

Molecularbiological wine analysis with real-time PCR

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.

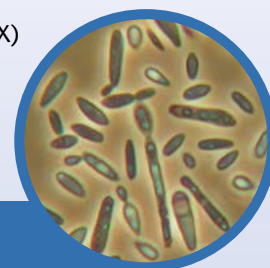
Usually, cellar technically controlled fermentation runs with the addition of selected yeasts mostly from *Saccharomyces* strains and possibly the use of *Oenococcus oeni* to control the optional malolactic fermentation.

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 Q002/Q371/Q372/Q373.

The new multiplex kit GEN-IAL QuickGEN First-Wine PCR Kit (Art. No. Q321/Q322/Q323/Q324) detects

- *Lactobacillus*, *Pediococcus*, *Oenococcus oeni* (FAM)
- Acetic acid 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*

© LWVO 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

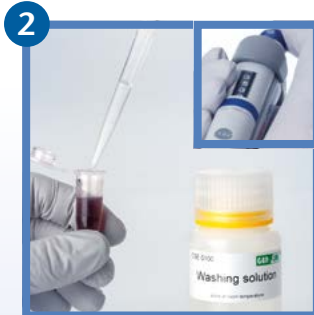
* 4plex assays for MyGo Pro requires a specific kit (Q324).

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

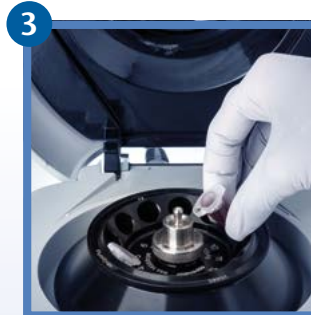
DNA preparation



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



2 Add 0.5 mL washbuffer of Q002 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

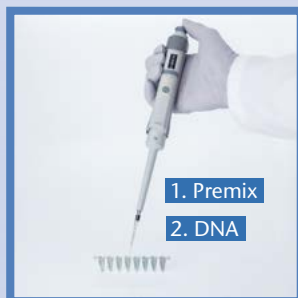


7 Add 100 µL Lysis buffer and mix/vortex: the DNA is ready to use

PCR set-up



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

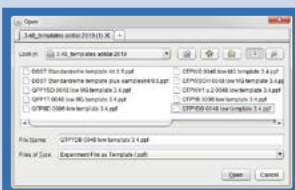


2. Remove bubbles by short centrifugation or shaking down

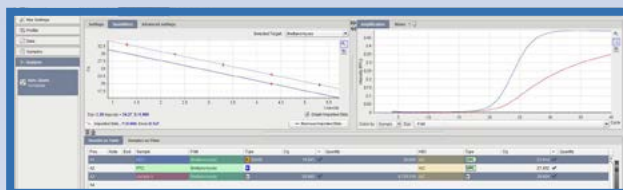


3. Start the qPCR

Software and data



Example: real time cyler 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 Q360). 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

Product	Description	No. of Tests/Amount	Art. No.
DNA preparation			
GEN-IAL® QuickGEN Sample Preparation Centrifugation	DNA preparation of beverage samples	100 reactions	Q002
GEN-IAL Simplex Easy Wine	DNA preparation of wine samples (no QuickGEN)	100 reactions	Q300
Qualitative real-time PCR			
GEN-IAL® QuickGEN Wine Screening	DNA screening and differentiation of wine spoilage bacteria and yeasts: <i>Lactobacillus</i> ; <i>Pediococcus</i> ; <i>Oenococcus oeni</i> ; acetic acid bacteria; yeast	48 reactions	Q321 Q322 Q323 Q324
GEN-IAL® QuickGEN Wine Screening without yeast	DNA Screening of wine spoilage bacteria: <i>Lactobacillus</i> ; <i>Pediococcus</i> ; <i>Oenococcus oeni</i> ; acetic acid bacteria	48 reactions	Q331 Q332 Q333 Q334
GEN-IAL® QuickGEN Oenococcus oeni	Specific DNA detection of <i>Oenococcus oeni</i>	48 reactions 50 reactions	Q351 Q352 Q353 Q354 Q355
GEN-IAL® Biogenic amines	Specific DNA detection of bacteria forming biogenic amines	50 reactions	Q345
GEN-IAL® QuickGEN Acetic acid bacteria	Specific DNA detection of acetic acid bacteria	48 reactions 50 reactions	Q511 Q512 Q513 Q515
GEN-IAL® QuickGEN Yeast Dekkera spp.	Specific DNA detection of <i>Dekkera</i> spp.	48 reactions 50 reactions	Q551 Q552 Q553 Q555
GEN-IAL® QuickGEN Yeast <i>Dekkera bruxellensis</i> quantitative	Specific DNA detection of <i>Dekkera bruxellensis</i>	48 reactions	Q370 Q371 Q372 Q373
GEN-IAL® QuickGEN Yeast <i>Zygosaccharomyces bailii</i>	Specific DNA detection of <i>Zygosaccharomyces bailii</i>	48 reactions	Q561 Q562 Q563
GEN-IAL® QuickGEN** First-Yeast PCR Kit Wild Yeast	DNA screening and differentiation of wild yeast	48 reactions	Q532 Q533
GEN-IAL® QuickGEN Yeast Differentiation	DNA screening and differentiation of 12 yeasts	96 reactions/ 12 samples	Q541 Q542 Q543
GEN-IAL® Dekkera anomala	Specific DNA detection of <i>Dekkera anomala</i>	50 reactions	Q929
Accessories			
Multiplex Screening			
GEN-IAL® Dekkera bruxellensis Standards	DNA standards for <i>Dekkera bruxellensis</i> quantification	200.000 cfu	Q360
Color Compensation Kit LightCycler® 480	Color compensation kit for multiplex assays	5 reactions	Q800
GEN-IAL® Simplex® Easy Wine-Washing Solution	Washing solution for SEW 0100	43 ml	Q301



Real-time PCR

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

* Compatible with the QuickGEN line.

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

Q**1 High profile: ABI 7500, Agilent MX 3005P
 Q**2 Low profile: MyGo Pro (2- and 3plex kits), ABI QuantStudio 5
 Q**3 White strips: Bio-Rad CFX96, LightCycler® 480
 Q**4 Low profile: MyGo Pro (4plex kits)
 Q**5 Liquid reagents without pre-coated strips

Other block cycler devices may be suitable as well. Information is available on request. Further parameters/species detection kits are available on request.