

## LYSE 'N EASY<sup>®</sup> -ROYAL Stage 1 - DNA Release

You need: microLYSIS-Plus (MLP) & cells

- Mix cells with 20  $\mu$ l microLYSIS-Plus
- Overlay with mineral oil if necessary
- Place in a Thermal Cycler

### Lysis Profile

- Step 1: 65°C for 15 mins  
(may need to be longer for very tough cells)
- Step 2: 96°C for 2 mins
- Step 3: 65°C for 4 mins
- Step 4: 96°C for 1 min
- Step 5: 65°C for 1 min
- Step 6: 96°C for 30 secs
- Step 7: 20°C hold

After lysis use in [Stage 2](#), or store at -20°C for future use

## Stage 2 - DNA Amplification

You need: MLP-DNA mix (MLP-D) from [Stage 1](#), MegaMix-Royal (MMR), Just Water & 2 PCR primers

Make a premix of the two PCR primers at 2.5 to 5 pmol/ $\mu$ l each  
Make up PCR mastermix as following, adding 1x volume extra for multiple tubes

	Volume for one	Volume for 4 (x5)
MMR	10 $\mu$ l	50 $\mu$ l
Primer Premix	1 $\mu$ l	5 $\mu$ l
Just Water	4 $\mu$ l	20 $\mu$ l
<b>Total</b>	<b>15 <math>\mu</math>l</b>	<b>75 <math>\mu</math>l</b>

Set up 4 PCRs as follows (guide only)

Tube	1	2	3	4
PCR Mastermix	15 $\mu$ l	15 $\mu$ l	15 $\mu$ l	15 $\mu$ l
MLP-D	5 $\mu$ l	2.5 $\mu$ l	1 $\mu$ l	- $\mu$ l
Just Water	- $\mu$ l	2.5 $\mu$ l	4 $\mu$ l	5 $\mu$ l
<b>Total</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>	<b>20 <math>\mu</math>l</b>

Program the Thermal Cycler as follows (guide only)

Initial denaturation step: 95°C for 5 mins

Then cycle 30 times:

Step 1: 95°C for 30 secs

Step 2: Annealing temp. of primers for 30 secs

Step 3: 72°C for 45 secs

After cycling, load samples directly on agarose gel. No loading buffer required.  
Make sure that the sample hasn't evaporated during cycling, as this will distort the results

Supplied as 3 solutions (microLYSIS-Plus, MegaMix-Royal and Just Water) in multiple of 1 ml aliquots  
Store at -20°C

For Research Only