



Institut Pasteur
de Montevideo

Get 2 for 1!: A dual reporter cell line for simultaneous detection of substances with estrogenic and/or androgenic activity

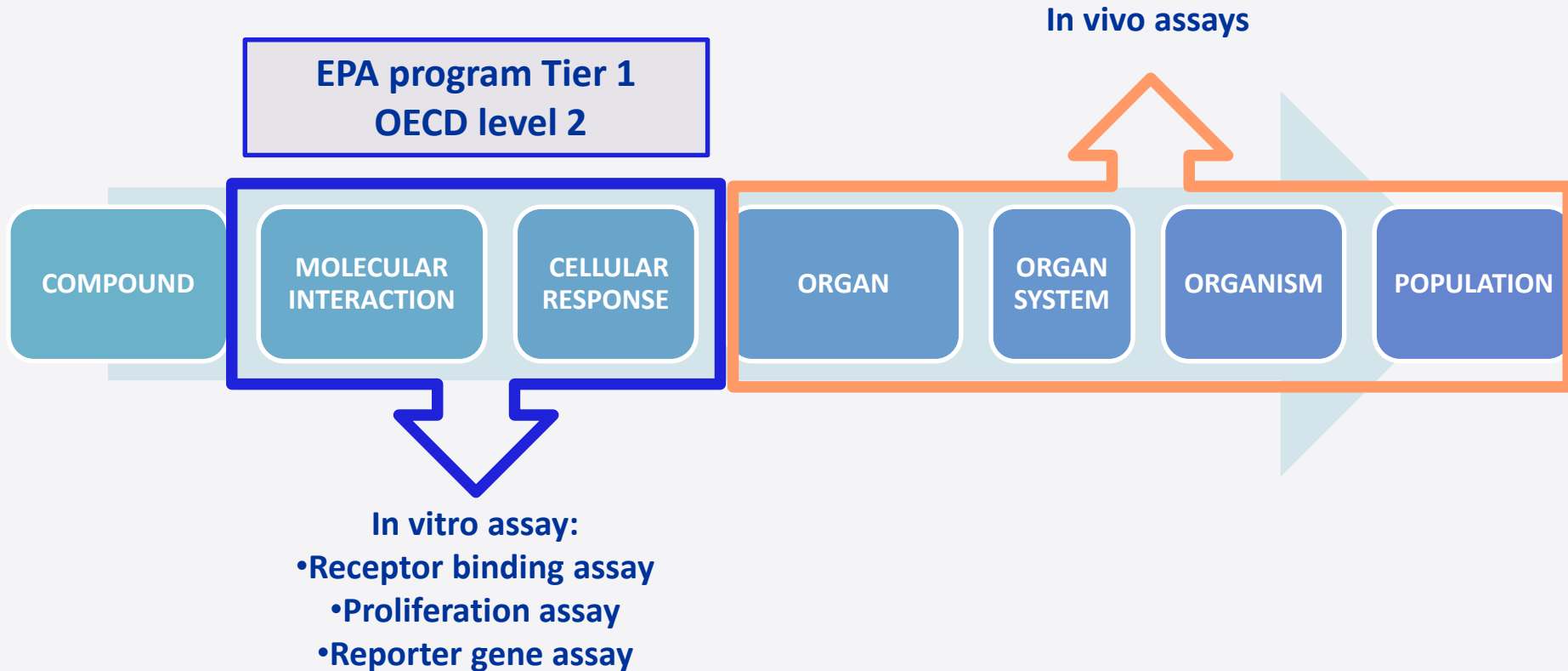
Romina Pagotto

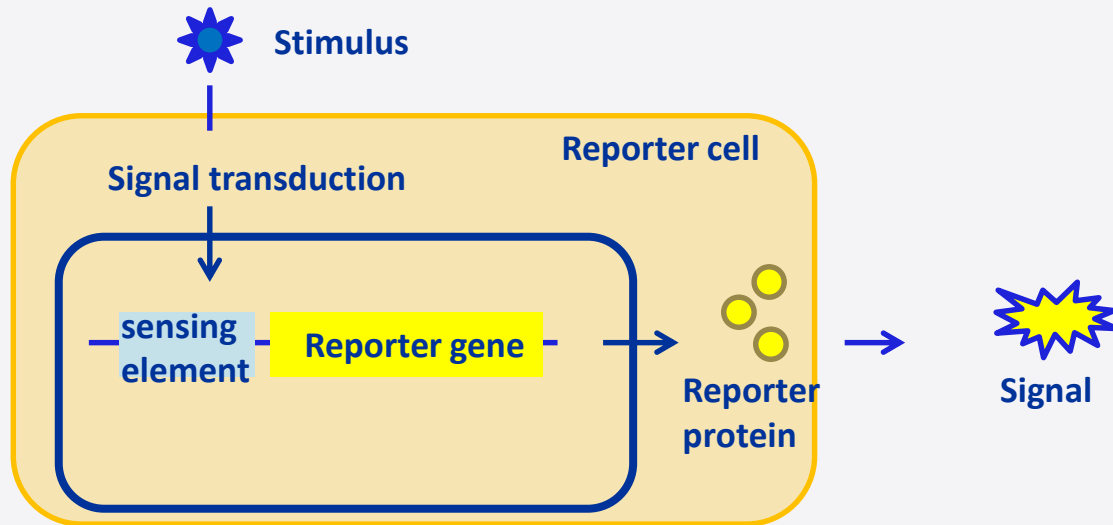
Institut Pasteur de Montevideo

Uruguay

2nd Panamerican Conference for Alternative Methods
Rio de Janeiro –August 2018

Screening battery of chemical for ED





MAMMALIAN REPORTER CELL LINES

ESTROGENS :

- MVLN
- ER- CALUX
- MELN
- HELM
- T47DKBluc

ANDROGENS:

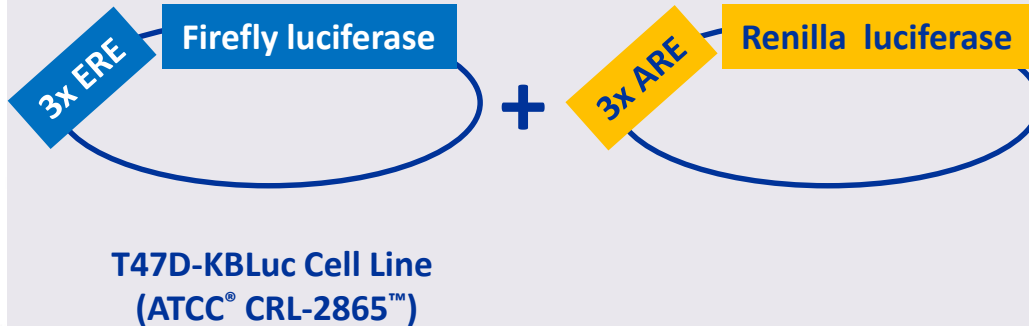
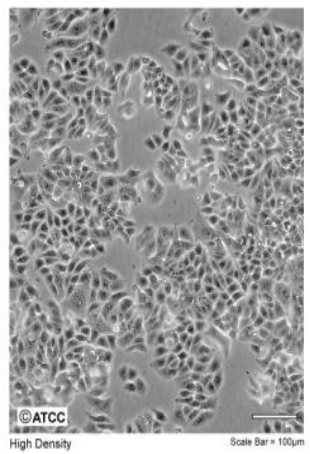
- PALM
- AR CALUX
- AR Lux
- MDA-kb-2

What about having



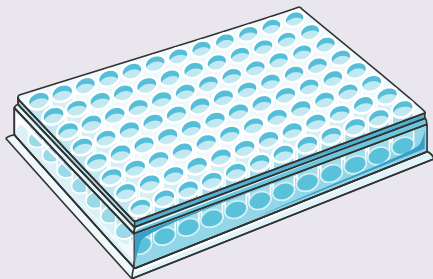
Generate and characterize a dual reporter cell line for the detection of estrogenic and/or androgenic substances.

Reporter cell line generation :



1. Transfection
2. Antibiotics selection
3. Dilution Cloning
4. Characterization
5. Amplification of responding clones

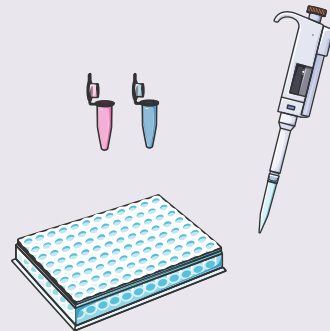
Reporter assay:



Seed the cells in RPMI
W/out phenol red and
5% DCC FBS



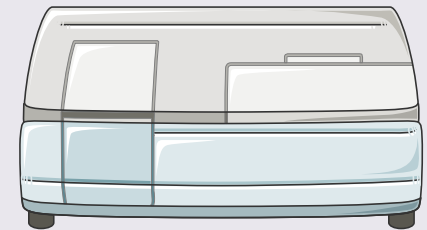
24 h



Add the compounds
to be tested

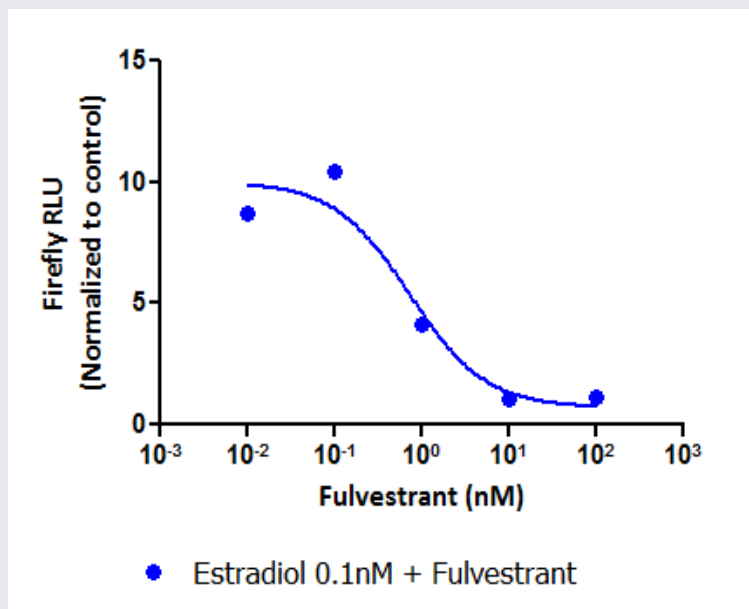
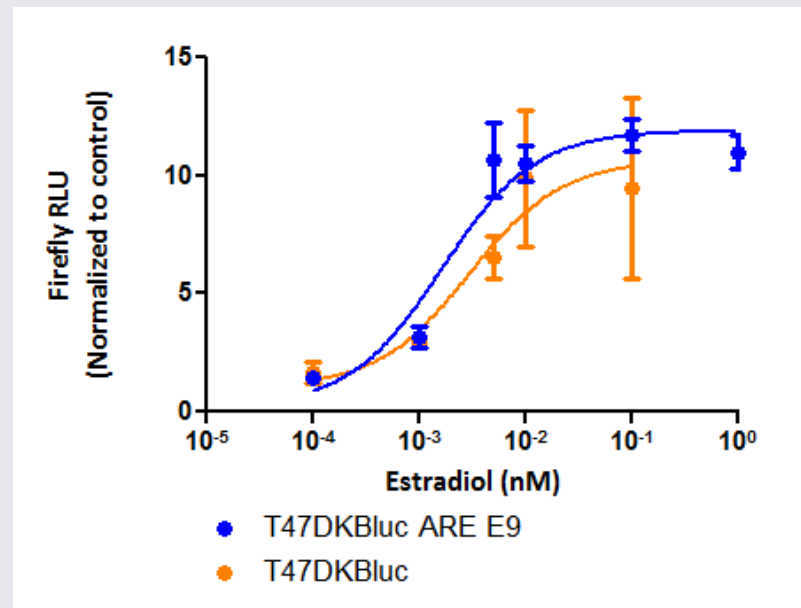
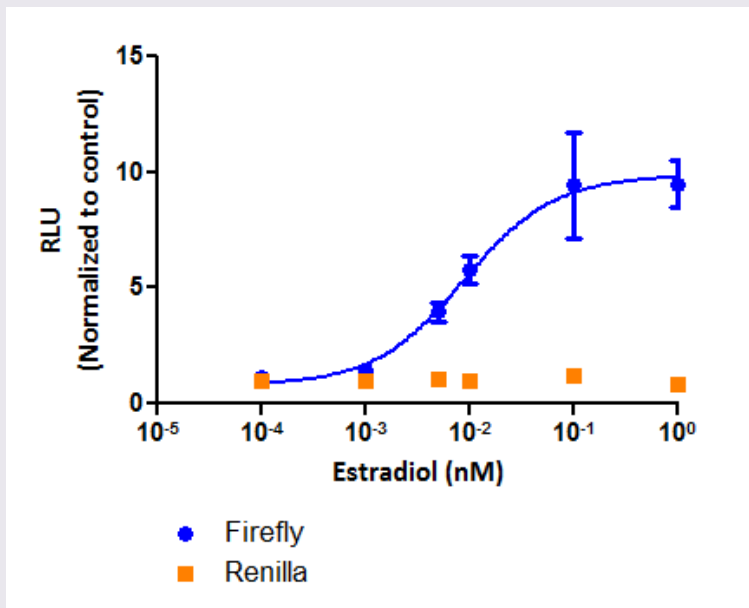


24 h



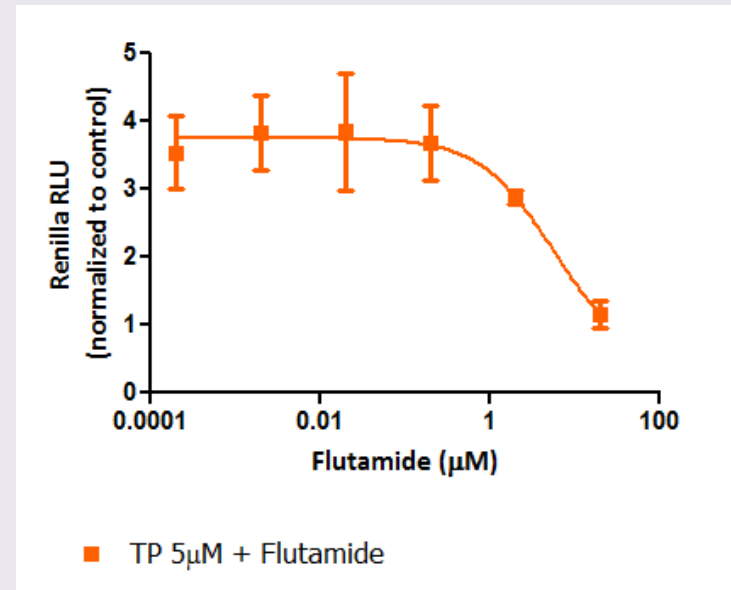
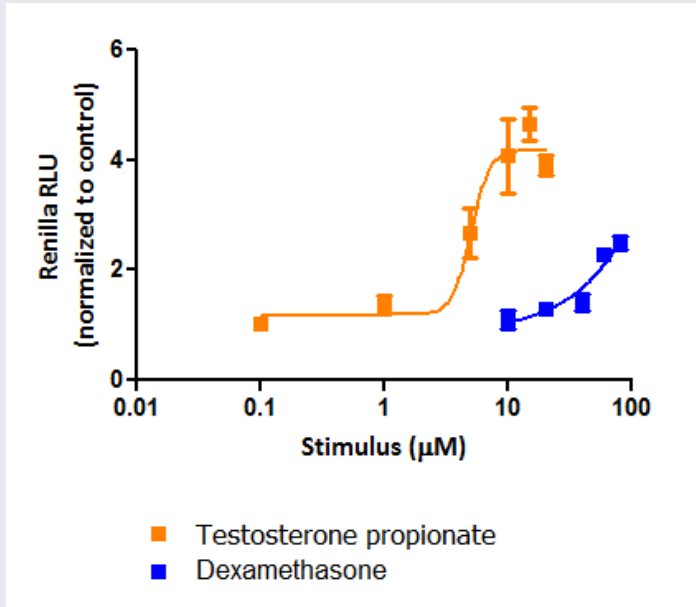
Cell lysis and quantification of luciferase
Firefly and *Renilla* activities

Clone T47DKBluc ARE E9 – ER response



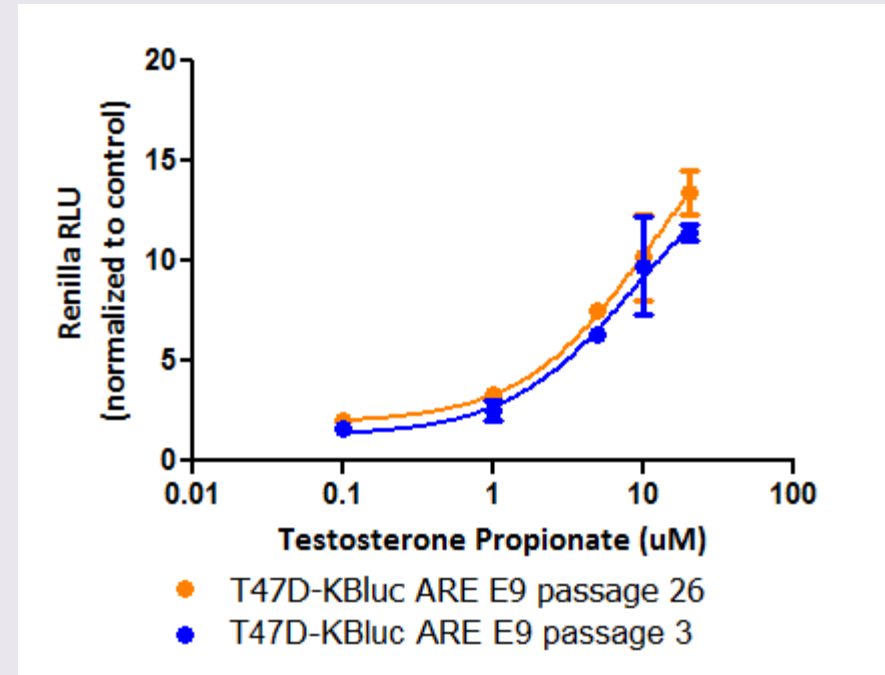
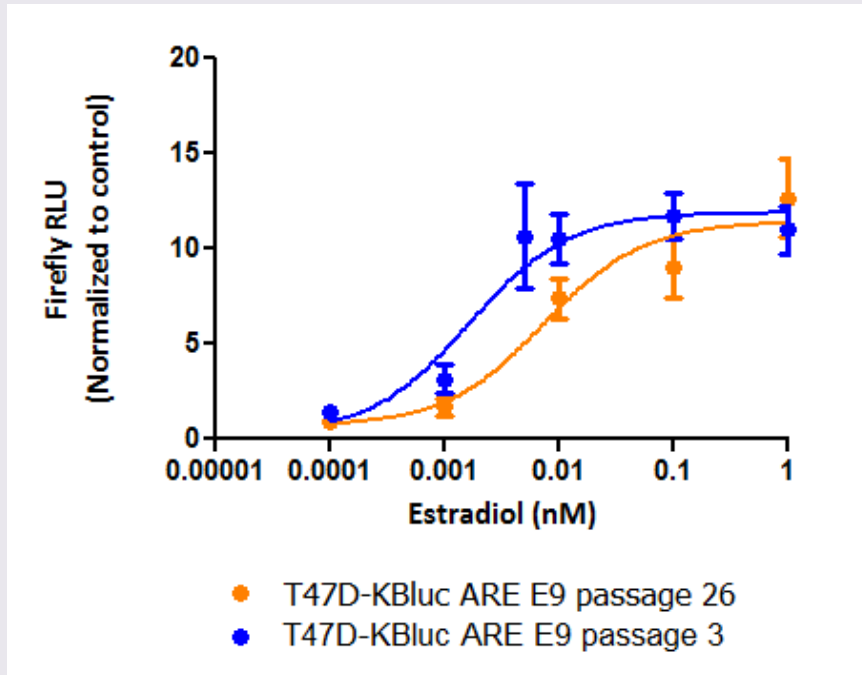
The ER response was comparable to that of the original cell line (T47DKBluc) EC50 ≈ 5 pM

● Clone T47DKBluc ARE E9 – AR response



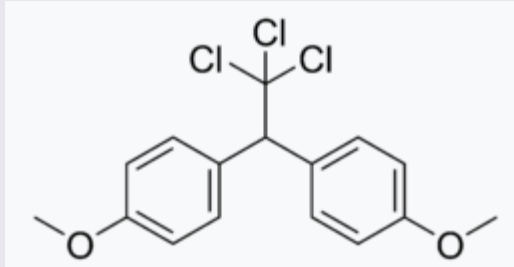
EC50 AR \approx 5 μM
Specific Response to androgens
Low sensitivity (AR endogenous)

Passage stability

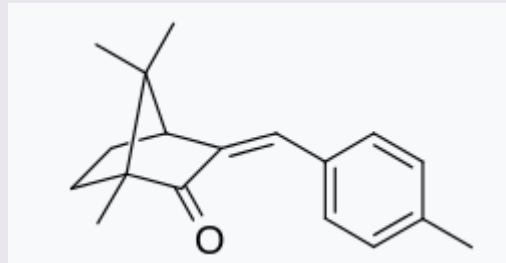


The response remained stable at least for 20 passages

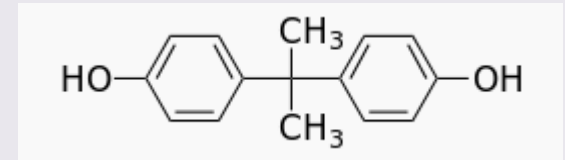
● Endocrine disruptors to be tested



Methoxychlor (MTX)



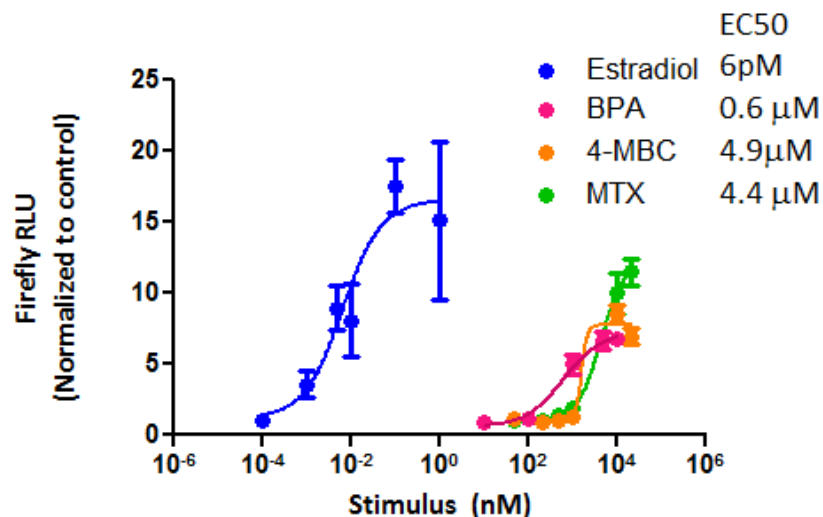
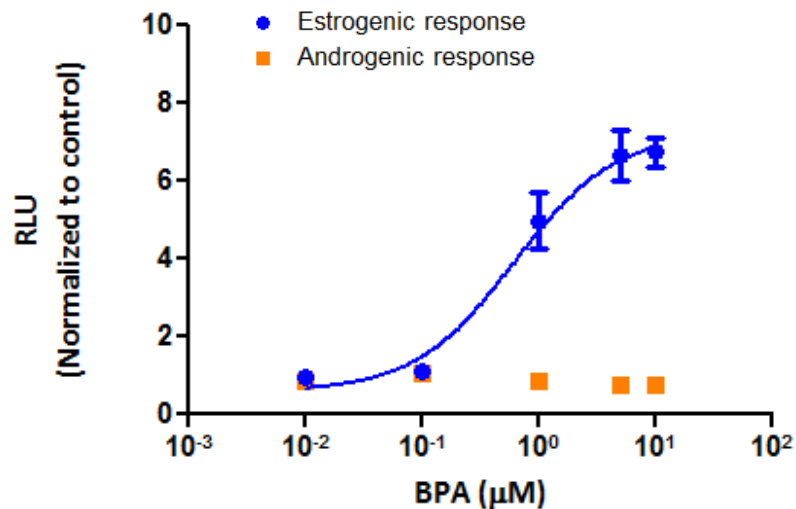
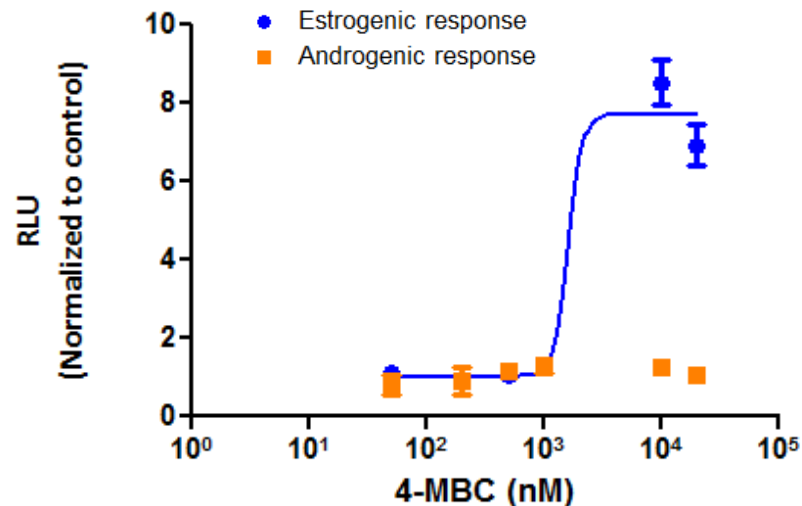
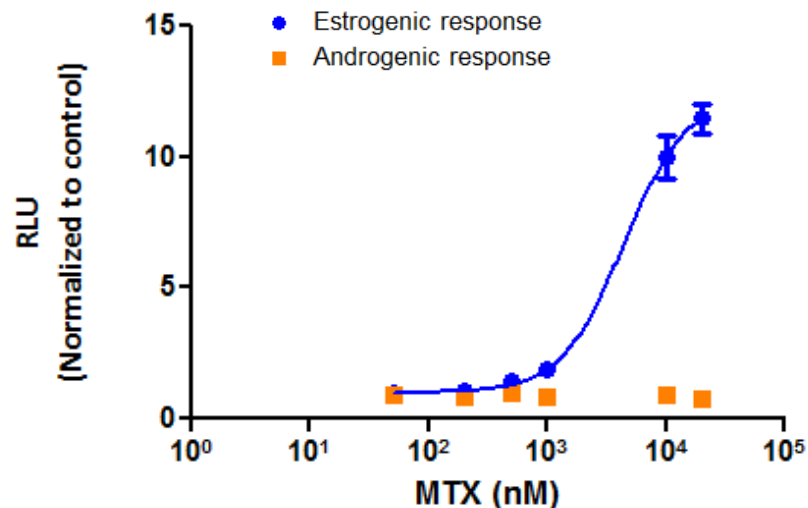
**4-methylbenzylidene camphor
(4-MBC)**



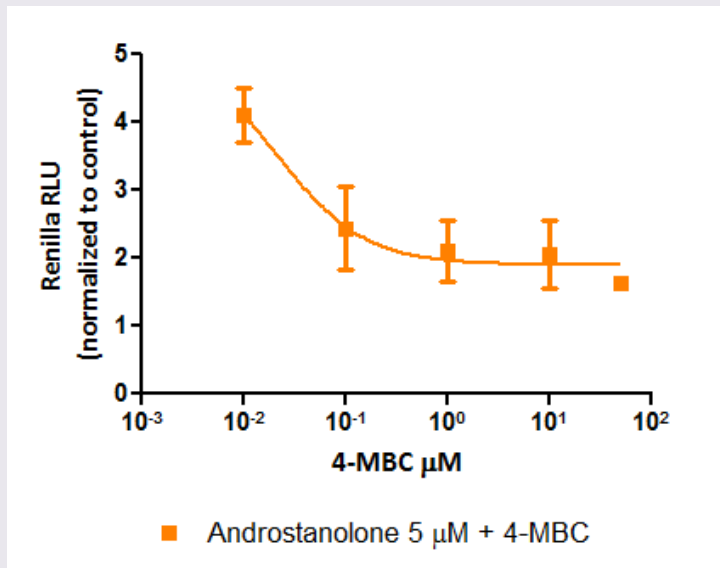
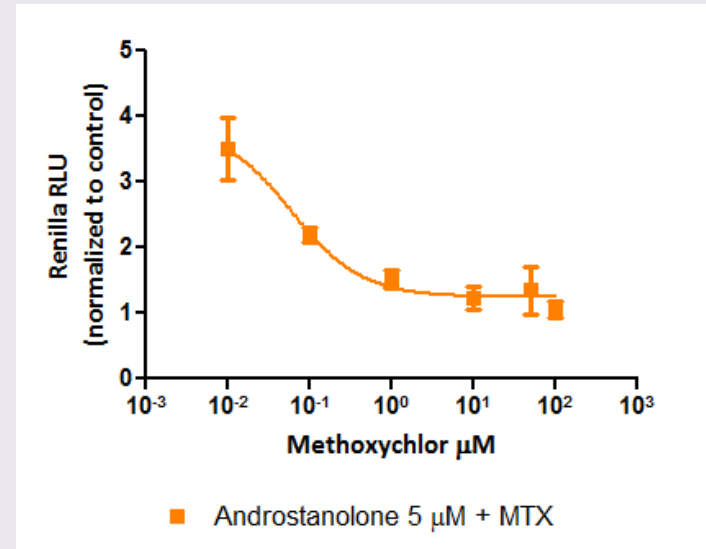
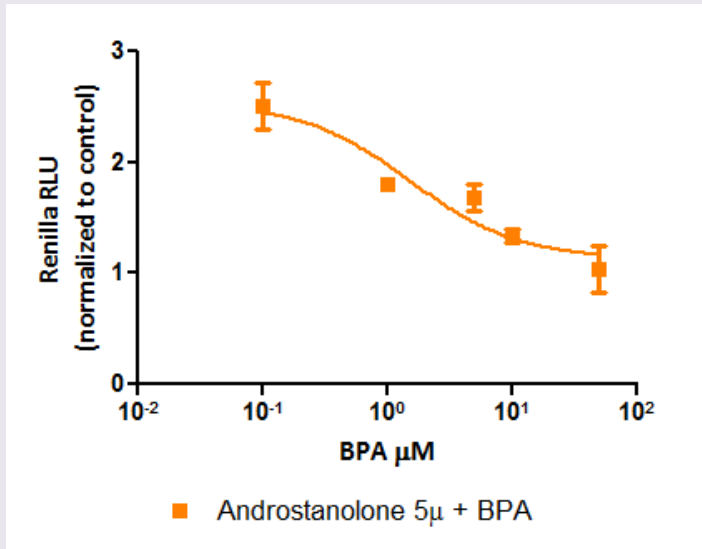
Bisphenol A (BPA)



Reporter assay evaluation of EDC



EDCs anti androgenic response



BPA, 4-MBC and MTX showed to have estrogenic and anti androgenic properties based on ARE E9 cell reporter assay

Dual reporter cell line T47DKBluc ARE E9 :

- Estrogenic activity (pM range)
- Androgenic activity (uM range)

- Was able to detect anti-androgenic activity from 4-MBC, BPA and MTX

Next challenges:

- Evaluate estrogenic and androgenic mixtures, complex putative EDCs and biological samples

- Compare ARE E9 performance with another available RG cell line

Institut Pasteur de Montevideo - Uruguay

Cell Biology Unit

Mariela Bollati

Karen Perelmuter

Vanesa Piattoni

Hellen Daghero

Tatiana Basika

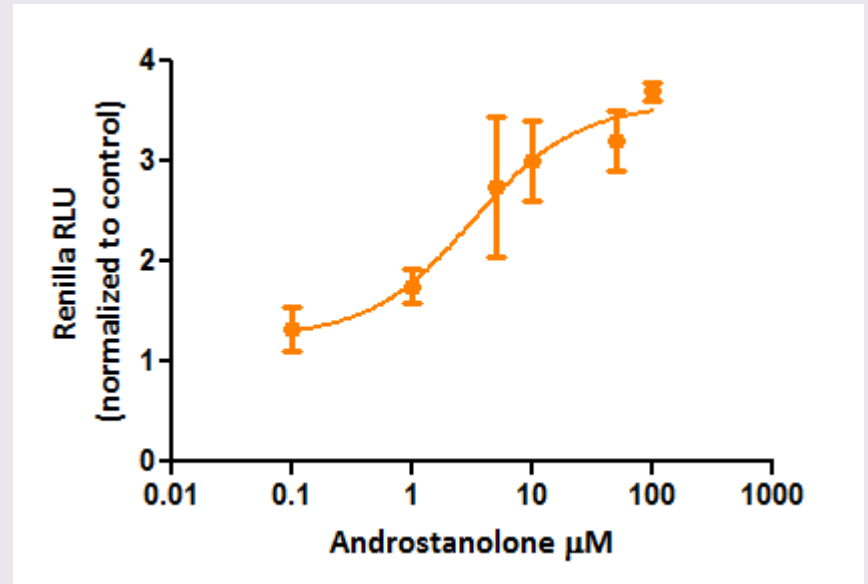
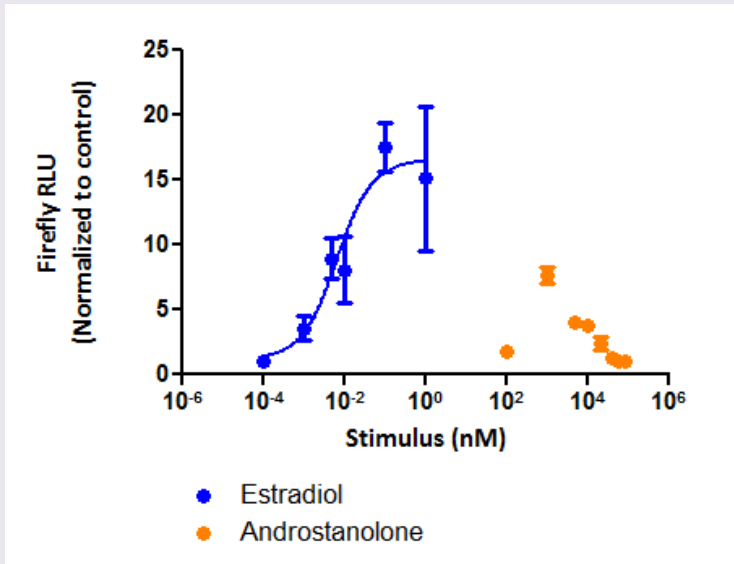
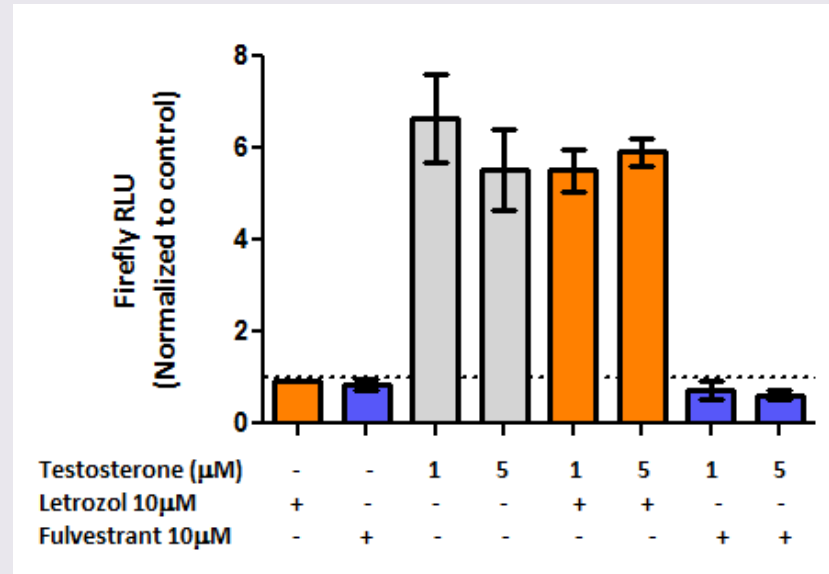
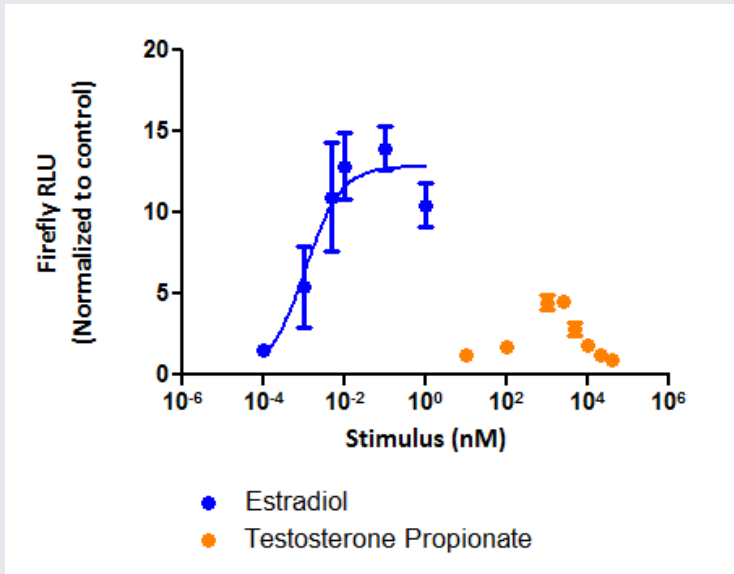
Paula Céspedes

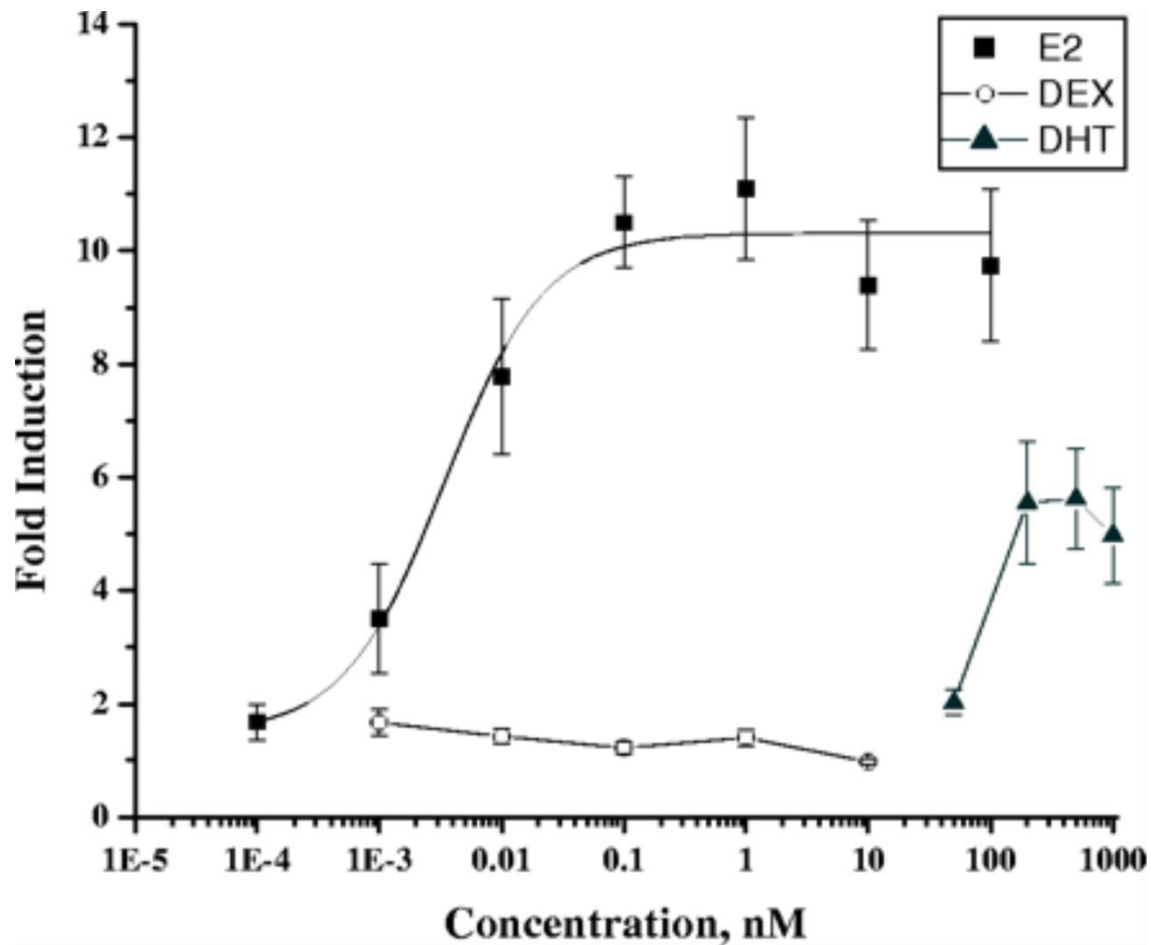
Romina Pagotto





Estrogenic response with androgenic stimuli

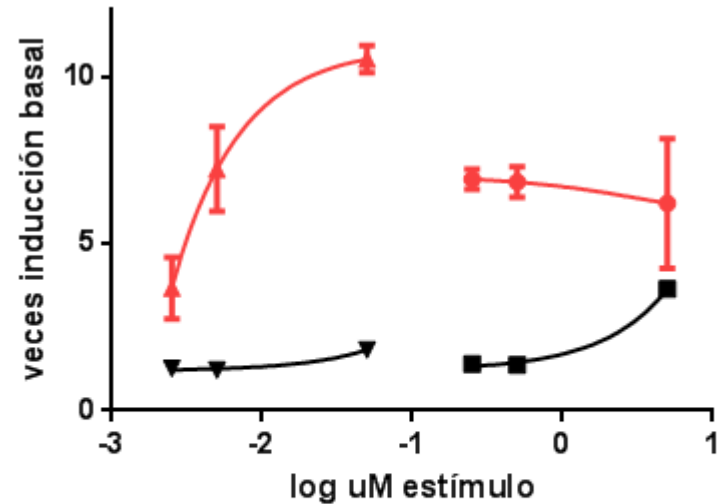
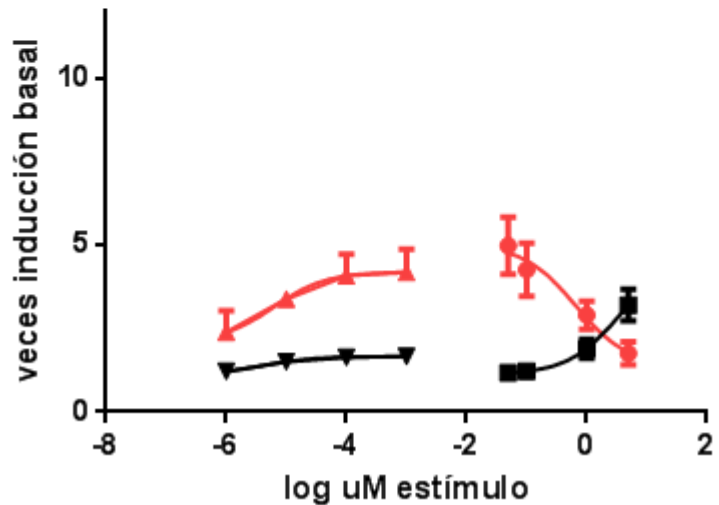
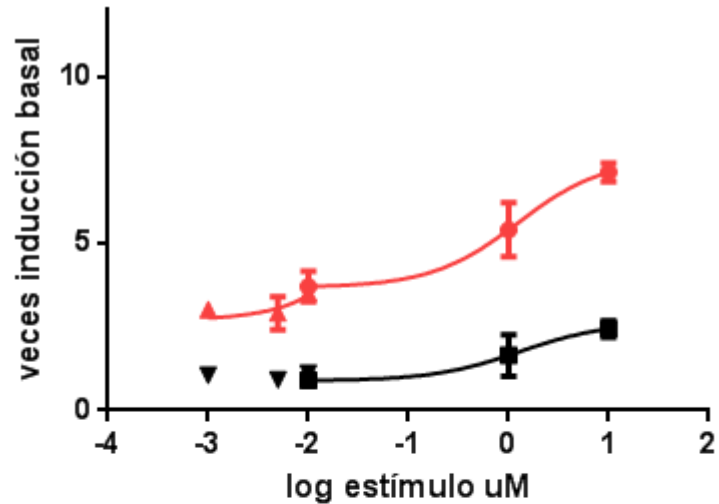




From: Development and Characterization of a Cell Line That Stably Expresses an Estrogen-Responsive Luciferase Reporter for the Detection of Estrogen Receptor Agonist and Antagonists

Toxicol Sci. 2004;81(1):69-77. doi:10.1093/toxsci/kfh180

Toxicol Sci | Toxicological Sciences vol. 80 no. 1 © Society of Toxicology 2004; all rights reserved.



- ARE E9 FIREFLY E2FIJO 0.005
- ARE E9 RENILLA E2FIJO 0.005
- ▲ ARE E9 FIREFLY TPFIJO 0.5
- ▼ ARE E9 RENILLA TPFIJO 0.5

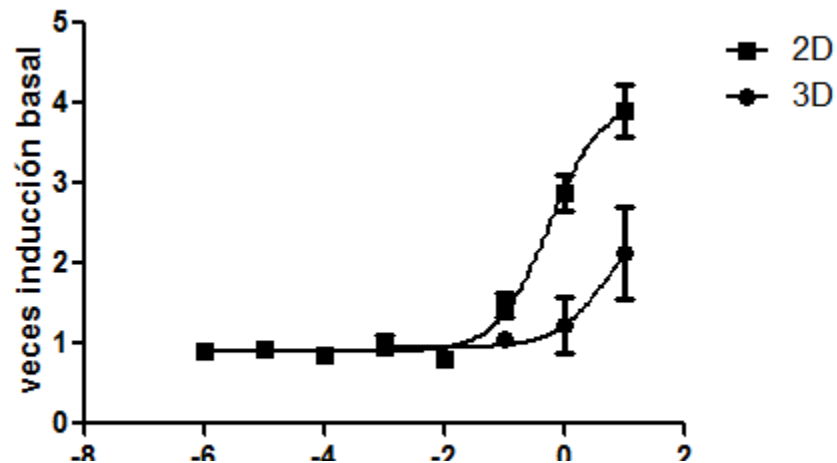
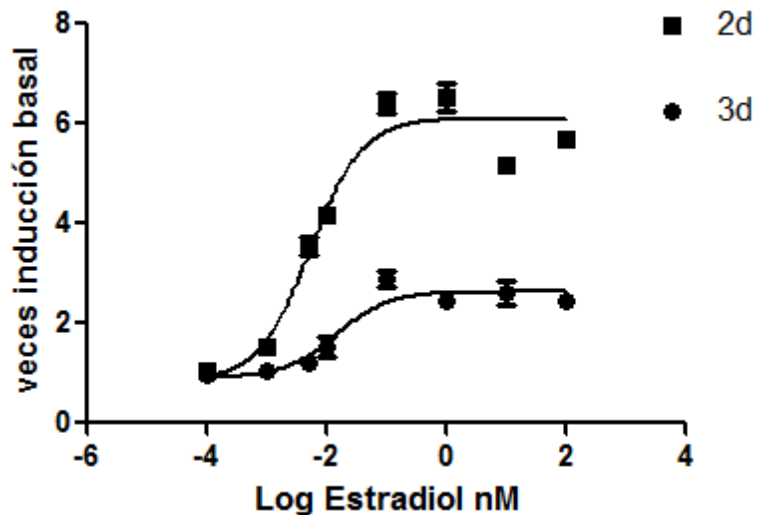
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AC**GTTACA**ATCT**GTTCTA**AGCTCTAGCTAGGGTATATAAT**AGATCT**CTTAGTTCCGATACTCGACTT
(134pb)

Baja sensibilidad a andrógenos por menor número de receptores? RT PCR /ICC

DHT por qué activa al reportero de estrógenos? Nueva droga a probar

Relevancia de esta línea en particular como detector de pE?

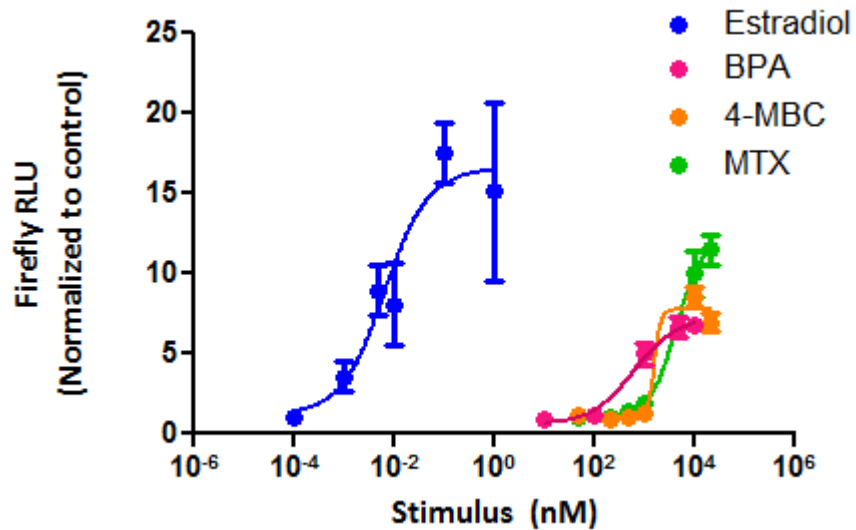
Prueba de concepto – utilidad para evaluar dos compuestos en un solo ensayo



Estradiol

2D EC50 : 0.0058+/- 0.0009 nM

3D EC50: 0.0153+/- 0.0022 nM



Screening battery of chemical for ED

EPA's 2009–2010 EDSP Tier 1 screening battery and putative Tier 2 definitive tests.

EPA EDSP Tier 1 in vitro test systems

Estrogen Receptor (ER) binding assay (rat uterine)
ER transcriptional activation assay (human cell line)
Androgen receptor binding assay (rat prostate)
Aromatase assay (human recombinant)
Steroidogenesis assay (human cell line)

EPA EDSP Tier 1 in vivo test systems

Uterotrophic assay (rat)
Hershberger assay (rat)
Male pubertal assay (rat)
Female pubertal assay (rat)
Fish short-term reproduction assay
Amphibian metamorphosis assay (frog)

EPA EDSP Tier 2 definitive tests

Two generation mammalian (rat) reproductive toxicity study
Amphibian (frog) development, growth and reproduction assay
Two generation avian reproductive toxicity study
Fish lifecycle toxicity study
Invertebrate (mysid) lifecycle toxicity study

OECD conceptual framework for the testing and assessment of chemicals for endocrine disruption.

Level 1

Sorting and prioritization based upon existing information

Level 2

In vitro assays providing mechanistic data

Level 3

In vivo assays providing data about single endocrine mechanisms and effects

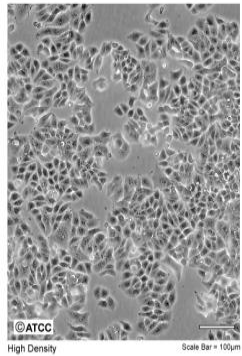
Level 4

In vivo assays providing data about multiple endocrine mechanisms and effects

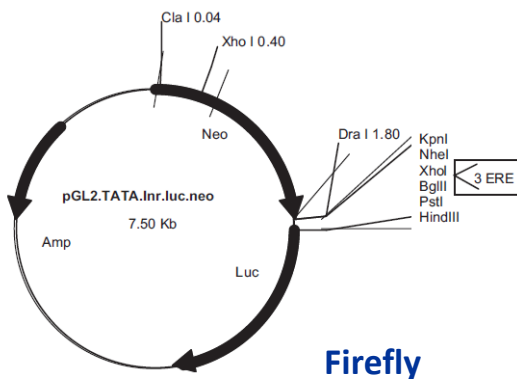
Level 5

In vivo assays providing adverse effects data from endocrine and other mechanisms

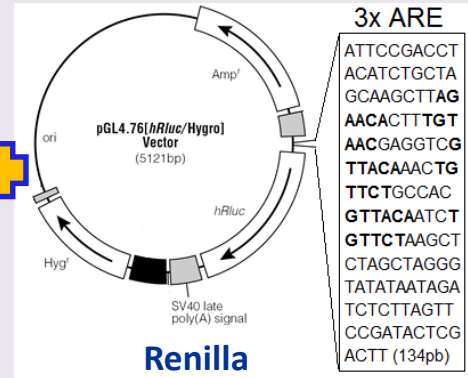
Reporter cell line generation :



T47D-KBLuc Cell Line
(ATCC® CRL-2865™)




Firefly



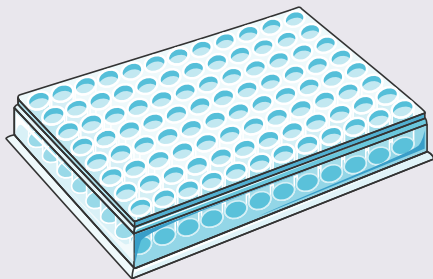
Renilla

3x ARE

```
ATTCCGACCT  
ACATCTGCTA  
GCAAGCTTAG  
AACACTTTGT  
AACGAGGTGG  
TTACAACTG  
TTCTGCCAC  
GTTACAATCT  
GTTCTAAGCT  
CTAGCTAGGG  
TATATAATAGA  
TCTCTTAGTT  
CCGATACTCG  
ACTT (134pb)
```

- 
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 2. Antibiotics selection
 3. Dilution Cloning
 4. Characterization
 5. Amplification of responding clones

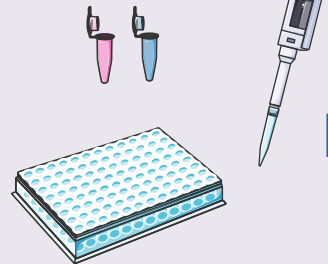
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5% DCC FBS



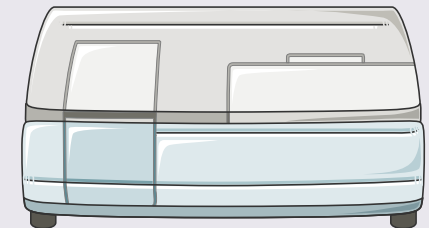
24 h



Add the compounds
to be tested



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Cell lysis and quantification of luciferase
Firefly and *Renilla* activities