



LightSwitch Multiplex Assays for Environmental Testing

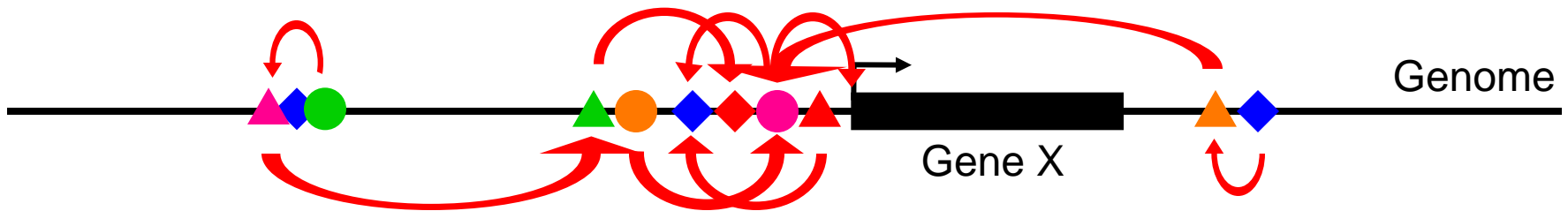
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SwitchGear Background

- Spun out of Stanford University in 2006
- Leading provider of functional genomics research tools with more than 1,000 customers world-wide
- LightSwitch Biomarker products (validated cell-based assays for biological pathways) are widely used in pharma high-throughput screening groups
- Environmental toxicology bioassay screening is a new application for LightSwitch Biomarker products

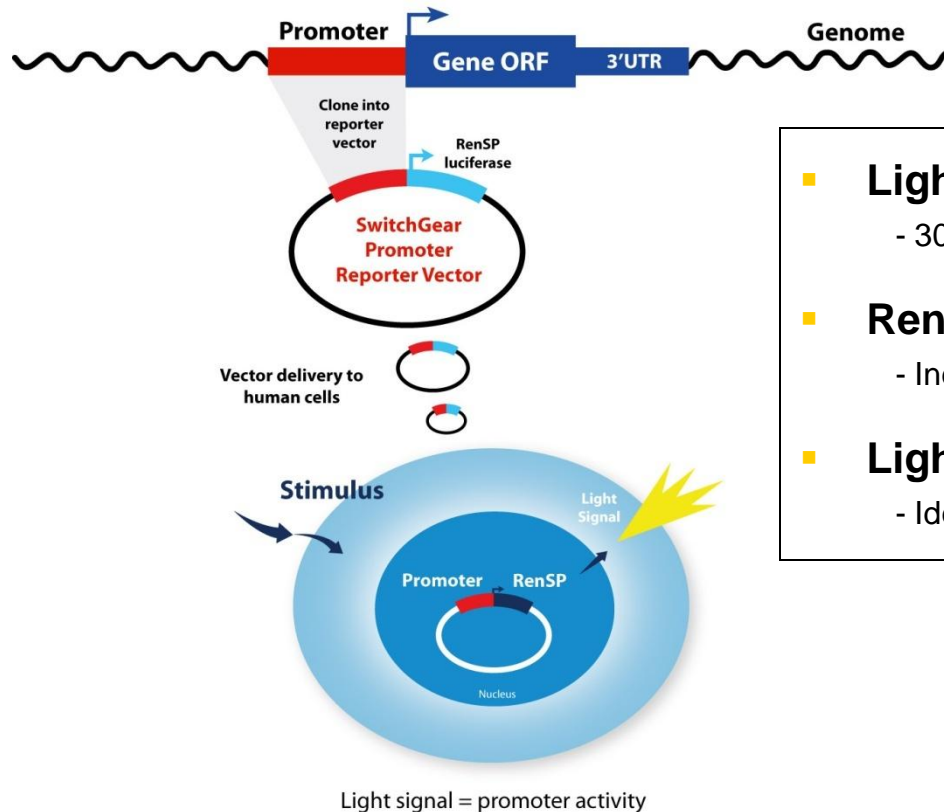
SwitchGear Background

- First wave of genomic technologies focused on genes
(3% of the human genome)
- SwitchGear is at the front of the next wave of genomic technologies focused on regulatory elements
(> 3% of the human genome)



LiGHTSWiTCH

Luciferase Reporter Assay System



- **LightSwitch GoClones™**
 - 30,000 cloned human regulatory elements
- **RenSP: next-gen luciferase gene**
 - Industry-leading brightness and sensitivity
- **LightSwitch Luciferase Assay Reagents™**
 - Ideal for high-throughput, plate-based screens

LiGHTSWiTCH

GoClone™ Collections

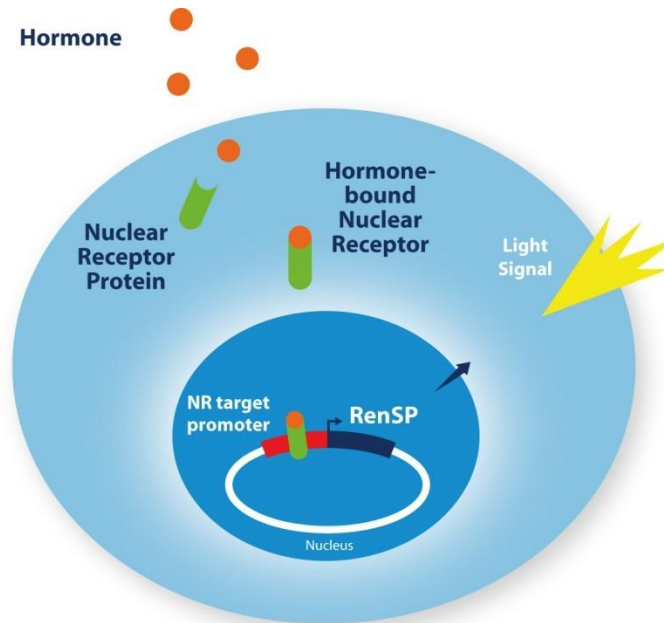
	Current
Human promoters	18,000
Human 3'UTRs	12,000
Synthetic elements	1,000
Validated biomarkers	800

- *Sold as:*
 - Individual constructs
 - Validated biomarker sets

LiGHTSWiTCH

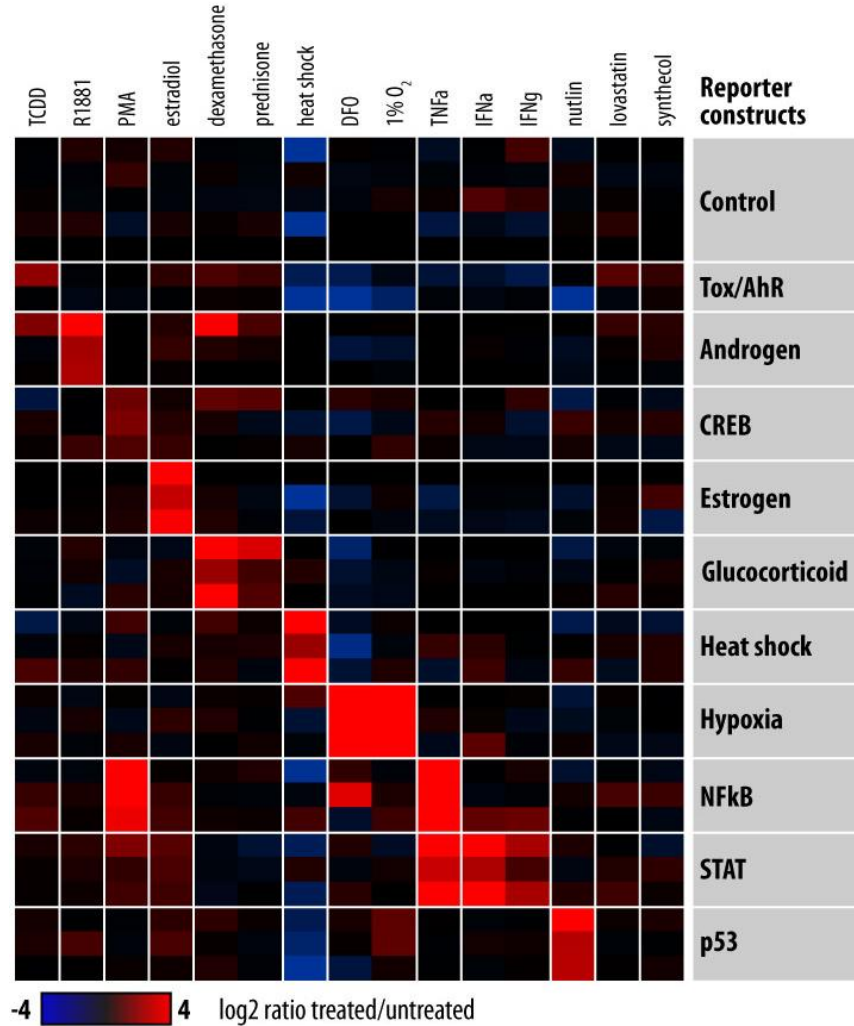
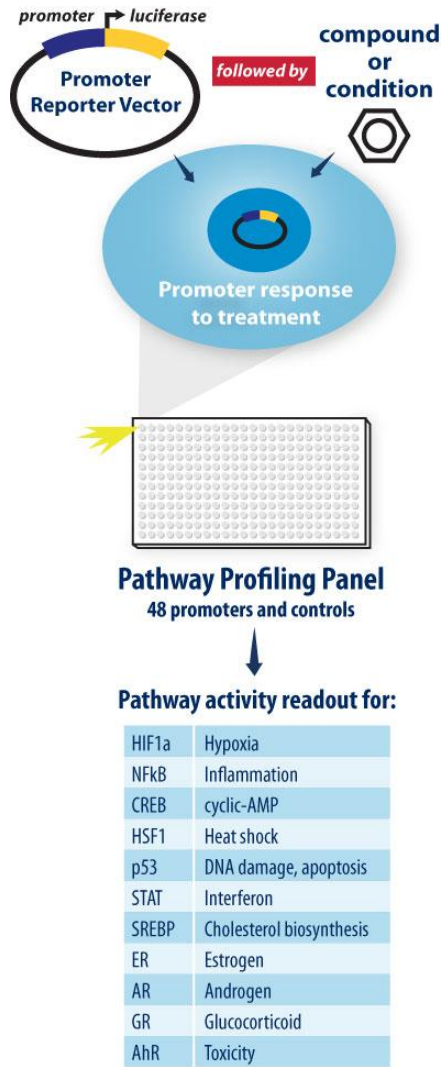
Luciferase Reporter Assay System

Nuclear receptor / hormone signaling



Light signal = hormone activity

LightSwitch Biomarkers



SwitchGear Assays for Environmental Testing

Biomarkers for Pharma HTS



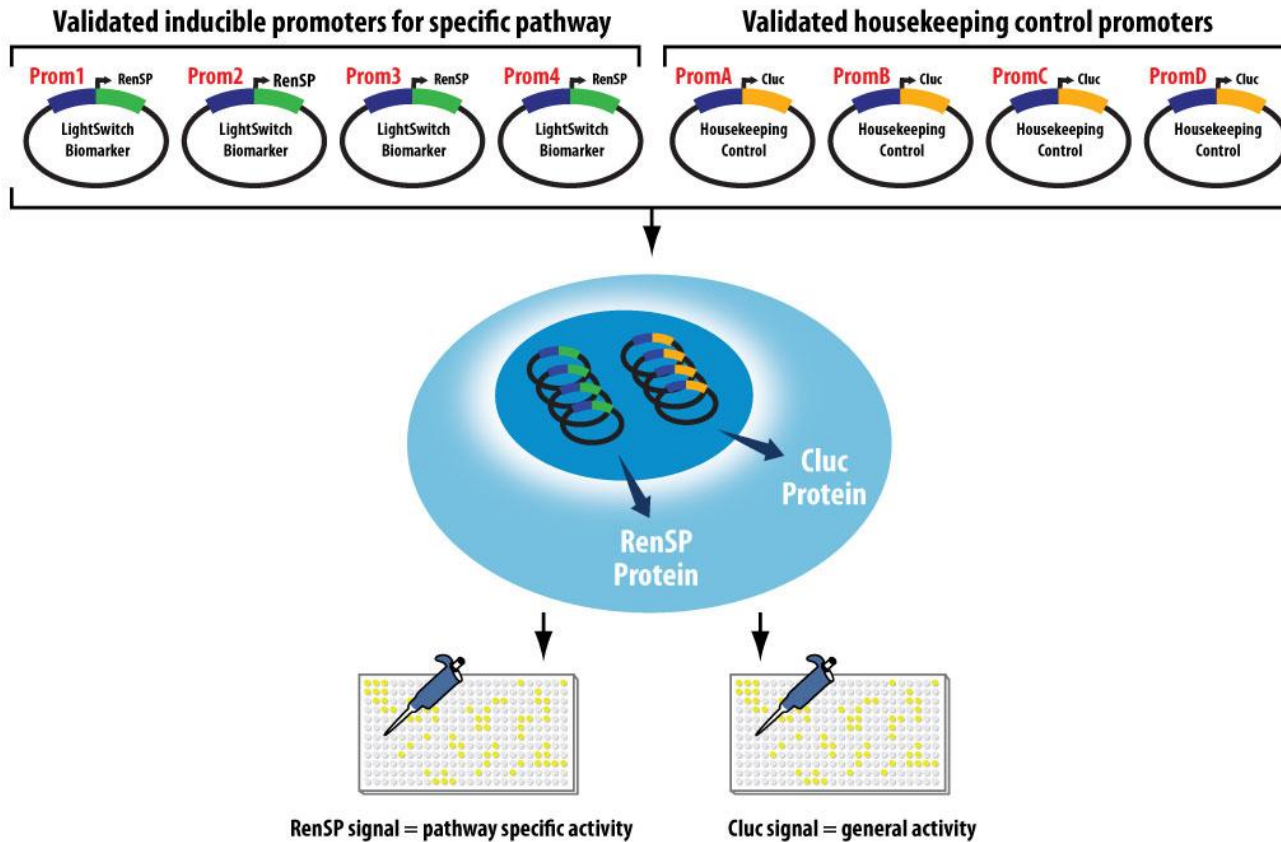
Biomarkers for Water Tox Screening

“LightSwitch Multiplex Assays”

- Androgen pathway
- Estrogen pathway
- Glucocorticoid pathway
- AhR / dioxin pathway
- Genotoxicity pathway

LightSwitch Multiplex Assay

Multiplex Assay = 1 well, 1 water sample



LightSwitch Multiplex Assay

Advantages

- **Multiplex: 1 well = 1 water sample**
 - Many assays available (ER, GR, AR, dioxin, genotox)
 - Customizable: 18,000 human elements available for new assay development
- **Multiple pathway targets per assay**
 - Multiple validated promoter biomarkers used to measure a pathway rather than relying on a single response element
- **Direct measure of human biology**
 - Measure the interaction of native human receptors on human DNA elements in human cells
- **Internal controls**
 - Panel of housekeeping promoters for assessing cell viability
- **Fully-integrated LightSwitch Assay System™**
 - Comprehensive kits and protocols from cell seeding to data collection
 - Tightly optimized and QC'd assay reagents provide highly reproducible results

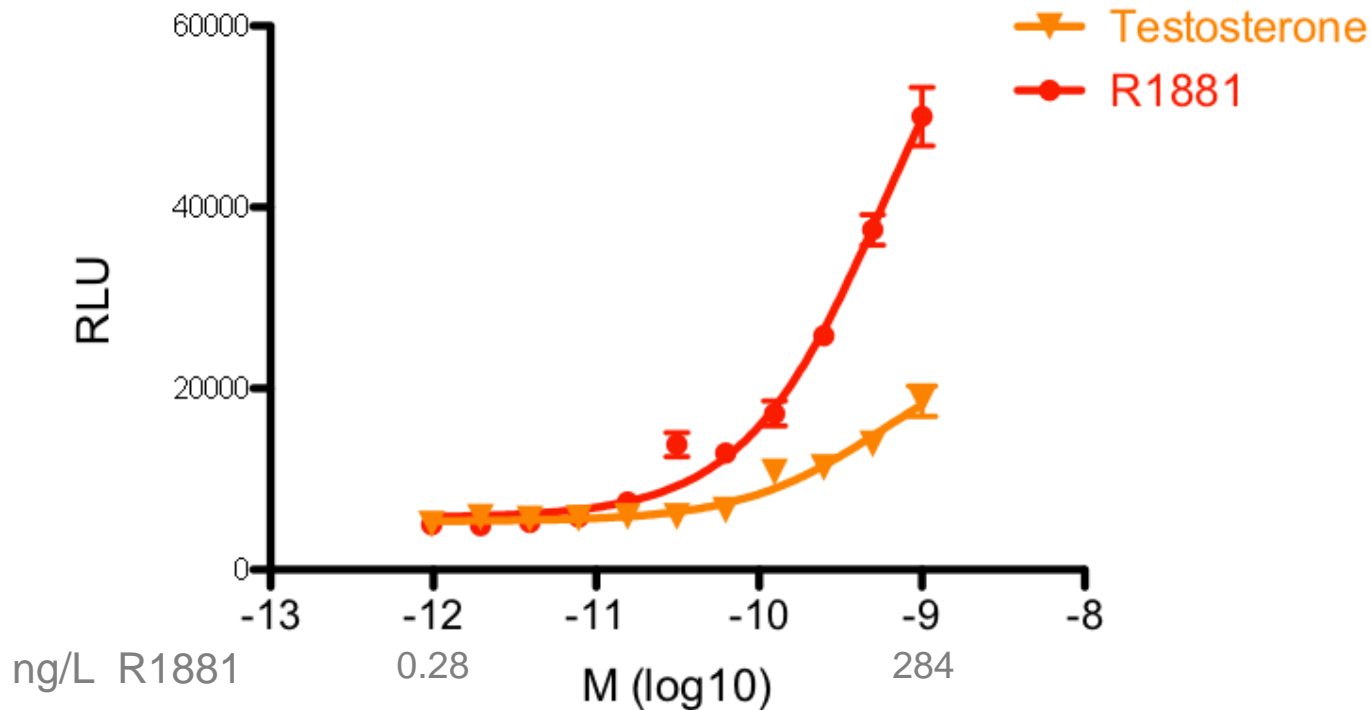
LightSwitch Multiplex Assay

Workflow

- **DAY 1**
 - Combine Multiplex Assay Mix with cells and seed in multi-well plate
- **DAY 2**
 - Add water or control samples to each well
- **DAY 3**
 - Add LightSwitch Assay Reagents and read light output
 - Determine cell-viability: Yes / No
 - Determine pathway activation: Yes / No

LightSwitch Multiplex AR Assay

Known androgen agonists



Lowest Detectable Dose

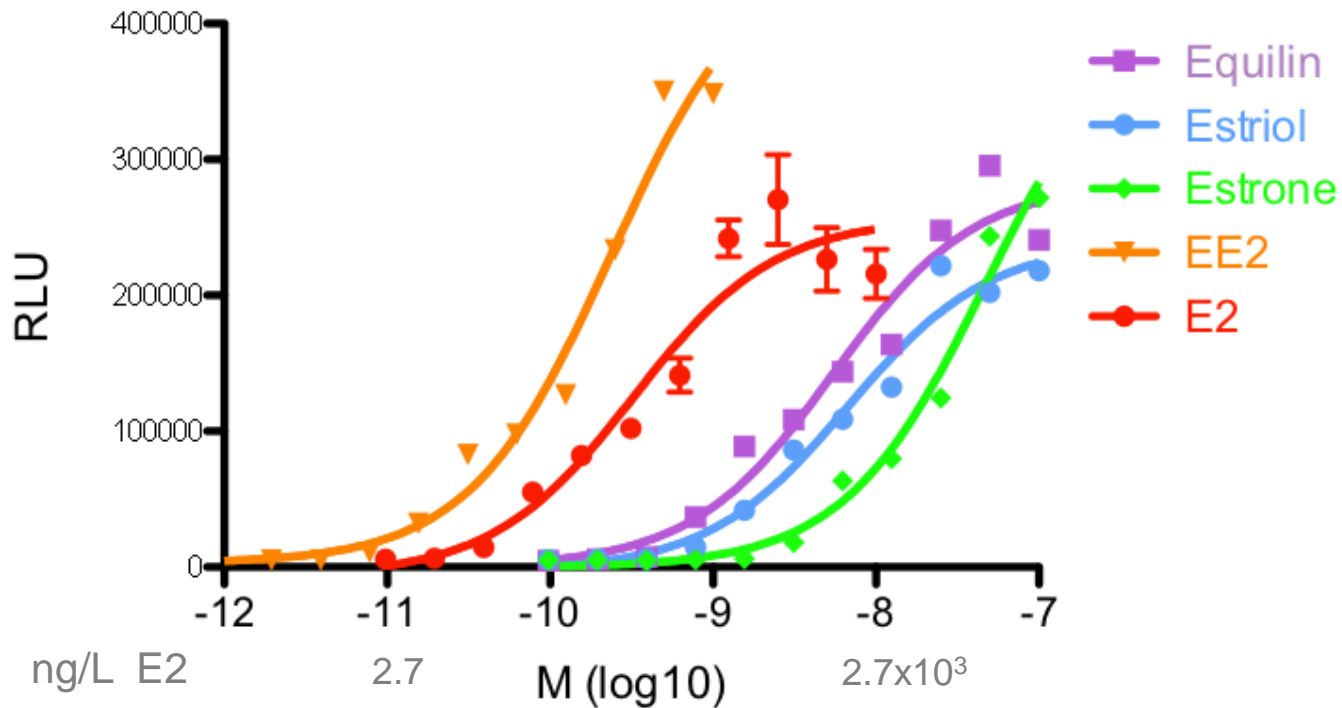
$\sim 1.5 \times 10^{-11}$ M (~ 4 ng/L)
R1881

Reproducibility within linear
range of R1881 curve

CV (stdev/mean) = 11%

LightSwitch Multiplex ER Assay

Known estrogen agonists



Lowest Detectable Dose

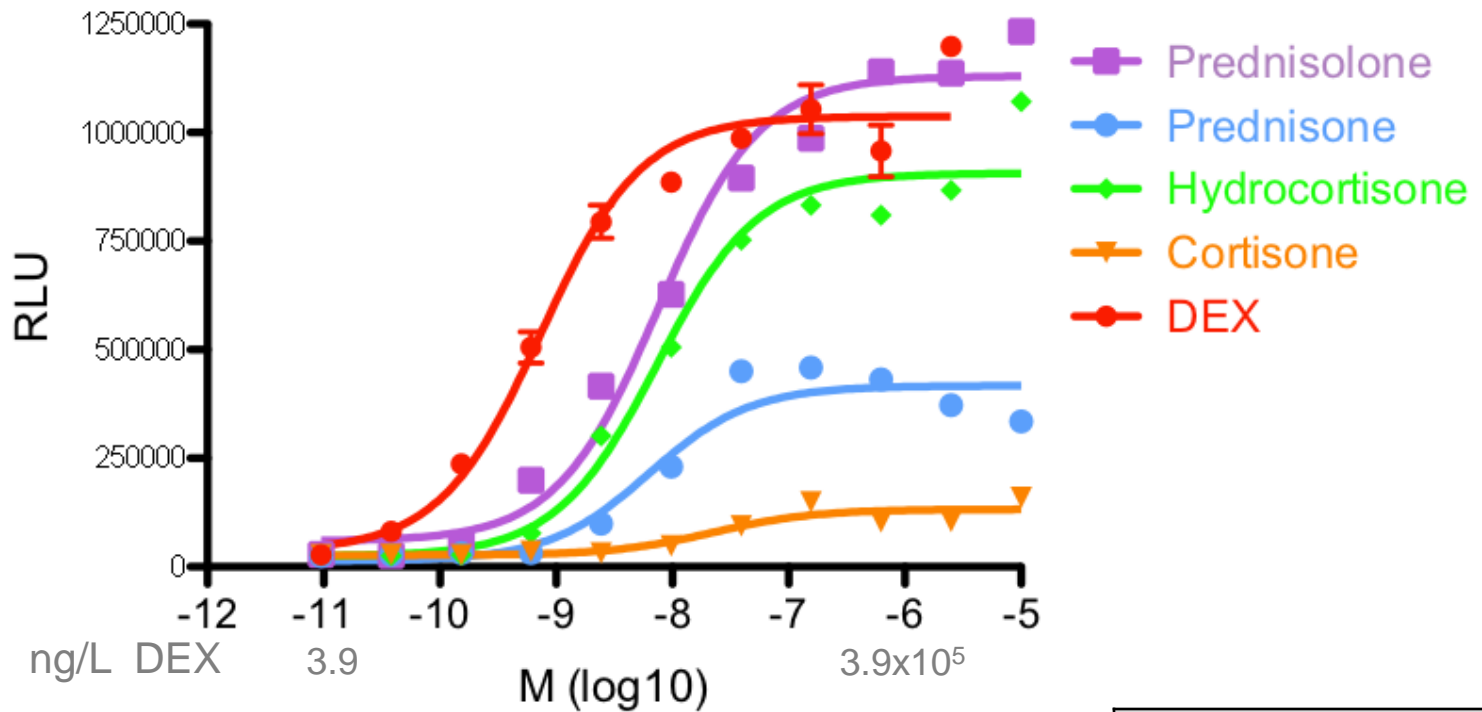
$\sim 7 \times 10^{-12}$ M (~ 2 ng/L)
EE2

Reproducibility within linear
range of E2 curve

CV (stdev/mean) = 10%

LightSwitch Multiplex GR Assay

Known glucocorticoid agonists



Lowest Detectable Dose

$\sim 3.5 \times 10^{-11}$ M (~ 13 ng/L)
DEX

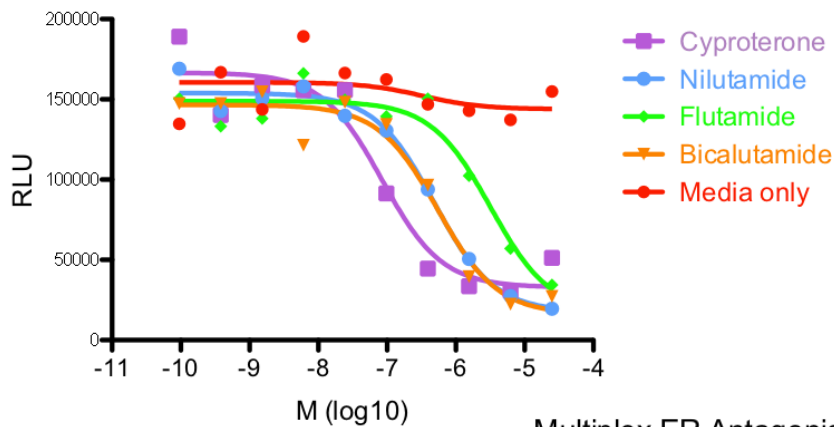
Reproducibility within linear
range of DEX curve

CV (stdev/mean) = 12%

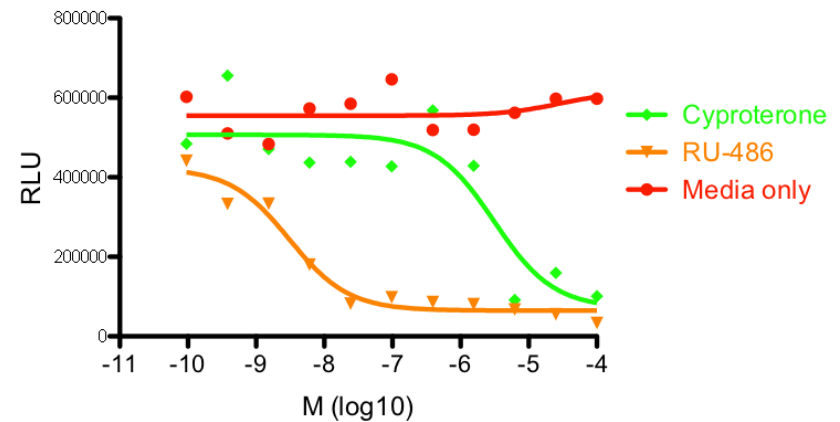
LightSwitch Multiplex Assays

Antagonist format

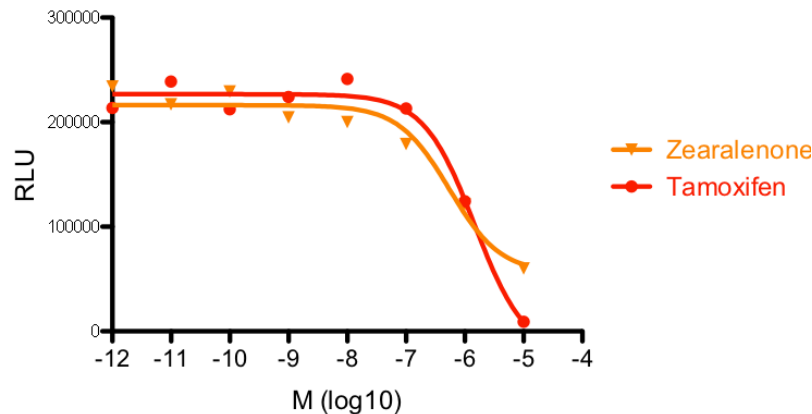
Multiplex AR Antagonist Assay (1nM R1881)



Multiplex GR Antagonist Assay (10nM DEX)



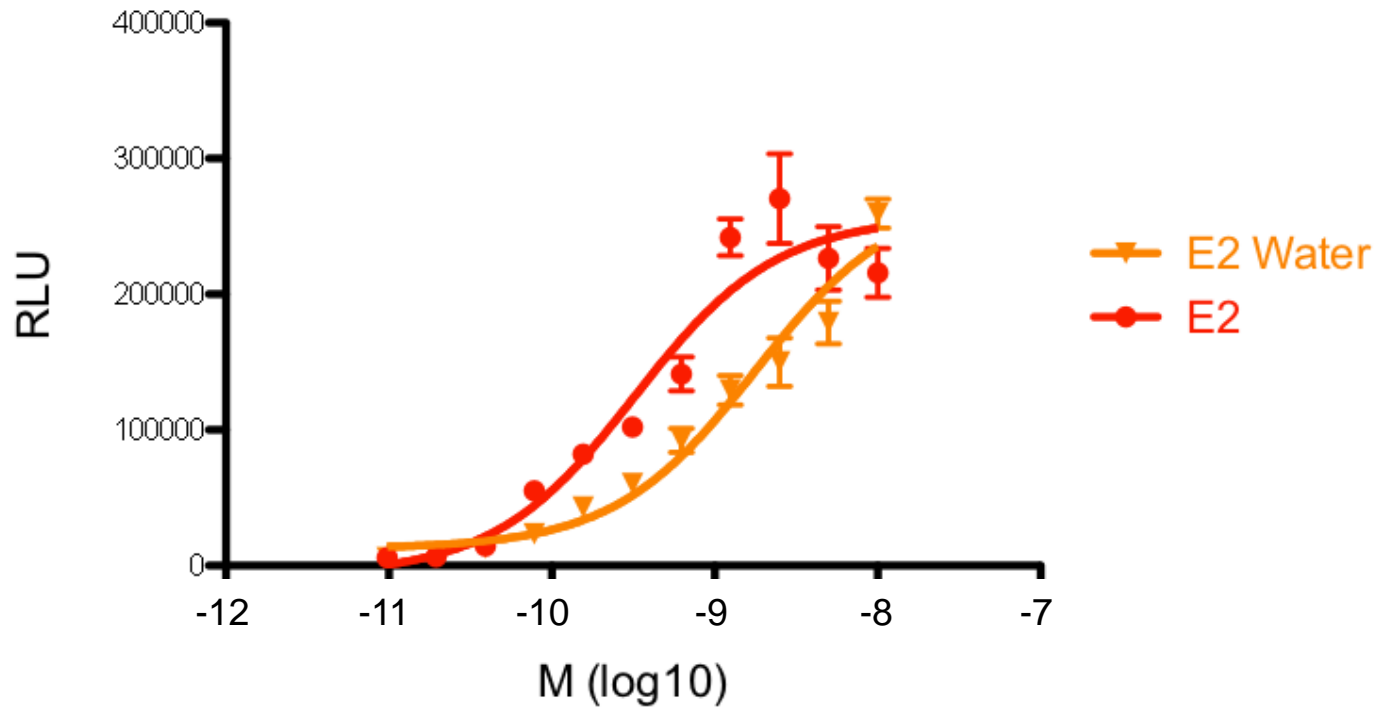
Multiplex ER Antagonist Assay (10nM E2)



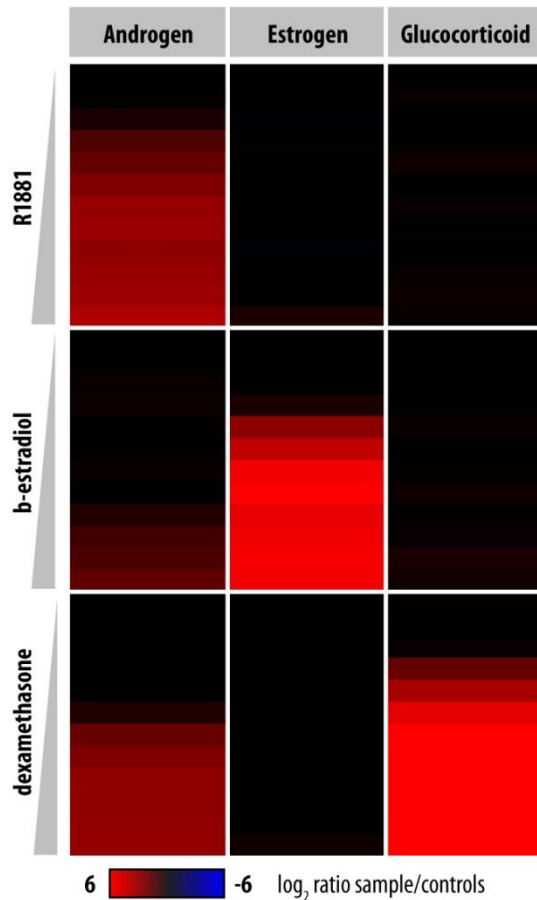
Dose-curves in spiked water samples

“E2”= E2 dilution series in ethanol

“E2 water”= E2 dilution series in Green Valley water sample



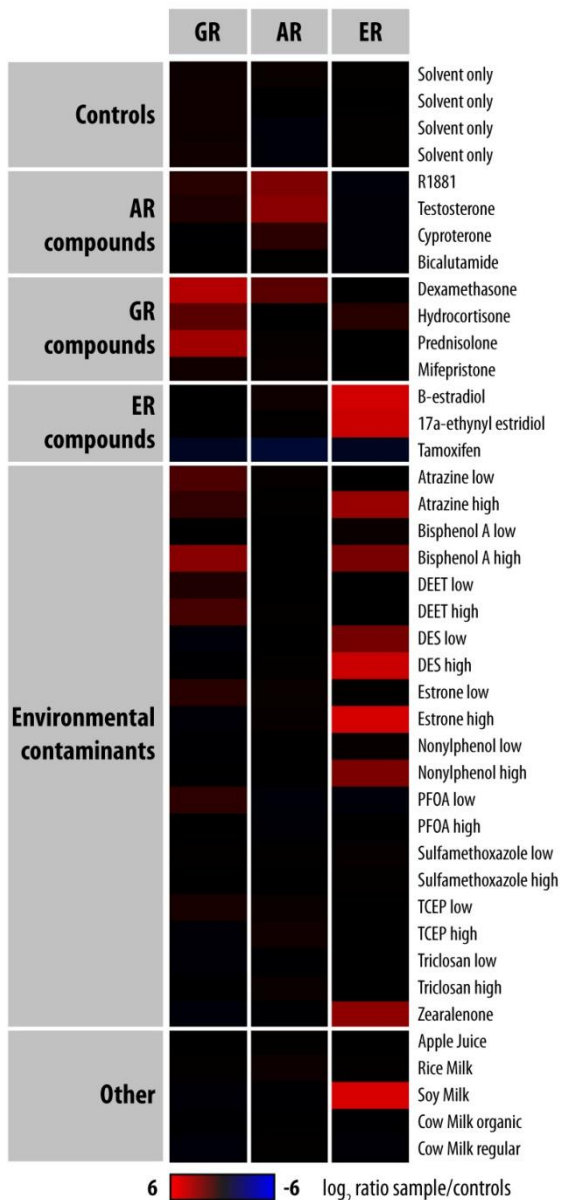
Dose-response as a heat map



Conclusions:

- The ALL-NR assay is able to robustly detect R1881, E2, and DEX
- The androgen receptor assay responds weakly to E2 and DEX at high doses (consistent with AR literature)

Multiplex screen of known environmental contaminants

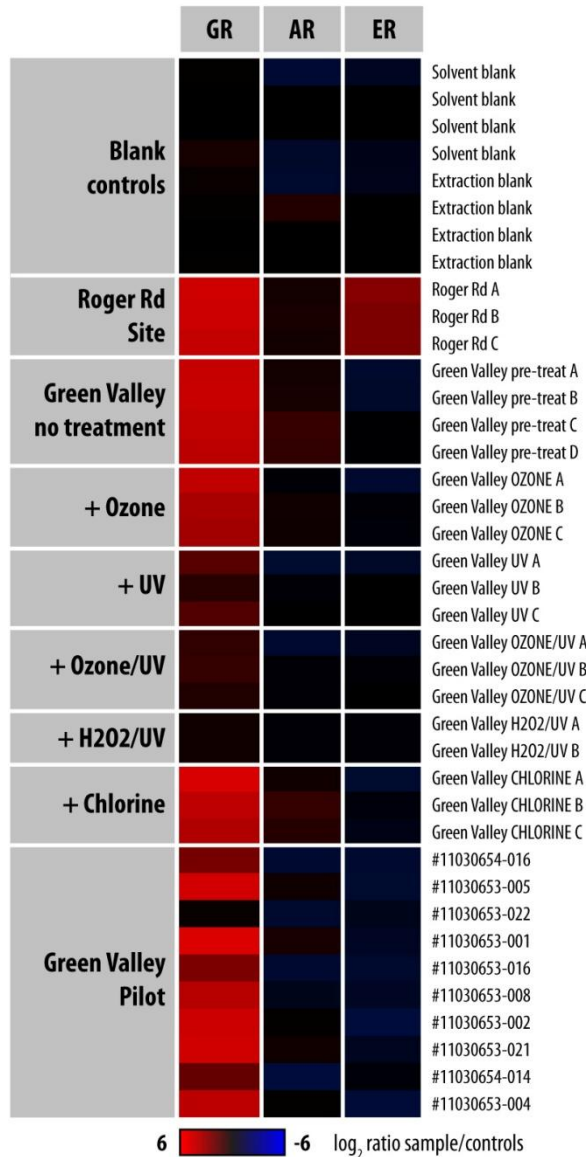


Conclusions:

- Known agonists are clear pathway activators
- 7 / 11 contaminants are identified as activators of ER or GR pathways
- Known estrogenic activity of soy milk is clearly identified

6 -6 log₂ ratio sample/controls

Multiplex screen of water samples: Snyder lab collaboration



Conclusions:

- Many pre-treated recycled water samples show glucocorticoid (GR) activity
- H₂O₂/UV is most effective at removing GR activity
- Chlorine and ozone alone are not effective at removing GR activity
- 9 / 10 Green Valley Pilot samples show significant GR activity

Summary of SwitchGear's Offering

- Validated Multiplex Assay Kits for **AR, ER, GR, AhR/dioxin, Genotoxicity (PR, TR coming soon)**
- Kits include everything you need:
 - ✓ Cells
 - ✓ Multiplex biomarker transfection mix
 - ✓ LightSwitch Assay Reagents
 - ✓ Cell-viability Assay Reagents
 - ✓ Analytical standards
- Huge collection of biomarker reporters for new assay development
- Screening services



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