Functional Analysis of ER Transcriptional Inhibition by CtIP Co-repressor Complex

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Estrogen Receptor (ER)

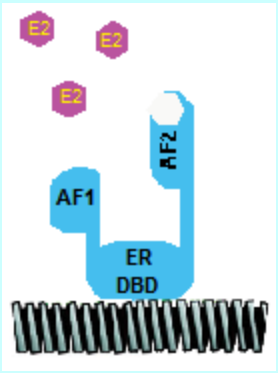
- ***ER belongs to family of proteins called nuclear receptors***
  - ***–Ligand initiated transcription factor***

- There are numerous nuclear receptor motifs that interact with ER, such as AP-1 and SP-1

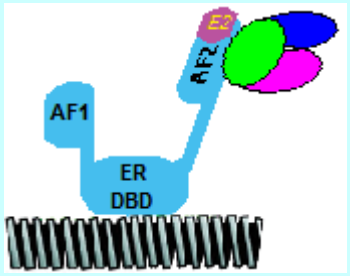
Estrogen and Breast Cancer

- Breast cancer was first recognized to be an E2-dependent disease in 1896.
- Prolonged exposure to E2 results in increased risk for breast cancer.
- E2 promotes breast cancer progression by stimulating malignant cell proliferation

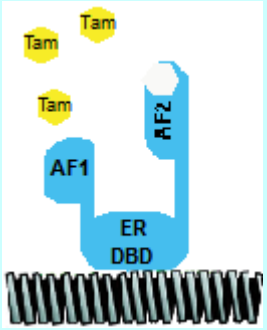
Generalized ER Regulated Transcription



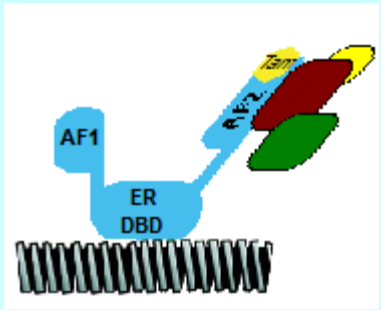
Conformation Change →



→ Estrogen induced transcription



Conformation Change →



→ Antagonistic inhibition of transcription

Therapy for Breast Cancer

- Blocking the action of E2 on its receptor
- Reducing circulating levels of E2

Tamoxifen

- Tamoxifen blocks the effects of E2 by binding to ER
- Conformation change = change in co-regulators

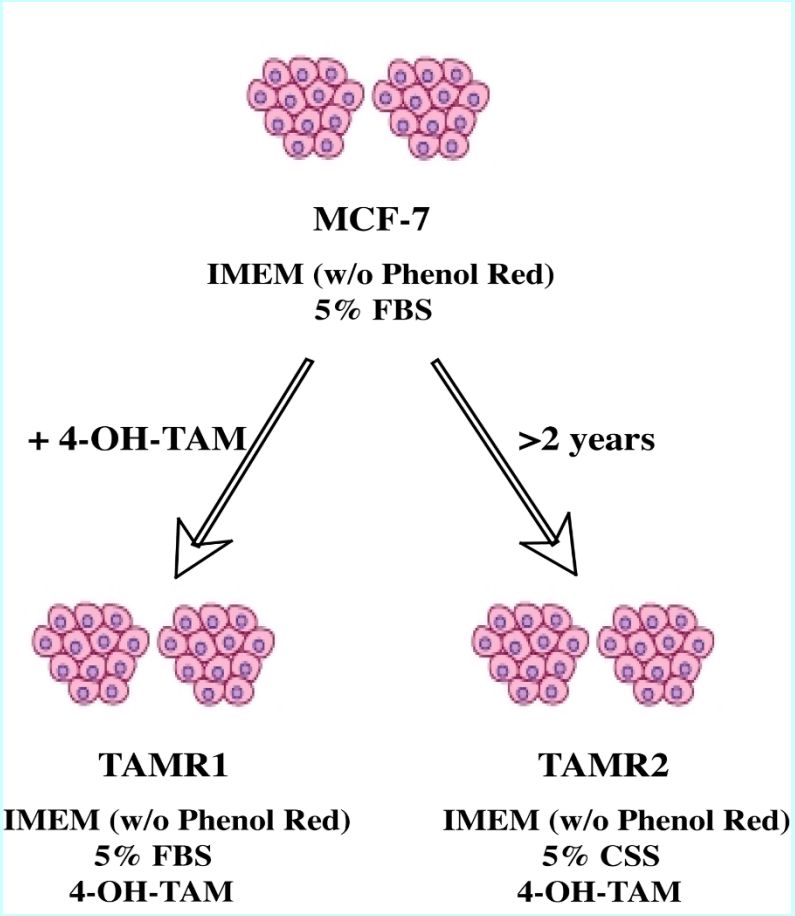
Suspected Mechanisms of Resistance

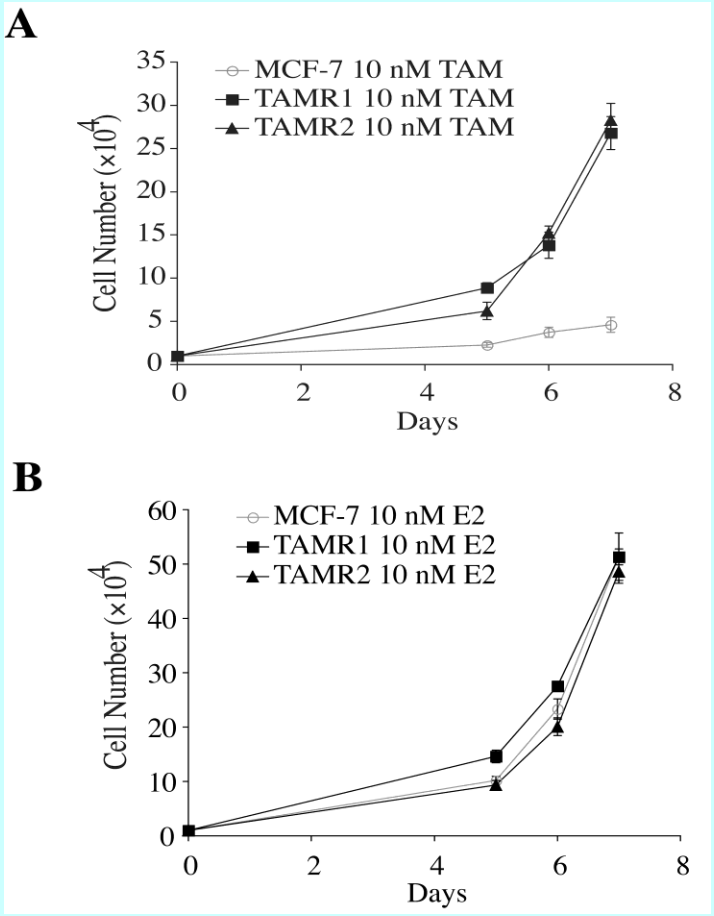
- Loss of expression or mutation of ER
- Altered expression of co-regulator proteins.

Tamoxifen Resistance

- Many tumors that respond to TAM eventually develop resistance
- Mechanisms of resistance are still poorly understood.

Development of Tamoxifen Resistant MCF-7 Breast Cancer Cell Line Variants

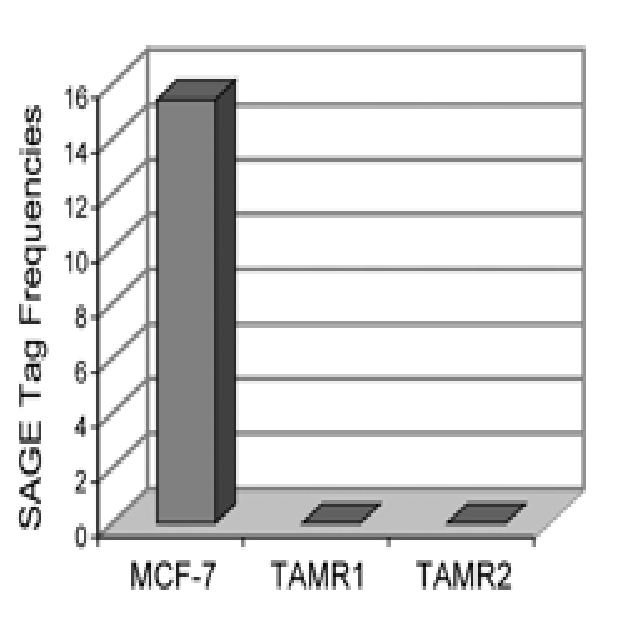


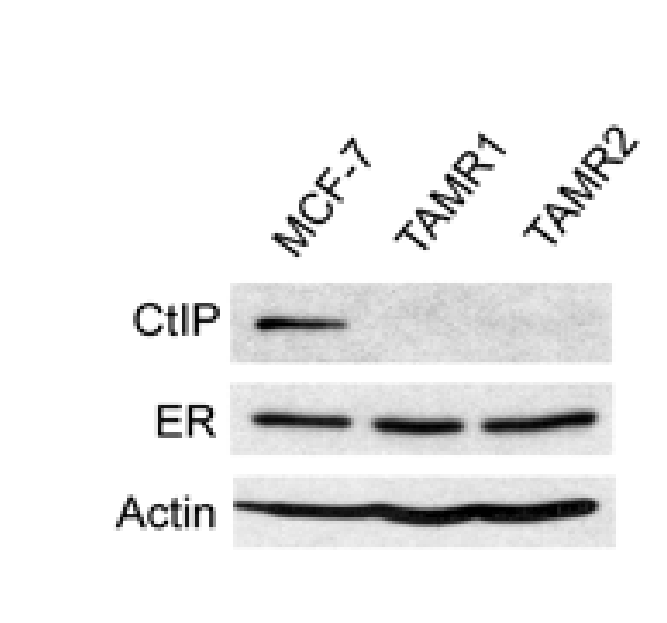


Serial Analysis of Gene Expression (SAGE)

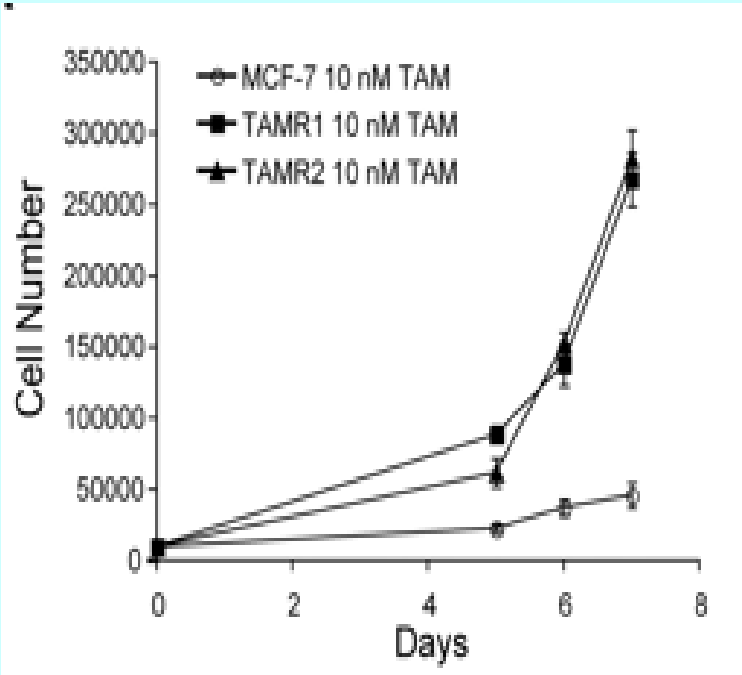
- SAGE in TAMR1 and TAMR2 identified many dysregulated genes
- CtIP was most significantly down regulated

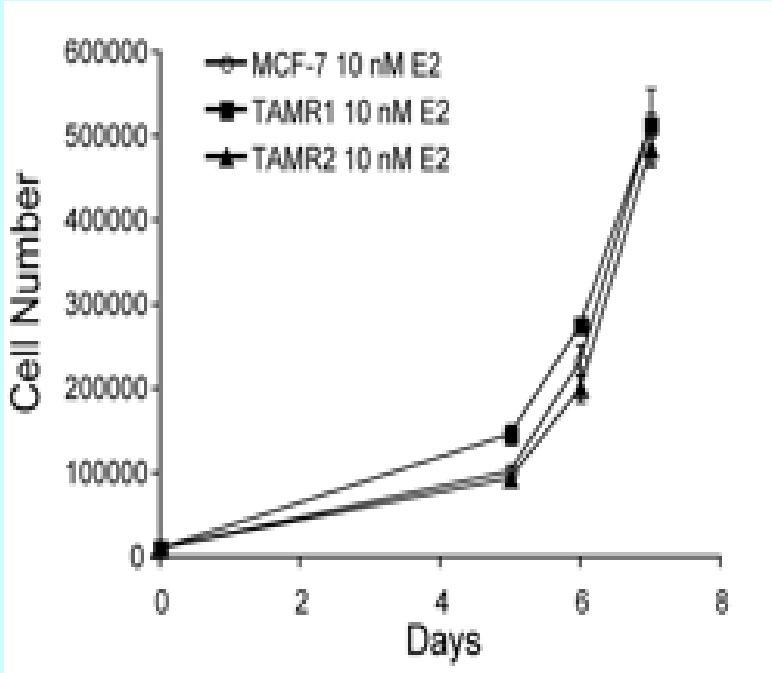
Loss of CtIP in MCF-7 Tamoxifen Resistant Cell Lines





Tamoxifen Resistant MCF-7 Cell Lines





Observation

- Developed Tamoxifen resistance by breast cancer cells
- CtIP most significantly down regulated protein

Hypothesis

- BRCA1 and CtIP are components of a multiprotein complex that functions as a corepressor of ER transcriptional activity.

- Disruption of this complex will result in increased transcription of ER-dependent genes in the absence of E2 stimulation

Additional Question

- Does tamoxifen act as an agonist in the absence of corepressors?

Investigation

- Experiments and Techniques
  - Growth Curve Determination (MTT Assay)
    - Metabolism of reagent by growing cells results in measurable change in absorbance
    - Change in absorbance is proportional to cell number
  - B-gal/Luciferase Assay
    - Utilizes ER-regulated firefly luciferase and beetle luciferine to generate luminescent signal
    - Measured luminescence indicates level of transcription
  - RT-PCR Gene Expression Assay
    - RT-PCR provides a quantitative approach to identifying relative levels of mRNA product
    - Allows us to measure differential gene expression within cells

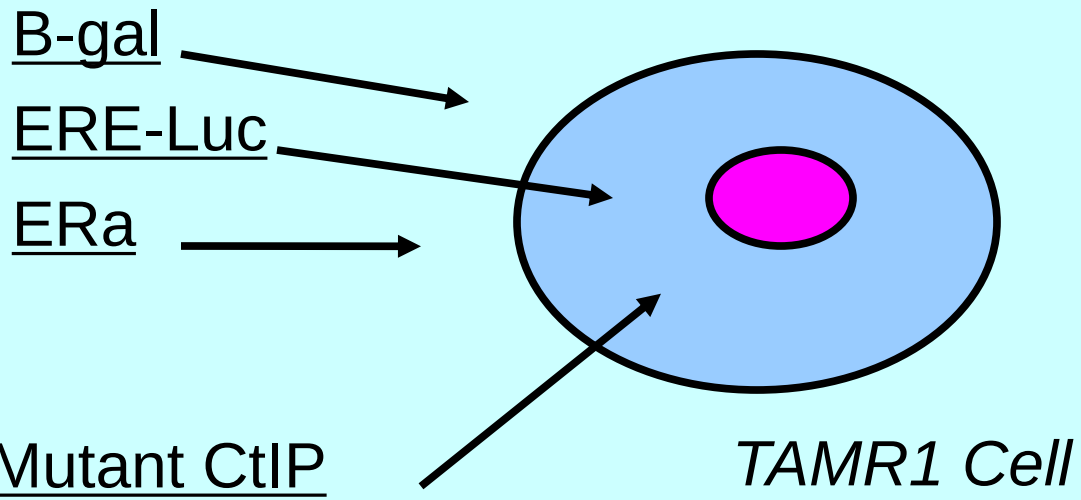
MTT Growth

- Insert graph showing tamr1 vs mcf-7 in css/tam, tamr1 css/tam vs. tamr1 css/etoh, this shows that tam is not acing as agonist in absence of CtIP it just lost its antagonistic properties.
- Show tamr1 in E2/tam/css vs. tam to show tam successfully blocks effects of E2

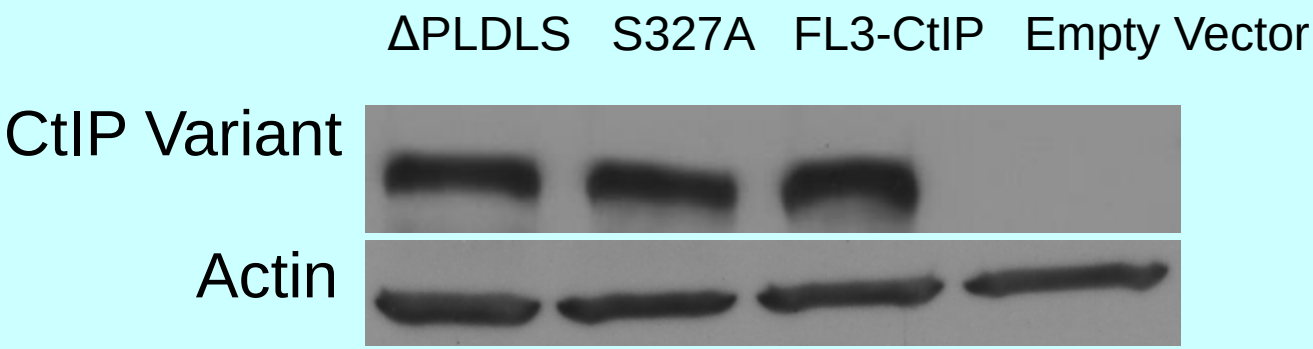
Luciferase Reporter System

- Luciferase Assay- used to quantify ER-regulated gene expression

Transfection of Plasmids

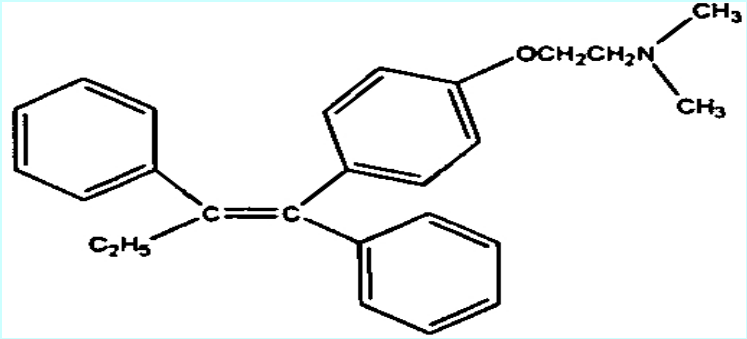


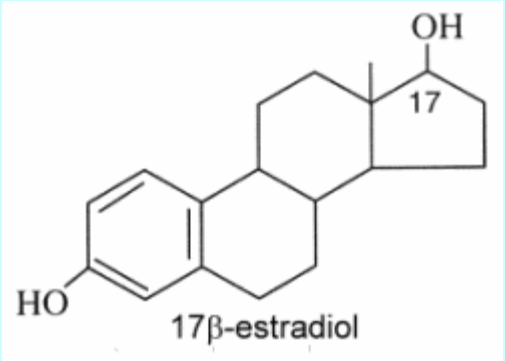
Expression of Transient CtIP



Treatments

- 1uM TAM
- 10nM E2
- 1uM TAM/10nM E2
- EtOH





Results

RT-PCR Gene Expression Assay

- Methods and Conditions
- Results(?)

Interpretation of Data

- Growth Curve Determination
  - Interpretation of Data

Interpretation of Data

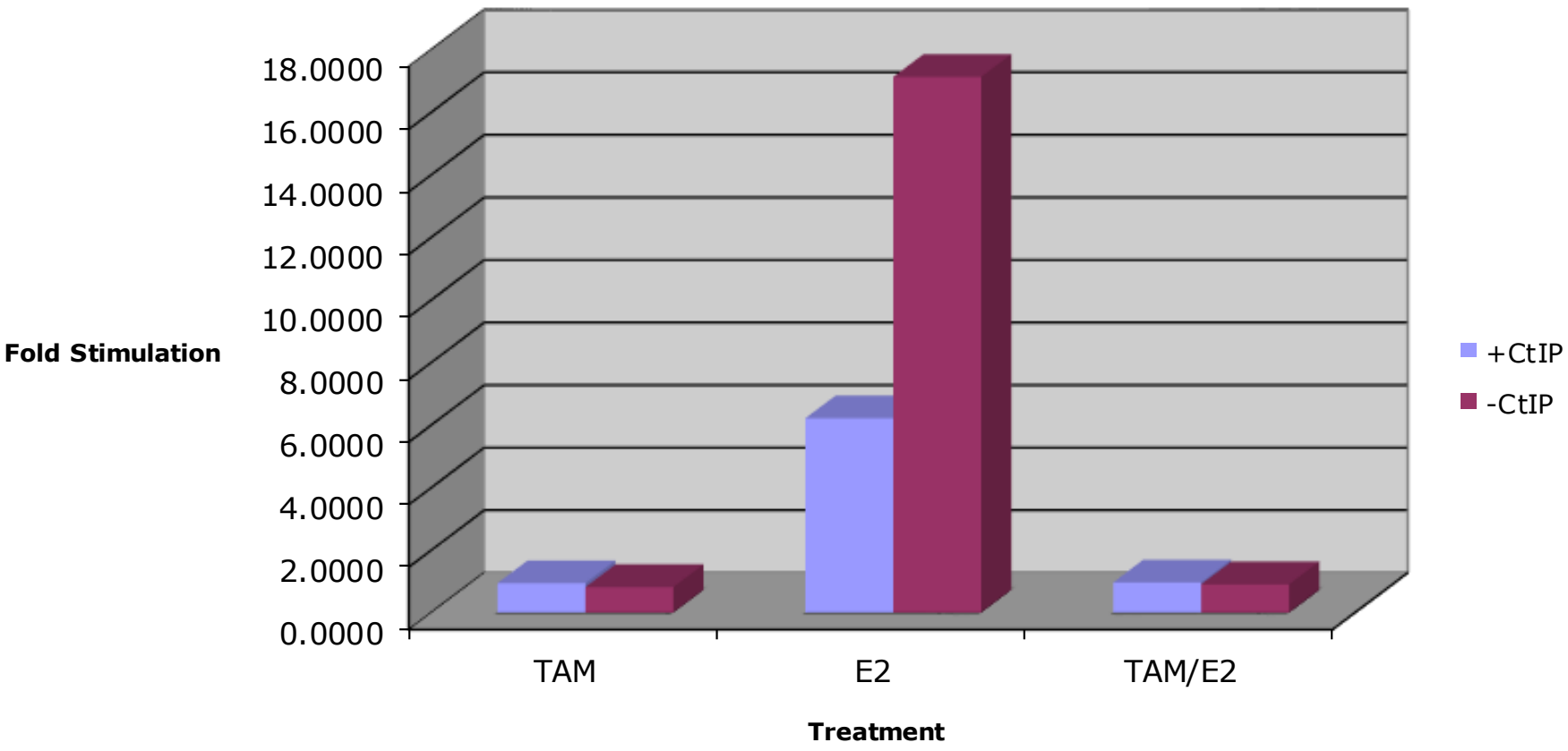
- B-gal Luciferase Assay
  - Interpretation of Data

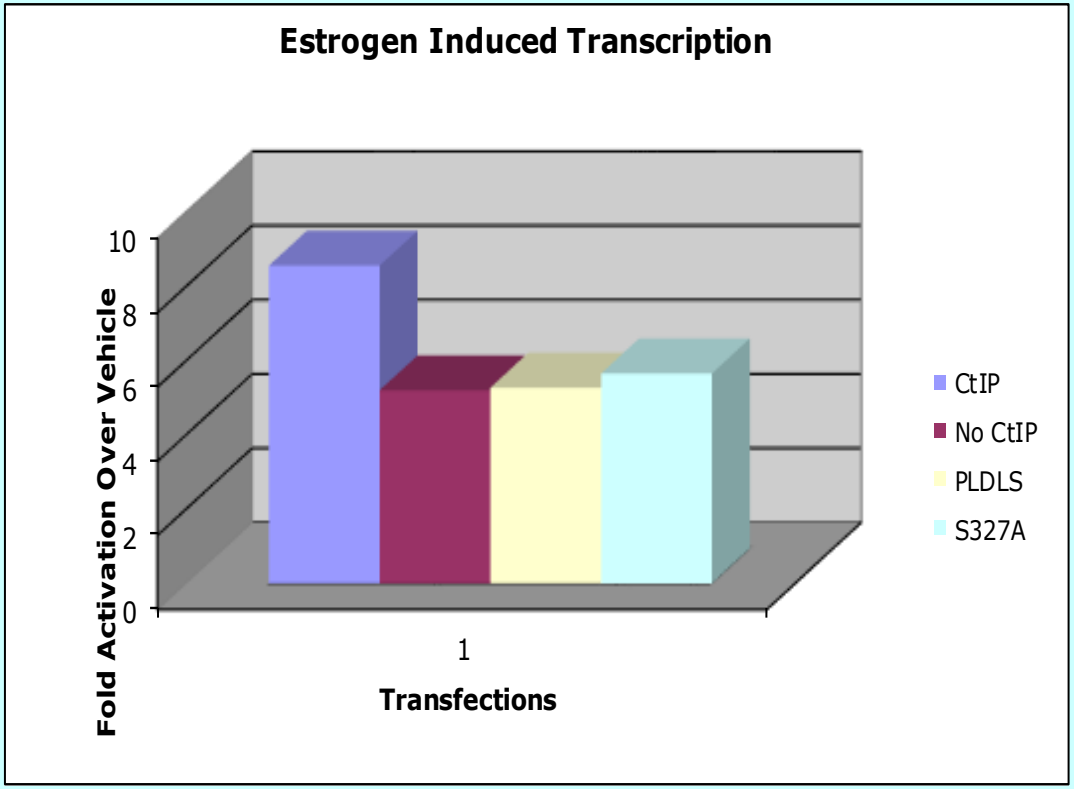
Interpretation of Data

- RT-PCR Gene Analysis
  - Interpretation of Data

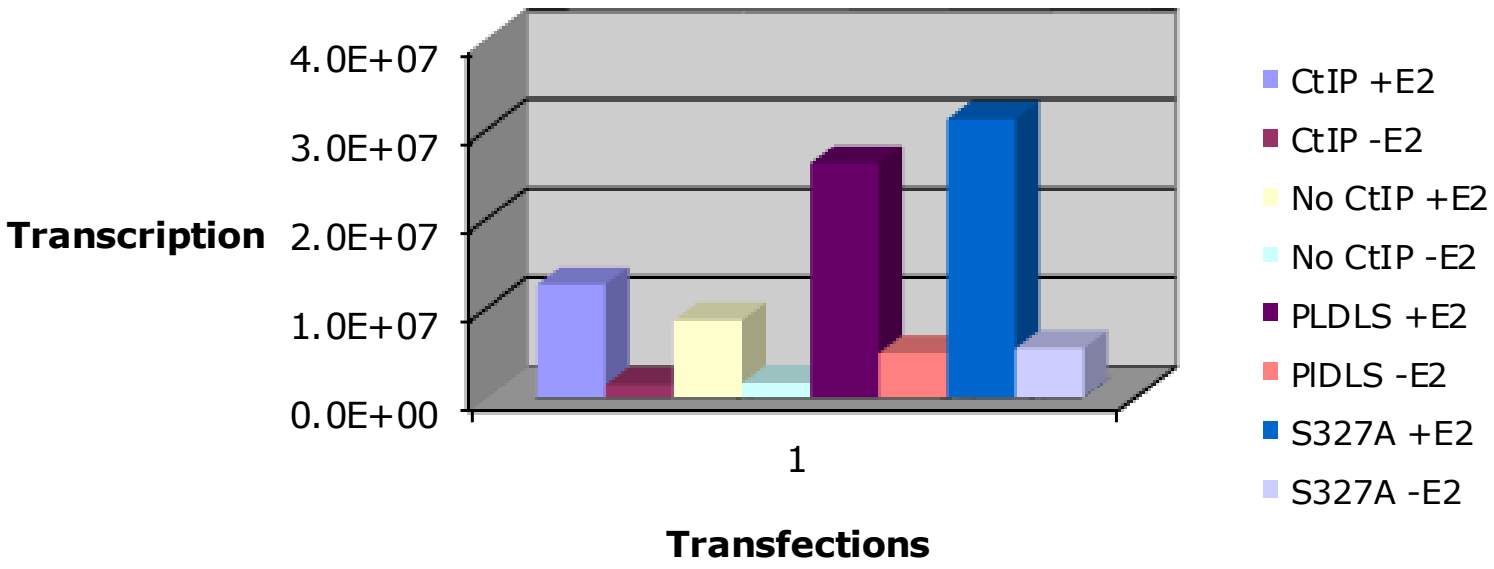
Conclusion

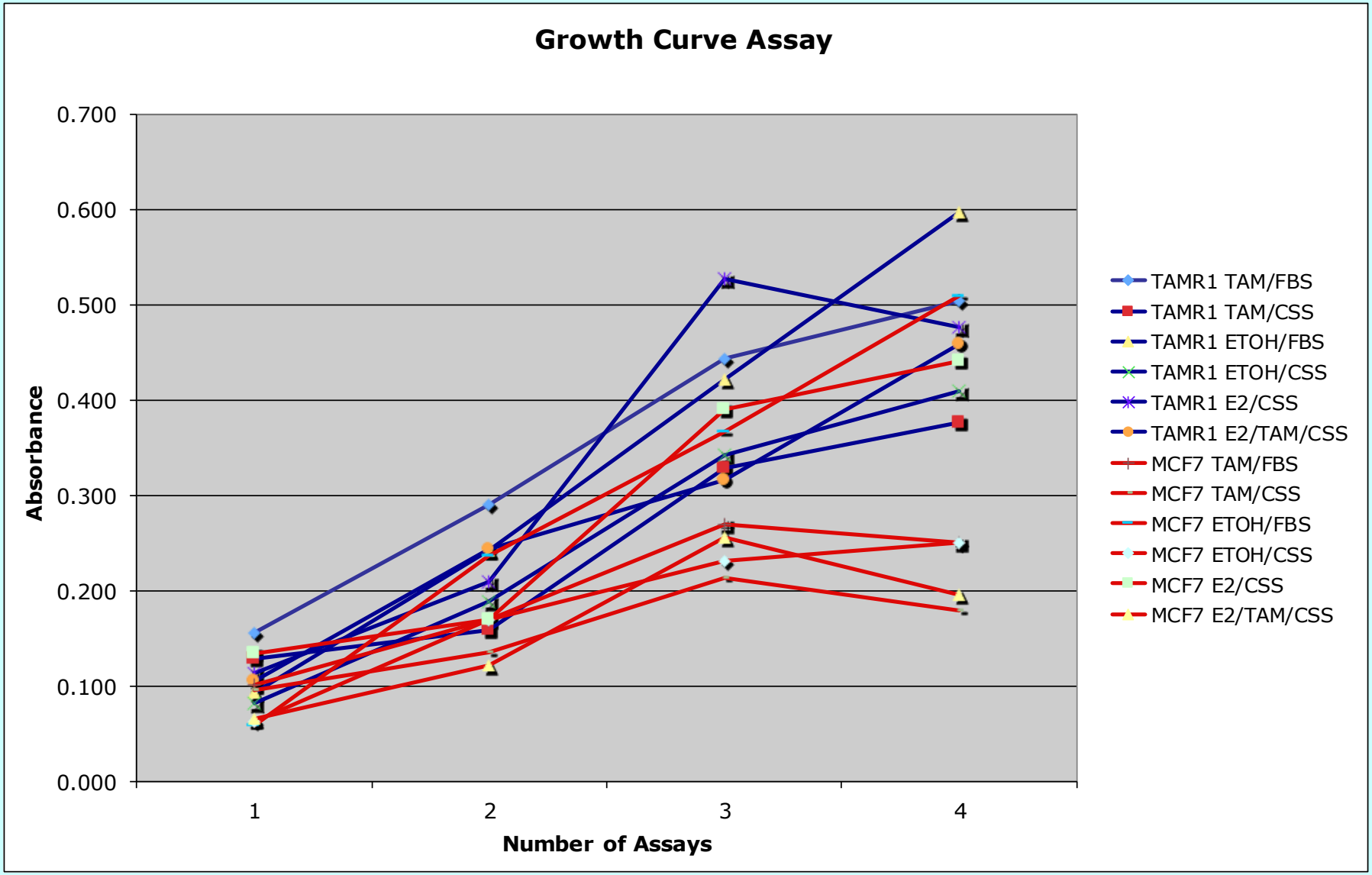
CtIP Repression of Estrogen Dependent Transcription




Estrogen Induced Transcription
Fold Activation Over Vehicle
10
8
6
4
2
0
1
Transfections
CtIP
No CtIP
PLDLS
S327A

Estrogen to Vehicle Comparison




Growth Curve Assay
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Absorbance
1
2
3
4
Number of Assays
TAMR1 TAM/FBS
TAMR1 TAM/CSS
TAMR1 ETOH/FBS
TAMR1 ETOH/CSS
TAMR1 E2/CSS
TAMR1 E2/TAM/CSS
MCF7 TAM/FBS
MCF7 TAM/CSS
MCF7 ETOH/FBS
MCF7 ETOH/CSS
MCF7 E2/CSS
MCF7 E2/TAM/CSS