

What can you do with a luciferase Reporter Assay?

Nuclear Receptor Assay Applications

Presented Fall 2009



Click the icon in the upper left hand corner to view speaker notes for slides.

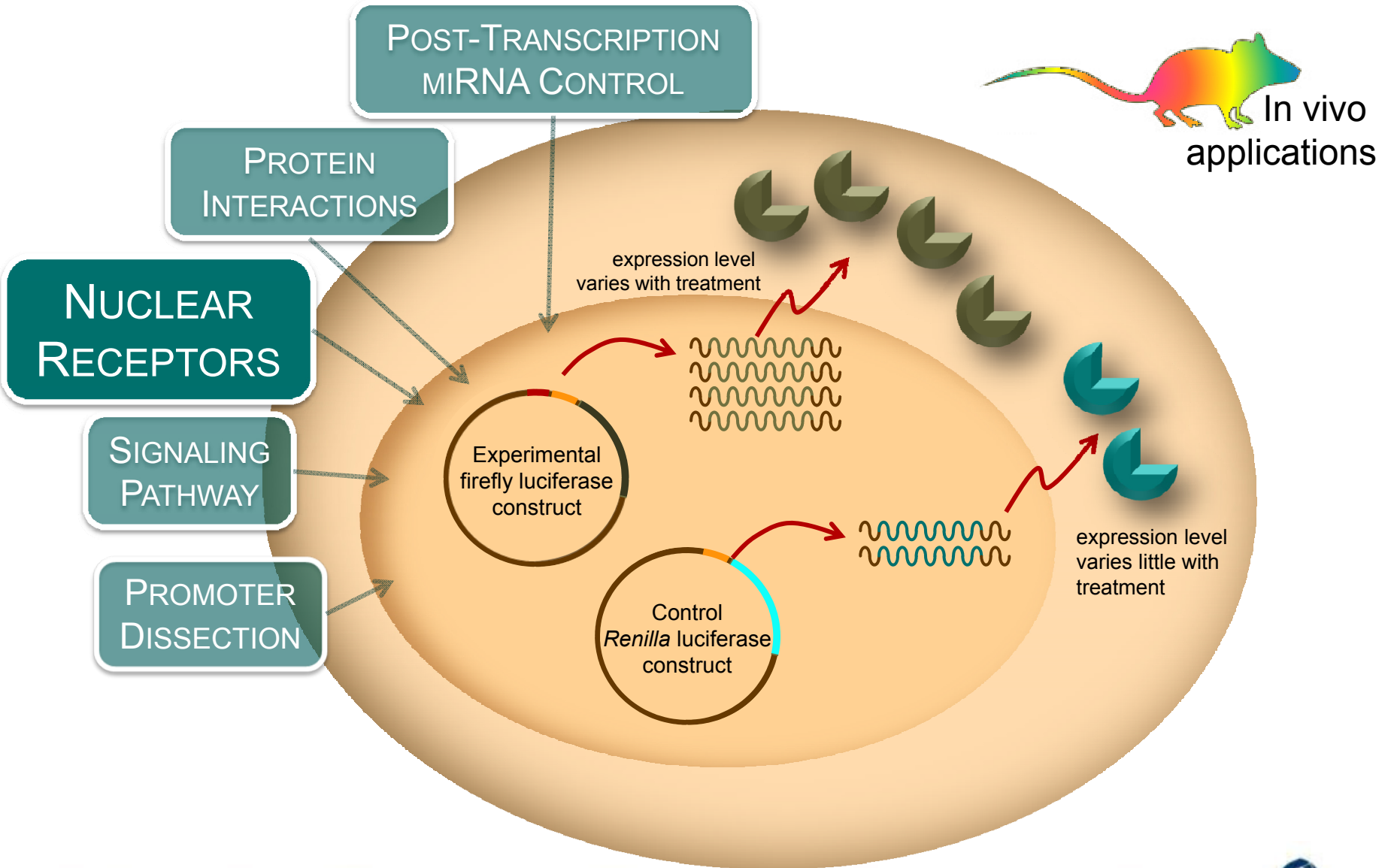


Have a question?
Ask a Scientist



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Application Overview

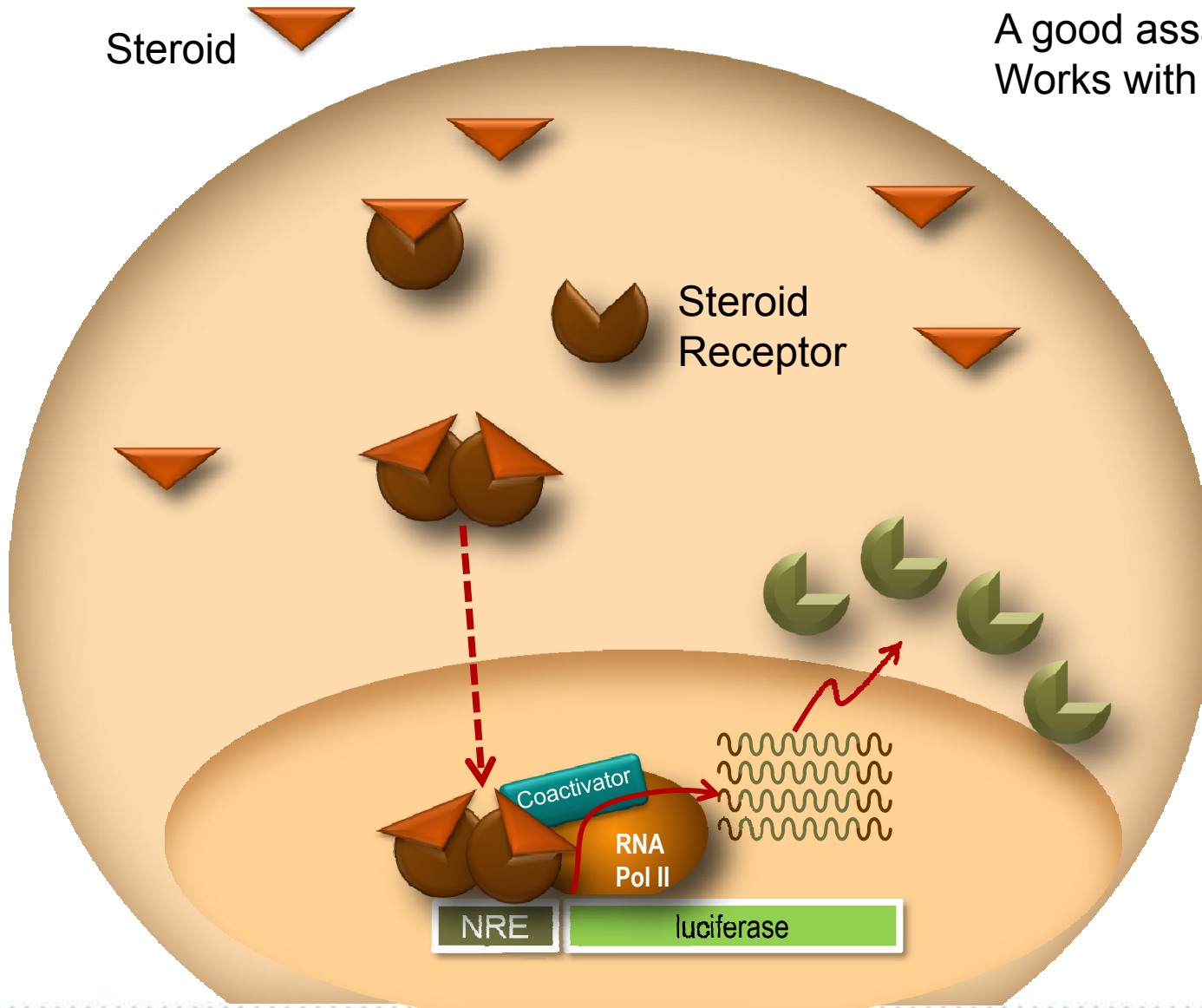




Traditional Nuclear Receptor Assays

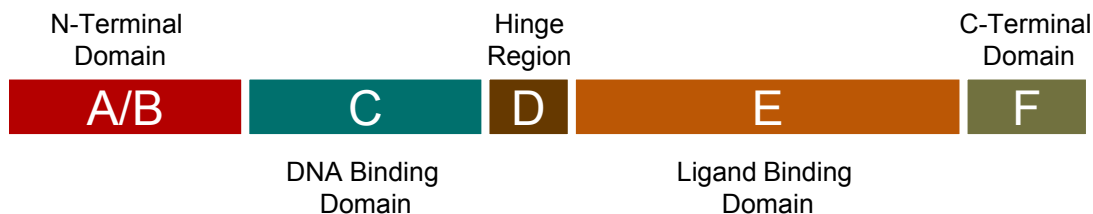
Steroid

A good assay
Works with endogenous receptors





Your expression analysis found an uncharacterized NR...



- Response elements are not specifically known
- Agonists are not known
- Coactivators are not known
- How can you do work on this nuclear receptor?

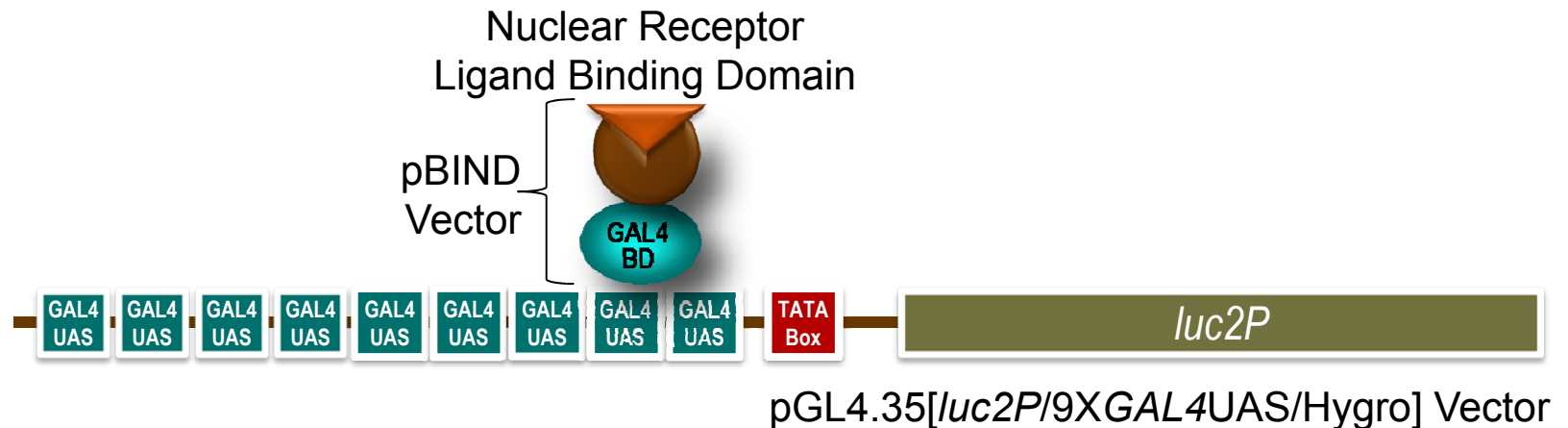
A One-Hybrid Luciferase Reporter Assay can help understand the nuclear receptor more fully



Universal Nuclear Receptor Assays

Ligand binding domain responsible for:

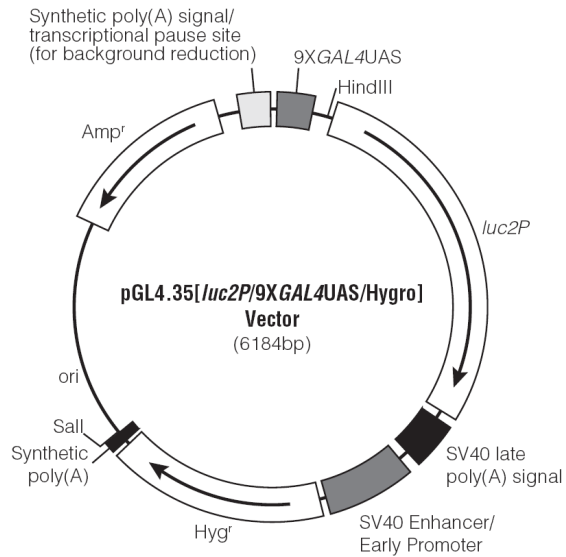
- homodimerization (class I receptors)
- heterodimerization (class II receptors)
- corepressor binding
- coactivator binding



What you need...



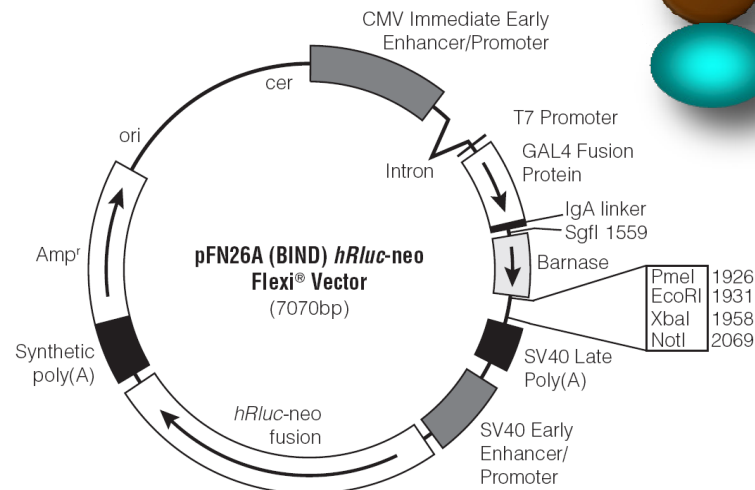
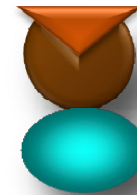
pGL4.35[*luc2P*/9XGAL4UAS/Hygro] Vector



Both vectors ready to make stable cells

- pGL4.35 → Hyg^R
- pFN26A → Neo^R

Nuclear Receptor Ligand Binding Domain



8172MA

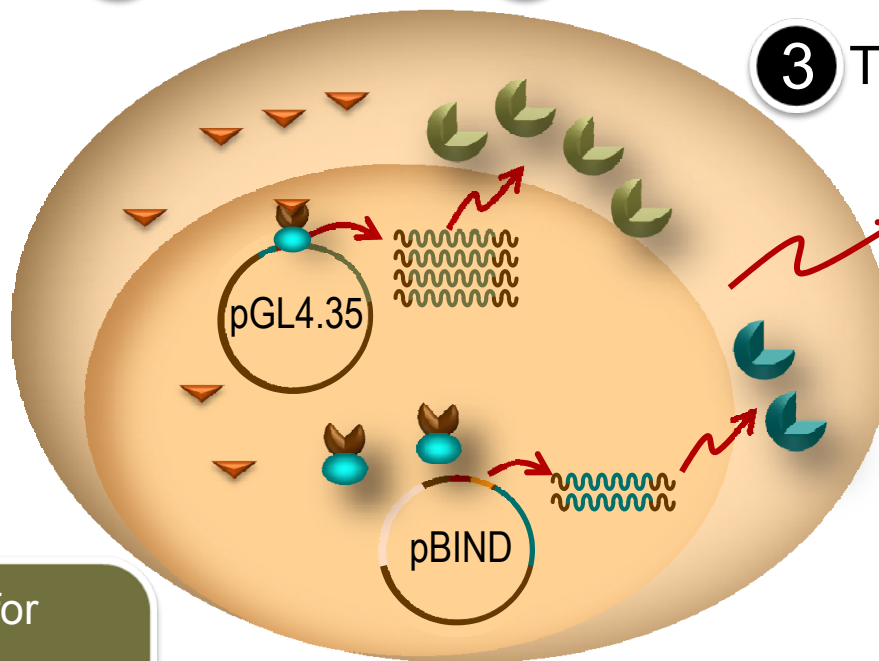


Nuclear Receptor Assay Principle

1 TRANSFECT 2 CULTURE 2-3 DAYS

3 TREAT

4 DUAL-LUCIFERASE[®] ASSAY



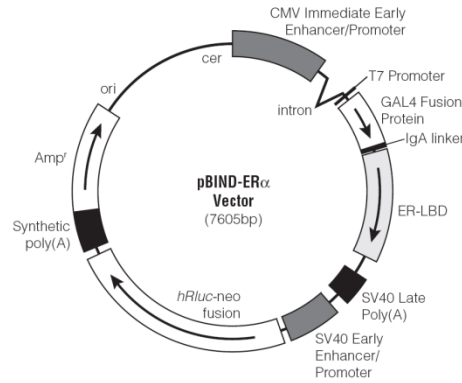
Screen for agonists, antagonists, co-activators, co-repressors

Make your own Nuclear Receptor Luciferase Reporter Cell line
• pGL4.35 Hyg^R

Perform mutagenesis on the ligand binding domain and assay in your responsive cell.

- No interference from endogenous receptor

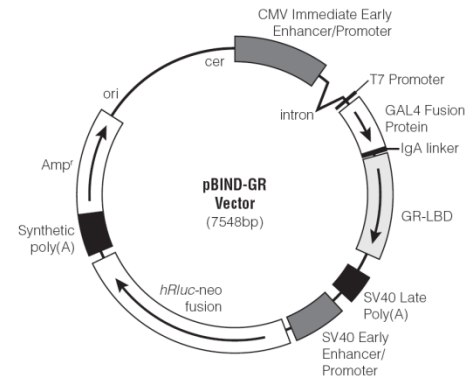
Pre-designed tools available for nuclear receptor assays



Pre-designed Estrogen Receptor α - & Glucocorticoid Receptor-GAL4 Binding Domain fusions available

GloResponse™
9XGAL4UAS-luc2P
HEK293 Cell Line

HEK 293 Hyg^R cell line
made with pGL4.35



Ready to use with the nuclear receptor or CheckMate™ Assays

Case Study: Coactivator of Vitamin D receptor



Vitamin D receptor (VDR) is a nuclear receptor that upon ligand binding displaces a co-repressor and allows co-activators to bind, allowing transcription.

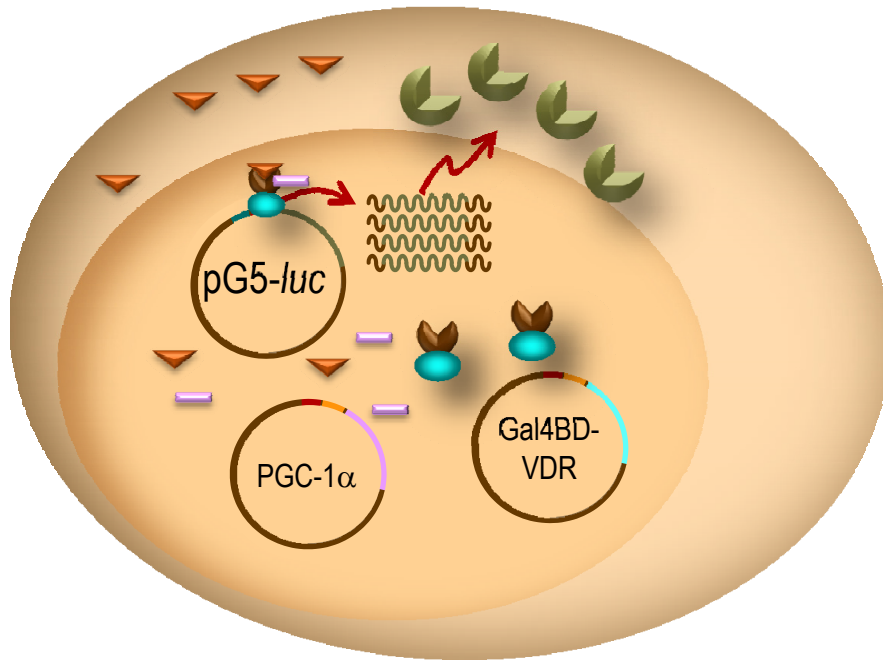
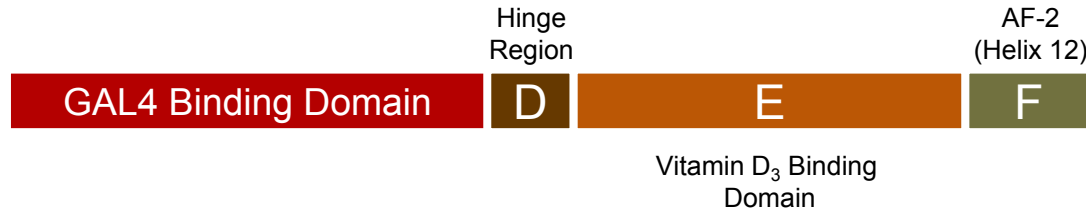
Central question:

- Peroxisome Proliferator-Activated Receptor γ coactivator-1 α (PGC-1 α) and the Vitamin D receptor have overlapping tissue distribution,
- Does PGC1 α function as a coactivator of VDR?

Savkur, R.S., et al. (2005) *Mol. Pharmacol.* **68**, 511-517.

Case Study:
Savkur, R.S. et al.

PGC-1 α is a coactivator of VDR



Constructs	DMSO	VitD ₃	Fold Increase Luc activity
Gal4BD-VDR + pG5-luc	+	-	1
	-	+	5
Gal4BD-VDR + PGC-1 α + pG5-luc	+	-	1
	-	+	up to 500
Gal4BD-VDR(Δ AF-2) + PGC-1 α + pG5-luc	+	-	1
	-	+	1

Conclusions supported by work with full receptor & mammalian two-hybrid work

More Information...



[Paquio, A., et al. \(2008\) Improvements to luciferase reporter assays for nuclear receptor function. PS065 presented at Society for Biomolecular Sciences meeting.](#)