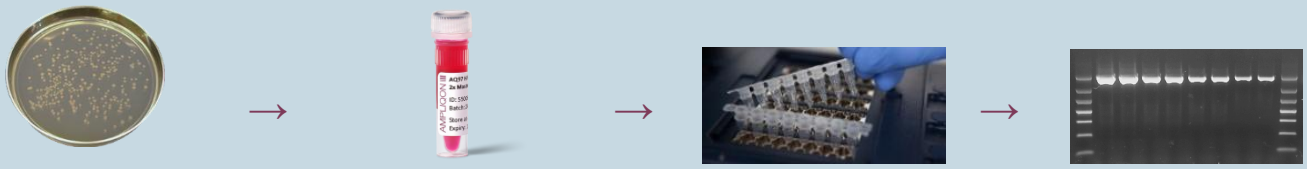
APPLICATION NOTE · DIRECT COLONY PCR

Rapid screening of bacterial and yeast colonies directly from crude samples

AQ97 HiFi Hot Start 2x Master Mix
AQ97 HiFi Hot Start 2x Master Mix RED

AMPLIQON
PCR ENZYMES & REAGENTS



SAMPLE PREPARATION

A Direct method

- Pick one single colony (1-2 mm in size).
- Transfer it directly into the PCR tube containing the reaction mix.

B Resuspension method

- Transfer one single colony into 10-20 µl PCR-grade water and vortex.
- Use 1 µl of the suspension per PCR reaction.

PCR PROTOCOL

Component	Vol./rxn	Final conc.
AQ97 HS HiFi MM	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
Template	1 µl*	-
TOTAL	25 µl	-

** For method A, add one colony (1-2 mm in size).*
For method B, add 1 µl of the bacteria suspension.

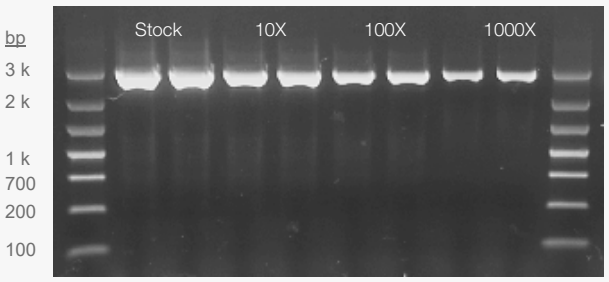


Figure 1. Colony PCR performed with method B.
A single colony was dissolved in 20 µl PCR-grade water and diluted 10×, 100× and 1000×. Specific, high-yield products were observed across all dilutions. Marker: Ampliqon Iqon PCR Ladder

PCR PROGRAM

Temp.	Duration	Cycles
98 °C	2-5 min *	1
98 °C 50-65 °C ** 72 °C	10 sec 30 sec 30 sec	25-35
72 °C	5 min	1

** Initial heating time can be adjusted based on the age of the colony.*
*** Annealing temperature depends on the primer set.*

GEL ELECTROPHORESIS

Load 10 µl of the PCR product directly onto an agarose gel. Select agarose percentage based on product size.

TIPS

Method A
- Use minimal colony material
- Resuspend one colony in ≥3× reaction volume
- Excess colony may inhibit PCR

Method B
- Test a 10x dilution alongside the undiluted stock.
- Dilution often resolves inhibition

Annealing temperature
- Use the Ampliqon T_m calculator or run a gradient

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