TYPS
Tübingen yeast progesterone screen
iGEM 2012 Tübingen University
“The prevalence of intersex is more widespread than anyone anticipated” Haseltine, U.S.G.S.
What are intersex fish?

- Male fish are concerned
- Less fertile
- Development of ovotestis

→ Endocrine disruptors
Water Contamination

- Medical waste
- Fertilizers, intensive farming
- Chemical industries
- Unknown factors
→ Seawage treatment?
Endocrine disruptors

- Biologically active in low concentrations
- Synergistic effects
- Difficult to detect, Bioassays

BPA

Progesterone

17β-Estradiol
TYPS (Tübingen yeast progesterone screen)

1. Receptor  2. Inverter  3. Reporter
The receptor: mPRα

- Extracellular
- Specific
- Heterologous expression in yeast
- Source organisms:
  a) *Danio rerio*
  b) *Xenopus laevis*

06/10/2012
Jessica L. Smith et. al: “Heterologous expression of human mPRα, mPRβ and mPRγ in yeast confirms their ability to function as membrane progesterone receptors” Steroids, 2008
The promoter: Pfet3

- Active in low-iron environment
- LIM (low-iron medium)
- Repressed by iron receptors of the PAQR superfamily
  → Negative signal
The inverter
The inverter

Pfet3 \rightarrow \text{Repressor}

P_{\text{Repressor}} \rightarrow \text{Reporter}
The inverter
The inverter

A) Mig1 / Psuc2
Glucose-dependent gene regulation

B) Rox1 / Panb1
Oxygen-dependent gene regulation

- Well characterized repression systems
- Knockout strains available
A) β-Galactosidase
- Several substrates available
- CPRG, high absorbption
- Used in YES-assays

B) Firefly-Luciferase
- Bioluminescence
- High sensitivity
- Signal sequence deleted, no transport to peroxisomes
TYPS (Tübingen yeast progesterone screen)

Plasmids:
- pRS313
- pRS315
- pRS316
Flexibility
Lab impressions
Project status

Promoters
- Padh1
- Pfet3
- Psuc2
- Panb1

Genes
- mPRα (*D. rerio*)
- mPRα (*X. laevis*)
- Mig1
- ROX1
- Luciferase
- LacZ

Terminator
- Tadh1
Human practice
Team Tübingen thanks...

- Advisors:
  Prof. Ralf-Peter Jansen and lab
  Prof. Alfred Nordheim
  Prof. Oliver Kohlbacher
  Prof. Lutz Heide
  Dr. Christian Apel

- Sponsors

06/10/2012
Measuring endocrine disruptors

Currently available assays

- Vitellogenin test (*in vivo*)
- Human breast cancer cells
- YES (yeast estrogen screen)-assays

→ TYPS (Tübingen yeast progesterone screen)
Measurement

SPE of water samples → Reference substances

TYPS assay culture → Incubation & Lysis → Read out