

## Yeast-2-Hybrid Assays

Phenex has cloned a comprehensive list of human Nuclear Receptors Ligand Binding Domains (LBDs) and a huge panel of Cofactors (CoF) that are described in the literature. This panel is used by Phenex to determine ligand-dependent Cofactor recruitment in the yeast system. For this purpose, Phenex employs the direct mating of respective NR and CoF interaction vectors harbored by a yeast  $\alpha$  and  $a$  type, respectively. Together with a robotized all-fluid phase dilution scheme, Phenex is able to assay for ligand dependency of Cofactor interactions in a dose response manner.

The Yeast-2-Hybrid direct mating system is ideal to obtain an overview which Cofactor (constructs) are recruited to a certain Nuclear Receptor in a ligand-dependent fashion. Each NR has its own distinct CoF binding pattern upon administration of its cognate native ligand. However, subtle differences between classical full agonists and partial agonists can be detected in this system. This makes it a good starting point to analyze SNuRM-effects of a set of NR binding drug candidates.

The following list of Yeast-2-Hybrid assays can be employed for identification of ligand dependent cofactor interactions for the respective Nuclear Receptors.

Nuclear Receptor; Trivial Name	Nuclear Receptor; Systematic Name	Assay with full length NR	Assay with LBD	Control Agonist
DAX1	NR0B1	X		-
SHP	NR0B2	X		-
TR $\alpha$	NR1A1		X	Triiodothyronine (T3)
TR $\beta$	NR1A2		X	Triiodothyronine (T3)
RAR $\alpha$	NR1B1	X	X	all-trans Retinoic Acid (ATRA)
RAR $\beta$	NR1B2	X	X	all-trans Retinoic Acid (ATRA)
RAR $\gamma$	NR1B3	X	X	all-trans Retinoic Acid (ATRA)
PPAR $\alpha$	NR1C1		X	GW7647
PPAR $\beta/\delta$	NR1C2		X	GW501516
PPAR $\gamma$	NR1C3		X	Rosiglitazone (RGZ)
Rev-Erba	NR1D1		X	-
Rev-Erb $\beta$	NR1D2		X	-
ROR $\alpha$	NR1F1		X	-

Nuclear Receptor; Trivial Name	Nuclear Receptor; Systematic Name	Assay with full length NR	Assay with LBD	Control Agonist
ROR $\beta$	NR1F2		X	-
ROR $\gamma$	NR1F3		X	-
LXR $\beta$	NR1H2	X	X	T0901317
LXR $\alpha$	NR1H3	X	X	T0901317
VDR	NR1I1	X	X	1,25-Dihydroxycholecalciferol (VitD3)
PXR (SXR)	NR1I2		X	SR12813
HNF4 $\alpha$	NR2A1		X	-
HNF4 $\gamma$	NR2A2		X	-
RXR $\alpha$	NR2B1	X	X	9-cis-Retinoic Acid (9cisRA)
RXR $\beta$	NR2B2	X	X	9-cis-Retinoic Acid (9cisRA)
RXR $\gamma$	NR2B3	X	X	9-cis-Retinoic Acid (9cisRA)
COUP-TF2	NR2F2		X	-
ER $\alpha$	NR3A1	X	X	17 $\beta$ -Estradiol (b-Est)
ER $\beta$	NR3A2	X	X	17 $\beta$ -Estradiol (b-Est)
ERR $\alpha$	NR3B1		X	Diethylstilbestrol (DES)
ERR $\gamma$	NR3B3		X	-
MR	NR3C2	X	X	Aldosterone (Ald)
PR	NR3C3	X	X	Progesterone (Prog)
AR	NR3C4	X	X	Dihydrotestosterone (DHT)
LRH-1	NR5A2		X	-