

LYSE 'N EASY[®] -GOLD Stage 1 - DNA Release

You need: microLYSIS-Plus (MLP) & cells

- Mix cells with 20 μl 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-Gold (MMG), Just Water & 2 PCR primers

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

| | Volume for one | Volume for 4 (x5) |
|---------------|----------------|-------------------|
| MMG | 10 μl | 50 μl |
| Primer Premix | 1 μl | 5 μl |
| Just Water | 4 μl | 20 μl |
| Total | 15 μl | 75 μl |

Set up 4 PCRs as follows (guide only)

| Tube | 1 | 2 | 3 | 4 |
|---------------|--------------|--------------|--------------|--------------|
| PCR Mastermix | 15 μl | 15 μl | 15 μl | 15 μl |
| MLP-D | 5 μl | 2.5 μl | 1 μl | - μl |
| Just Water | - μl | 2.5 μl | 4 μl | 5 μl |
| Total | 20 μl | 20 μl | 20 μl | 20 μl |

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, process according to detection protocol.
Make sure that the sample hasn't evaporated during cycling, as this will distort the results

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

For Research Only