Evaluation of *S. cerevisiae* promoters during growth on xylose

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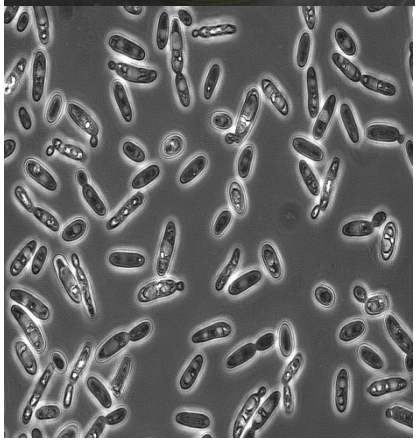
Motivation

- Identify a promoter that is strongly induced during growth on xylose
- Increase production of recombinant enzymes needed during the degradation of lignocelluloses
- Recombinant enzymes such as xylanases, beta-glucosidase, cellulases etc

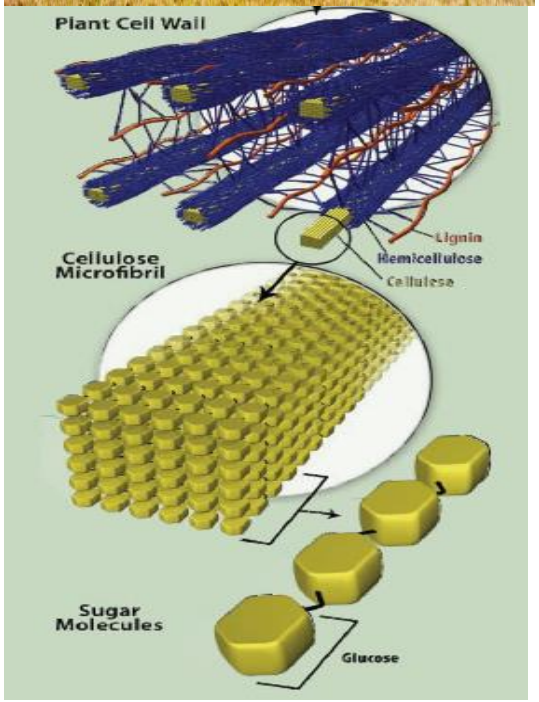
Introduction



- *S.cerevisiae* has been used for years in the production of recombinant proteins
- *S.cerevisiae* is a widely used organism
  - fast cell growth
  - tolerate high ethanol concentration
  - tolerate wide spectrum of inhibitors
  - well-characterised physiology & genetics
  - GRAS status

Introduction

- Glucose is the most abundant sugar in nature (cellulose)
- *S. cerevisiae* is a Crabtree positive yeast
- Its produces ethanol on glucose under aerobic conditions (little biomass)
- High expression level of recombinant protein is linked to the amount of biomass obtained during fermentation (Ferndahl *et al.*, 2010)

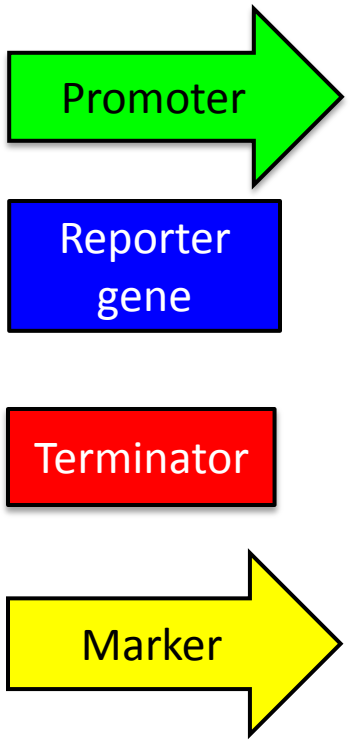


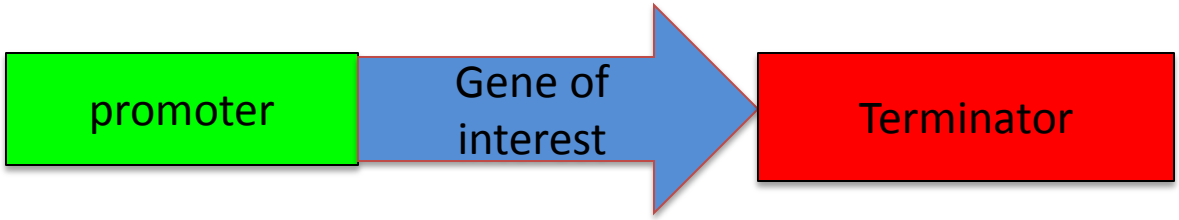
Introduction

- Xylose is the second most abundant sugar in nature and considered a waste in most industries
- *S. cerevisiae* cannot utilise xylose as a carbon source
- *S.cerevisiae* engineered to grow on xylose has been reported to produce more biomass on xylose
- Xylose is the more suitable carbon source in recombinant protein production

Introduction

- Promoter
  - ENO1, ENO2, YG100, PGK1, ADH2,GPD3
- Reporter gene
  - Xylanase gene
- Terminator
  - ENO1, ENO2, YG100, PGK1, ADH2,GPD3
- Episomal selectable marker
  - URA3



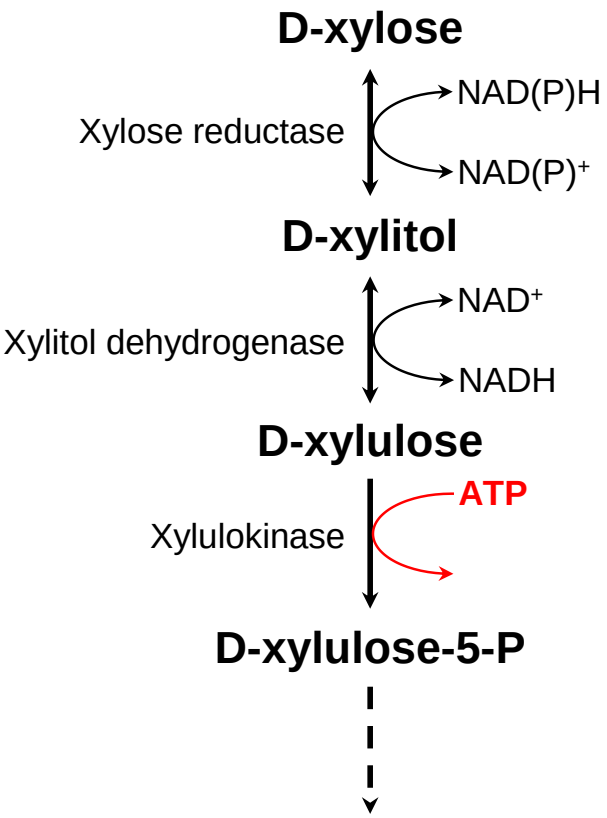


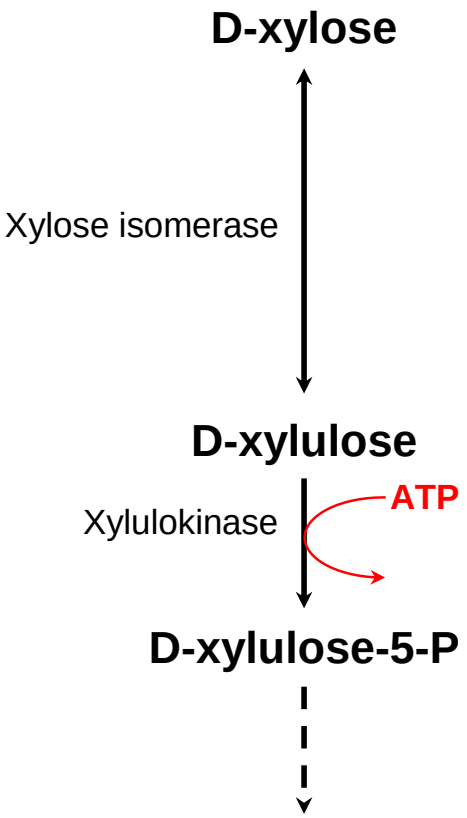
Engineering *S.cerevisiae*(Y294)

sXI gene (*B.thetaiotaomicron*

XYL3 (P. stipitis)

~~*GRE3*~~





Restriction map of the plasmids

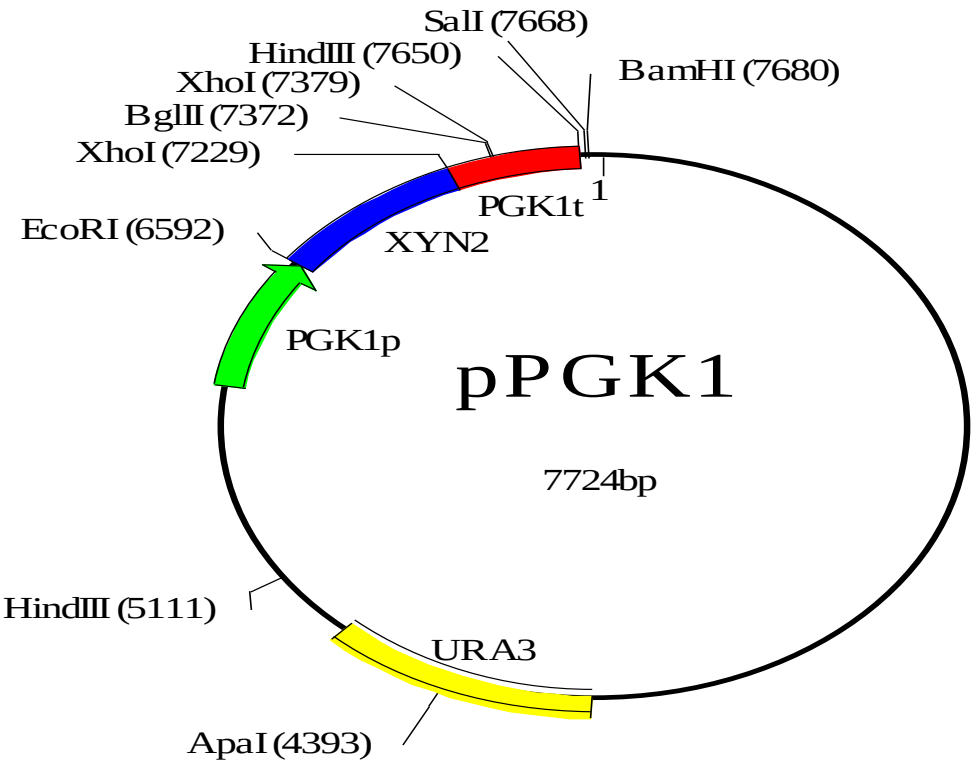


Figure 1. pPGK1 used as base for the construction of all episomal plasmids.

Results and Discussion

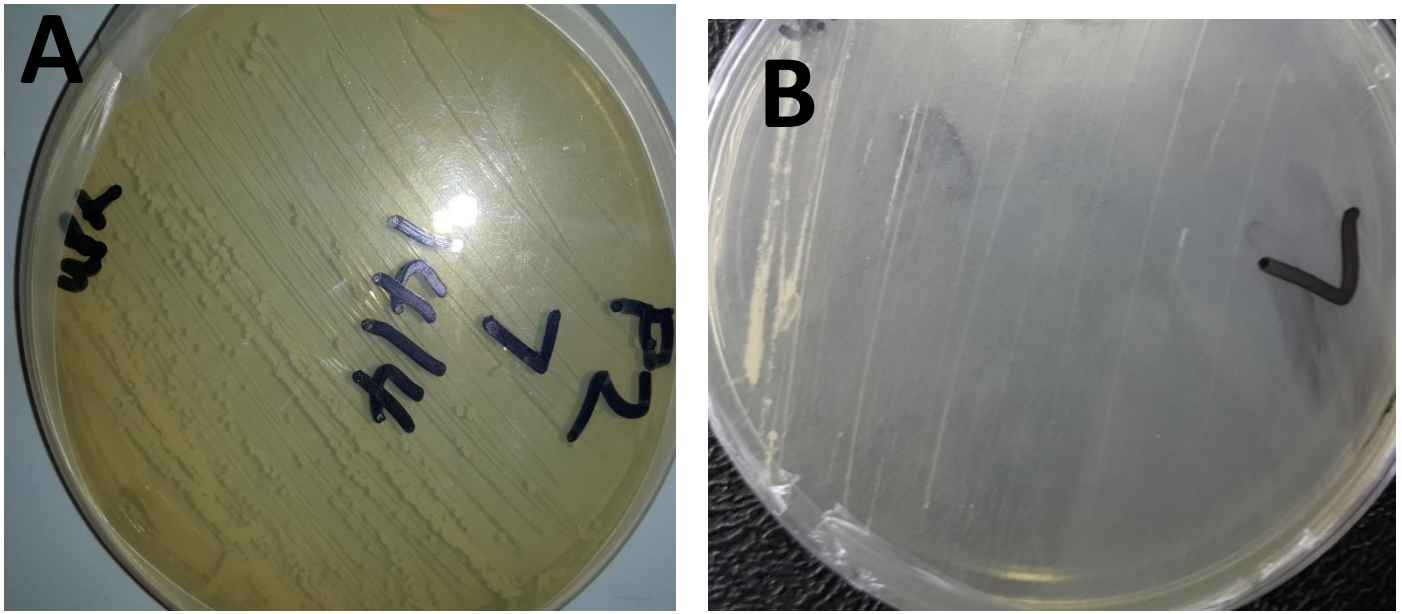
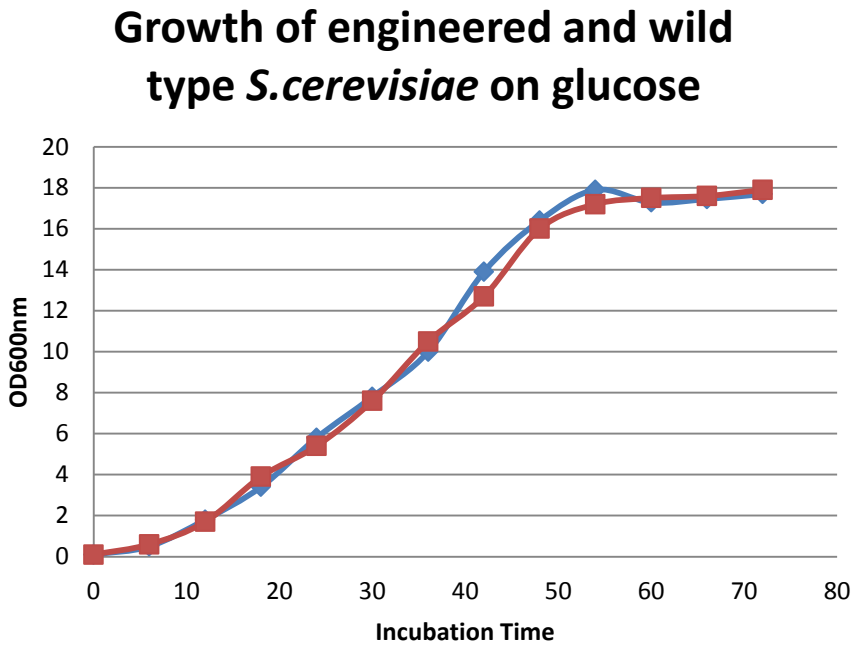


Figure 2. Engineered *S.cerevisiae* on xylose and SC –URA3 with glucose.

Results and Discussion



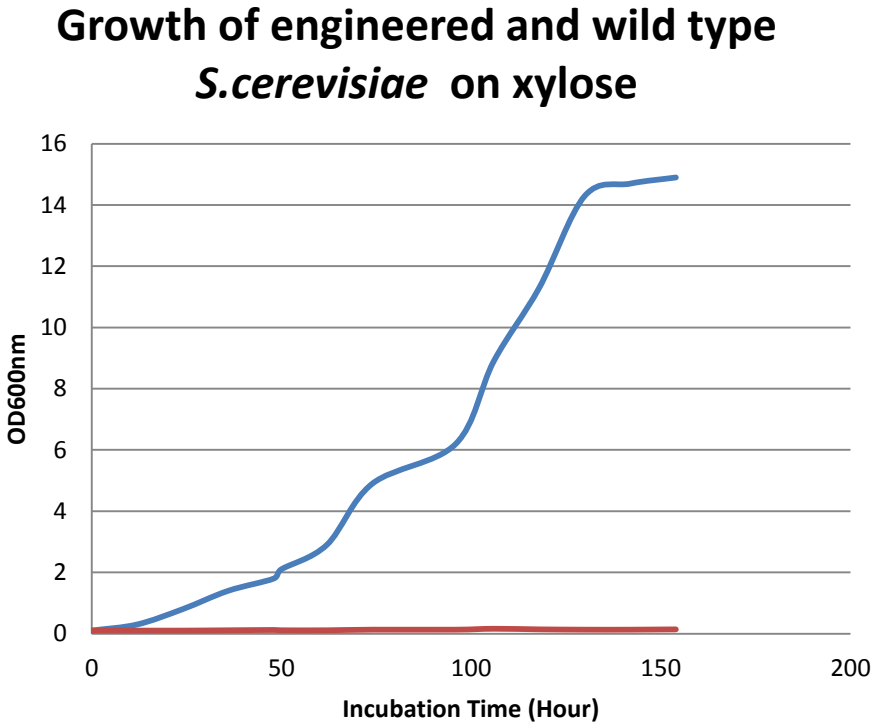


Figure 3.Growth curve of the wild type and engineered yeast on glucose and on xylose

Results and Discussion

- Transformants spotted on RBB-xylan plates with different carbon source

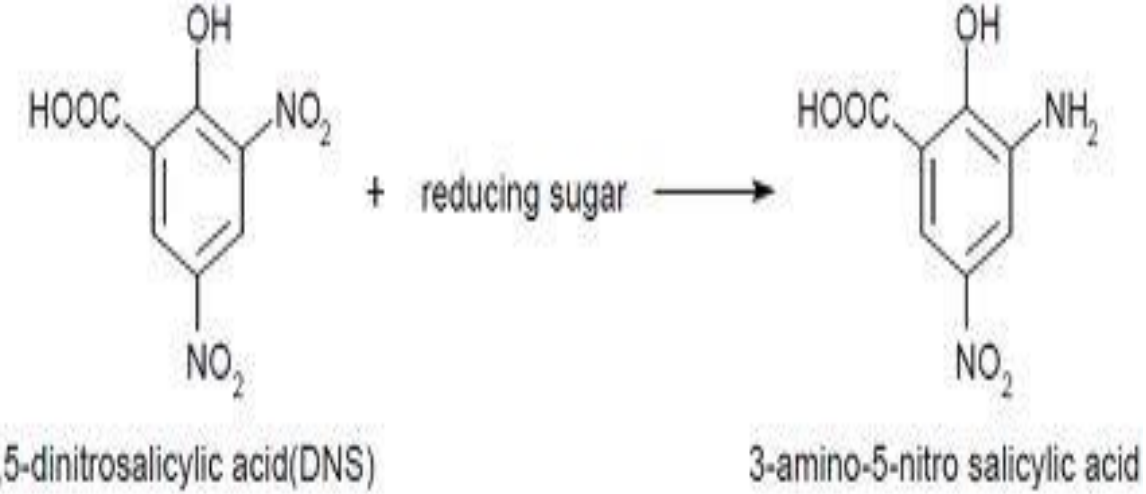
RBB-xylan xylose

RBB-xylan glucose

Figure 4. The RBB-xylan plates used to confirm xylanase activity

Results and Discussion

- DNS ASSAY was done to determine the xylanase activity following the method by (Bailey *et al*, 1992)



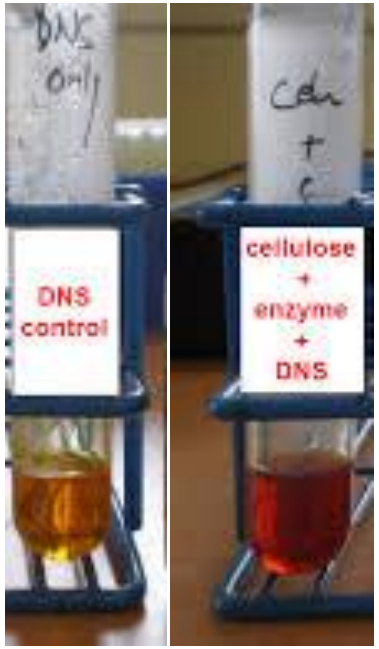


Figure 5. Determination of the amount of xylanase enzyme produced using DNS assay

Results and Discussion

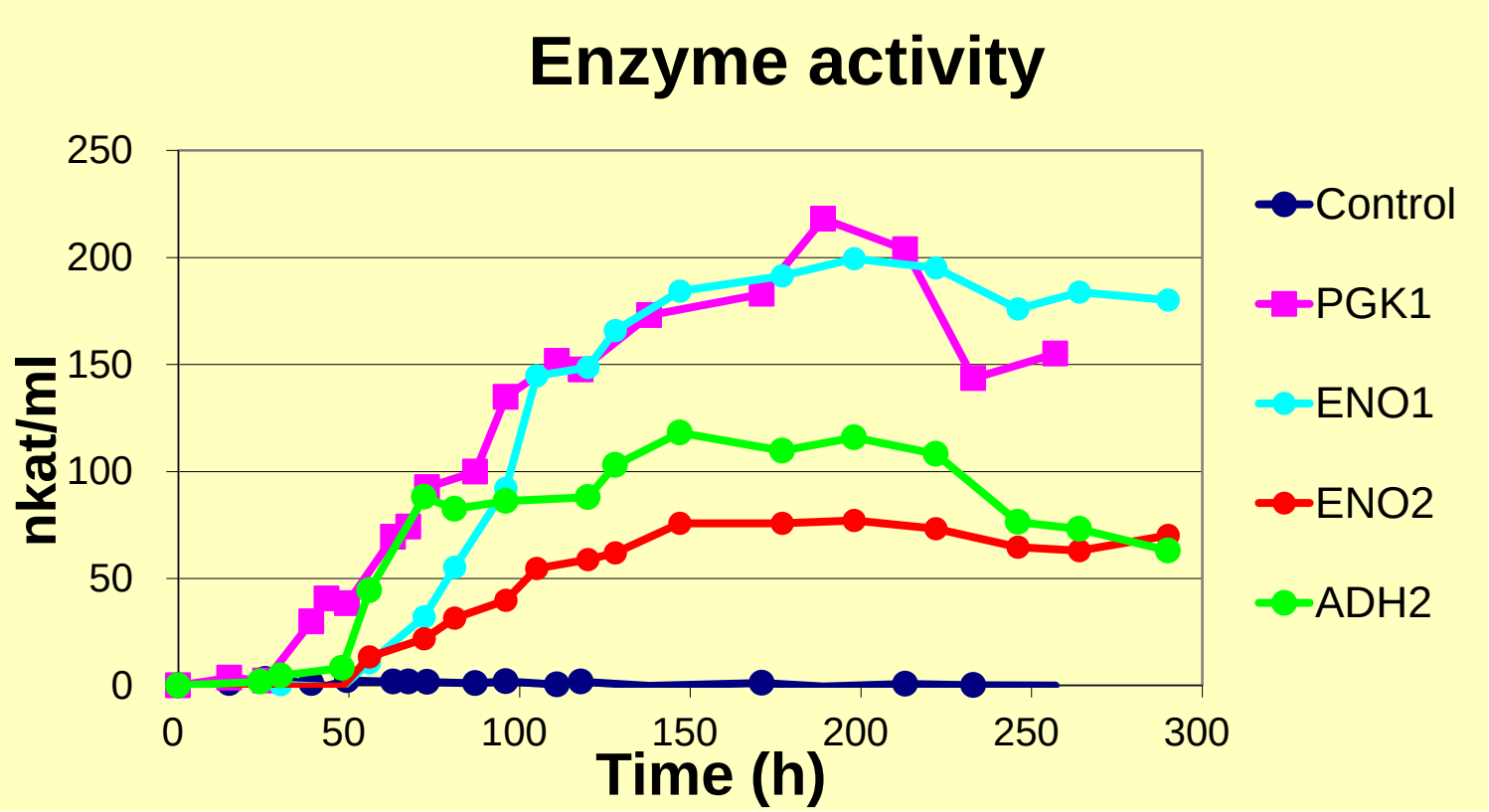
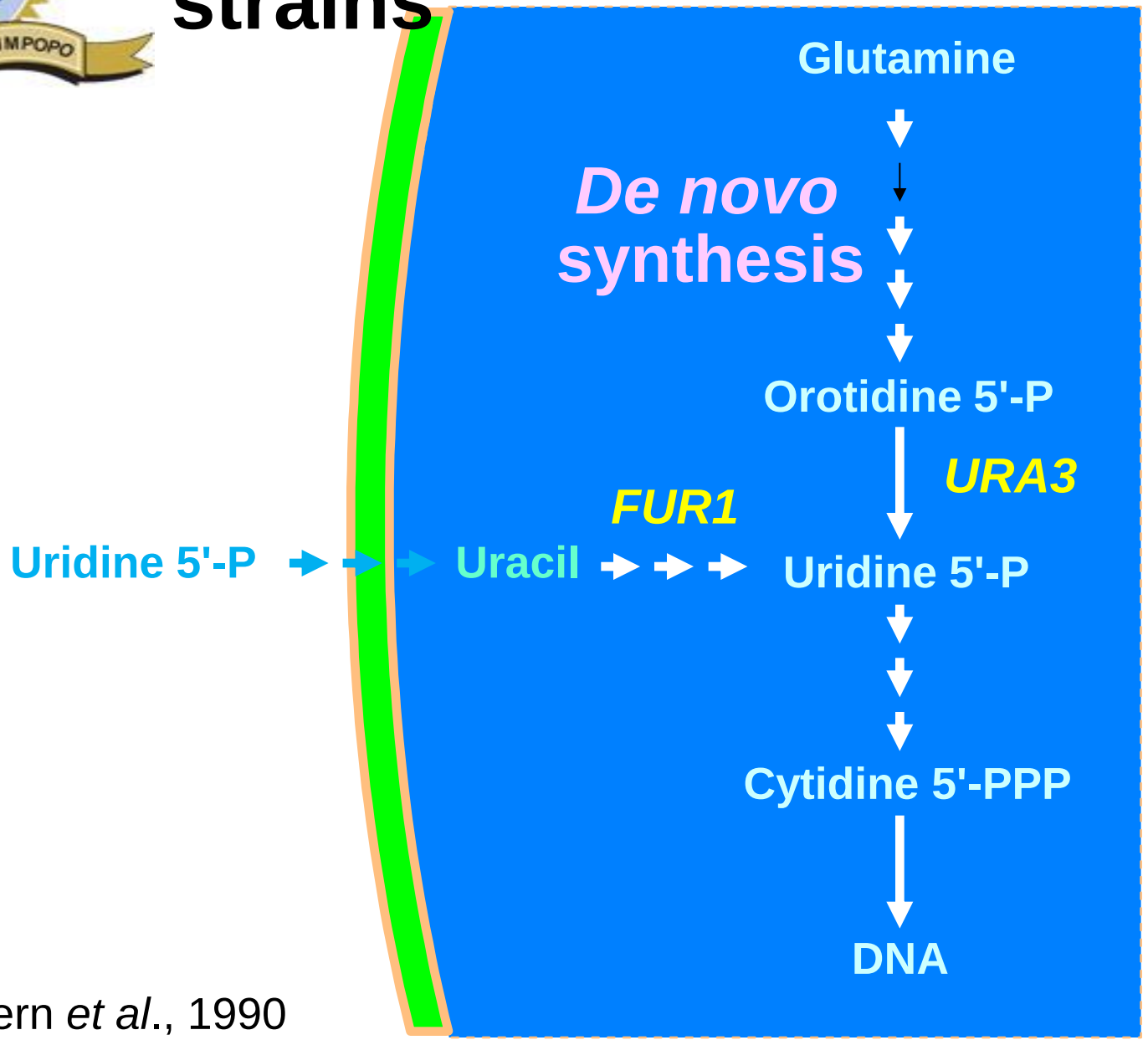


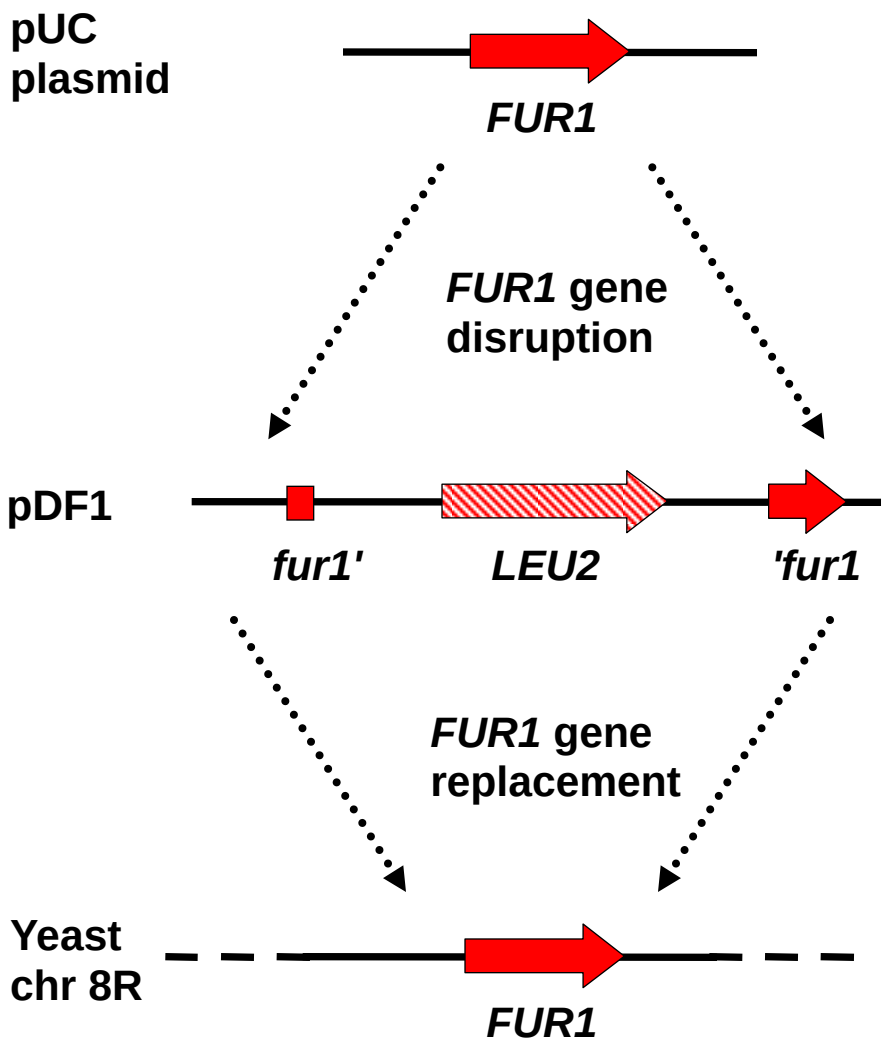
Figure 6. Determination of the amount of xylanase enzyme produced using DNS assay

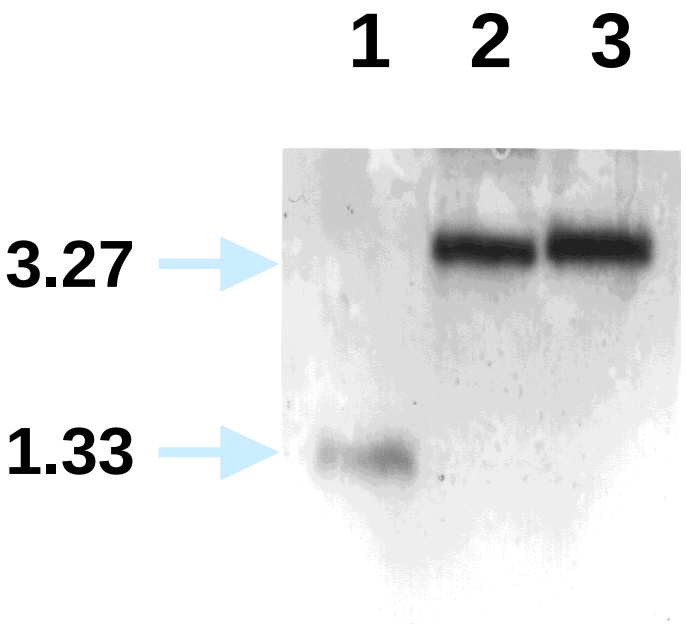
Construction of *fur1::LEU2* strains



Kern *et al*., 1990

Construction of *fur1::LEU2* strains





FUTURE WORK

- Evaluation of episomal promoters during growth on xylose
- Determine metabolic burden during growth on xylose in the bioreactor

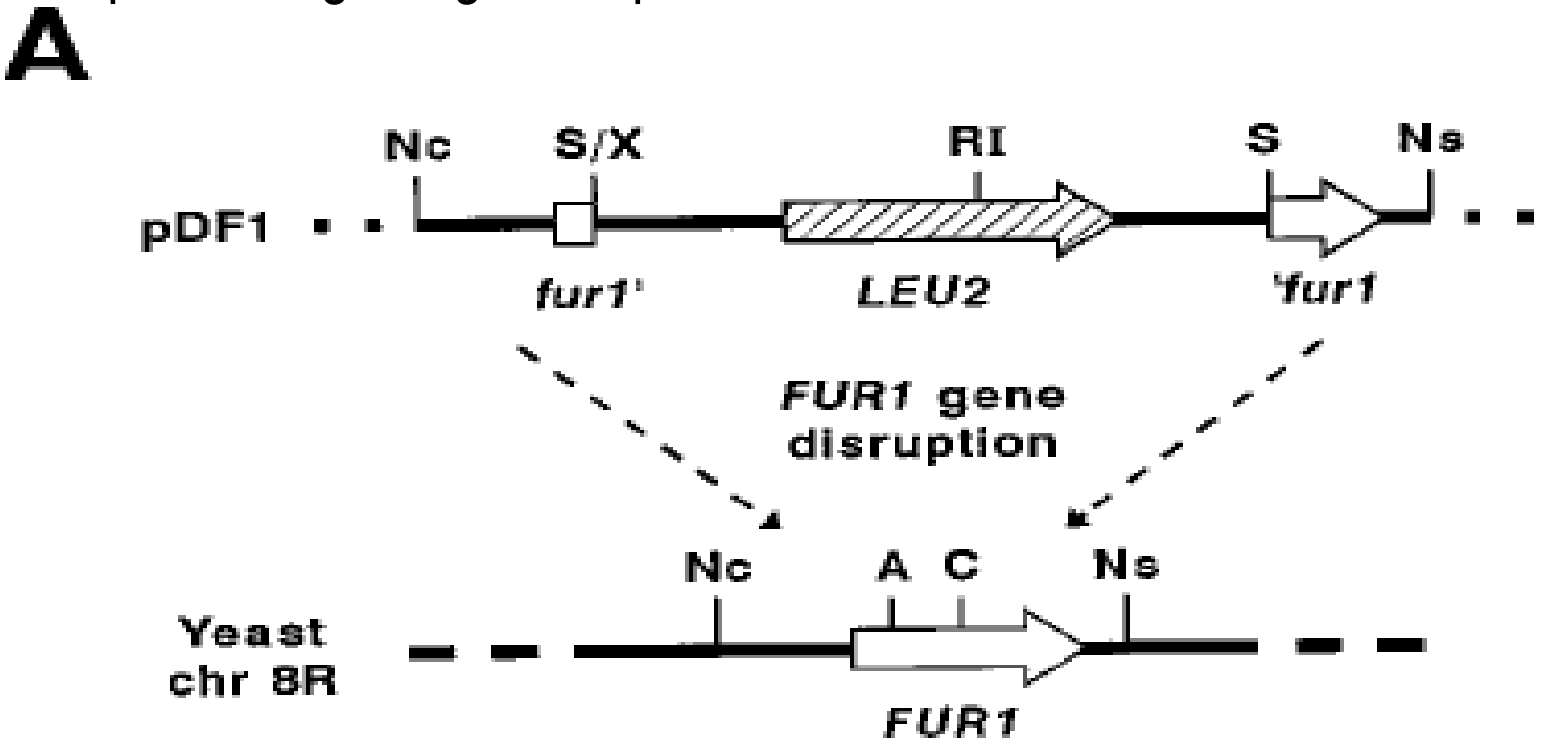
ACKNOWLEDGEMENT

- Many thanks Dr. La Grange
- Prof Ncube and Prof van Zyl
- Dr V. Mbazima
- Van Zyl lab (Stellenbosch University)
- Department of BMBT (University of Limpopo)
- RSES for financial assistance

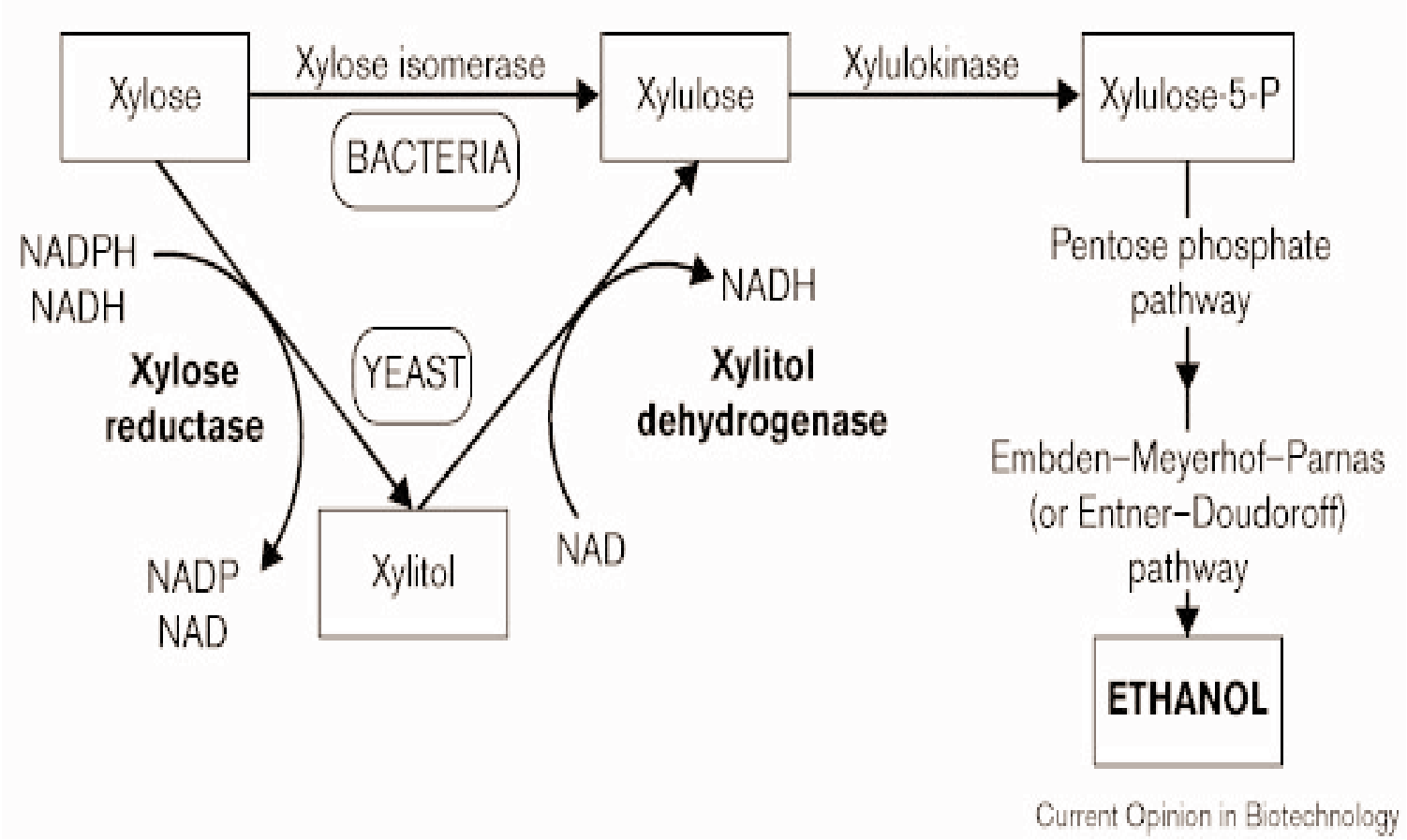
Thank you ……

Fur1 Disruption

- fur1::LEU2 allele was Isolated as 3.27kb from pDF1 plasmid
- NcoI-NsiI restriction enzymes
- Disrupted using the gene replacement method



Introduction



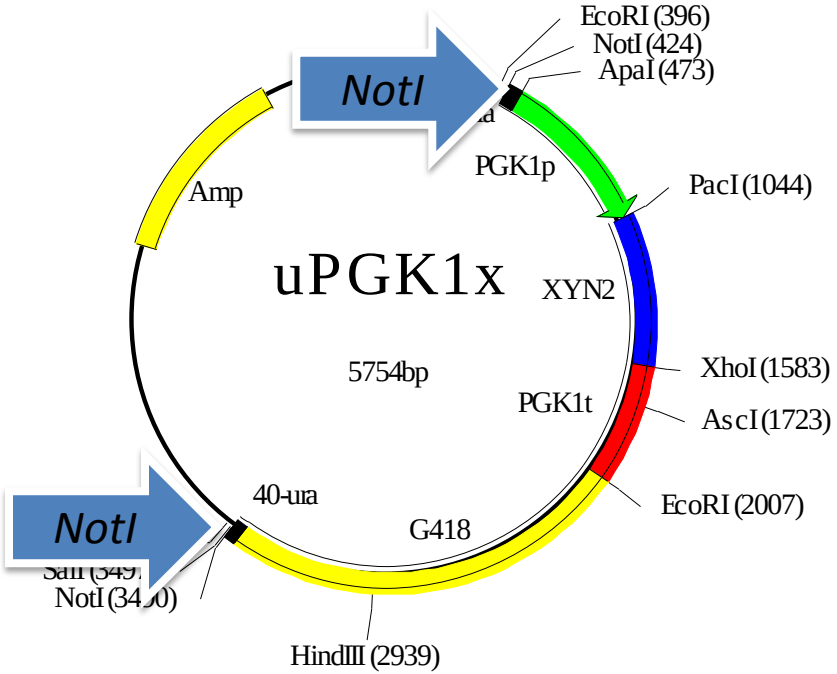
(Aristidou and Pentila,2000)

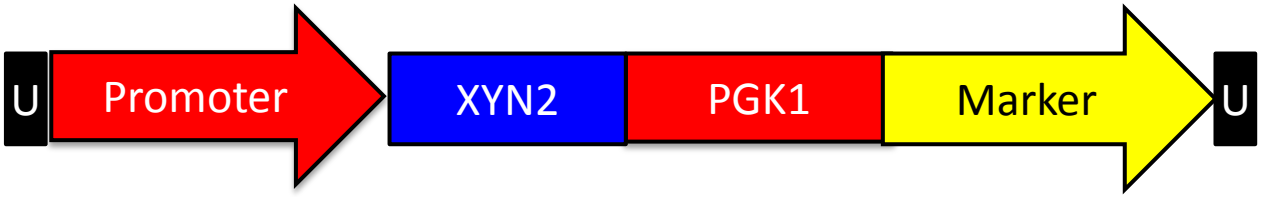
Transformation of integrated plasmids

- **Transformation**
  - Transformation in *S. cerevisiae*

S228c strain

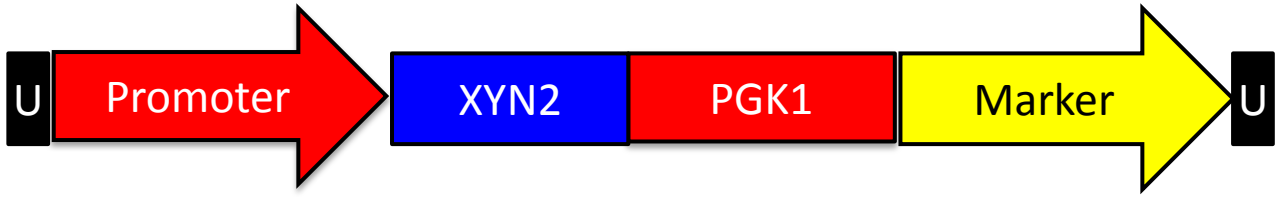
  - Transformants selected on geneticin (G418)



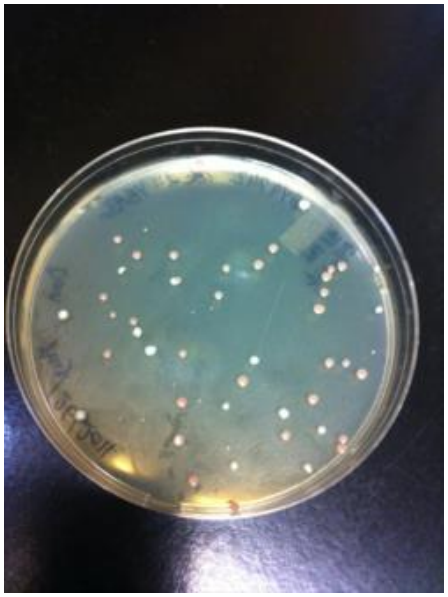


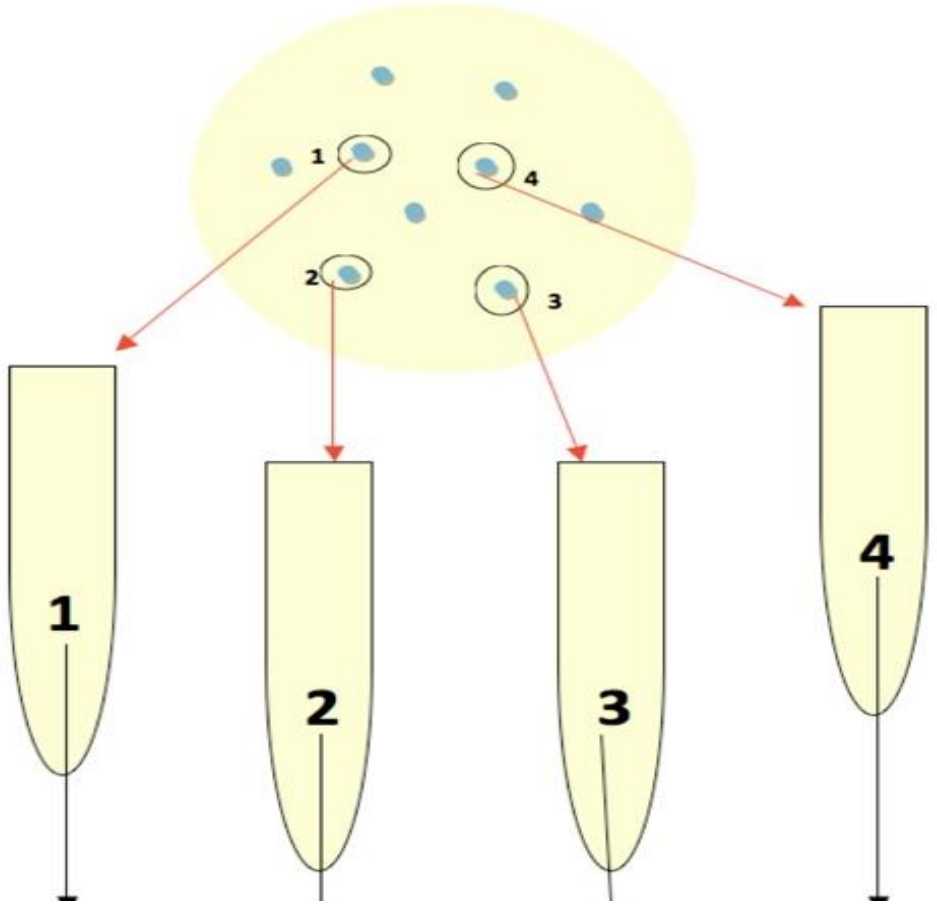
Integrating expression cassettes

- *PGK1p* and *PGK1t* isolated from plasmid by restriction digest
- G418 isolated from plasmid by PCR
- 40bp *URA3* overhangs added to expression cassette (target cassette to *URA3*-locus)
- Expression cassette cloned into pUC19



Transformation





Rapid genomic DNA isolation,
"Bust and Grab" method

Construction of the strain that grows on xylose

- *S. cerevisiae* Y294 (Matα *leu* 2-3, 112 *ura* 3-52,*his* 3,*trp*1-289)

- Transformation with pMJM121 with Synthetic codon optimised xylose isomerase
  - *B. thetaiotaomicron* XI
  - Selective marker (zeocin)

- Disruption cassette (gre3::Xyl3Hygromycin) was used to knock out *GRE3* gene

RESULTS

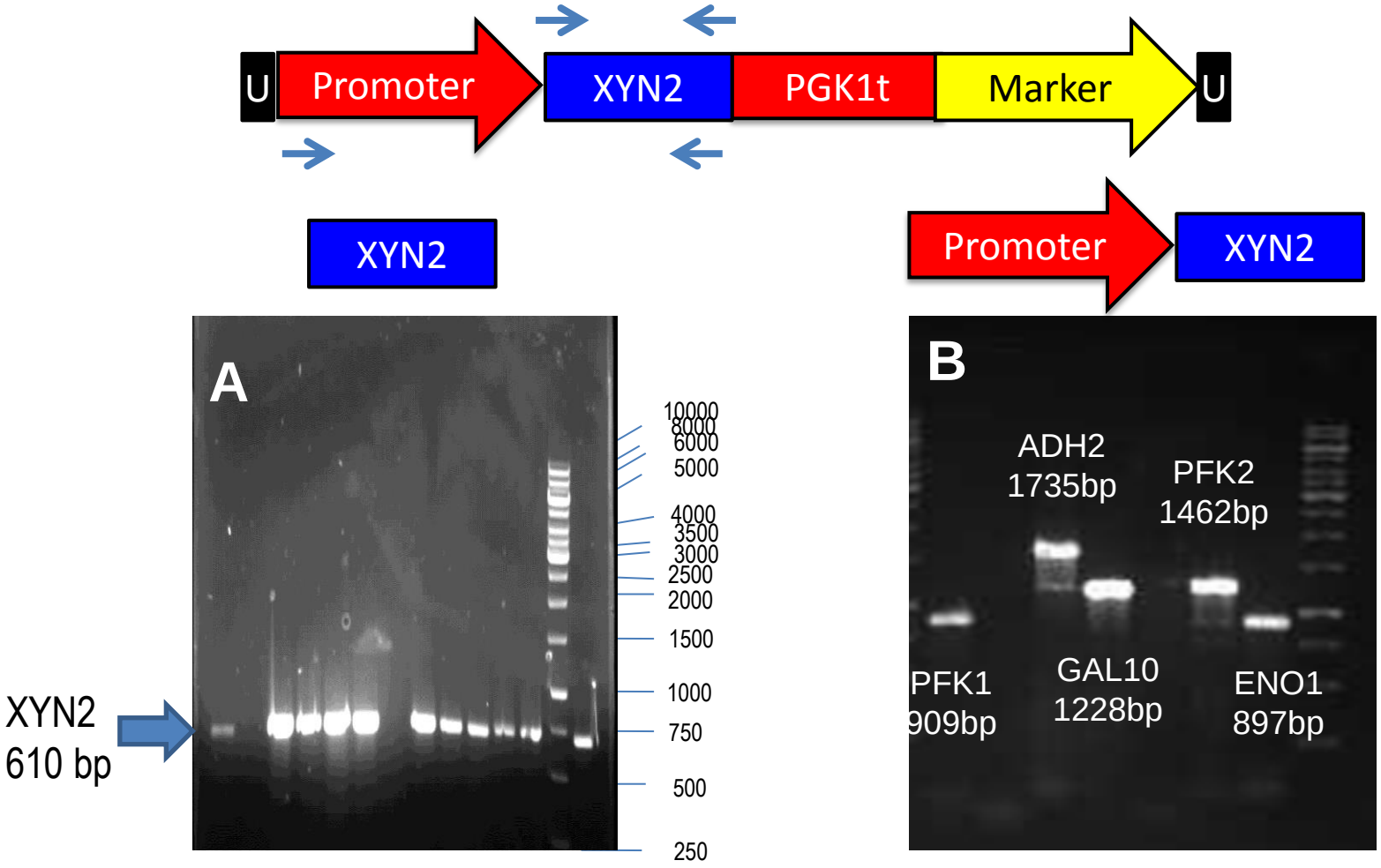


Figure 2: (**A**) Agarose gel electrophoresis of the PCR with XYN2 primers (**B**) Gel electrophoresis of PCR with promoter specific primer and XYN2 right primer