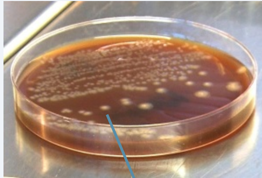
Simple protocol

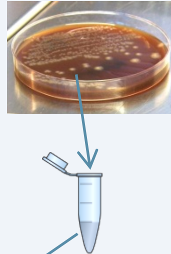
AMPLIQON
PCR ENZYMES & REAGENTS

Screening of bacterial and yeast colonies:
TEMPase 2x Master Mix BLUE

A



B



1. Preparation of the bacteria or yeast DNA

A. Direct Method

No preparation needed.

→ One single colony* is picked (1-2 mm in size) and transferred into the PCR tube containing 24 µl reaction mix.

B. Resuspension Method

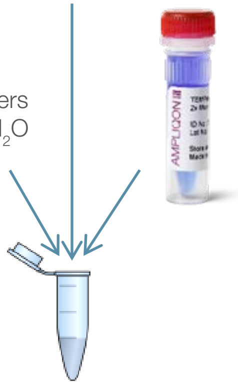
→ Transfer one single colony* into a tube containing 10 – 20 µl PCR grade water.

→ Mix well (vortex).

→ Use 1 µl of the suspension for PCR

*Yeast colonies need to be used fresh

+ primers
+ PCR grade H_2O



2. PCR protocol

Pipet the following reaction mix.

For more than one sample, scale the volumes up and add extra 10 % volume.

Component	Vol./reaction	Final concentration
Blue TEMPase 2X Master Mix	12.5 µl	1X
PCR-grade H_2O	10.5 µl	-
Forward primer (10 µM)	0.5 µl	0.2 µM
Reverse primer (10 µM)	0.5 µl	0.2 µM
TOTAL volume	24 µl	Final reaction volume: 25 µl

Distribute 24 µl reaction mix into each tube.

Add 1 colony or 1 µl of bacteria suspension to the reaction mix and run the PCR.

3. PCR program

Temperature	Duration of cycle	Cycles
95 °C	15 min	1
95 °C	20 sec	25 - 35
50 – 65 °C*	30 sec	
72 °C	30 sec	
72 °C	5 min	1

* the annealing temperature depends on the primer set

4. Gel electrophoresis

Load 10 µl of the PCR product directly on an agarose gel.

The percentage of the agarose depends on the expected product size.

5. Analysis and result

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