



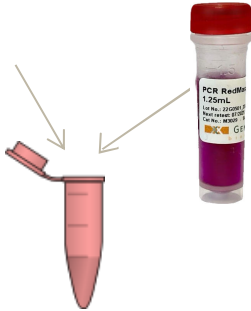
## Protocol

# Sequencing of coloured PCR products using PureIT ExoZAP PCR CleanUp:



## RedMastermix (2X) // SuperHot MasterMix Blue (2X)

Template DNA  
Primers  
PCR grade H<sub>2</sub>O



Distribute 25µL reaction mix into each tube.

### 1. PCR protocol

→ Pipet the following reaction mix:

Component	Vol./reaction	Final concentration
2X Master Mix	12.5µL	1X
PCR-grade H <sub>2</sub> O	11.5µL – 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)	0.5µL	0.2µM
Reverse primer (10 µM)	0.5µL	0.2µM
<b>TOTAL volume</b>	25µL	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

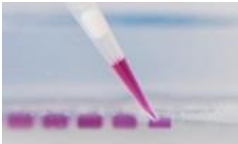
\* 5 min for Taq 2x Master Mix RED \*\* the annealing temperature depends on the primer set



### 3. Gel electrophoresis

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

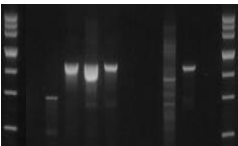
The percentage of the agarose depends on the expected product size.



### 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

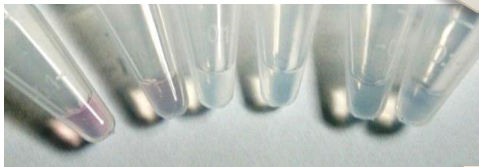


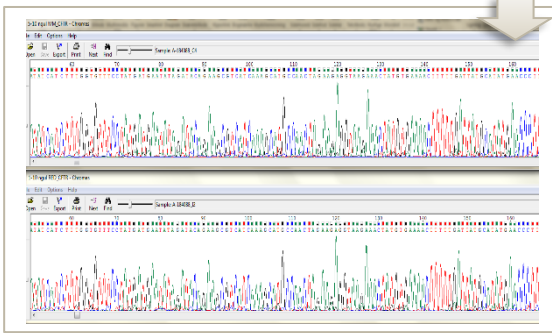
### 6. Dilution before sequencing

→ 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: RedMastermix 8x diluted, RedMastermix 16x diluted, PCR Mastermix 8x diluted, PCR Mastermix 16x diluted, SuperHot MasterMix Blue 16x diluted and SuperHot MasterMix Blue 8x diluted.*





## 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 – PCR Master Mix 2X without loading dye*

*Bottom: Sequencing post PCR - RedMastermix (2X)*

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