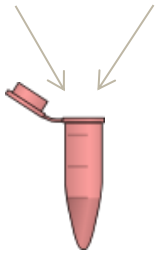
Protocol

Sequencing of coloured PCR products using PureIT ExoZAP PCR CleanUp:

AMPLIQON
PCR ENZYMES & REAGENTS

Taq 2x Master Mix RED / TEMPase 2x Master Mix A BLUE

Template DNA
Primers
PCR grade H_2O



Distribute 25 µl reaction mix into each tube.

1. PCR protocol

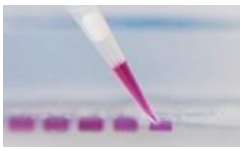
→ Pipet the following reaction mix:

Component	Vol./reaction	Final concentration
2X Master Mix	12.5 µl	1X
PCR-grade H_2O	11.5 µl – x µl	-
Template DNA	x µl	Genomic DNA: 50 ng (10 – 500 ng) Plasmid DNA: 0.5 ng (0.1 – 1 ng) Bacterial DNA: 5 ng (1 – 10 ng)
Forward primer (10 µM)	0.5 µl	0.2 µM
Reverse primer (10 µM)	0.5 µl	0.2 µM
TOTAL volume	25 µl	Final reaction volume: 25 µl

2. PCR program

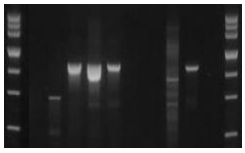
Temperature	Duration of cycle	Cycles
95 °C	15 min*	1
95 °C	20 sec	
50 – 65 °C**	30 sec	25 - 35
72 °C	30 sec	
72 °C	5 min	1

* 5 min for Taq 2x Master Mix RED ** the annealing temperature depends on the primer set



3. 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.

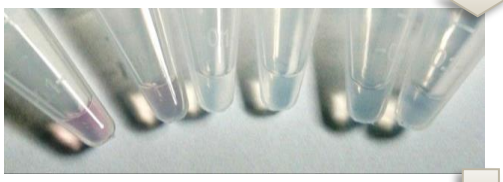


4. Analysis and result

→ Check product bands for correct size.
→ Estimate DNA concentrations.

5. Product purification

→ Spin column purification: Follow the kits protocol.
→PureIT ExoZAP PCR CleanUp: Follow the protocol

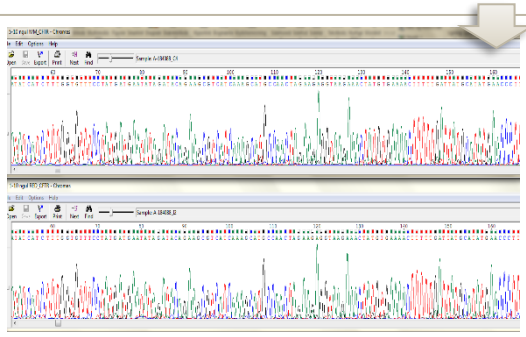


6. Dilution before sequencing

→ Dilute the purified PCR products to the appropriate concentration needed for sequencing.
→ When using PureIT ExoZAP PCR CleanUp dilute the samples at least 8 times.

From left to right: Taq Master Mix RED 8x diluted, Taq Master Mix RED 16x diluted, Taq Master Mix 8x diluted, Taq Master Mix 16x diluted, TEMPase Master Mix BLUE 16x diluted and TEMPase Master Mix

Ampliqon A/S, Stenhuggervej 22, DK-5230 Odense M, Denmark. Phone: +45 70201169 Fax: +45 70201179 info@ampliqon.com www.ampliqon.com



7. Sequencing and ID

→ Send the samples for sequencing.

→ Perform a BLAST search at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Sequencing electropherograms

Top: Sequencing post PCR – Taq 2x Master Mix without loading dye

Bottom: Sequencing post PCR - Taq 2x Master Mix RED

NB. This protocol is also suitable for sequencing of PCR products when using 5x PCR Buffer RED

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