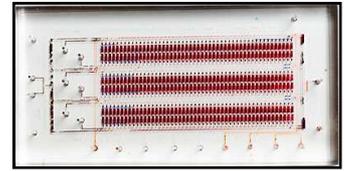




International Research News

High-throughput microfluidic single-cell RT-qPCR

Single-cell analysis is emerging as the gold standard of genetic research because samples usually contain a mixture of cells – the most important of which may be present in only very small numbers and impossible to distinguish. However, the method has been too costly to become widespread in research. Now Canadian researchers have demonstrated the implementation of scalable and quantitative single-cell gene expression measurements on an integrated microfluidic system (right). The device allows individual cells to fall into place like balls in a pinball machine. Once isolated into their separate chambers, the cell's RNA can be extracted and replicated for further analysis. The device's ease of use and cost-effectiveness arise from its integration of almost the entire process of cell analysis – not just separating the cells, but mixing them with chemical reagents to highlight their genetic code and analyzing the results by measuring fluorescent light emitted from the reaction.



The device performs 300 high-precision single-cell RT-qPCR measurements per run, surpassing previous microfluidic systems by a factor of 100 in throughput, and with further scaling the device has the potential to carry out over 1 000 measurements per run. With the device, the researchers have established a dynamic range of at least 10^4 , measurement precision of better than 10%, single molecule sensitivity, and specificity capable of discriminating the relative abundance of alleles differing by a single nucleotide. Compared to tube-based single-cell RT-qPCR, microfluidic processing provides improved reproducibility, precision, and sensitivity, all of which may be critical in identifying subtle differences in cell populations. Nanolitre volume also results in a 1 000-fold reduction in reagent consumption, thereby enabling cost effective analysis of large numbers of single cells. More complex fluid routing to distribute cell contents across multiple chambers will in the future allow for the multiplexed measurements of tens of targets across hundreds of cells, and for combining this technology with single molecule detection by digital PCR. The microfluidic system could also be used for single-cell processing and preamplification, followed by high-throughput microfluidic qPCR or sequencing. <http://dx.doi.org/10.1073/pnas.1019446108>

Genome of 'Brett' sequenced

Scientists in Australia have sequenced the genome of *Dekkera bruxellensis* (*Brettanomyces*), the yeast organism commonly known as 'Brett'. *Brettanomyces* is well known to winemakers throughout the world as a so-called 'spoilage yeast'. Its metabolic products can impart 'sweaty saddle leather', 'barnyard', 'burnt plastic' or 'band-aid' aromas to wine. Some winemakers consider it a desirable addition to wine, but New World vintners generally consider it a defect. Some authorities consider Brett to be responsible for 90% of the spoilage problems in premium red wines. The possibility of Brett developing sulphite resistance was one reason why the researchers set out to crack its genetic code. They have already found the most important gene responsible for sulphite tolerance in Brett. The breakthrough should offer Australian winemakers a competitive advantage in managing the troublesome yeast. www.infowine.com/docs/20111123-brett_genome.pdf

High-power ultrasonics (HPU) for sanitization of wine barrels

If not cleaned correctly, barrels can accumulate tartrate deposits and wine spoilage organisms, which can be detrimental to wine quality. High-power ultrasonics (HPU) in conjunction with hot water of at least 60°C has been shown to completely remove tartrate deposits. Whether or not the treatment reduces the microbial load in the barrel is not known, and *Dekkera bruxellensis* (*Brettanomyces*) cells (the spoilage yeast) have been recovered from oak at a depth of up to 8 mm. In a new study, both 1- and 3-year-old oak were treated with HPU in hot water (60°C) and no culturable Brett cells were detected on the surface (0 to 2 mm) or subsurface (2 to 4 mm) of the oak after the treatment. To check whether the HPU treatment affected the barrels adversely, wines stored over a 12-month period in barrels initially cleaned with hot water, cold water, or HPU, did not differ in their extraction of oak compounds, nor could these wines be differentiated by a sensory panel. Thus, HPU did not adversely affect oak extraction into wine. <http://www.ajevonline.org/content/62/4/519>

Wine and Health

The current issue of the *Journal of Wine Research* (Volume 22, Issue 2, 2011 www.tandfonline.com/toc/cjwr20/current) is entirely devoted to the topic of Wine and Health, and features papers presented at Winehealth 2010 which was held in Italy. The editorial states that the epidemiological data presented, together with the data from existing epidemiological studies, suggest and support that light to moderate wine consumption without heavy drinking bouts provides:

- an approximately 30% reduced risk of cardiovascular diseases such as hypertension, myocardial infarct and ischaemic stroke in both diabetic and non-diabetic, and hypertensive and normotensive individuals—myocardial injury from an infarct is also limited; an approximately 30–40% reduced risk of diabetes mellitus;
- a reduced risk for certain cancers such as for non-Hodgkin's lymphoma, aero-digestive tract and lung cancers (by approximately 20–40%), as well as colorectal cancer at three subsites (by approximately 20%);
- an up to 50% reduced risk of developing dementias;

- and an approximately 20% reduced risk of decline in cognitive function; which equates to an approximately 10% reduced risk of death from all causes as compared with life-long abstainers and heavier consumers. The reduction in risk is attributable to 10–20 g of alcohol per day, which equates to one to two glasses of wine per day. <http://dx.doi.org/10.1080/09571264.2011.603907>

A review on the same subject in the current issue of the *American Journal of Enology and Viticulture* concludes that there is ample evidence supporting the health benefits associated with regular and moderate consumption of wine, particularly polyphenol-rich red wine. These are decreased risks of cardiovascular conditions, type-2 diabetes, and many types of cancer. The alcohol component increases HDL cholesterol levels, inhibits platelet aggregation, and reduces systemic inflammation. Polyphenols present in wine independently provide antioxidant protection, decrease platelet aggregation, and increase endothelial function. Wine polyphenols also act through a variety of mechanisms to prevent and attenuate inflammatory responses, thereby serving as possible cardioprotective, neuroprotective, and chemopreventive agents. <http://www.ajevonline.org/content/62/4/471>

Using a colloidal silver complex in place of sulphur dioxide

The use of sulphur dioxide in the food industry has come increasingly into question because it can lead to pseudo-allergies. A preliminary investigation into substituting SO₂ with a colloidal silver complex (CSC) at doses of 1g/kg of grape has shown promise. The concentration of silver concentration in finished white and red wines was well below the legal limits. Wine composition was slightly affected, but CSC wines had a lower alcoholic degree and acetaldehyde content than SO₂ wines. A major drawback was found to be its lack of antioxidant activity in white wines. <http://dx.doi.org/10.1016/j.foodcont.2011.06.014>

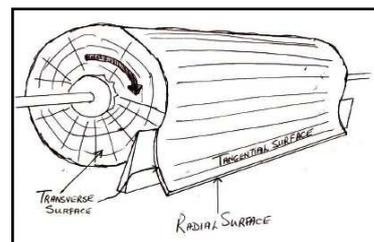
Local Research News

Characterization of *Pythium* and *Phytophthora* species associated with grapevine decline

Grapevine decline and replant diseases cause great economical losses in the grapevine industry. Such diseases are caused by a complex of root pathogenic organisms which not only decrease water and nutrient uptake of plants, resulting in poor growth, but also induce stress that could enhance trunk diseases. Many *Pythium* species (commonly called water moulds), along with their close relatives, *Phytophthora* species are such plant pathogens. In South Africa, *Pythium* spp. are often isolated from diseased grapevine roots, where they are thought to play an important role in replant diseases of young vines, as well as in the decline of older vines where they destroy a substantial amount of root mass. In a study of *Pythium* (*P.*) and *Phytophthora* (*Ph.*) species it was found that the most common infections in nurseries were caused by *P. vexans* (16.7%), followed by *P. ultimum* var. *ultimum* (15.0%) and *P. irregulare* (11.7%). In established vineyards, *P. irregulare* (18.0%) and *P. vexans* (6.2%) were also among the three most prevalent species, along with *P. heterothallicum* (7.3%). Of the three *Phytophthora* species identified in vineyards, *Ph. cinnamomi* (5.1%) and *Ph. sp. niederhauserii* (1.1%) predominated. Pathogenicity studies on rootstock 101-14 Mgt showed that some *Ph. sp. niederhauserii* and *P. vexans* isolates had comparable virulence comparable to the known grapevine pathogens *Ph. cinnamomi* and *P. irregulare*. Sensitive qPCR assays were developed for the detection of *P. ultimum* var. *ultimum*, *P. irregulare*, *P. vexans* and the genus *Phytophthora*. These assays will be invaluable in limiting pathogen dispersal through screening of nursery material, since pathogenic species were isolated from symptomless nursery vines. www.sawislibrary.co.za/dbtextimages/Winetech2010_15.pdf

Alternative oak products

The SA wine-maker's ability to produce higher quality wines with alternative oak products (AOPs) is limited by the fact that the product quality and product performance of the available AOPs do not match the winemaker's requirements of consistency, repeatability and predictability. This is because AOPs, such as tank inserts, barrel inserts, staves, planks, segments, slats, rods, balls, blocks, cubes, chips, granules, powder, extract, etc. are currently made from log and barrel residues, i.e. wood material ultimately not suitable for barrel manufacture. A study posed the question 'How can an oak tree be harvested, the log break-down accomplished and the wood processed so that the extraction of the chemical flavourants from the oak wood (or release of oak fragrances, etc. into the wine) is at an optimum?' As a result, a new production process for the manufacture of AOPs has been developed and is being commercialised. It includes rotary peeling (right), a technique used in the making of veneers. www.sawislibrary.co.za/dbtextimages/Winetech2010_17.pdf



Other News

Improving refrigeration efficiency

As refrigeration can account for as much as 50-70% of winery electricity consumption, the Australian Wine Research Institute (AWRI) has undertaken a project to see how Australian wineries can improve refrigeration efficiency and save on electricity costs. A reference guide has been produced which contains an overview of winery refrigeration concepts, together with 19 improvement opportunities. Case studies have been performed at two wineries to specifically investigate some of the possibilities identified in this reference guide. The reference guide and the two case studies may be downloaded from www.awri.com.au/commercial_services/winemaking_operations/refrigeration/

Winetech Scan is available on the Winetech website www.winetech.co.za

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