I. Direct amplification from various strains of filamentous fungi

A. Reaction setup

Reactions were performed in 50 µl volumes, containing crude sample (filamentous fungi – sampled as for colony PCR – *see below for details*) supplied by the Institute for Wine Biotechnology, University of Stellenbosch. Final concentrations of reaction components were as follows:

Component	Volume (µl)	Final concentration
PCR grade water	20.6	-
2x KAPA3G Plant PCR Buffer (containing dNTPs and $MgCl_2$)	25.0	1X (1.5 mM $MgCl_2$)
25 mM $MgCl_2$	1.0	0.5 mM (2.0 mM total)
10 µM Forward primer	1.5	0.3 µM
10 µM Reverse primer	1.5	0.3 µM
Template DNA	As required[1]	N/A
2.5 U/µl KAPA3G Plant DNA Polymerase	0.4	1.0 U per reaction
TOTAL	**50.00**	

[1] Colony PCR sampling method: To each PCR tube containing the PCR reaction, a small amount (just a touch) of colony was added using a 10 µl pipette; stirring of the tip in the reaction to ensure sufficient transfer of the cells.

B. PCR protocol

Cycling was performed with an Eppendorf MasterCycler ep*gradientS* thermal cycler (100% ramp rate), using the following parameters:

Step	Temperature	Time	
Initial denaturation	95 °C	15 min	
Denaturation	95 °C	20 sec	x 45
Annealing	**50 – 65 °C**	15 sec	
Extension	72 °C	45 sec	
Final extension	72 °C	1 min	
HOLD	4 °C	∞	

Cycling time = 1 h 30 min

C. Analysis and Results

After the addition of 6X KAPA Loading Dye (10 µl per 50 µl PCR product), 25 µl of each sample was analysed in a 1.0% TBE-agarose gel. Results were visualized by ethidium bromide staining.

Products of direct amplification from individual strains of plated filamentous fungi

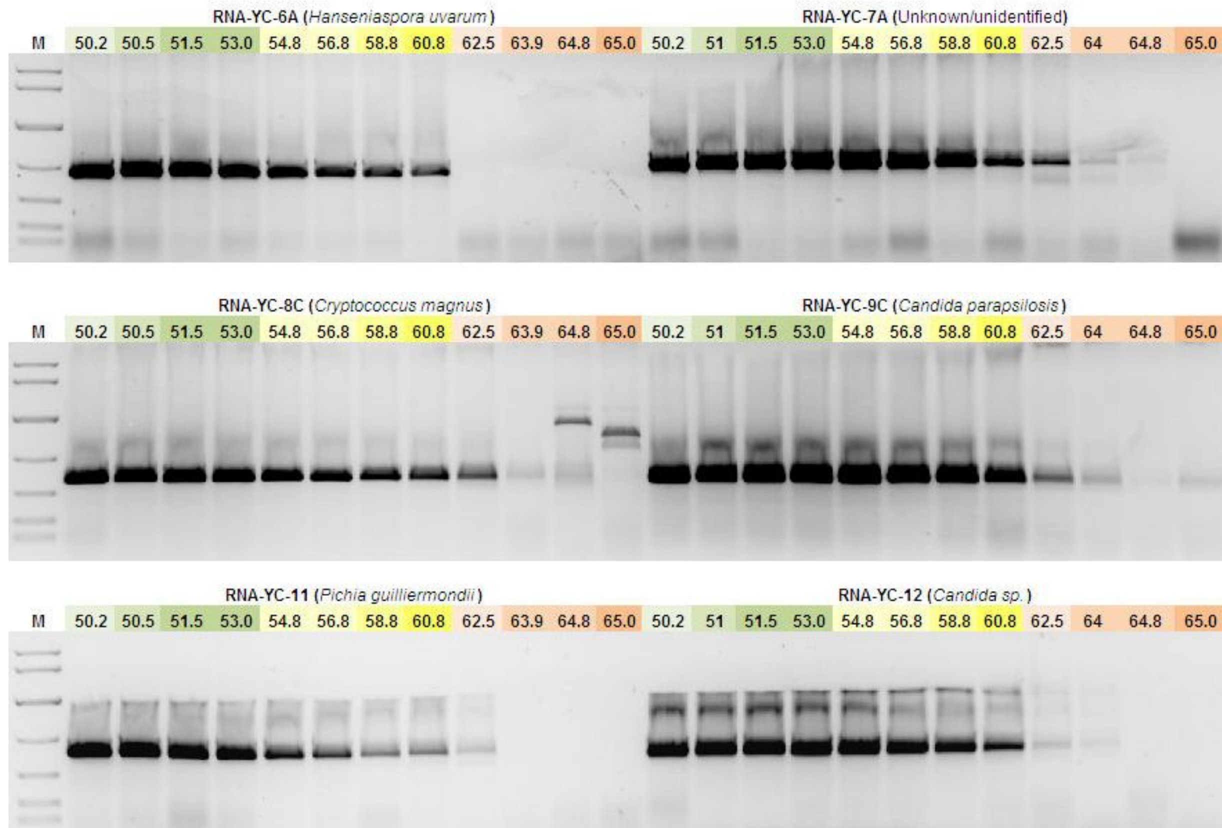

Figure 1. Products of direct amplification from individual strains of plated filamentous fungi. Reactions were performed in 50 µl volumes, containing crude sample (filamentous fungi – sampled as for colony PCR) as template. Final concentrations of reaction components were as follows: 1X PCR Buffer (containing 0.2 mM of each dNTP), 2.0 mM $MgCl_2$, 0.3 µM of each primer and 1 U KAPA3G Plant DNA Polymerase. Cycling was performed in the Eppendorf ep*gradient*S thermocycler, using a standard 3-step cycling protocol (95 °C for 15 min, 45 x [95 °C for 20 sec, 50 – 65 °C for 15 sec, 72 °C for 45 sec], 72 °C for 1 min). PCR products were analyzed by agarose gel electrophoresis (in a 1% agarose-TBE gel) and visualized by ethidium bromide staining. KAPA Express Ladder (5 µl per lane) was used as DNA ladder.

II. Direct amplification from a single of filamentous fungi in the presence of red wine and grape juice

A. Reaction setup

Reactions were performed in 50 µl volumes, containing crude sample (filamentous fungi – sampled as for colony PCR – as described before) supplied by the Institute for Wine Biotechnology, University of Stellenbosch. Final concentrations of reaction components were as follows:

Component	Volume (µl)	Final concentration
PCR grade water	Up to 50 µl	-
2x KAPA3G Plant PCR Buffer (containing dNTPs and $MgCl_2$)	25.0	1 x (1.5 mM $MgCl_2$)
25 mM $MgCl_2$	1.0	0.5 mM (2.0 mM total)
Red wine / red grape juice (1–5 µl)	As required	-
10 µM Forward primer	1.5	0.3 µM
10 µM Reverse primer	1.5	0.3 µM
Template DNA (as supplied)	As required	N/A
2.5 U/µl KAPA3G Plant DNA Polymerase	0.4	1.0 U per reaction
TOTAL	**50.00**	

B. PCR protocol

Cycling was performed with an Eppendorf MasterCycler ep*gradientS* thermal cycler (100% ramp rate), using the following parameters:

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	15 min	
Denaturation	95 °C	20 sec	x 45
Annealing	**53 °C**	15 sec	x 45
Extension	72 °C	45 sec	x 45
Final extension	72 °C	1 min	
HOLD	4 °C	∞	

Cycling time = 1 h 30 min

C. Analysis and Results

After the addition of 6X KAPA Loading Dye (10 µl per 50 µl PCR product), 25 µl of each sample was analysed in a 1.0% TBE-agarose gel. Results were visualized by ethidium bromide staining.

Products of direct amplification from a single strain of plated filamentous fungi in the presence of increasing volumes of red wine or red grape juice.

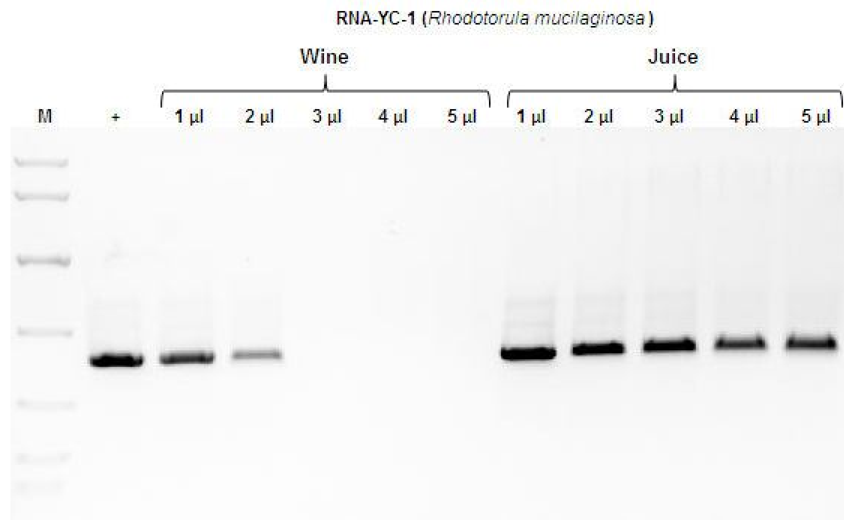

Figure 2 Products of direct amplification from a single strain of plated filamentous fungi in the presence of increasing volumes (1 – 5 µl per 50 µl PCR) of red wine or red grape juice, respectively. Reactions were performed in 50 µl volumes, containing crude sample (filamentous fungi – sampled as for colony PCR) as template. Final concentrations of reaction components were as follows: 1X PCR Buffer (containing 0.2 mM of each dNTP), 2.0 mM $MgCl_2$, 0.3 µM of each primer and 1 U KAPA3G Plant DNA Polymerase. Cycling was performed in the Eppendorf ep*gradient*S thermocycler, using a standard 3-step cycling protocol (95 °C for 15 min, 45 x [95 °C for 20 sec, 53 °C for 15 sec, 72 °C for 45 sec], 72 °C for 1 min). PCR products were analyzed by agarose gel electrophoresis (in a 1% agarose-TBE gel) and visualized by ethidium bromide staining. KAPA Express Ladder (5 µl per lane) was used as DNA ladder.