A new high-throughput method for the rapid extraction of yeast DNA

XXXVI Jornadas Portuguesas de Genética

30.may.2011

Vieira E.[1,2] [a] ,Drumonde-Neves J.[1,3] [a],Lima M.T.[3] ,Araújo I.[2], Casal M.[1] and Schuller D.[1]

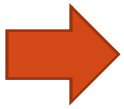
[1] CBMA (Centre of Molecular and Environmental Biology) / Department of Biology / University of Minho, Portugal
[2]Vinalia - Biotechnology Solutions for Viticulture and Enology, *Spin-off* of the University of Minho, Braga
[3]Research Centre for Agricultural Technology, University of Azores, Angra do Heroísmo
[a] **contributed equally**

Universidade dos Açores
Departamento de Ciências Agrárias
Centro de Investigação de Tecnologias Agrárias dos Açores

Introduction

- Ecological, evolutionary and population genetic studies of yeasts require the processing of **high numbers of isolates**;

- The current molecular methods for yeast identification and characterization require **time-consuming** and **labor intensive** DNA extraction protocols.

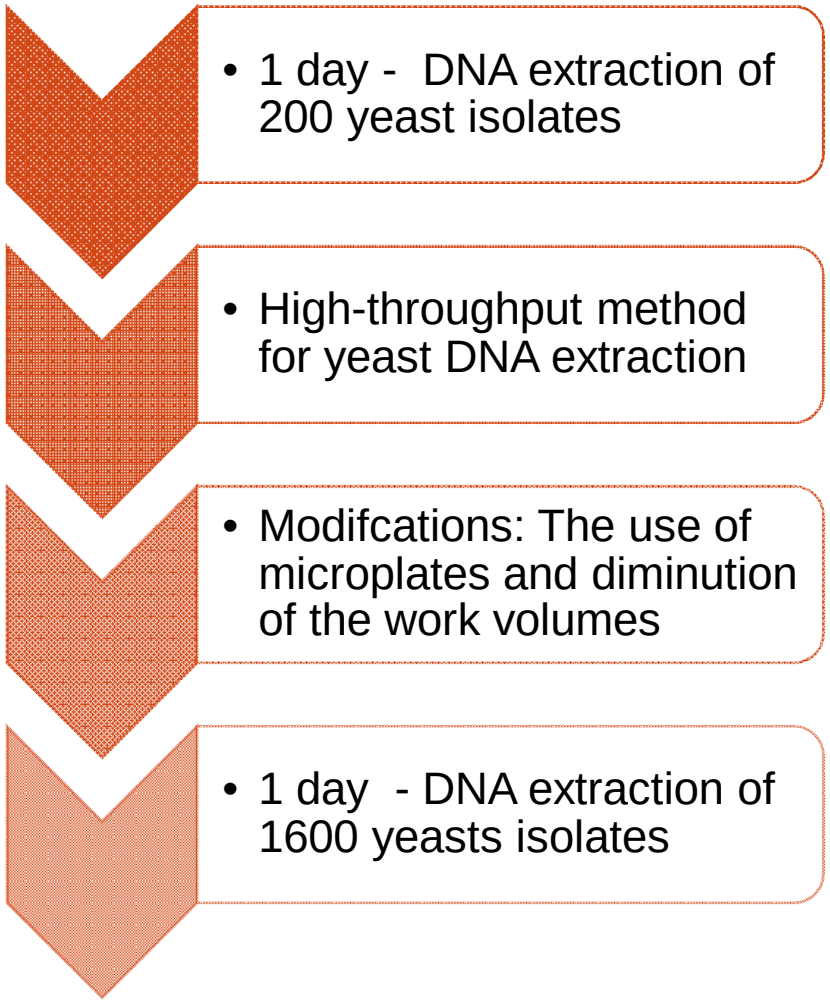


High-throughput method for yeast DNA extraction

Introduction

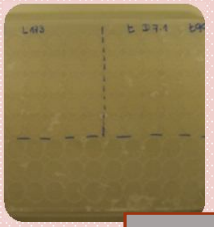
1. Cell collection
2. Ressuspension and incubation in buffer with sorbitol and lyticase
3. Addition of sodium dodecyl sulphate (incubation 5 min, 65 ºC)
4. Addition of potassium acetate (incubation 5 min, -20 ºC)
5. Centrifugation and precipitation (isopropanol, ethanol)

(Lopez *et a*l 2001; Schuller *et al*., 2004)



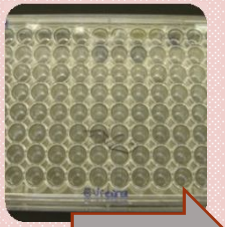
Introduction

Strategy

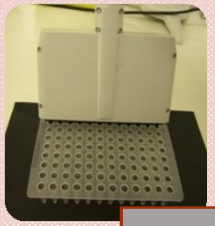


glycerol stocks of yeast isolates (microplates)

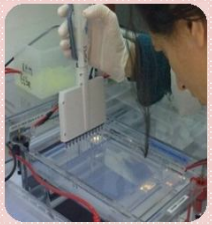
Growth in microplate with YPD



Microplate DNA extraction



96 wells plate PCR amplif.



Electrophoresis

agarose gel or capillary electrophoresis

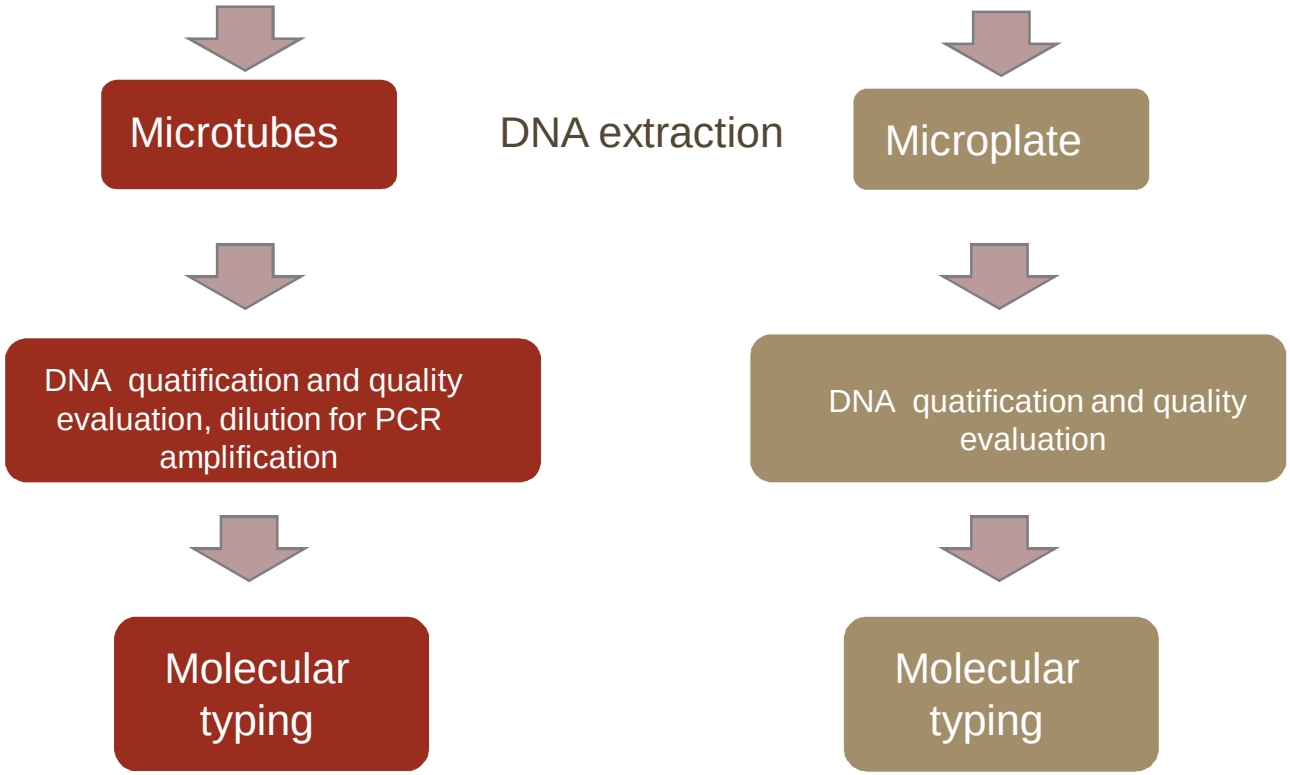
Materials and Methods

Optimization of microplate-based DNA extraction

1. **Cell collected by centrifugation of X volume to test (2 min, 4000 rpm)** ⟵ **30, 50, 100 and 150 µl**
2. Ressuspend in a (sorbitol 1 M, EDTA-Na_2 0,1 M, pH 7.5) + lyticase 3,3U/µl volume proportional to *X*
3. Incubate for 30 min at 37 ºC
4. Ad (Tris-HCl 50 mM, EDTA-Na_2 20 mM, pH 7.4) + SDS 10%(w/v) volume proportional to *X*
5. Incubate for 5 min at 65 ºC
6. Ad of potassium acetate 5 M proportional *X*
7. Place for 5 min at -20 ºC
8. **Centrifuge at 4 ºC (Y min, 4000 rpm)** ⟵ **5, 10,15, 30, 45 and 60 min**
9. Transfer the supernatent to Isopropanol volume proportional to *X*
10. Incubate for 5 min at room temperature
11. Wash with etanol 70% (v/v)
12. Air-dry the pellets
13. Ressuspend in 100 µl of TE (1:10)

Materials and Methods

Validation of microplate-based DNA extraction



Materials and Methods

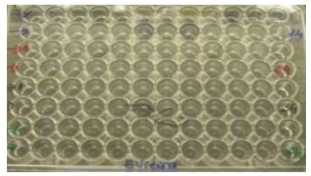
Yeasts strains and species

4X DNA extraction of 12 yeasts species e 12 *S. cerevisiae* strains

Species	Strain [a] designation
S.cerevisiae	L507
S.cerevisiae	L508
S.cerevisiae	L509
S.cerevisiae	L510
S.cerevisiae	L517
S.cerevisiae	L528
S.cerevisiae	L589
S.cerevisiae	L590
S.cerevisiae	L591
S.cerevisiae	L592
S.cerevisiae	L593
S.cerevisiae	L594

[a] From the CBMA collection

Species	Species designation
Kluyveromyces marxianus	L101
Zygosaccharomyces bailii	L110
Candida tropicalis	L140
Hanseniaspora uvarum	L141
Saccharomwodes ludwigii	L145
Zygosaccharomyces rouxii	L146
Torulaspora delbrueckii	L149
Metschnikowia pulcherrima	L151
Dekkera anomala	L161
Candida vini	L163
Candida stellata	L164
Saccharomyces pretoriensis	L215



Materials and Methods

DNA Quantification and quality evaluation

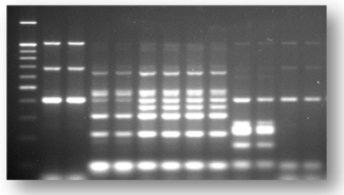
- Concentration (ng/µl)
- 260/280 Ratio

DNA amplification

S.cerevisiae strains

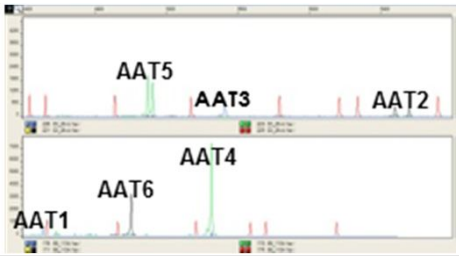
- Interdelta sequence analysis

(Legras and Karst 2003; Schuller *et al*., 2004)



- Microsatellite analysis

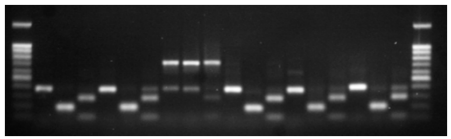
(Perez *et al.,* 2001*;* Legras *et al*., 2005)



Yeasts species

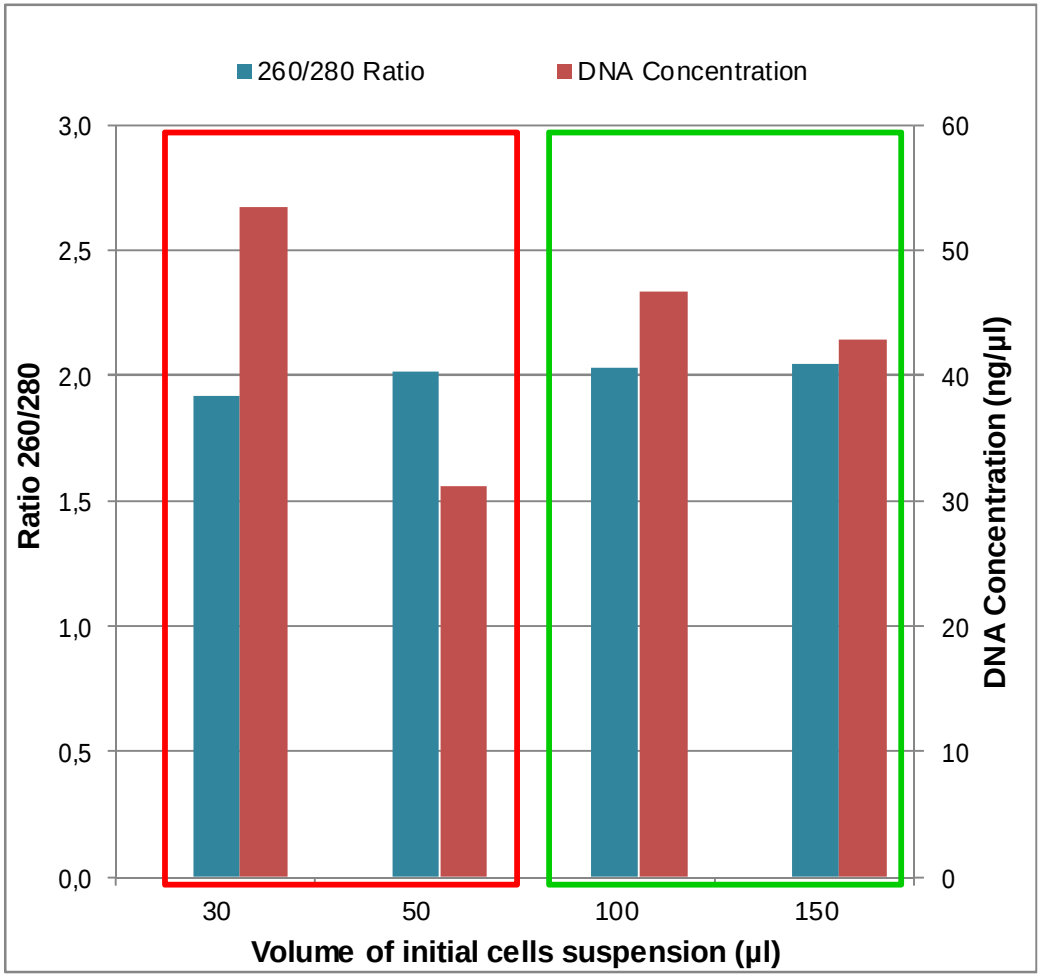
- ITS sequences - DNA restriction fragment length polymorphism (RFLP)

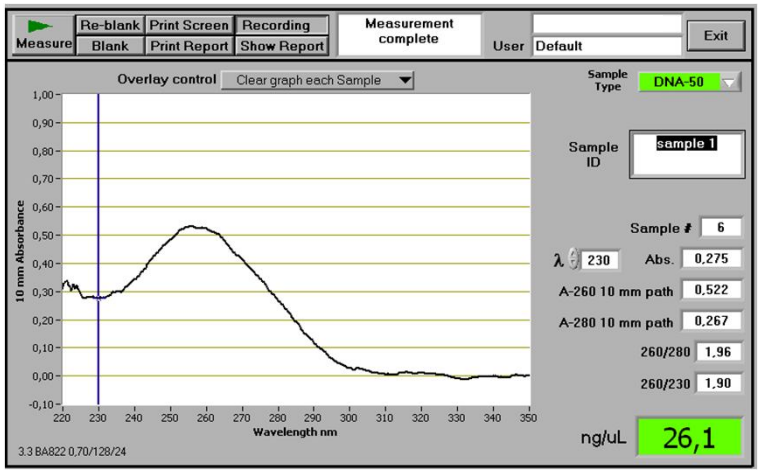
(Esteve-Zaroso *et al*., 1999; Fernandez-Espinar *et al*., 2000)



Results

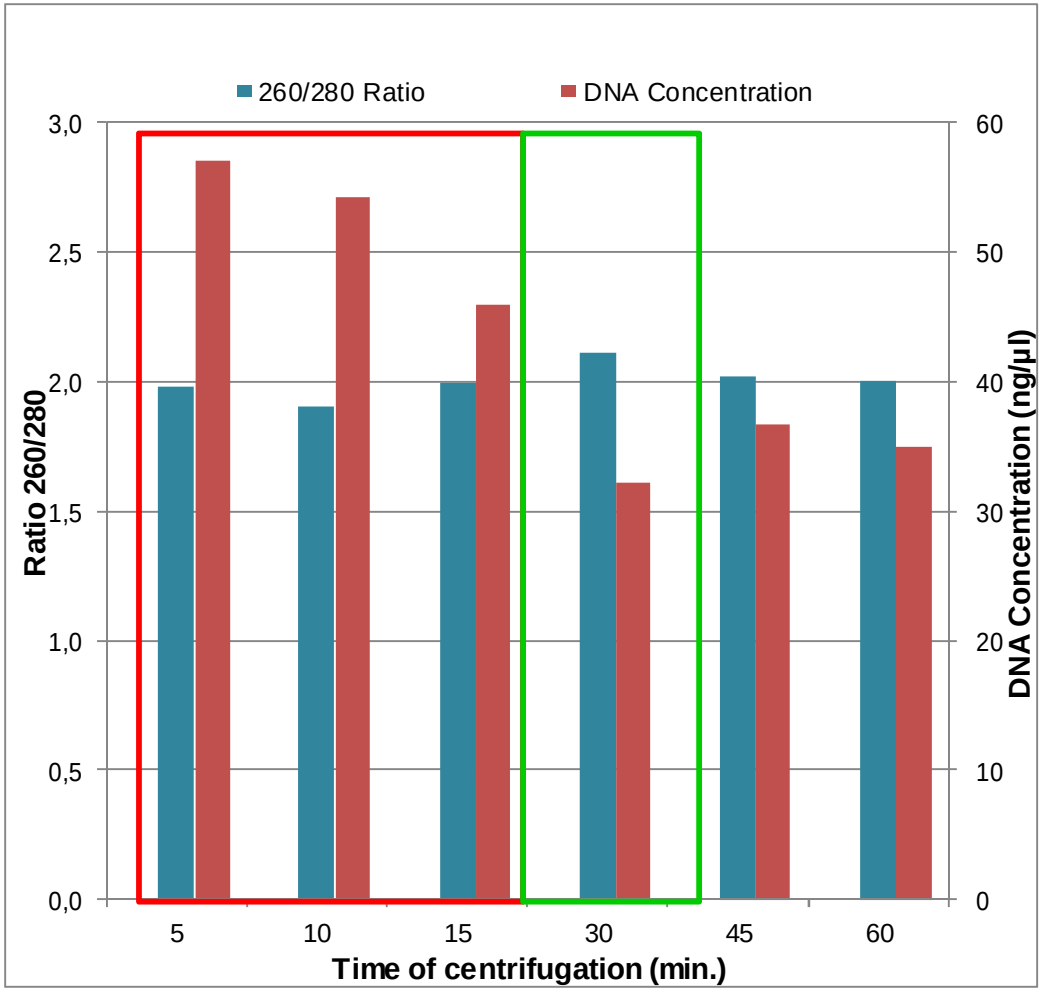
Optimization of microplate-based DNA extraction

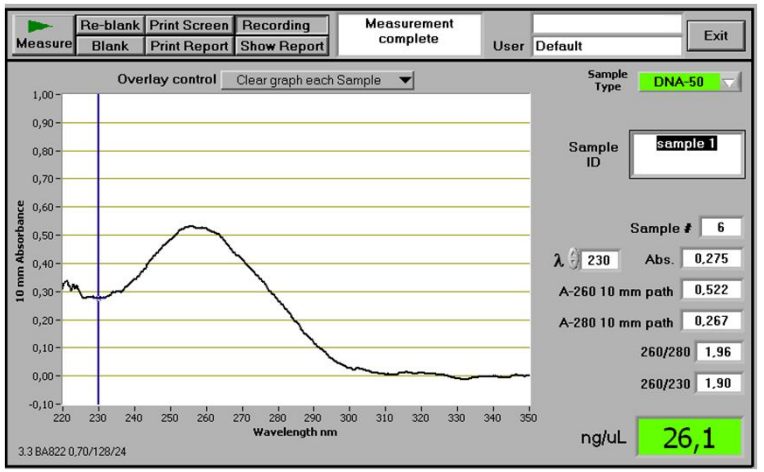




Results

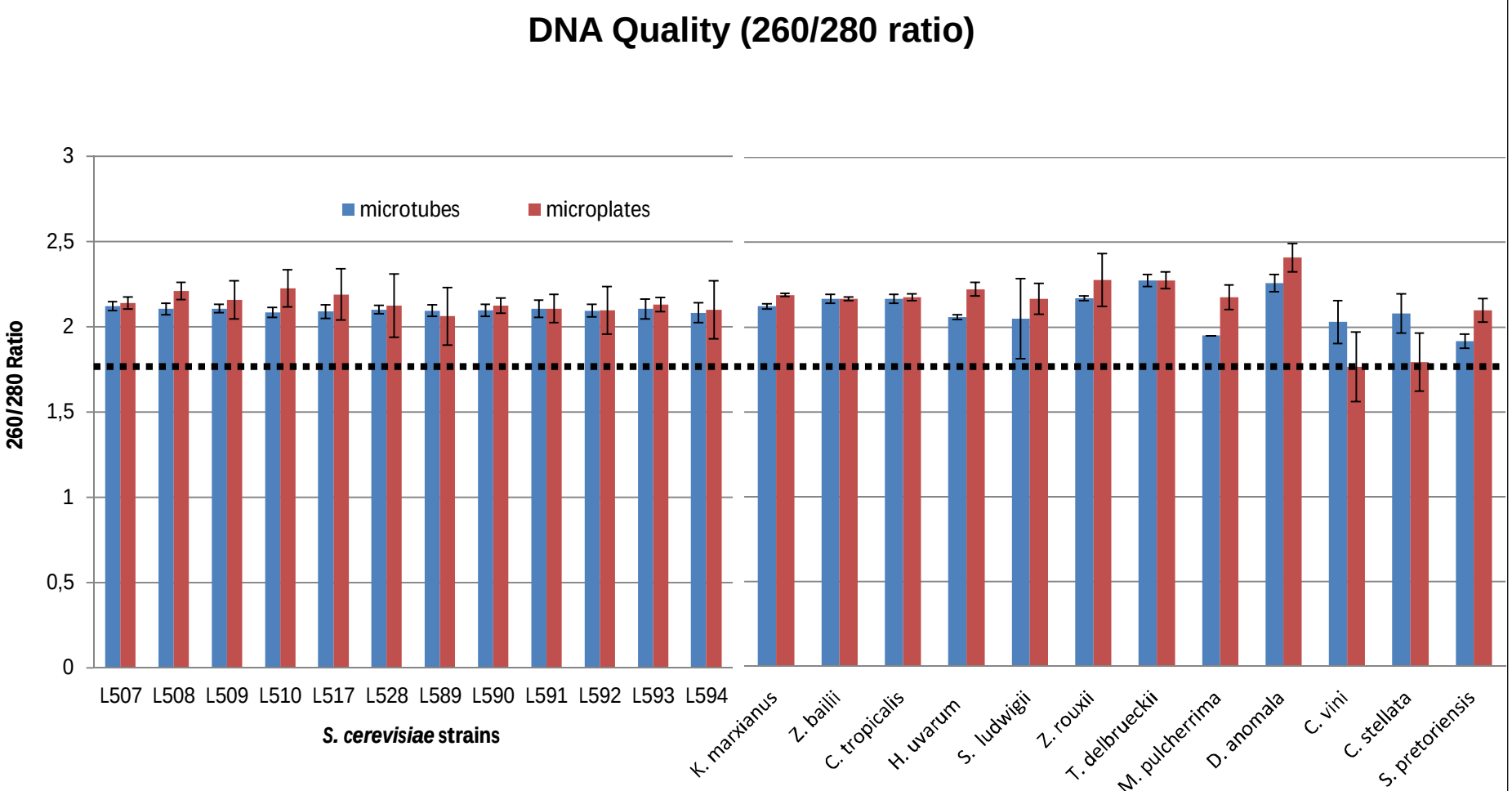
Optimization of microplate-based DNA extraction





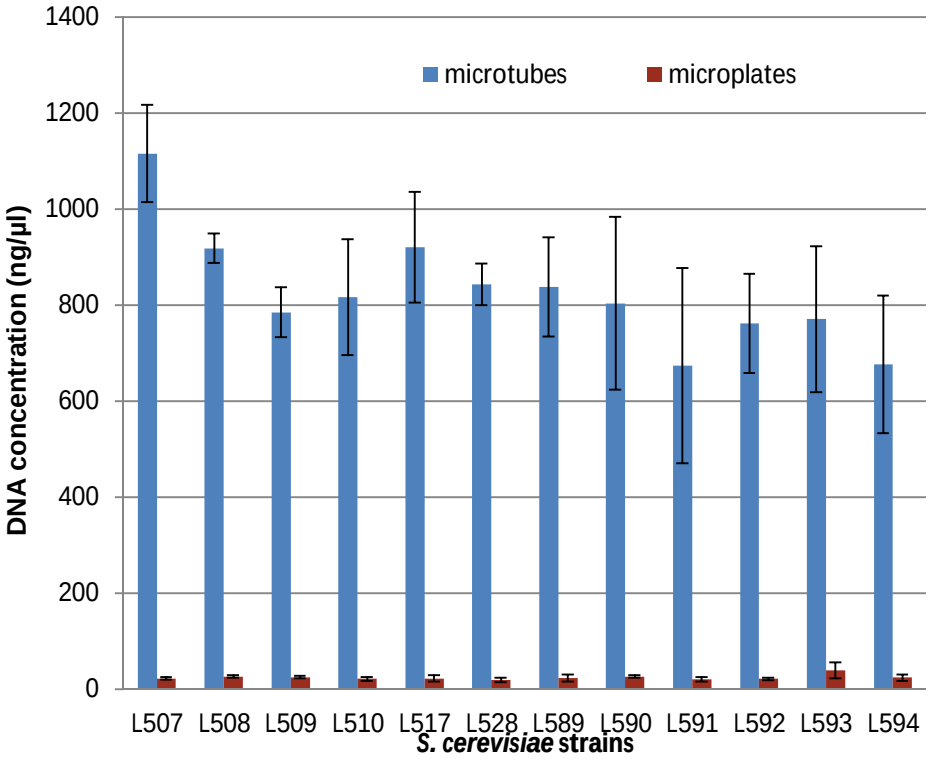
Results

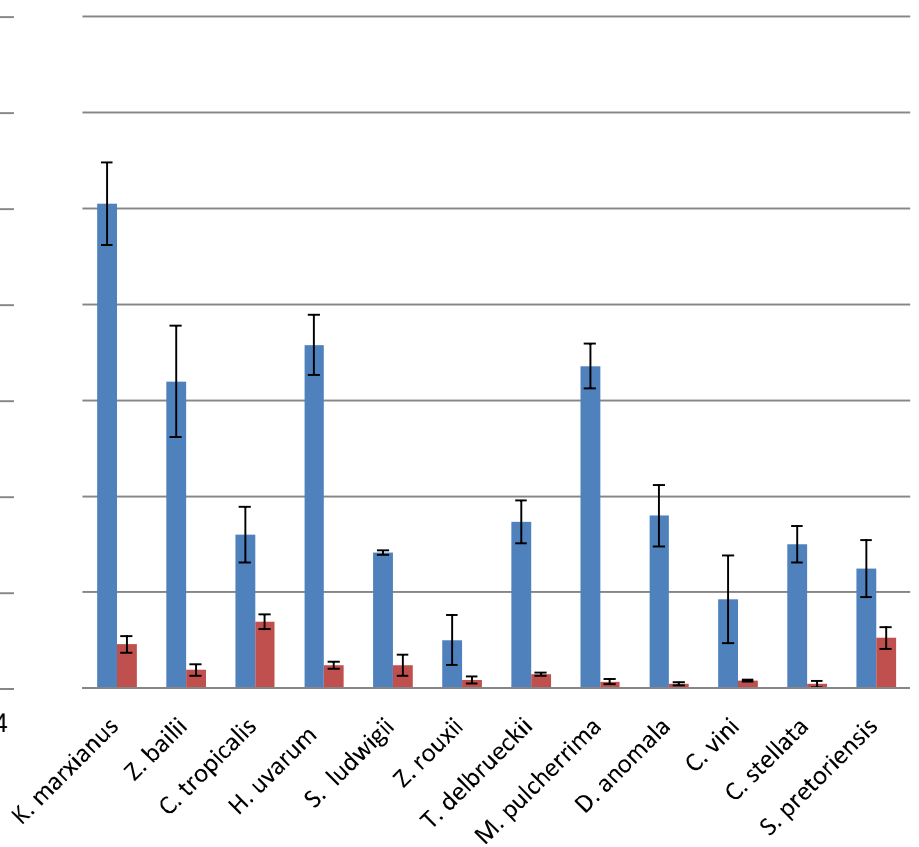
Validation of microplate-based DNA extraction

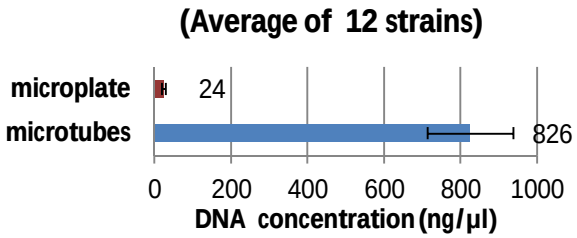


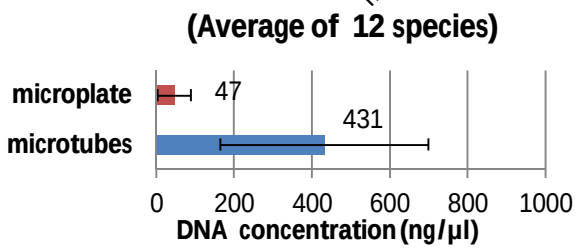
Results

Validation of microplate-based DNA extraction





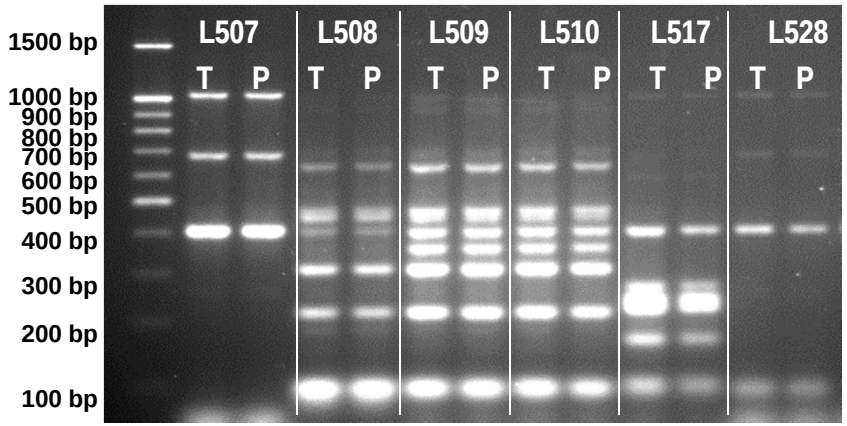




Results

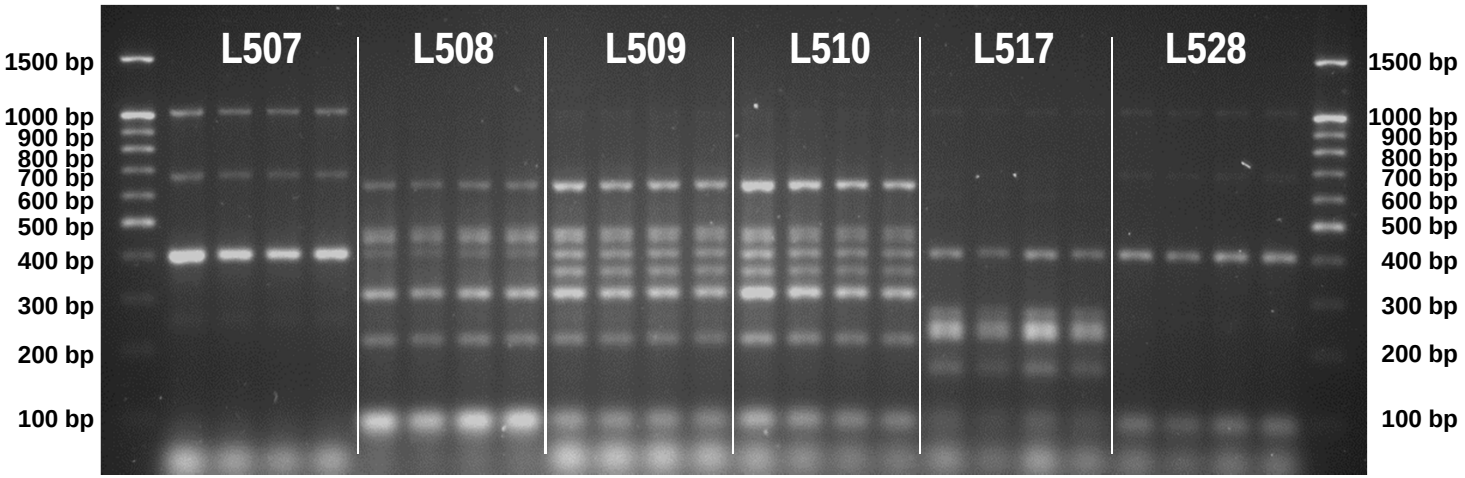
PCR-based interdelta sequence amplification for the distinction of *S.cervisiae* strains

(Legras and Karst 2003; Schuller, *et al*. 2004)



T microtube-based DNA extraction

P microplate-based DNA extraction



Results

PCR-based microsatellites amplification for the distinction of *S.cervisiae* strains

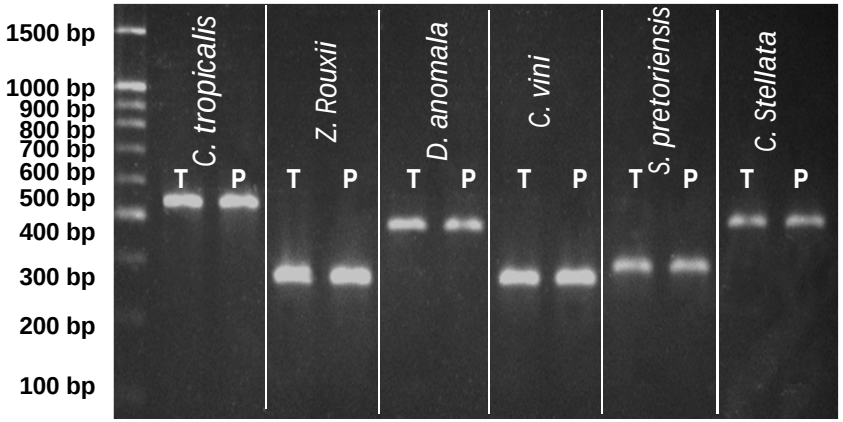
(Perez *et al*., 2001; Legras *et al*., 2005)

		S. cerevisiae strains											
		microtube-based DNA extraction						microplate-based DNA extraction					
Microsatellite	Allele	L507	L508	L509	L510	L528	L589	L507	L508	L509	L510	L528	L589
ScAAT1	1	201	189	189	186	216	219	201	189	189	186	216	219
	2	201	189	201	186	219	220	201	189	201	186	219	220
ScAAT2-1	1	372	375	375	375	372	375	372	375	375	375	372	375
	2	372	375	375	375	378	375	372	375	375	375	378	375
ScAAT4-1	1	305	302	302	302	329	329	305	302	302	302	329	329
	2	305	302	329	302	329	329	305	302	329	302	329	329
ScAAT5-1	1	216	219	222	219	216	222	216	219	222	219	216	222
	2	216	222	222	222	219	222	216	222	222	222	219	222
ScAAT6-1	1	255	250	253	256	256	256	255	250	253	256	256	256
	2	255	250	255	256	259	256	255	250	255	256	259	256
C5-1	1	115	117	117	115	113	113	115	117	117	115	113	113
	2	133	125	125	124	129	131	133	125	125	124	129	131
C11-1	1	191	187	187	187	209	197	191	187	187	187	209	197
	2	191	211	211	211	211	197	191	211	211	211	211	197
YPL009-1	1	305	274	274	274	277	319	305	274	274	274	277	319
	2	305	286	286	286	277	319	305	286	286	286	277	319
YOR267c-1	1	335	305	311	311	289	324	335	305	311	311	289	324
	2	335	311	311	311	302	324	335	311	311	311	302	324

Results

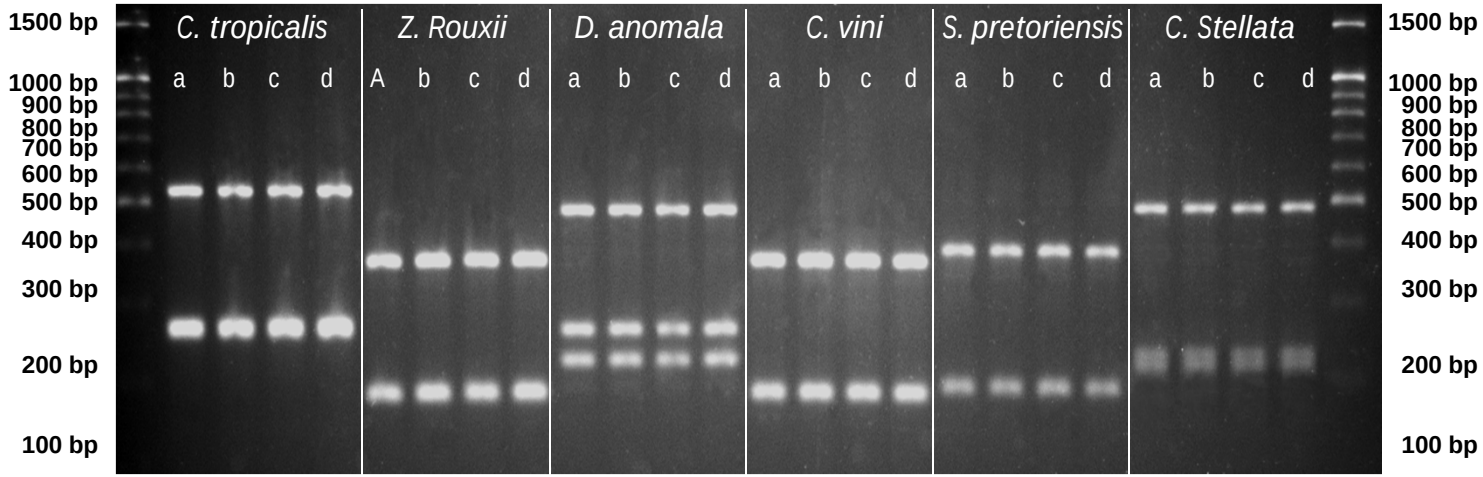
PCR-based ITS sequences amplification and RFLP yeast species identification

(Esteve-Zaroso *et al*., 1999; Fernandez-Espinar *et al*., 2000)



T microtube-based DNA extraction

P microplate-based DNA extraction



Conclusions

- Allows the processing of **1600 yeast isolates per day**, which is a eight-fold increase compared to the conventional method;
- DNA final concentration obtained with the modified protocol was in the range of **20-50 ng/µL**;
- **DNA quality** is suitable for the usual DNA amplification protocols;
- Reagents volumes were **reduced by 90%.**

Acknowledgements

University of Minho

Dorit Schuller
Margarida Casal

Vinalia

Isabel Araújo

University of Azores

Mª Teresa Lima

This work was funded by the fellowship SFRH/BDE/33672/2009 and Vinalia (Eugénia Vieira) and M.1.2/F/006/2008 (João Drumonde), as well as by the projects PTDC/BIA BCM/64745/2006, PTDC/AGR-ALI/103392/2008 and FP7/2007-2013 (nº 232454).