

## OVERVIEW

- A multiplexed, multi-receptor transactivation assay was developed to target nuclear receptors from a diverse set of ecological species.
- Screening a targeted library showed correlation between species' receptor sequence similarity and chemical response.

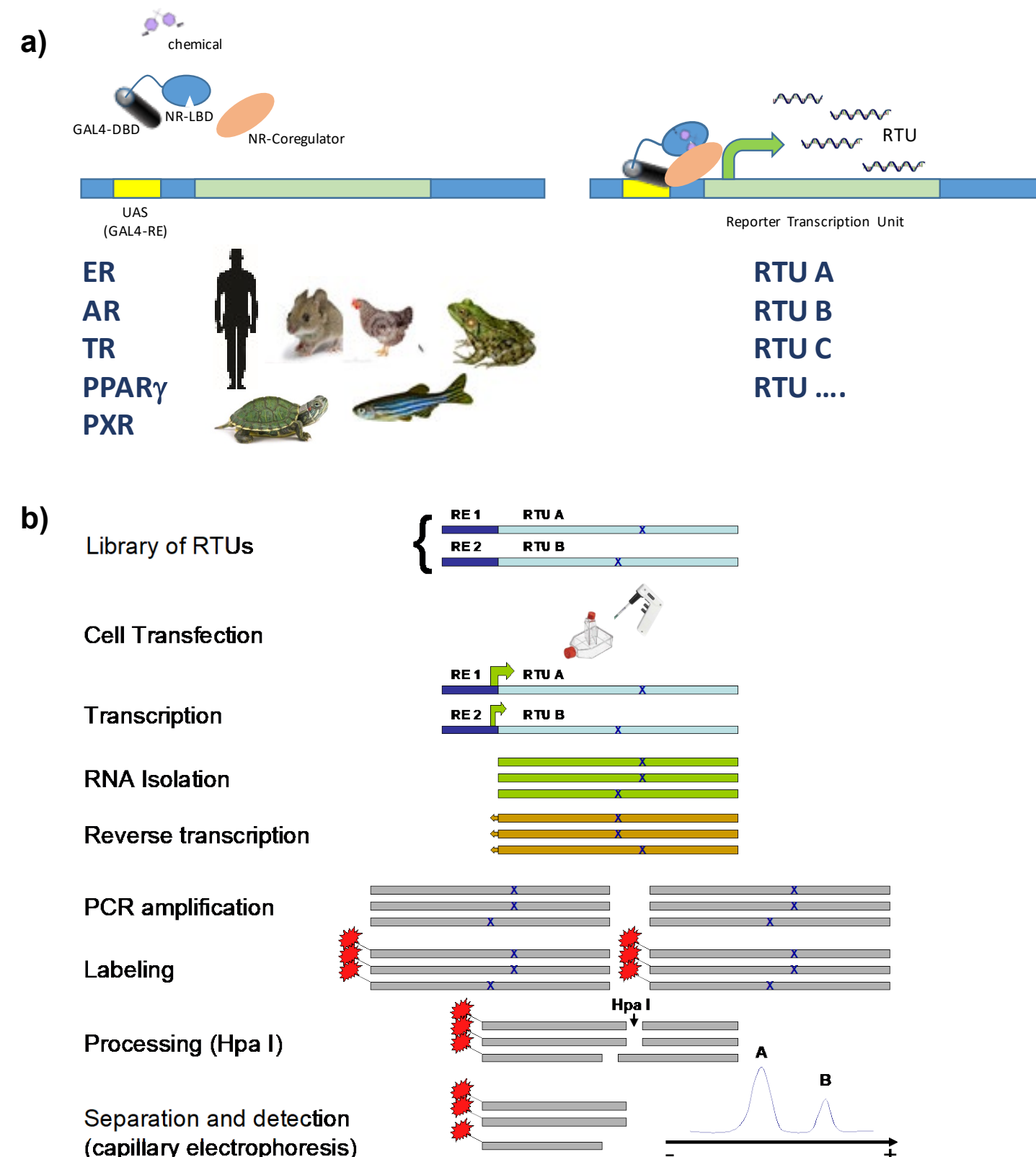
## INTRODUCTION

Screening technologies have been developed to identify xenobiotic chemicals that bind nuclear receptors and thus have potential for adverse health effects through disruption of endocrine function. However, the focus has been on human receptors despite environmental exposure to a huge diversity of other species. We evaluated a multiplexed transactivation assay providing the ability to screen for effects across multiple species and nuclear receptors.

## METHODS

**Table 1. Receptors & Species**

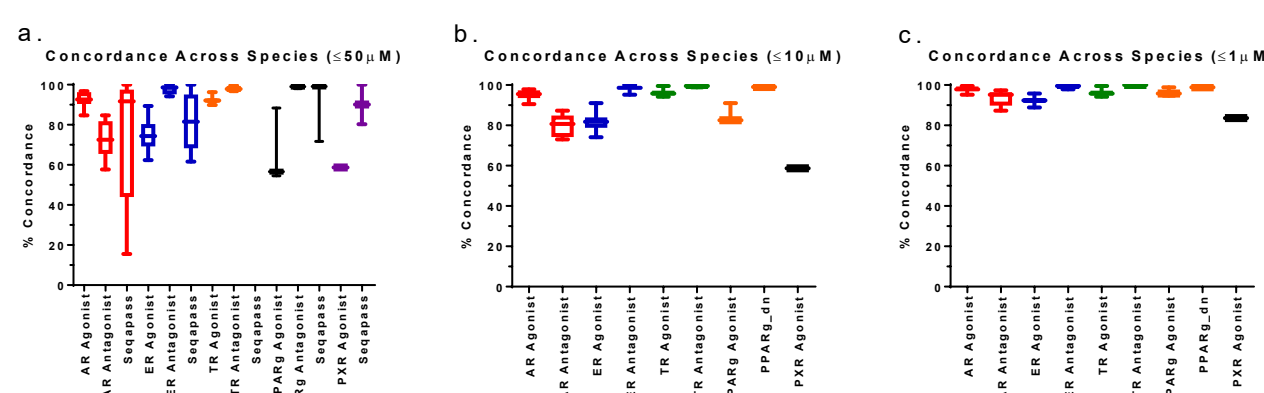
Abbreviation	Gene	ID	Species
ER1-Zf	Estrogen receptor-1 Zebrafish	BC162466	<i>Danio rerio</i>
ER2a-Zf	Estrogen receptor-2A Zebrafish	BC044349	<i>Danio rerio</i>
ER2b-Zf	Estrogen receptor-2B Zebrafish	BC086848	<i>Danio rerio</i>
ER1-Fr	Estrogen receptor-1 Frog	NM_001089617	<i>Xenopus laevis</i>
ER2-Fr	Estrogen receptor-2 Frog	NM_001130954	<i>Xenopus laevis</i>
ER1-Tr	Estrogen receptor-1 Turtle	NM_001282246	<i>Chrysemys picta</i>
ER1-Ch	Estrogen receptor-1 Chicken	NM_205183	<i>Gallus gallus</i>
ERa-Hu	Estrogen receptor-alpha Human	NM_000125	<i>Homo sapiens</i>
ERb-Hu	Estrogen receptor-beta Human	NM_001437	<i>Homo sapiens</i>
AR-Zf	Androgen receptor-Zebrafish	NM_001083123	<i>Danio rerio</i>
AR-Fr	Androgen receptor-Frog	NM_001090884	<i>Xenopus laevis</i>
AR-Tr	Androgen receptor-Turtle	XM_005279527	<i>Chrysemys picta</i>
AR-Ch	Androgen receptor-Chicken	NM_001040090	<i>Gallus gallus</i>
AR-Hu	Androgen receptor-Human	NM_000044	<i>Mus musculus</i>
TRa-Zf	Thyroid receptor-alpha Zebrafish	BC096778	<i>Danio rerio</i>
TRb-Zf	Thyroid receptor-beta Zebrafish	BC163114	<i>Danio rerio</i>
TRa-Fr	Thyroid receptor-alpha Frog	NM_001088126	<i>Xenopus laevis</i>
TRa-Tr	Thyroid receptor-alpha Turtle	XM_005294120	<i>Chrysemys picta</i>
THRa	Thyroid receptor-alpha Human	NM_199334	<i>Homo sapiens</i>
THRb	Thyroid receptor-beta Human	NM_000461	<i>Homo sapiens</i>
PPARg-Zf	PPARg Zebrafish	NM_131467	<i>Danio rerio</i>
PPARg-Ms	PPARg Mouse	NM_001127330	<i>Mus musculus</i>
PPARg-Hu	PPARg Human	BC006811	<i>Homo sapiens</i>
PXR-Hu	Pregnane X receptor Human	O75469	<i>Homo sapiens</i>
PXR-Ms	Pregnane X receptor Mouse	O54915	<i>Mus musculus</i>
GAL4 (control)	GAL4 Yeast DBD	P04386	<i>Saccharomyces cerevisiae</i>



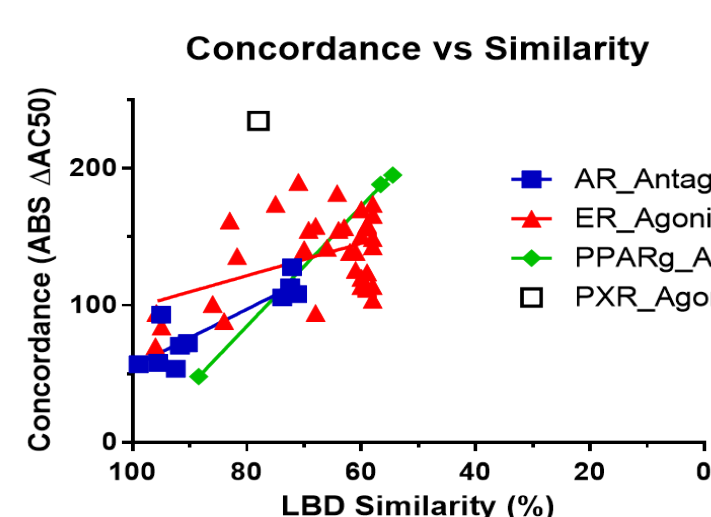
**Fig 1. Assay Platform.**

a) The ligand-binding domains of nuclear receptors shown in Table 1 were cloned into a mammalian one-hybrid system. b) Receptors were co-transfected with unique RTU reporter genes into HepG2 cells, pooled and treated with chemicals for 24 hours, and separated for reporter expression quantitation.

## RESULTS



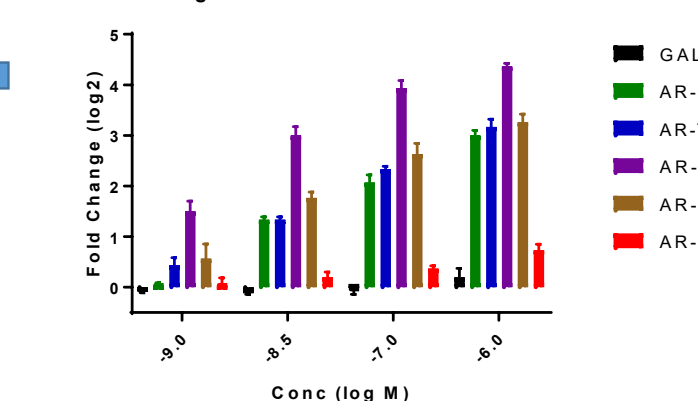
**Fig. 2. Concordance** across species was calculated by percent agreement (hit or inactive) at the designated potency cutoff: **a)** 50  $\mu$ M, **b)** 10  $\mu$ M and **c)** 1  $\mu$ M.



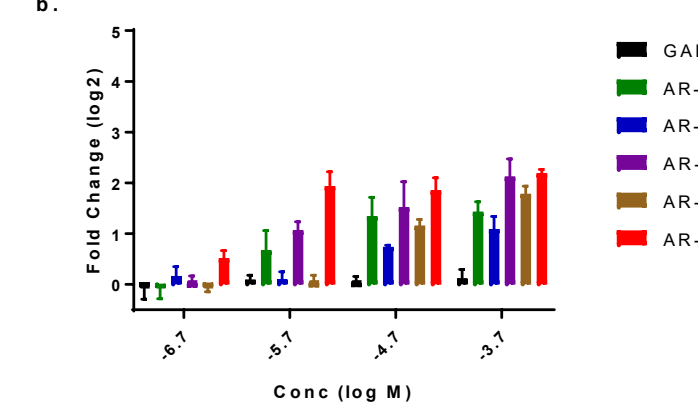
**Fig. 3.** Amino acid sequence similarity between species was calculated and compared to Concordance Value calculated by summing the absolute differences of AC50 values for each chemical.

## RESULTS

**a. AR Agonist: 6-fluoro-testosterone**



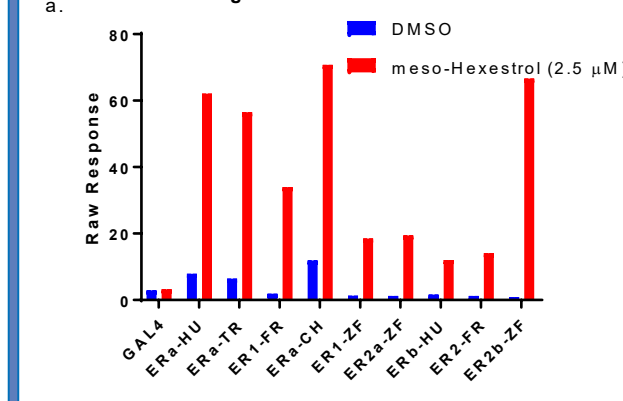
**b. AR Agonist: Norgestrel**



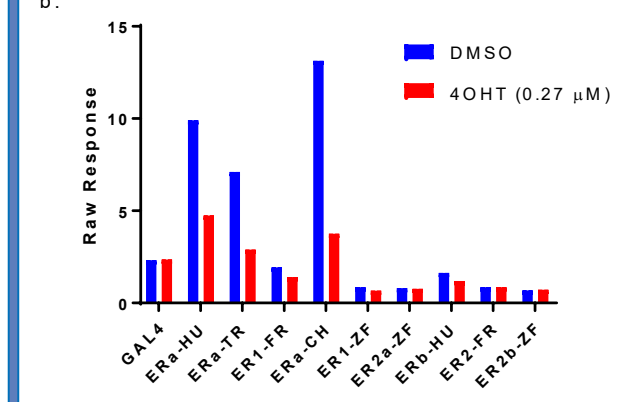
**Fig. 4 AR Response**

a) Initial testing with 2 nM 6-fluoro-testosterone for AR antagonist detection showed weak response in zebrafish. b) Norgestrel was shown to stimulate zebrafish AR and subsequent assays included both norgestrel and 6FT.

**a. ER Agonist: meso-Hexestrol**



**b. ER Antagonist: 4-OH-tamoxifen**

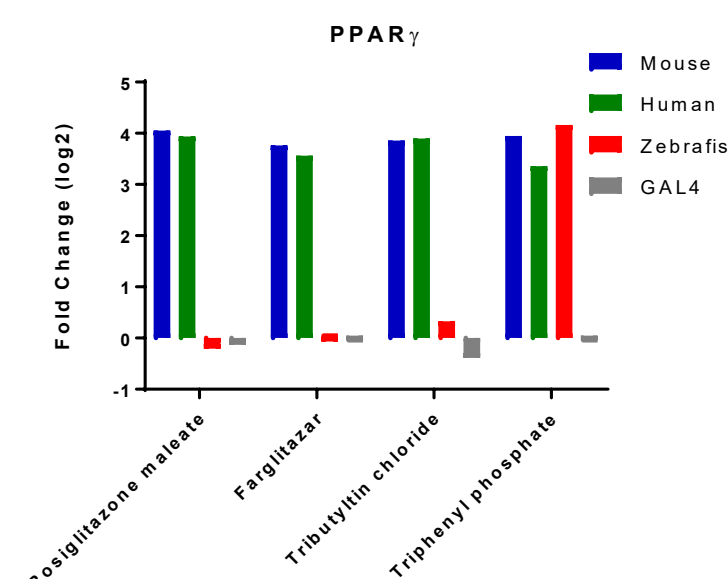


**Fig. 5 ER Response**

a) All ERs responded to meso-Hexestrol as an agonist. b) 4-OH-tamoxifen reduced basal ER activity for some of the ERs, permitting detection of ER antagonists without added agonist.

**Fig. 6. PPAR $\gamma$  Response.**

Human and mouse receptors responded as expected to known agonists; however, ZF receptor was unresponsive. All 3 were strongly activated by the flame retardant triphenyl phosphite.



## CONCLUSIONS

- All receptors responded to reference compounds, indicating human host cell was competent for diverse species
- High potency compounds were similar across species but less similar for lower potencies
- Differences found for some potent compounds, particularly for zebrafish
- LBD similarity between species correlates with compound sensitivity
- Flexible platform readily adaptable to screen multiple receptors/species of interest

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