

Molecularbiological wine analysis with real-time PCR Yeasts and Bacteria

New

- Precoated PCR tubes
- Integrated yeast lysis
- Reduced pipetting steps



Yeasts analysis for wine becoming increasingly important

Scientific methods are now applied in modern wine production. Wine-making starts with grapes and the production of grape juice, followed by maceration, fermentation, aging, clarification and stabilization before ending with the bottled wine. Many parameters must be or can be tested during wine production in order to allow decision-making at different stages.

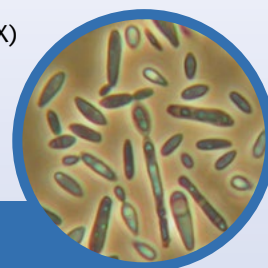
Usually, cellar technically controlled fermentation runs with the addition of selected yeasts mostly from *Saccharomyces* strains and possibly the use of *Oenococcus oeni* to control the optional malolactic fermentation.

The analyses of the yeast *Brettanomyces* (*Dekkera bruxellensis*) is becoming increasingly interesting. It is the most important wild yeast in wine, forming phenolic metabolic products 4-EP (4-ethylphenol) and 4-EG (4-Etylgujacol). Mainly, it causes blemished smells described as leather, sweat and/or horse. *Brettanomyces*, however, can also be specifically used in low concentrations to achieve a stronger sensory effect. This can be easily quantified using CSE0100/QTPYDB0048.

The new multiplex kit GEN-IAL QuickGEN First-Wine PCR Kit (Art. No. QTPWS0096 high/low/white) detects

- *Lactobacillus*, *Pediococcus*, *Oenococcus oeni* (FAM)
- Acetic acid forming bacteria (ROX)
- Yeasts (Cy5)
- Internal Amplification Control (HEX)

and enables a fast overview of the microbiological status of the wine.



Microscopic picture of *Dekkera bruxellensis*

© LVWO Weinsberg

Desirable or undesirable bacteria

For a controlled **malolactic fermentation**, *Oenococcus oeni* is added to the wine. It effects the transformation of malic acid into milder lactic acid. However, depending on the desired type of

wine, the presence of *Oenococcus oeni* may also be undesirable. Undesirable bacteria include these ones forming acetic acid.

Precoated PCR strips – a unique solution for convenient handling

Different combinations of screening and identification possibilities are available, thus allowing a cost effective and customized routine analysis.

The QuickGEN kits contain 8-well strips which are precoated with the reagents for up to 4 different parameters per tube. 8-well strips detecting one to four parameters can be used for 8 samples/reactions. As extension of this multiplexing each tube of a 8-well strip may contain different parameters – this allows a multiplex panel for up to 4 x 8 parameters for one sample in a 8-well strip.

Templates are available for MyGo Pro, CFX96™, and qTOWER³. These prepared templates contain the settings for dedicated kits and allow a direct start of the real-time PCR without the need to program the settings.

High	Low	White
Agilent MX3005P	IT-IS MyGo Pro*	Roche Lightcycler® 480 II
Applied Biosystems® ABI 7500 or higher	Agilent AriaMX	BioRad CFX96™
ThermoFisher QuantStudio®5 or higher	BioRad CFX96™	Analytik Jena qTOWER ³

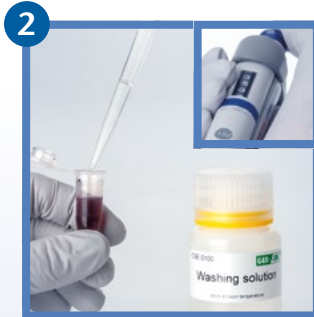
* 4plex assays for MyGo Pro requires a specific kit (QTPWS0096lowMG).

Quantification of yeast/bacteria in wine made easy using QuickGEN kits (example *Brettanomyces*)

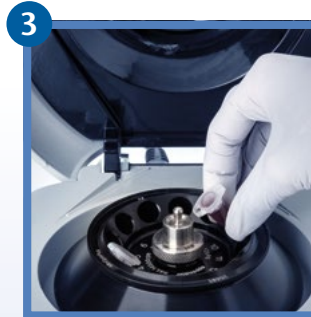
DNA preparation



1 Add 1 ml wine into a conic (not round shaped) 1.5ml tube



2 Add 0.5 ml washbuffer of CSE 0100 and mix



3 Centrifuge 5 min at 14.000 rpm. Always place the tubes in the same direction to identify the position of a nonvisible cell pellet later on



4 The non visible pellet will be at the side with the joint of the lid



5 Remove the supernatant carefully and completely from the opposite side



6 Start a short centrifugation spin and remove the remaining liquid completely without touching the pellet



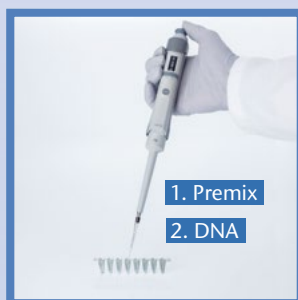
7 Add 100 µl Lysis buffer and mix/vortex: the DNA is ready to use

PCR set-up

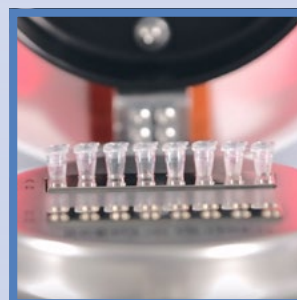


1. Premix
2. DNA

Add 1. Premix into the pre-coated tubes and add 2. DNA or controls.

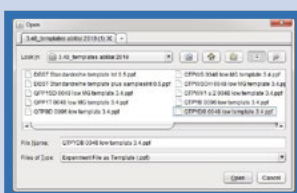


Remove bubbles by short centrifugation or shaking down.

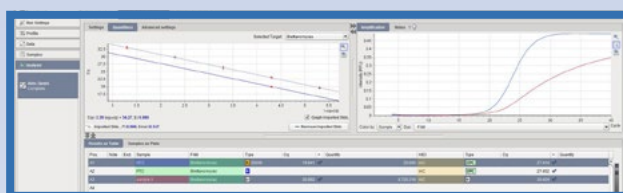


Start the qPCR.

Software and data



Example: real time cycler MyGO Pro for *Brettanomyces*. Open the relevant template and insert the sample names, start run



When the run is finished upload a previous generated standard curve (using DBST0100). Multiply the quantified results with a factor

40 (2.5 µl of 100 µl DNA were used) and achieve the quantitative result as cfu/ml wine. The entire process takes less than 2 hours

GEN-IAL® Products

DNA preparation			
QuickGEN Sample preparation centrifugation	DNA preparation of beverage samples	100 reactions	CSE 0100*
Qualitative real-time PCR			
GEN-IAL® QuickGEN** First-Wine PCR Kit	DNA screening and differentiation of wine spoilage bacteria and yeasts: <i>Lactobacillus</i> ; <i>Pediococcus</i> ; <i>Oenococcus oeni</i> ; acetic acid bacteria; yeast	96 reactions	QTPWS0096 high QTPWS0096 low QTPWS0096 low MG QTPWS0096 white
GEN-IAL® QuickGEN* First-Wine PCR Kit	DNA Screening of wine spoilage bacteria: <i>Lactobacillus</i> ; <i>Pediococcus</i> ; <i>Oenococcus oeni</i> ; acetic acid bacteria	96 reactions	QTPWSOH0096 high QTPWSOH0096 low QTPWSOH0096 white
GEN-IAL® QuickGEN* First-Oenococcus Oeni	Specific DNA detection of <i>Oenococcus oeni</i>	48 reactions	QTPOE0048 high QTPOE0048 low QTPOE0048 white
GEN-IAL® First-Wine Screening Biogene Amine	Specific DNA detection of bacteria forming biogenic amines	50 reactions	BAM0050
GEN-IAL® QuickGEN* Acetic acid bacteria	Specific DNA detection of acetic acid bacteria	48 reactions	QTPA0048 low QTPA0048 white
GEN-IAL® QuickGEN** First-Yeast PCR Kit Dekkera spp.	Specific DNA detection of <i>Dekkera</i> spp.	48 reactions	QTPYD0048 high QTPYD0048 low QTPYD0048 white
GEN-IAL® QuickGEN** Dekkera bruxellensis	Specific DNA detection of <i>Dekkera bruxellensis</i>	48 reactions	QTPYDB0048 high QTPYDB0048 low QTPYDB0048 white
GEN-IAL® QuickGEN** Zygosaccharomyces bailii	Specific DNA detection of <i>Zygosaccharomyces bailii</i>	48 reactions	QTPYZB0048 high QTPYZB0048 low QTPYZB0048 white
GEN-IAL® QuickGEN** First-Yeast PCR Kit Wild Yeast	DNA screening and differentiation of wild yeast	96 reactions	QTPWY0048 low QTPWY0048 white
GEN-IAL® QuickGEN** First-Yeast differentiation PCR Kit	DNA screening and differentiation of 12 yeasts	96 reactions	QYDIF0096 high QYDIF0096 low QYDIF0096 white
GEN-IAL® Dekkera anomala	Specific DNA detection of <i>Dekkera anomala</i>	50 reactions	TPYDA 0050
GEN-IAL® Pichia anomala	Specific DNA detection of <i>Pichia anomala</i> (<i>Wickerhamomyces anomalus</i>)	50 reactions	TPYPA 0050
Accessories			
Multiplex Screening			
GEN-IAL® Dekkera bruxellensis Standards	DNA standards for <i>Dekkera bruxellensis</i> quantification	200.000 cfu	DBST 0100
Color Compensation Kit LightCycler® 480	Color compensation kit for multiplex assays	5 reactions	PP1TCC 0005
Color Compensation Kit LightCycler® 2.0	Color compensation kit for multiplex assays	5 reactions	CCFH 0005
Washing solution	Washing solution for SEW 0100	43 ml	WS 0100

* Compatible with the QuickGEN line.

** Lyticase is already lyophilized in PCR tubes already submitted.

Real-time PCR

- Quick results (approx. 2 hours)
- Highly specific
- Easy handling: Lyticase precoated PCR tubes
- QuickGEN reduces the pipetting steps: less effort and increased safety
- Even performable in small wine laboratories