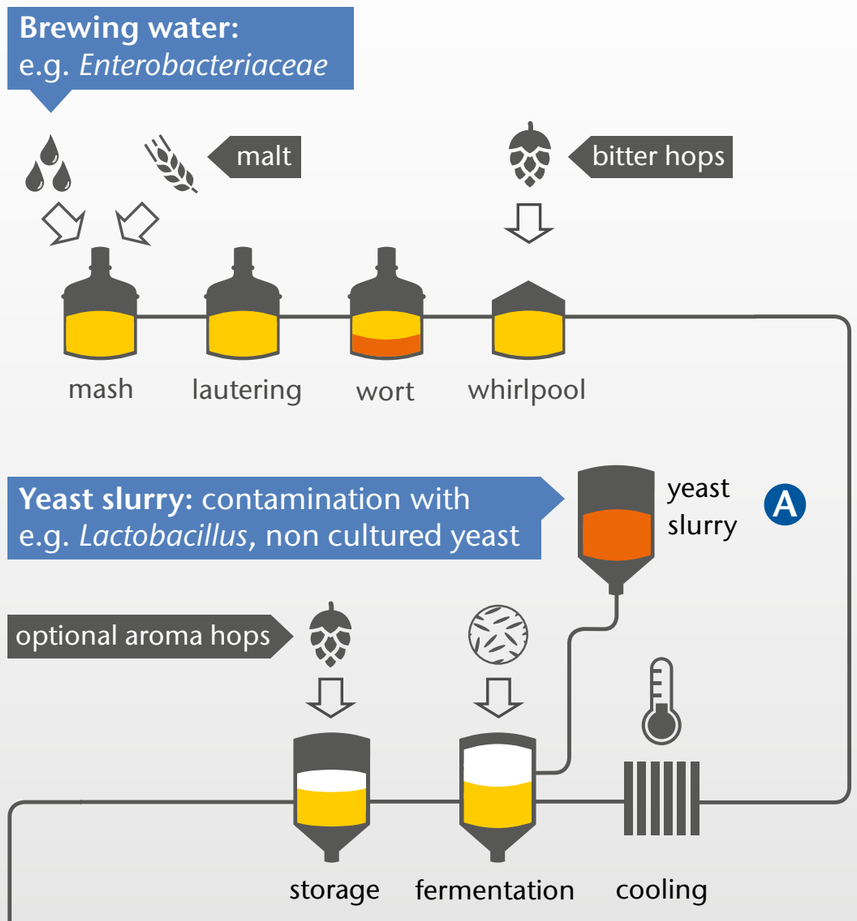
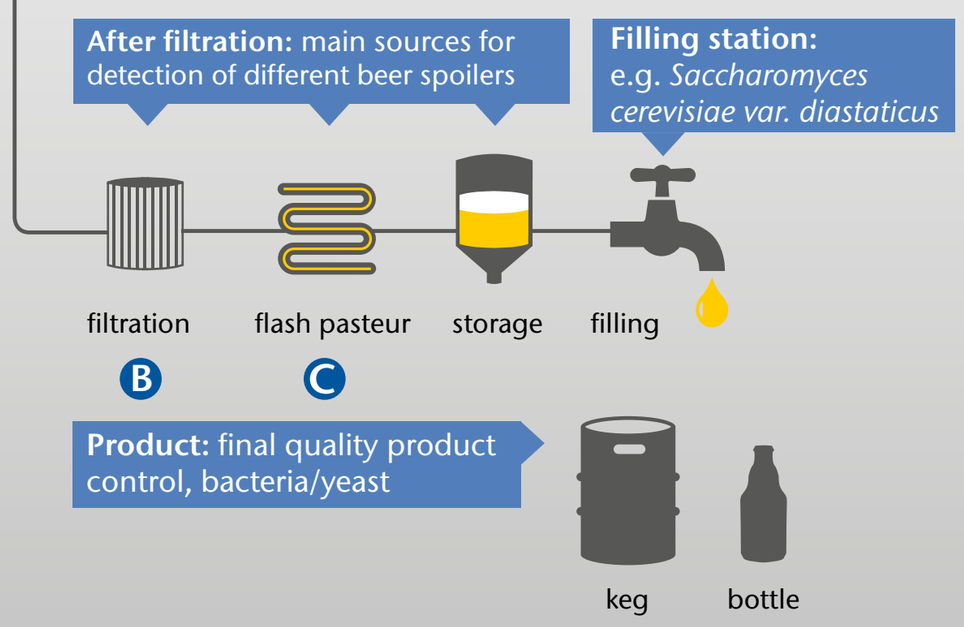
Suggested algorithm for the detection of beer spoilage organisms using GEN-IAL qPCR kits

5 potential sources of microbiological spoilage in beer brewing

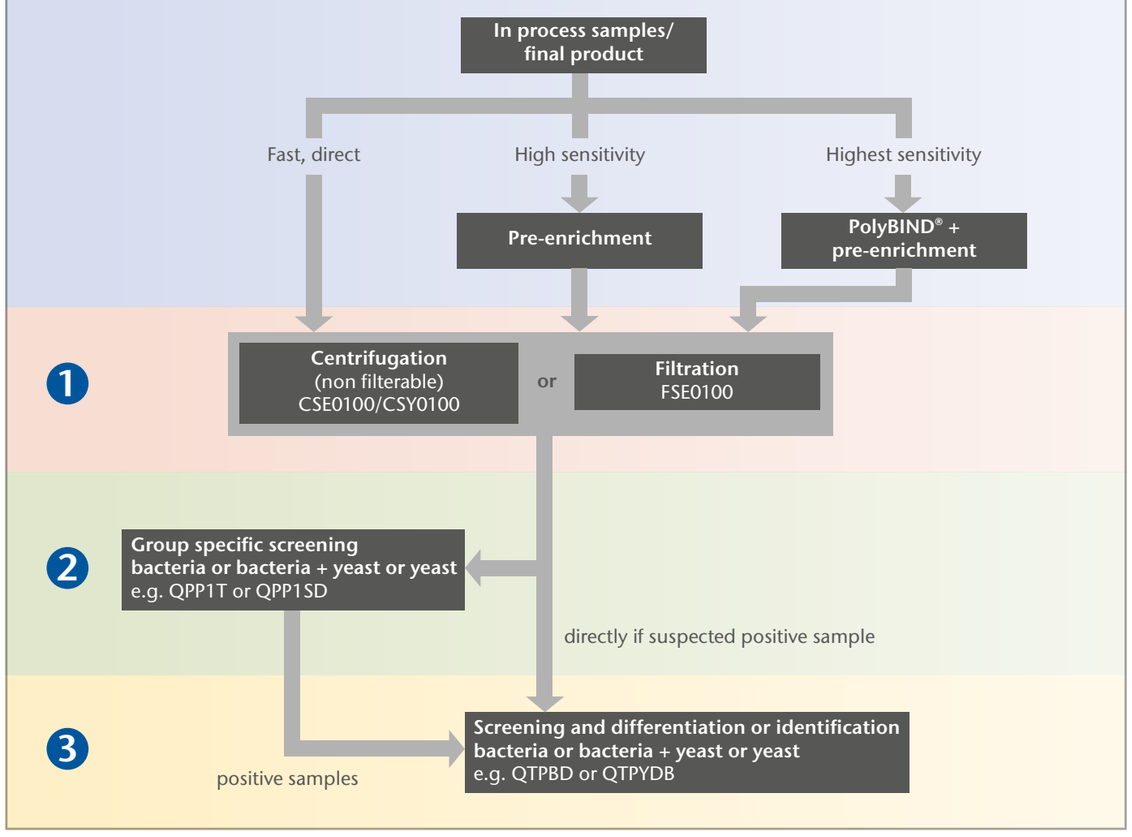
Potential sources of primary contamination



Potential sources of secondary contamination

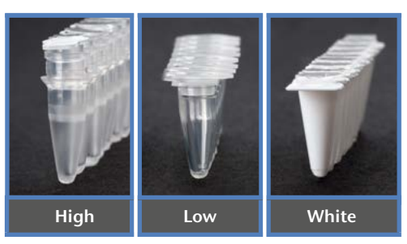


Algorithm for in process samples and final product



Other block cycler devices may be suitable as well. Information is available on request. Different kits are available for different real-time thermocyclers:

Tube profile	Real-time device
high profile	ABI 7500, Agilent MX3005P, Quant Studio 5 (0.2 mL)
low profile	Agilent Aria MX, BioRad CFX96, MyGo Pro, ABI 7500 Fast, Quant Studio 5 (0.1 mL)
white strips	BioRad CFX96, LightCycler® 480 II, Analytik Jena qTower³



1 DNA sample preparation

	DNA extraction kit	Intended use	Method	Sample volume
A	CSY 0100 GEN-IAL® QuickGEN* Sample preparation in yeast	For beverage samples mainly containing yeast	Centrifugation	1 mL sample
B	CSE 0100 GEN-IAL® QuickGEN Sample preparation centrifugation	Enriched or non-filterable samples & colonies	Centrifugation	1 - 50 mL sample
C	FSE 0100 GEN-IAL® QuickGEN Sample preparation filtration	All filterable samples with and without enrichment	Filtration	Up to 1 L sample

2 Group specific screening + differentiation of bacteria and yeast

	Channel/Target				
Art. No./Test kit	FAM	ROX	HEX	Cy5	Comments
QPP1T0050 GEN-IAL® QuickGEN P1 Screening	*Lactobacillus* spp., *Pediococcus* spp.	*Megasphaera* spp., *Pectinatus* spp.	Yeast	IAC	Possible 1st screening step: Group wise detection of all relevant beer spoilage organisms
QTPYB0096* GEN-IAL® QuickGEN First-Beer Yeast and Bacteria Differentiation	*Entero-bacteriaceae*, wild yeast 1, wild yeast 2	Acetic acid bacteria, *Pediococci* IAC	*Lactobacilli*, *Pediococci*, Bottom-fermented yeast, Top-fermented yeast		For 24 samples; Tubes are precoated with the qPCR reagents and contain lyticase – for easier handling and a more convenient lysis step
QTPBF0050 GEN-IAL® QuickGEN First-Biofilm	*Lactococcus lactis*, *Leuconostoc mesenteroides*	IAC	*Pichia anomala*		Biofilm forming species
QPP1SD0050 GEN-IAL® QuickGEN P1	*Lactobacillus* spp., *Pediococcus* spp.	*Megasphaera* spp., *Pectinatus* spp.	*Saccharomyces cerevisiae var. diastaticus*	IAC	Group wise detection of all relevant beer spoilage bacteria and *Saccharomyces cerevisiae var. diastaticus*, The latter can cause a secondary fermentation

Screening of bacteria

	Channel/Target				
Art. No./Test kit	FAM	ROX	HEX	Cy5	Comments
QPP1HR0048* GEN-IAL® QuickGEN P1 Screening	*Lactobacillus* spp., *Pediococcus* spp.	*Megasphaera* spp., *Pectinatus* spp.	Hop resistance genes horA/horC	IAC	Additional risk assessment for hop restistance

Screening of yeast

	Target		
Art. No./Test kit	FAM	HEX	Comments
QTPYD0050 GEN-IAL® QuickGEN First-Yeast PCR Kit Dekkera spp.	*Dekkera* spp.	IAC	Including: *D. bruxellensis, D. anomala, D. custersiana, D. naardenensis* & *D. nanus*
TPYUG0050 GEN-IAL® Bottom fermented yeast	Bottom fermenting yeast	IAC	Detection of bottom-fermenting yeasts to monitor the purity of yeasts in the quality control process
TPYOG0050 GEN-IAL® Top fermented yeast	Top fermenting yeast	IAC	Detection of top-fermenting yeasts to monitor the purity of yeasts in the quality control process

3 Identification of bacteria and yeast in one assay

	Target			
Art. No./Test kit	FAM	ROX	HEX	Comments
QTPBD0096* GEN-IAL® QuickGEN First-Beer Differentiation PCR Kit	*Enterobacteriaceae, P. damnosus, Pectinatus* spp., *L. brevis/ L. brevisimilis/ L. parabrevis, L. buchneri/ L. parabuchneri, L. plantarum/ L. paraplantarum, L. acetotolerans*	IAC, PTC *Saccharomyces cerevisiae var. diastaticus, P. claussenii, L. rossiae, L. casei/ L. paracasei, L. perolens/ L. harbinensis,*	*Pichia anomala, Pediococcus* spp. *(P. acidilactici, P. parvulus, P. inopinatus, P. pentosaceus), Megasphaera* spp., *L. lindneri, L. collinoides/ L. paracollinoides, L. coryniformis, L. backii*	Detection of 30 species and identification of 19 species For 12 samples; Tubes are precoated with the qPCR reagents and contain lyticase – for easier handling and a more convenient lysis step

Identification of bacteria only

	Target			
Art. No./Test kit	FAM	ROX	HEX	Comments
QTPHR0050 GEN-IAL® QuickGEN hop resistance genes horA and horC / hitA and orf5 TaqMan™	hor A / hor C	IAC	hit A, ORF 5	Hop resistance genes as genetic markers for beer-spoilage microorganisms
TPPMD0050 GEN-IAL® Pectinatus spp./Megasphaera spp.	*Pectinatus* spp.	IAC	*Megasphaera* spp.	Strictly anaerobic bacteria with high beer spoilage potential

Identification of yeast only

	Target		
Art. No./Test kit	FAM	HEX	Comments
QYDIF0096* GEN-IAL® QuickGEN First yeast differentiation PCR Kit	*Rhodotorula* spp., *Candida* spp., *Saccharomycodes ludwigii, Torulaspora delbrückii, Klyveromyces marxianus, Dekkera* spp., *Pichia* spp.	IAC, PTC *Saccharomyces exiguus, Saccharomyces cerevisiae var. diastaticus, Saccharomyces bayanus, Hanseniaspora* spp., *Debaromyces hansenii, S. pastorianus*	12 yeast species For 12 samples; Tubes are precoated with the qPCR reagents and contain lyticase – for easier handling and a more convenient lysis step
TPYDA0050 GEN-IAL® Dekkera anomala	*Dekkera anomala*	IAC	
TPYPA0050 GEN-IAL® Pichia anomala	*Pichia anomala (Wickerhamomyces anomalus)*	IAC	
TPYSD0050 GEN-IAL® Saccharomyces diastaticus	*Saccharomyces cerevisiae var. diastaticus*	IAC	Can cause a secondary fermentation due to its amylolytic property.
TPYPM0050 GEN-IAL® Pichia membranaefaciens	*Pichia membranaefaciens*	IAC	
QTPYDB0048* GEN-IAL® QuickGEN First-Yeast PCR Kit Dekkera bruxellensis	*Dekkera bruxellensis*	IAC	Precoated tubes containing the qPCR reagents as well as lyticase – for easier handling and a more convenient lysis step
QTPYZB0048 GEN-IAL® QuickGEN First-Yeast PCR Kit Zygosaccharomyces bailii	*Zygosaccharomyces bailii*	IAC	Troublesome spoiling yeast species due to its exceptional tolerance to various stressful conditions Precoated tubes containing the qPCR reagents as well as lyticase – for easier handling and a more convenient lysis step

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