Synthetic gene expression perturbation systems with rapid, tunable, single-gene specificity in yeast

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Carles Boix

Background

R. Scott McIsaac,
Benjamin L.
Oakes, Xin Wang,
Krysta A.
Dummit, David
Botstein and
Marcus B. Noyes

- ▶ System to perturb the expression of a single gene only.

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- Tool to understand complex regulatory networks.

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- ▶ Use DBDs that do not have multiple locations in the genome ($\sim$ 9 bp)

Journal Club 1

R. Scott McIsaac,
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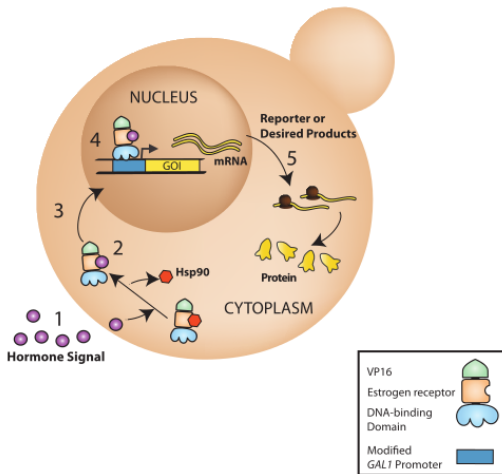Botstein and
Marcus B. Noyes

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Create a system which is:

Fast

Tightly regulated

Gratuitous (no effect on other genes)

Gradable

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This is the β-estradiol system which we are using.

DNA-binding domain (DBD)

Human estrogen receptor (ER)

VP16 activation domain

Use specific zinc fingers (Z_3 and Z_4)

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Figure : Synthesized ATF system

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Oakes, Xin Wang,
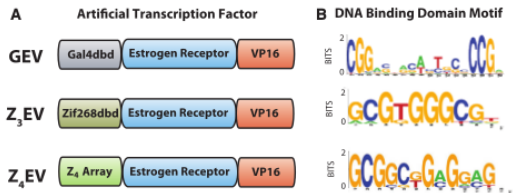Krysta A.
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Figure : Constructed ATFs and Binding Motifs

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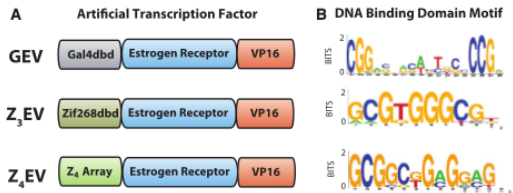Krysta A.
Dummit, David
Botstein and
Marcus B. Noyes

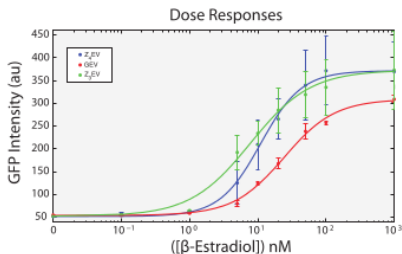**Figure :** Constructed ATFs and Binding Motifs

Figure : GFP level with reporter plasmid.

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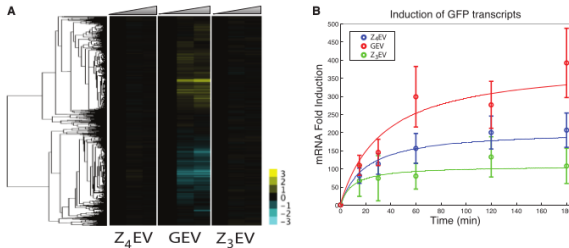Background

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Figure : Gene expression levels and GFP mRNA levels.

More than 50-fold induction in 15 minutes.

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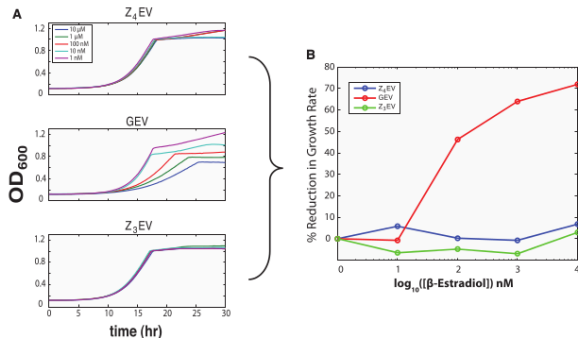Krysta A.
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Figure : Growth defect present in GEV strains.

Increasing β-estradiol decreases GEV growth rate.
70% Growth rate decrease from 10nM to 10μM.

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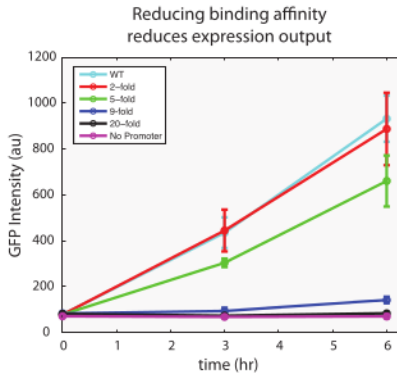**Figure :** Grading output by binding affinity

R. Scott McIsaac,
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Oakes, Xin Wang,
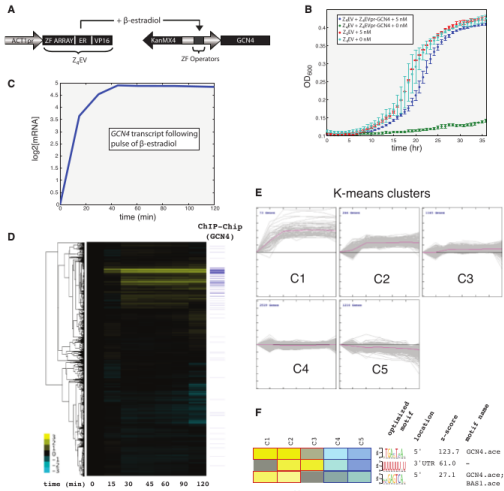Krysta A.
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Figure : *GCN4* (GOI) is a transcriptional activator of enzymes required for production of *aa*.

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The β-estradiol system:

- ▶ Is gradable
- ▶ Gratuitous
- ▶ Fast (50 fold in 15 min)
- ▶ Does not have a growth defect
- ▶ Can be moderated by binding affinity

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Have shown utility with a case study: *GCN4*. Authors find $\sim 200 - 300$ genes repressed and enriched (116 genes known before from ChIP data).