-1.23	-7.83	228.24
	3	23	19.97	-0.81	-7.41	169.61
	4	36	20.59	-0.64	-7.24	151.50
					Mean $\pm$ S.D.	141.3 $\pm$ 89.8

### 5C. CYP3A4 transcript with intron-6 retention

Fetal	Sample No.	EGA # (days)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	170	23.51	5.03	-0.37	1.30
	2	191	23.80	5.48	0.08	0.95
	3	204	22.68	4.60	-0.80	1.74
	4	227	27.63	9.82	4.42	0.05
					Mean $\pm$ S.D.	1.0 $\pm$ 0.7
Neonatal /pediatric	Sample No.	PNA # (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	4 days	24.40	6.86	1.46	0.36
	2	3	25.24	5.55	0.15	0.90
	3	4	30.57	11.27	5.87	0.02
	4	7	25.98	7.43	2.03	0.25
					Mean $\pm$ S.D.	0.4 $\pm$ 0.4
Adult	Sample No.	Age (years)	Cq*	$\Delta Cq$ ^	$\Delta\Delta Cq$ (Minus Fetal)	Fold of Change (Power)
	1	17	30.39	10.89	5.49	0.02
	2	23	26.19	4.96	-0.44	1.35
	3	23	23.36	2.59	-2.81	7.02
	4	36	25.43	4.20	-1.20	2.29
					Mean $\pm$ S.D.	2.7 $\pm$ 3.0

# EGA, estimated gestational age; PNA, postnatal age.

\* Cq values of technical triplicates were averaged.

^ Minus the mean of two reference genes (GAPDH and ACTB).