Validation Progress of The LUMI-CELL™ ER Recombinant Bioassay for Rapid Evaluation of Chemicals for Potential Estrogenic Activity

Presented by:

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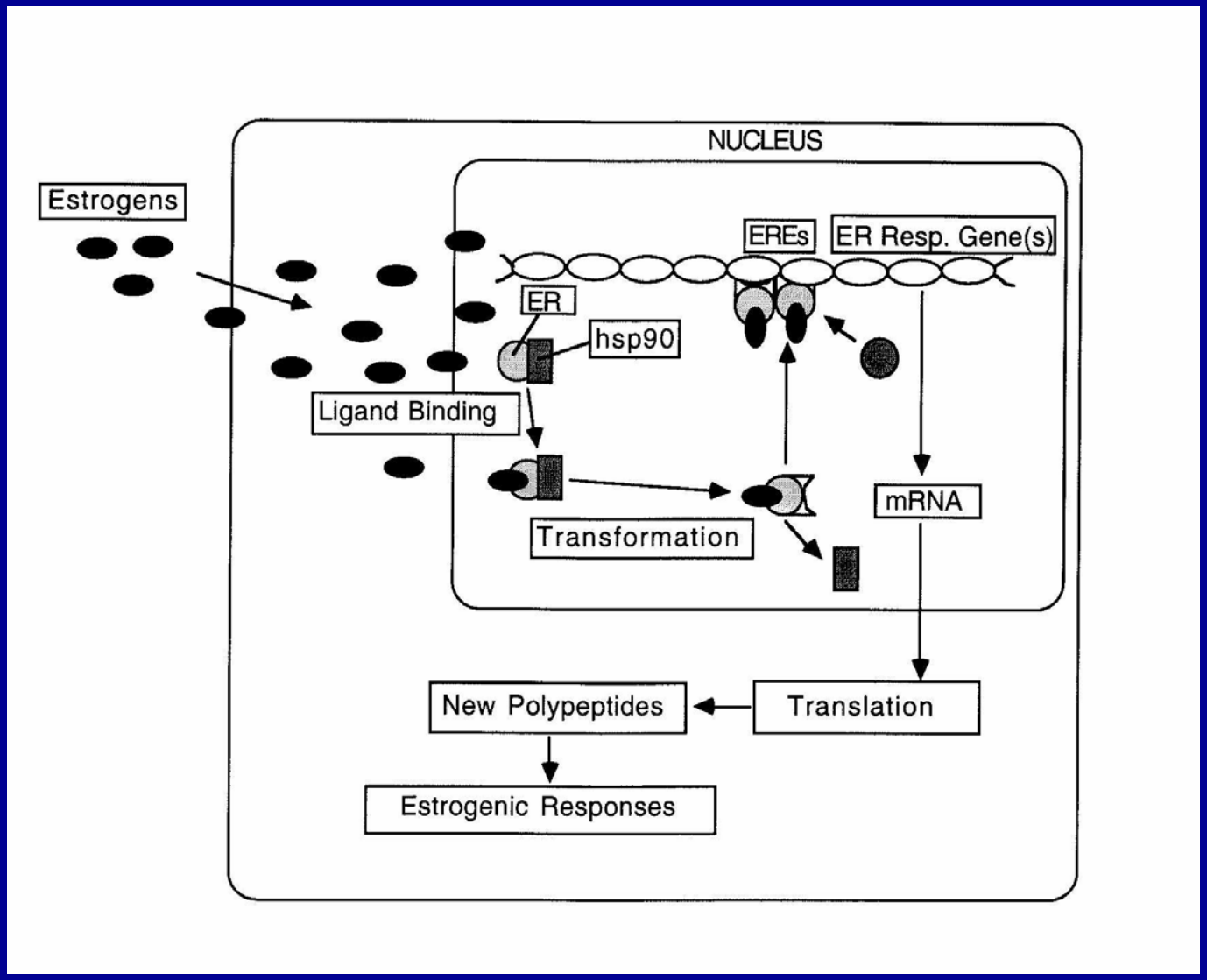
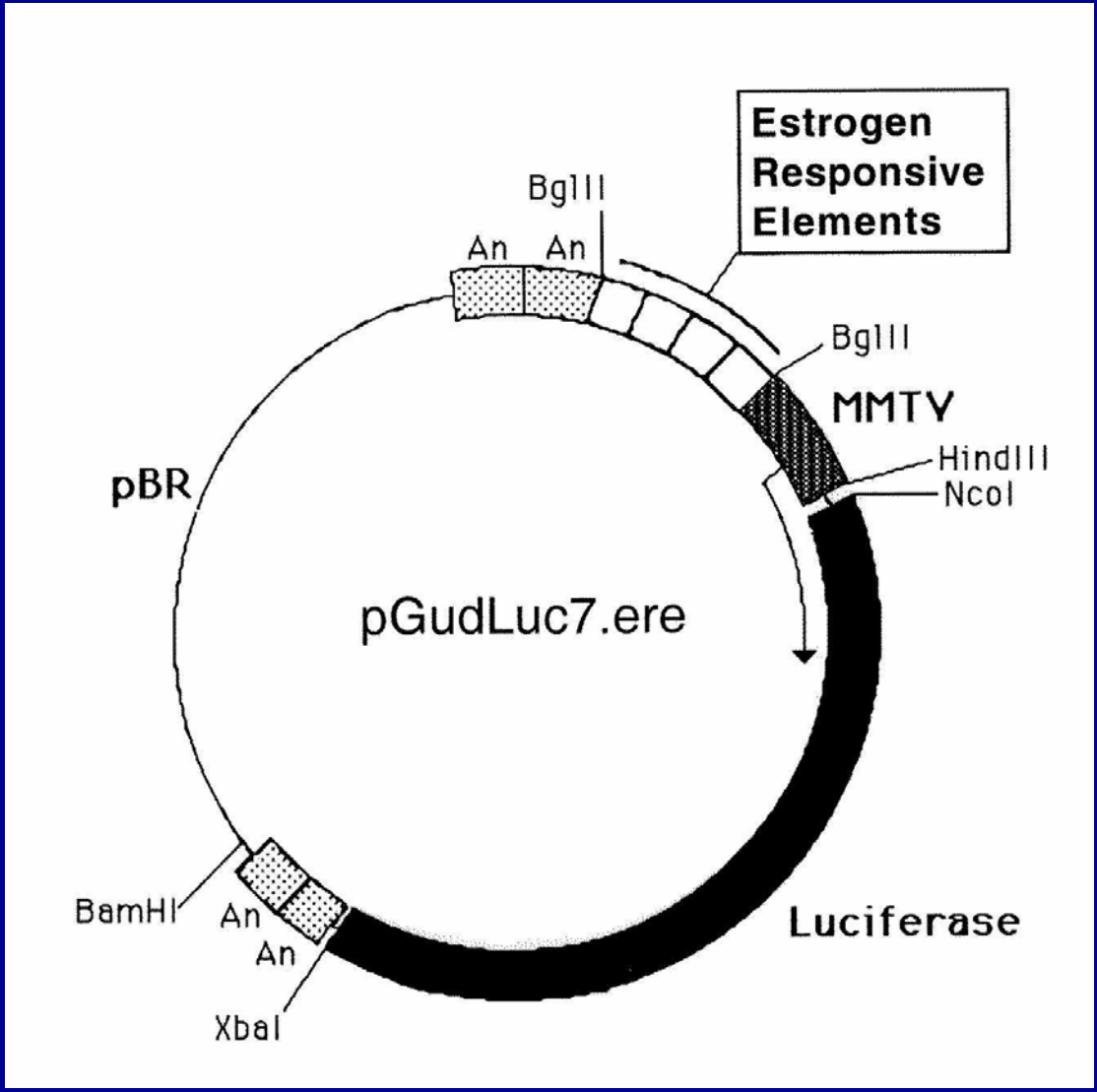
Introduction (continued)

- **Xenobiotics Detection System (XDS) Inc. developed the LUMI-CELL™ ER bioassay.**
- **BG-1 cells were stably transfected with an estrogen-responsive luciferase reporter gene plasmid (pGudLuc7ere).**
- **The resulting cell line (BG1Luc4E2), responds to estrogenic chemicals in a time-, dose dependent- and chemical-specific manner.**
- **BG1Luc4E2 cells, were able to detect as little as 0.1pM of 17β-estradiol.**

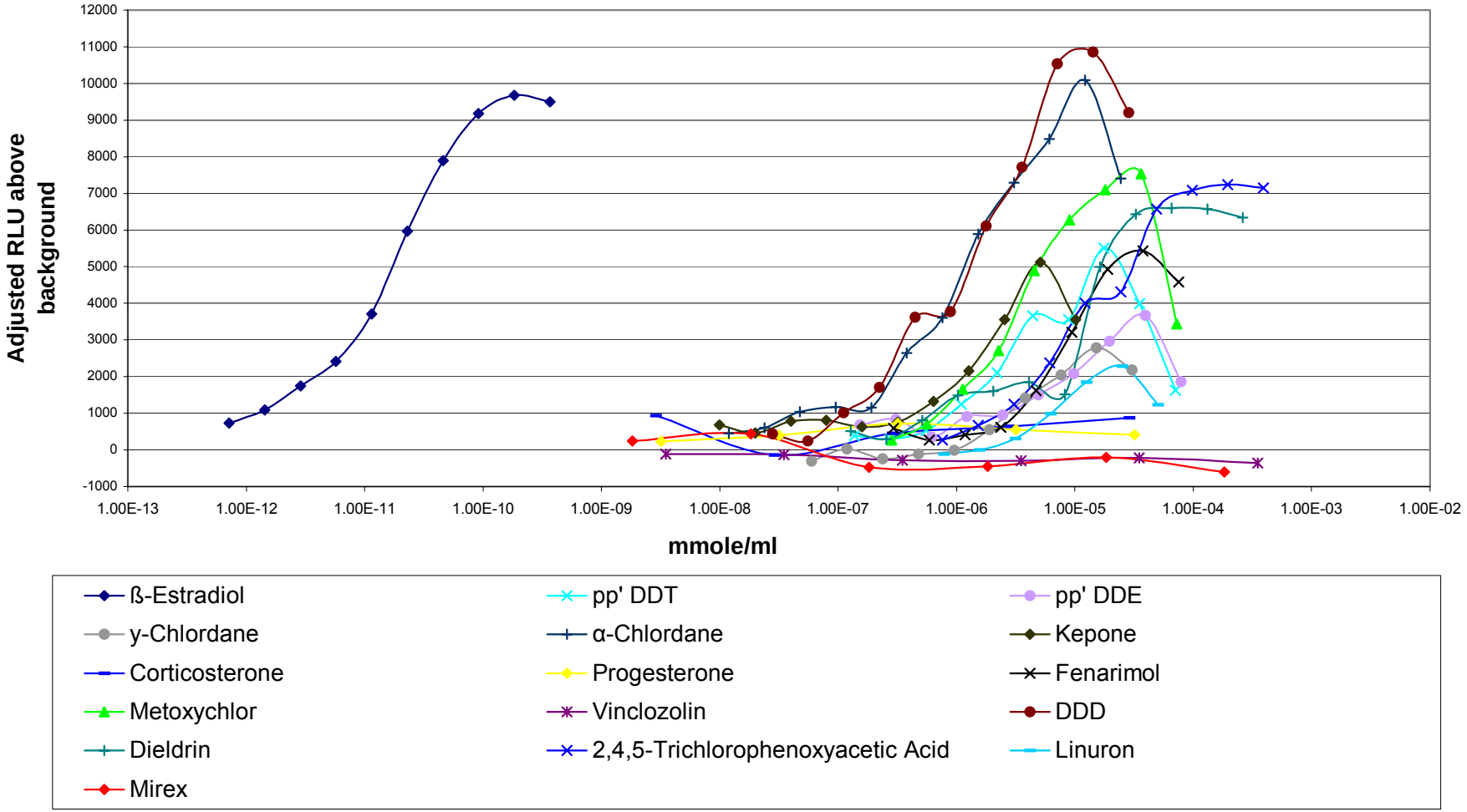
Endocrine Disruptor Model

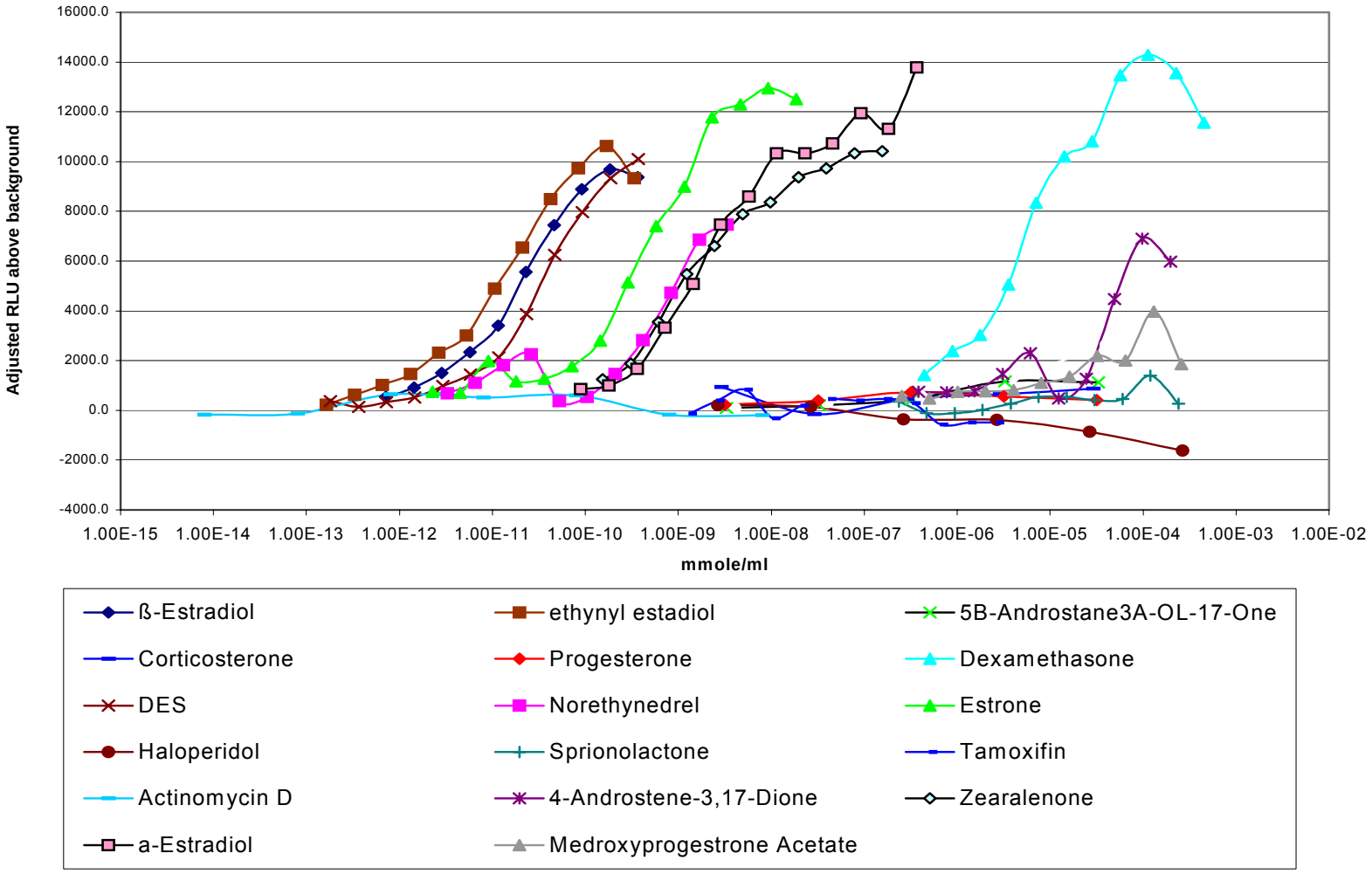


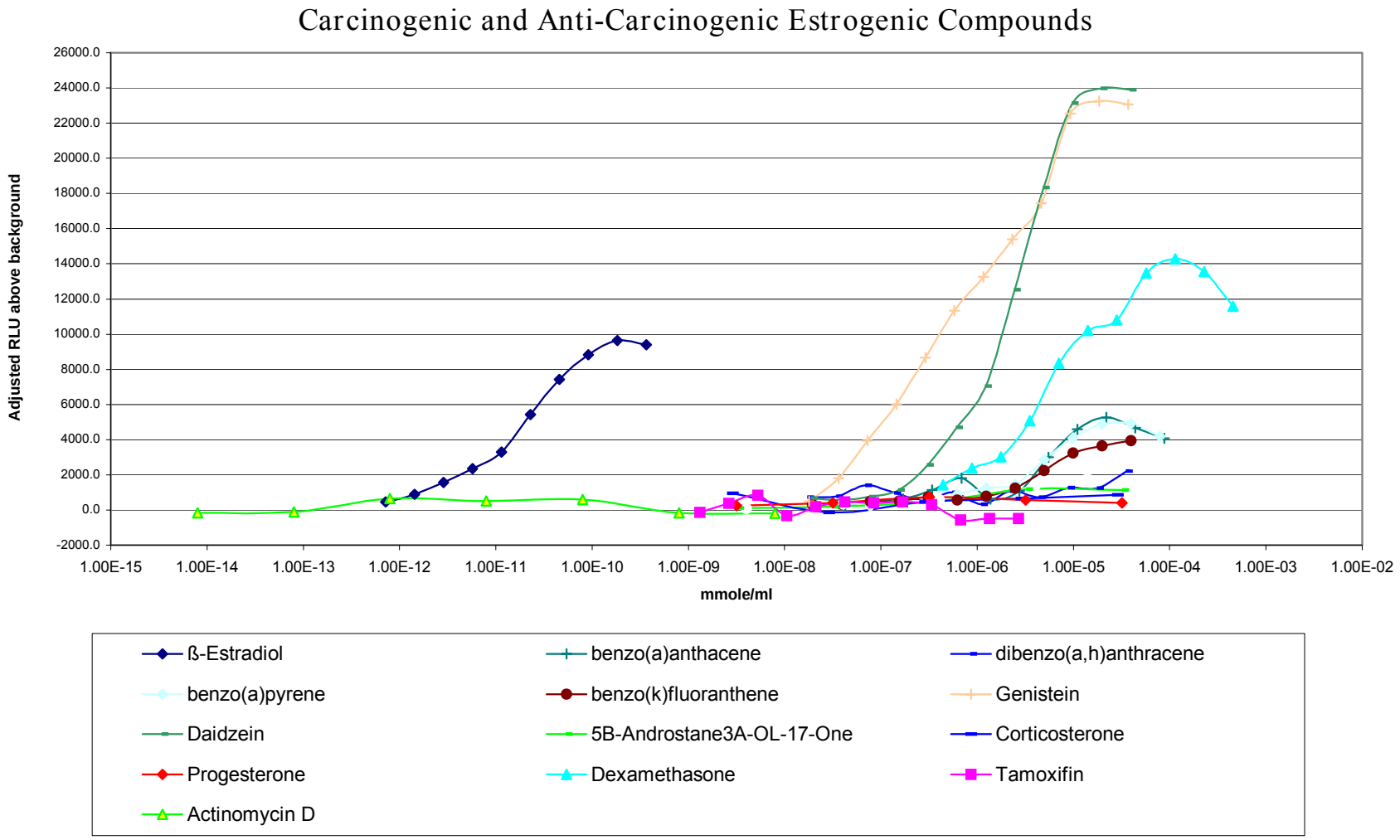
Plasmid Construct



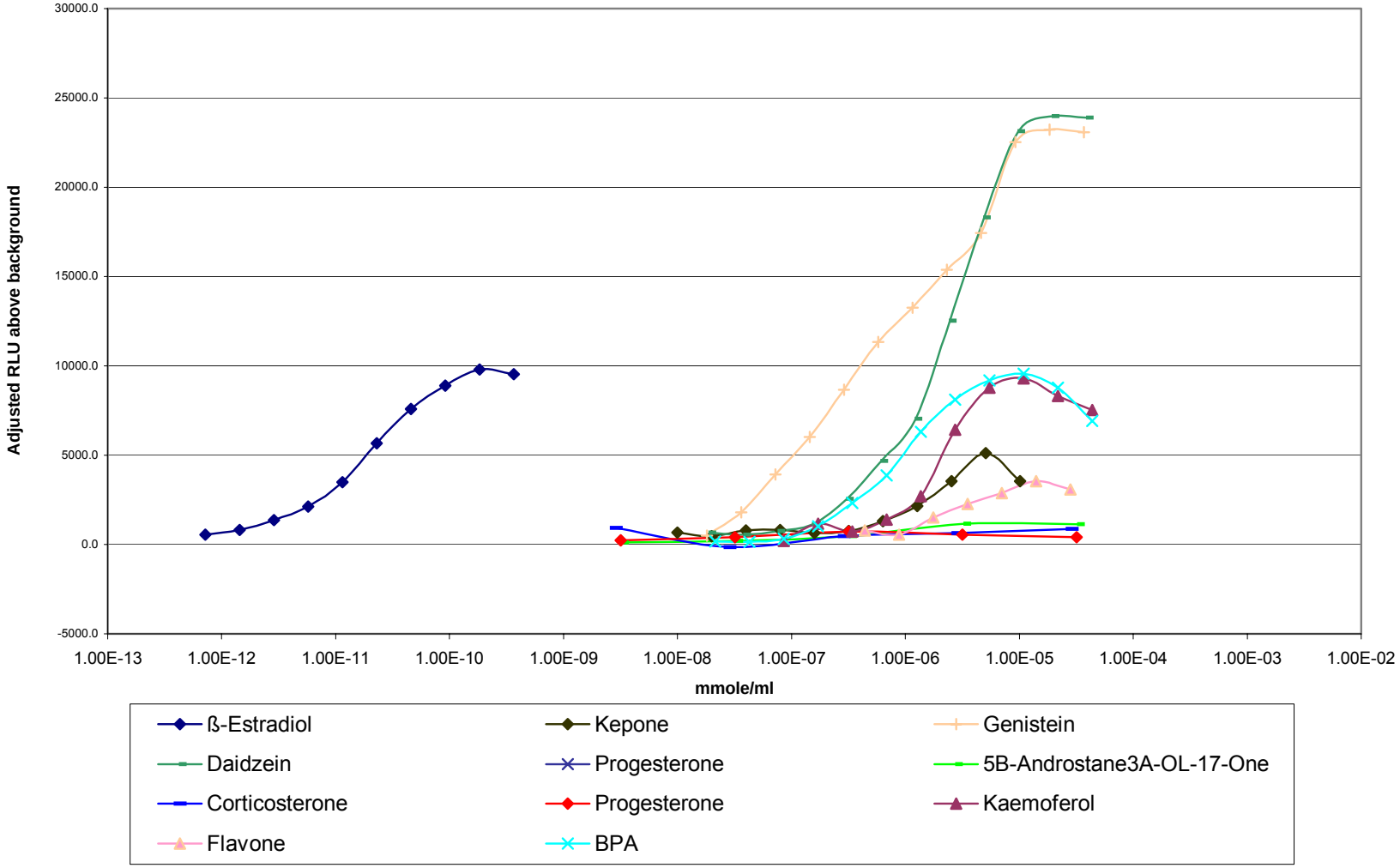
Organochlorine Pesticide Estrogenic Compounds



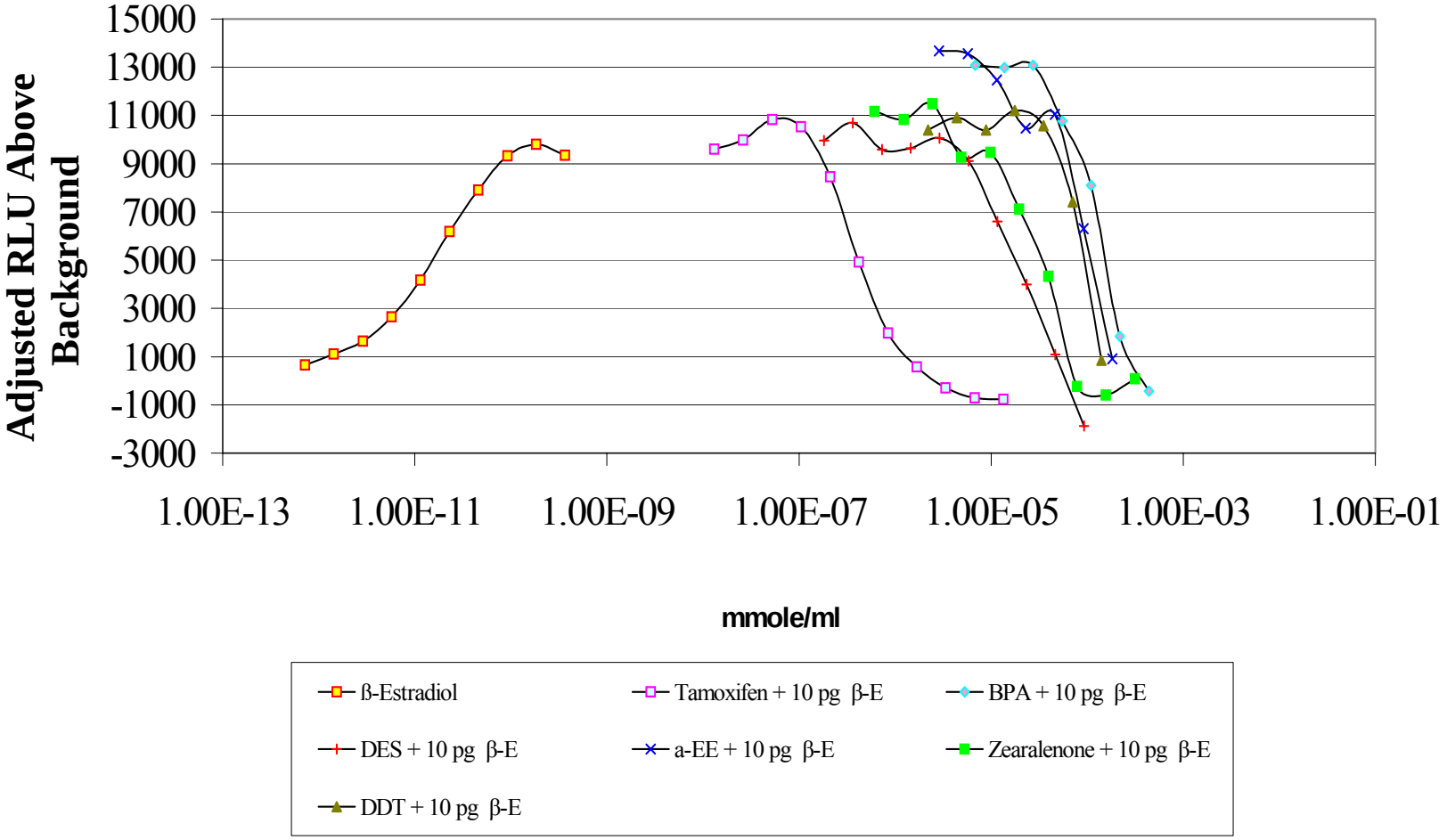

Pharmaceutical and Steroid Estrogenic Compounds
Adjusted RLU above background
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
-2000.0
-4000.0
1.00E-15
1.00E-14
1.00E-13
1.00E-12
1.00E-11
1.00E-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
1.00E-04
1.00E-03
1.00E-02
mmole/ml
ß-Estradiol
ethynyl estadiol
5B-Androstane3A-OL-17-One
Corticosterone
Progesterone
Dexamethasone
DES
Norethynedrel
Estrone
Haloperidol
Sprionolactone
Tamoxifin
Actinomycin D
4-Androstene-3,17-Dione
Zearalenone
a-Estradiol
Medroxyprogestrone Acetate


Carcinogenic and Anti-Carcinogenic Estrogenic Compounds
Adjusted RLU above background
26000.0
24000.0
22000.0
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
-2000.0
1.00E-15
1.00E-14
1.00E-13
1.00E-12
1.00E-11
1.00E-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
1.00E-04
1.00E-03
1.00E-02
mmole/ml
ß-Estradiol
benzo(a)anthacene
dibenzo(a,h)anthracene
benzo(a)pyrene
benzo(k)fluoranthene
Genistein
Daidzein
5B-Androstane3A-OL-17-One
Corticosterone
Progesterone
Dexamethasone
Tamoxifin
Actinomycin D

Flavone Estrogenic Compounds



Antagonistic Estrogenic Compounds



Results and Discussion (continued)

- This data clearly demonstrates that XDS's LUMI-CELL™ ER high-throughput bioassay system is:

  - Fast

  - Reliable

  - Sensitive

  - Reproducible

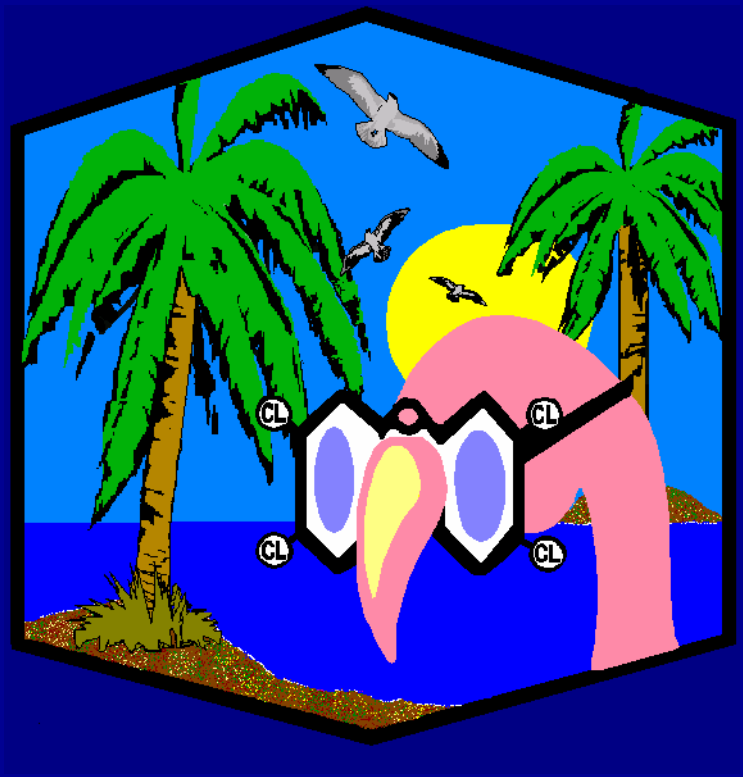
  - Agonist and Antagonist responses

  - Wide dynamic range to estimate EC_{50}s

  - Relatively inexpensive

  - Convenient

  - Meets many of the requirements mandated by the EPA and ICCVAMs Tier I (screening) requirements for EDC detection assays.



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Selected compounds tested using XDS's LUMI-CELL™ ER HTPS bioassay system for EDCs.

Name	CAS RN	Estradiol Positive at 10 µg/ml	Estradiol Positive at 1 µg/ml	EC 50 mmol/ml	Relative Induction
17β-Estradiol *	50-28-2	+	++	1.43E-11	1.00E+00
17alpha-Ethinyl Estradiol *	57-63-6	+	++	1.57E-11	9.11E-01
α-Zearalenol	36455-72-8	+	++	3.52E-11	4.06E-01
Kepone *	143-50-0	+	++	1.40E-06	1.02E-05
α–Chlordane	57-74-9	+	++	1.32E-06	1.08E-05
Coumesterol *	479-13-0	+	++	1.27E-06	1.13E-05
Genistein *	446-72-0	+	++	7.28E-07	1.96E-05
Dexamethasone *	50-02-2	+	++	5.10E-06	2.80E-06
Daidzein *	486-66-8	+	++	2.57E-06	5.56E-06
Kaempferol *	520-18-3	+	++	1.91E-06	7.49E-06
DDD	72-54-8	+	++	1.71E-06	8.36E-06
Corticosterone *	50-22-6	Cell Toxicity	++	In Process	In Process
Progesterone *	57-83-0	-	-	Antagonist	N/A
5b-Androstane3A-OL-17-One	53-42-9	-	-	Non-Active	N/A

*** ICCVAM proposed substances for validation of estrogen and androgen receptor binding and transcriptional assays.**