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Synthetic promoters

for recombinant protein expression in yeasts

Applied Synthetic Biology in Europe, February 7th, 2012

Das Kompetenzzentrum ACIB wird im Rahmen von COMET – Competence Centers for Excellent Technologies durch BMVIT, BMWFJ, die Länder Steiermark, Tirol und Wien gefördert. Das Programm COMET wird durch die FFG abgewickelt.

D Synthetic promoters

- Pichia pastoris expression platform
- Why new promoters
- How to design new promoters
- How to use new promoters

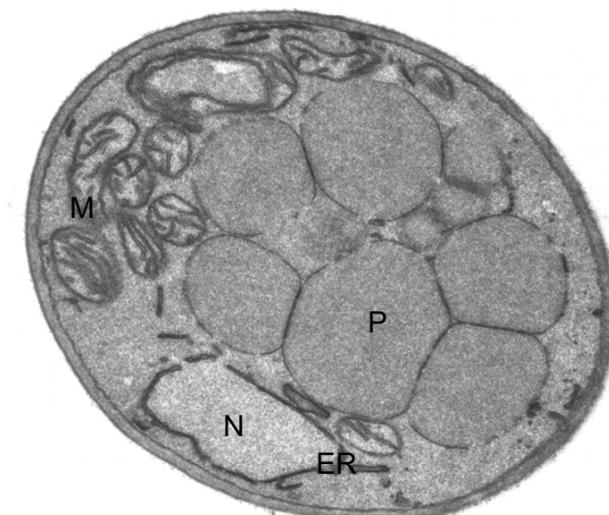
D *Pichia pastoris*

- Methylotrophic yeast
- Strong AOX1 promoter
- Microbial, high cell densities
- High intracellular protein yields (>20 g/L)
- Efficient “pure” secretion (>13 g/L)

- ① Protein production
- ② Whole cell biocatalysis
- ③ Metabolite production

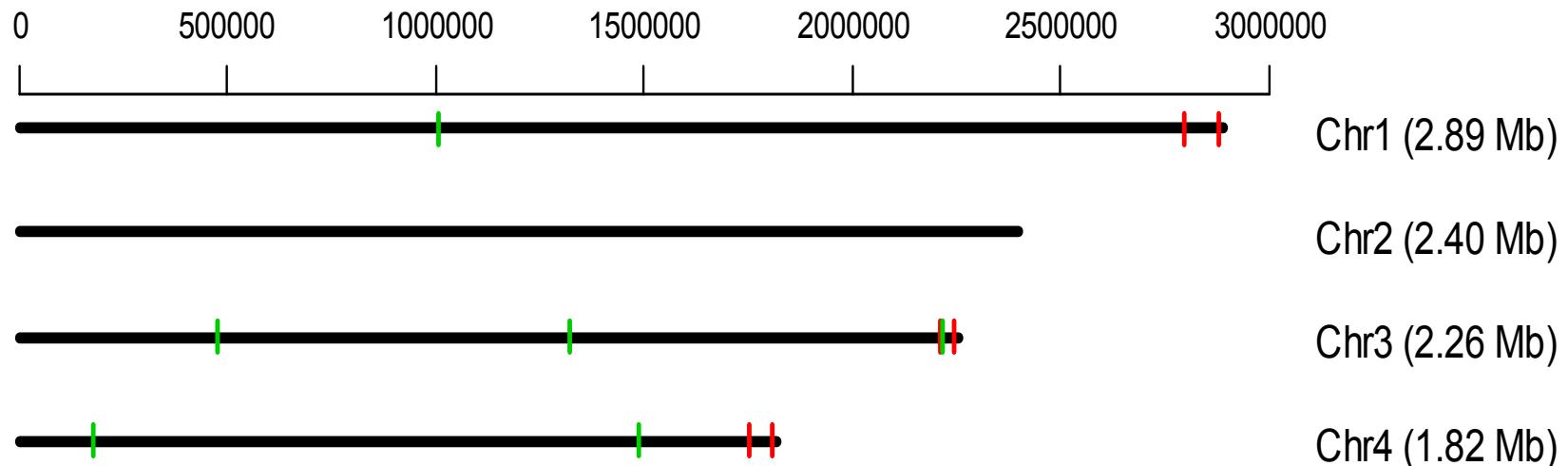
Hasslacher *et al.* 1997, Protein Expr Purif 11, 61-71.

Werten *et al.* 1999, Yeast 15, 1087-96.





Full Genome Sequence of *P. pastoris* CBS7435 (9.35 Mbp)



- 454 pyrosequencing - Genome sequencer FLX Roche
- 3 kbp mate-pair sequencing - Illumina Genome Analyzer IIx
- Sanger sequencing of PCR amplicons

...in collaboration with CeBiTec Bielefeld

Küberl *et al.* 2011, J Biotechnol 154, 312-320.

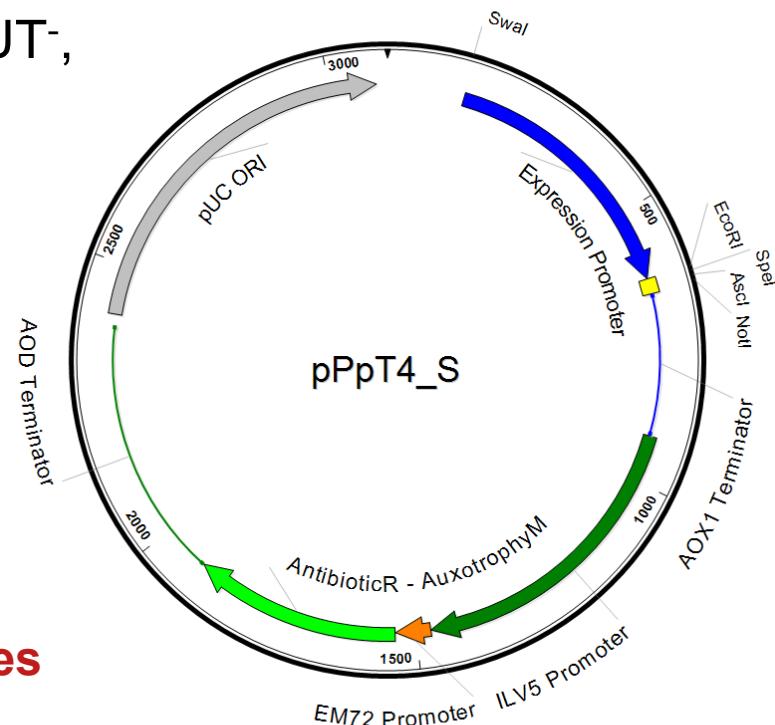
D Strains and Plasmids

Host strains: Mut^s, His⁻, *Arg, *GUT⁻,
ΔKU70

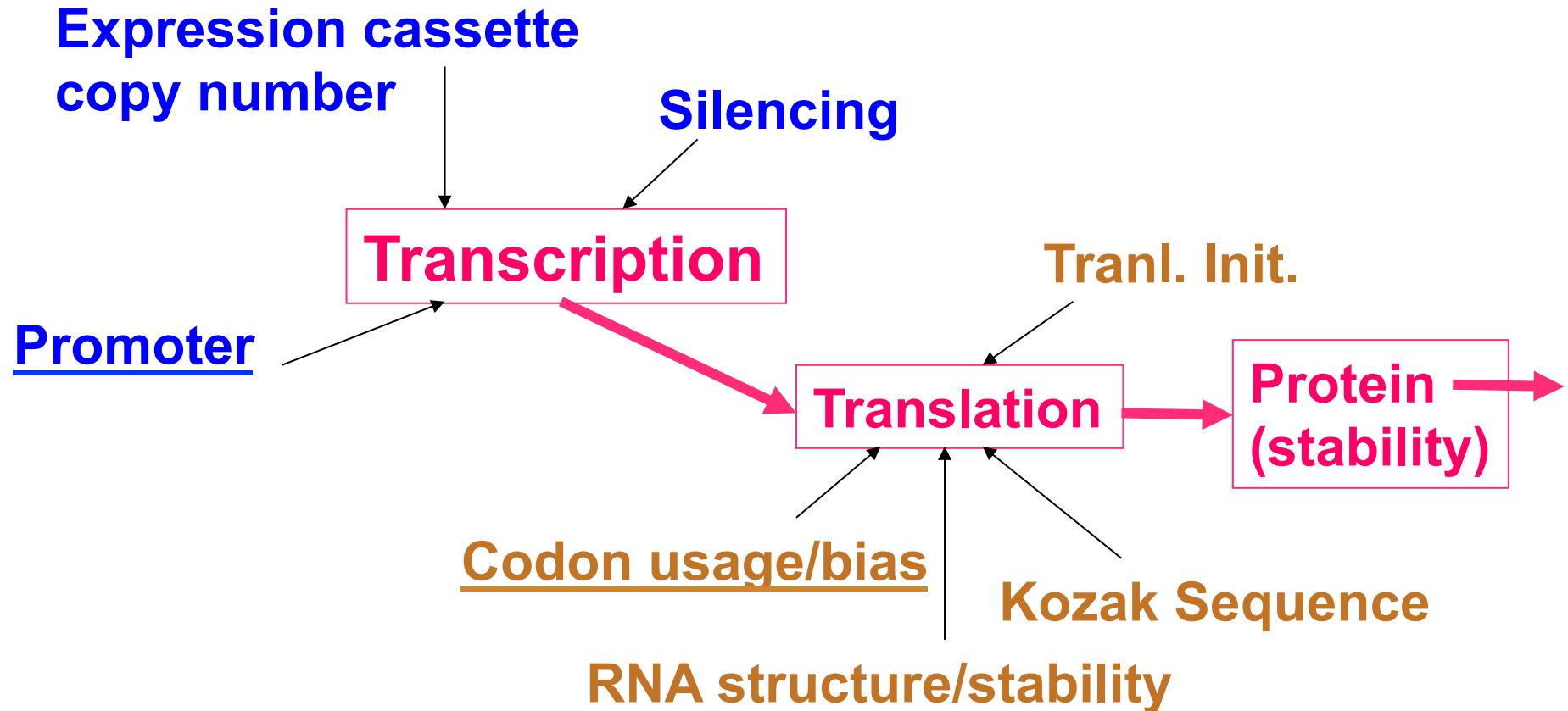
Plasmids: P_{GAP}, P_{AOX1}, His, Arg,
Zeo^R, Kan^R

*not yet available

- Available from TU Graz
- Single & multi copy vectors
- Genes cloned into *Pichia* vectors available from DNA2.0
- **Project collaborations and services at ACIB & VTU based on FTO expression platform**

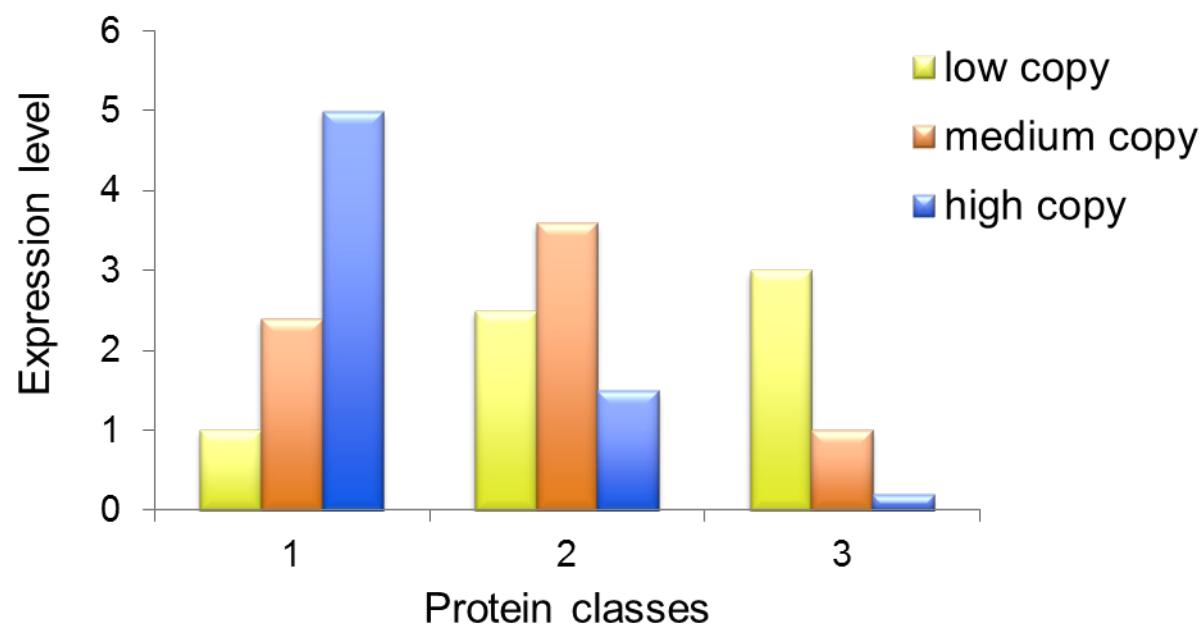


D Expression optimization



D Copy Number

- Special vectors for low and high copy numbers
- Colony (re-)growth on plates with high conc. of antibiotics





New synthetic promoters

desired properties for protein and synthetic pathway expression:

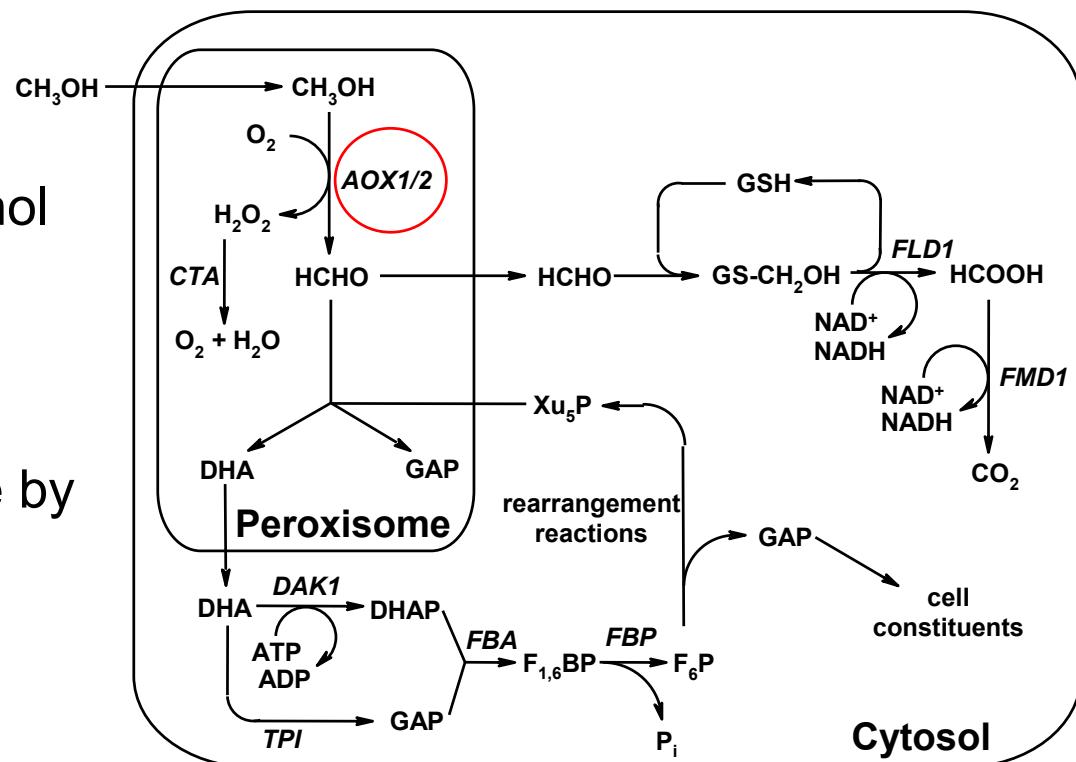
- ① Strong and weak, ideally tunable
- ② Not too long for simple cloning
- ③ Tightly regulated
- ④ Simple scalable induction procedure
- ⑤ Non toxic induction
- ⑥ (Innovative)
- ⑦ Several promoters with similar regulatory profile but different sequence (stable mini pathways)



Methanol utilisation pathway AOX1 Promoter

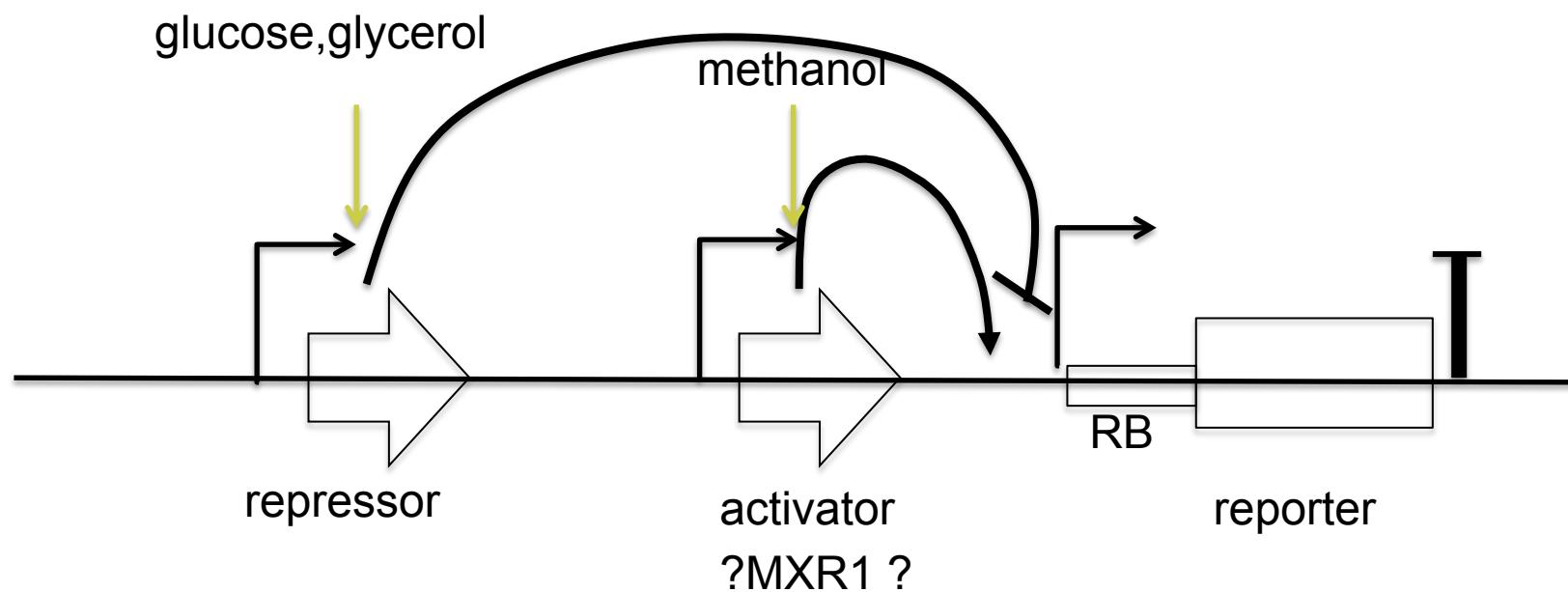
tightly regulated

- AOX1 : majority of alcohol oxidase
- Repressed by glucose, glycerol, ethanol, ...
- P_{AOX1} : Strongly inducible by methanol



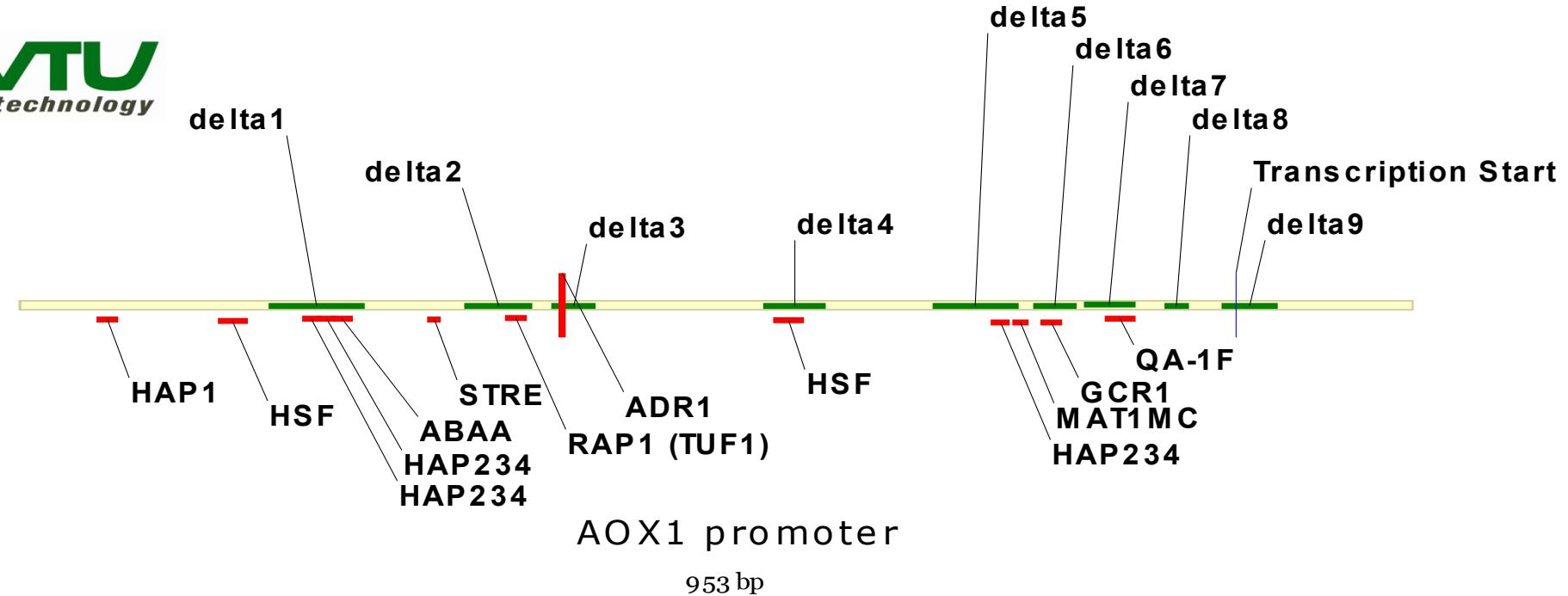
Tschopp, J.F., et al. Nucleic Acids Res 15 (1987).

D AOX1 Promoter



initial simplified model

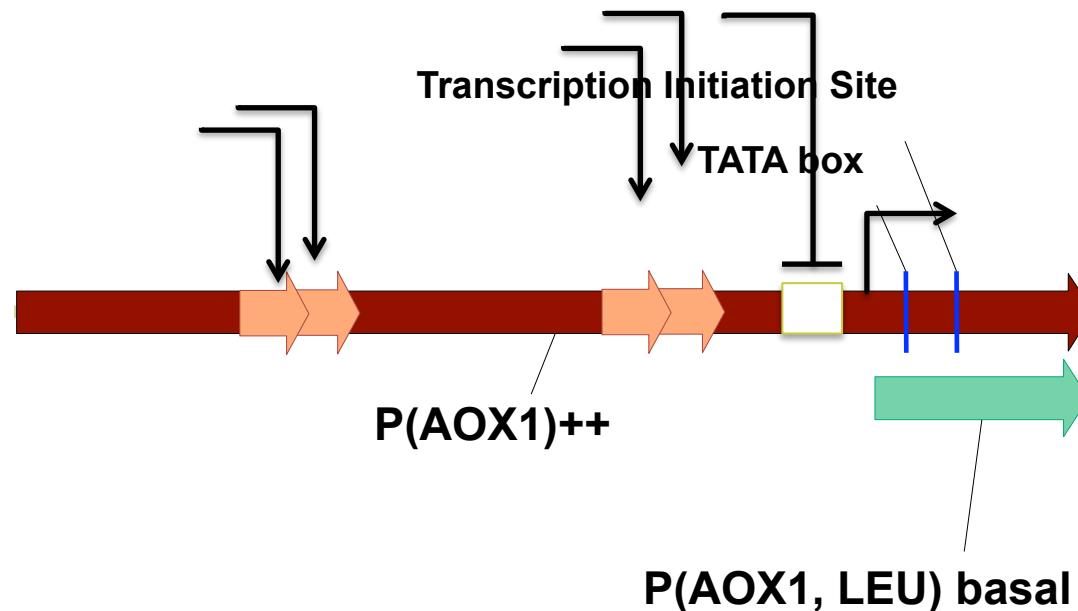
PAOX1 sequence analysis



Transcription factor binding sites:
HSF.....heat shock factor
STRE.....stress response element
HAP.....O₂ and glucose regulation
GCR....regulator of glycolysis
ADR....inductor of ScADH2 and peroxisomal genes

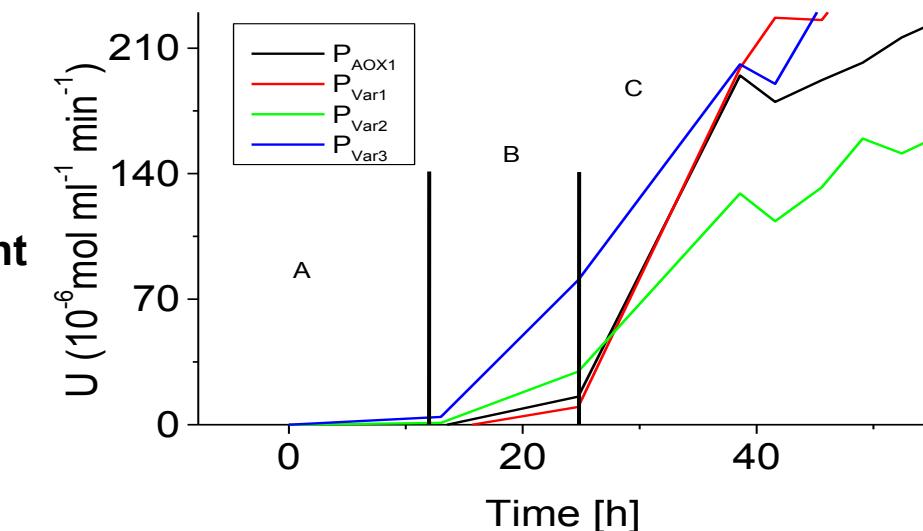
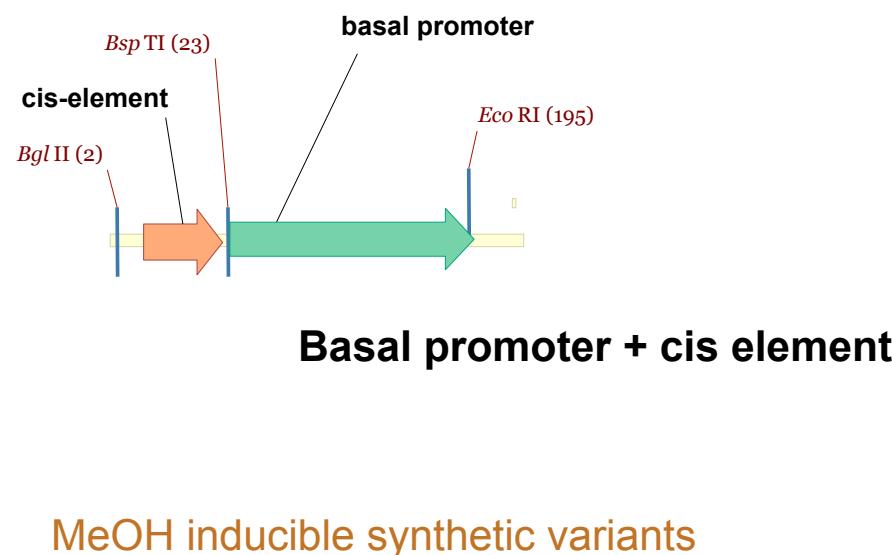
➤ **Primary promoter variant library:**
short deletions (5-60 bp), covering putative transcription factor binding sites

D Engineered promoters



Increased activity without MeOH

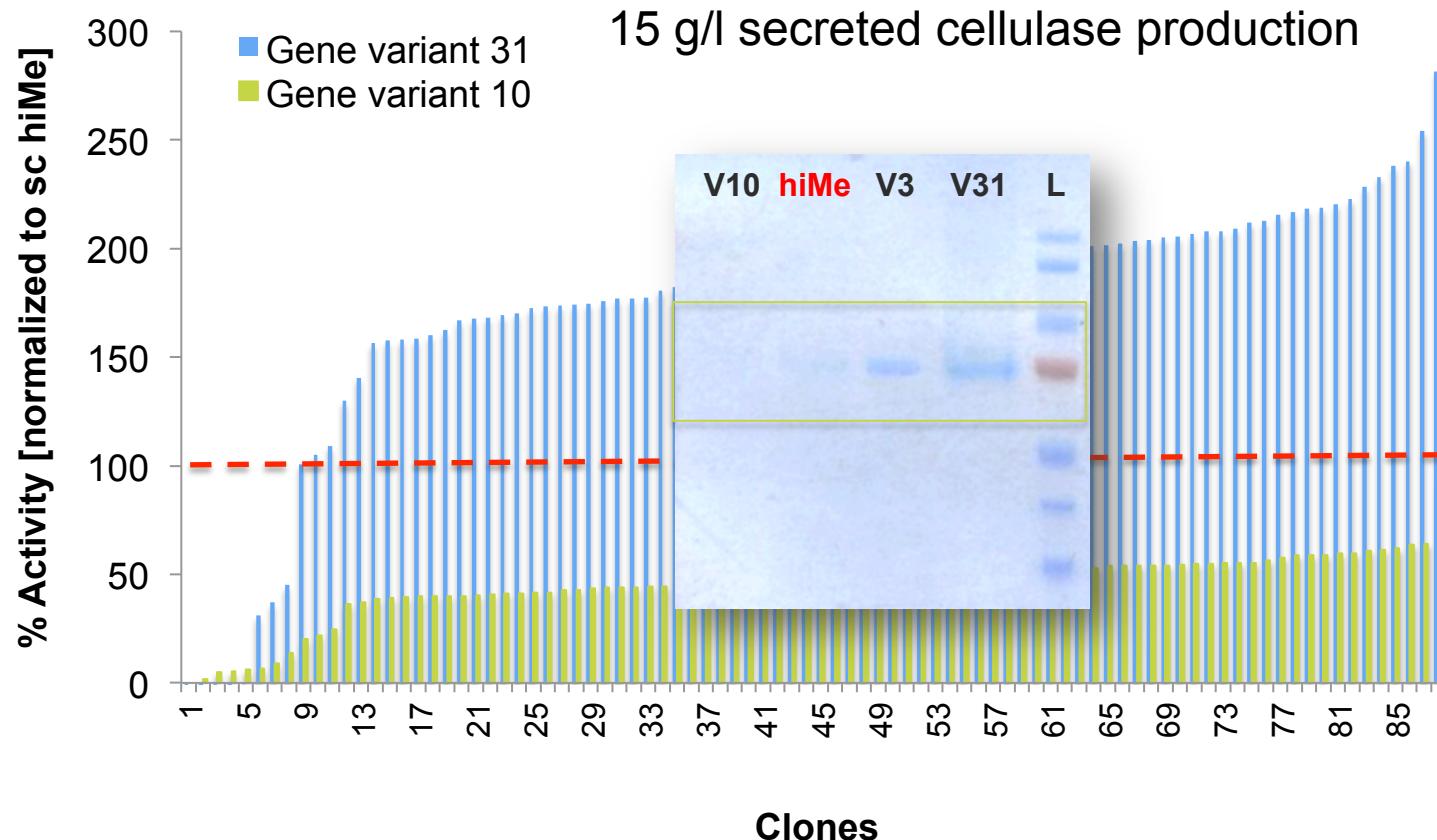
D Engineered promoters



WO2006/089329, Hartner et al., NAR 2008



Optimized *CBH2* by DNA2.0 Model



New Record for *Pichia* Secretion!!

Single copy technology applied

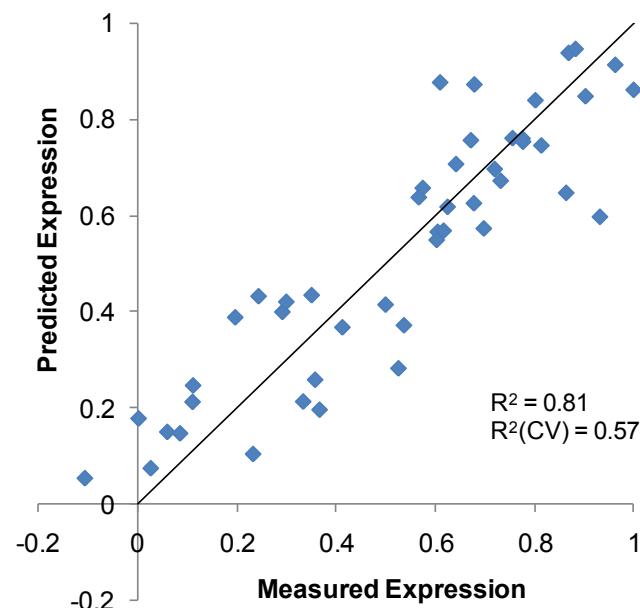
hiMe: high MeOH codon usage (single copy level)

Mellitzer et al., manuscript in preparation.

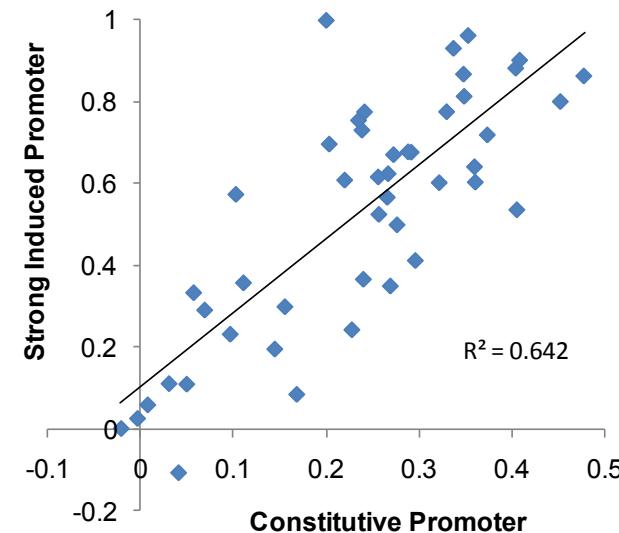


DNA2.0 Analysis

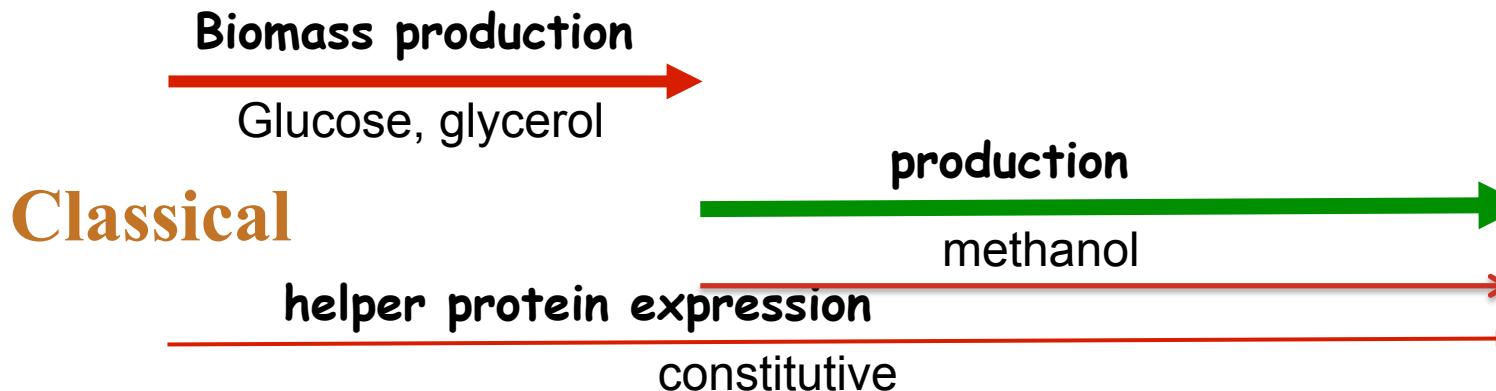
PLS Model for *P. pastoris*



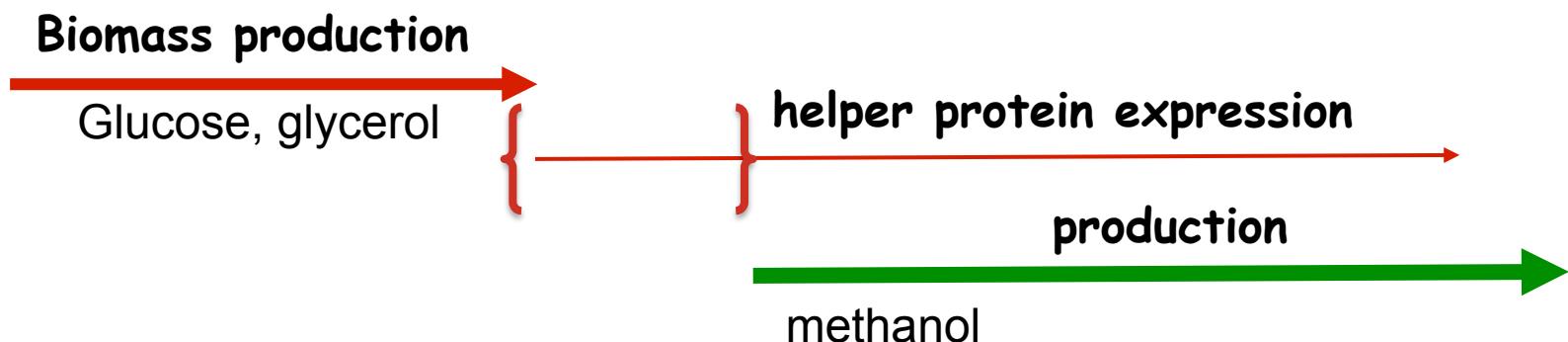
Promoter comparison



Cascade expression with new derepressed promoters

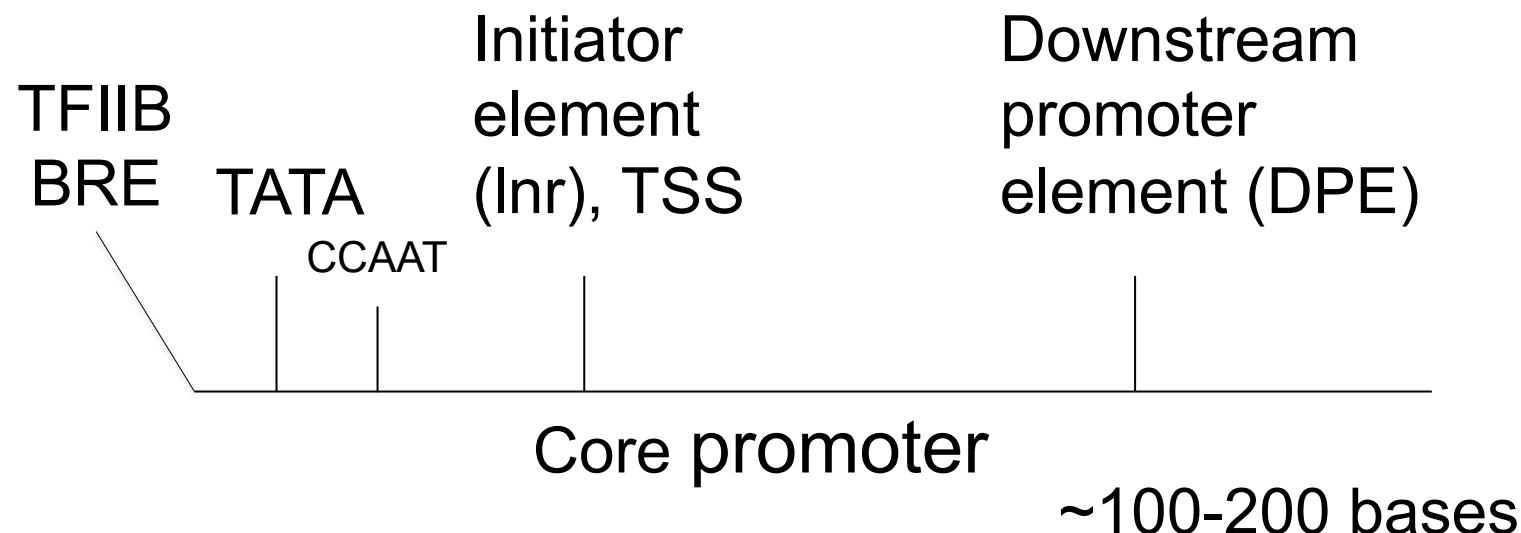


Cascade



D Small synthetic promoters

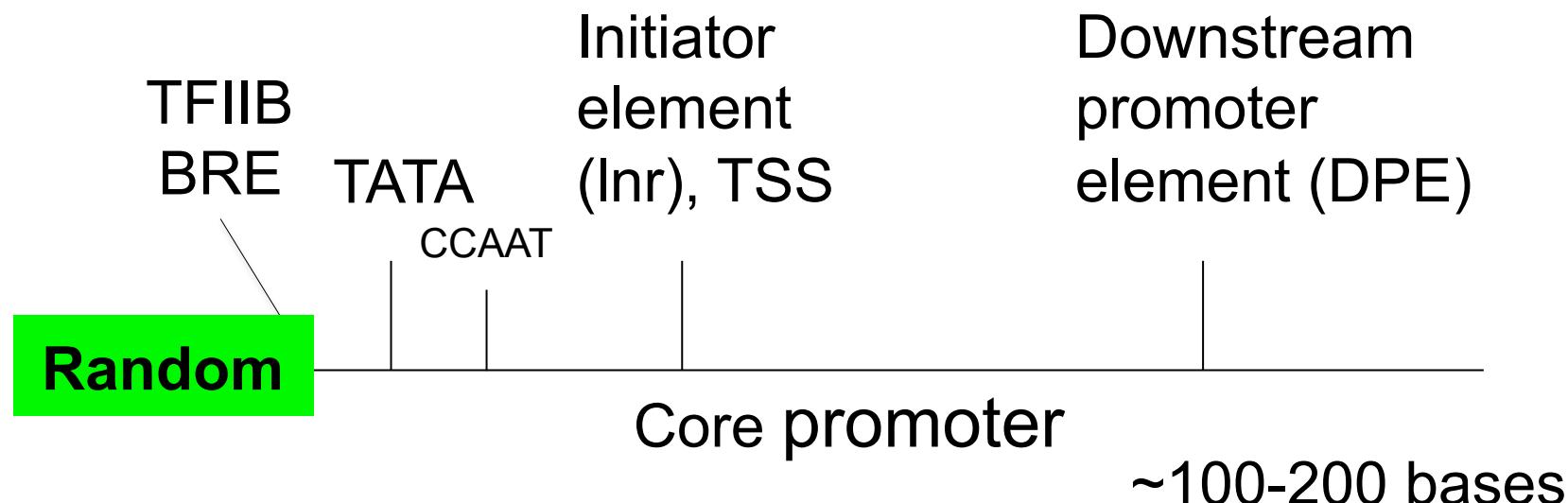
general eukaryotic promoter design



Replace regulation by fully synthetic core promoter

D Small synthetic promoters

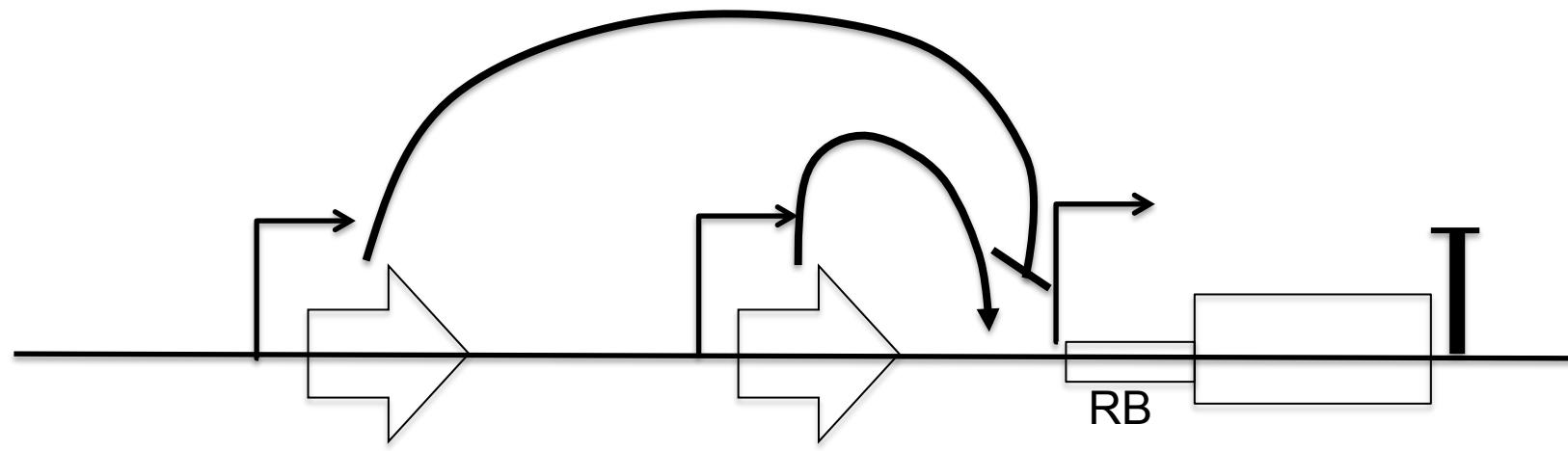
general eukaryotic promoter design



Replace regulation by fully synthetic core promoter



AOX1 Promoter



Repressor
(several !)

(6 MXR1 ! + others)
+ core promoter regulation

Initial model is much too simple



Acknowledgements

Roland Weis

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Tanja Hajek

Spotlight on *Pichia*



Pichia conference: Alpbach, Feb 29 – Mar 3, 2012
hands-on lab course: Graz, Feb 26 – 29, 2012



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and technology