LABORATORY OF MOLECULAR PATHOLOGY

PCR for metagenomics

Using Platinum II polymerase for 16S, ITS and mcrA gene analysis

Prep:

- ☐ 01 Thaw an aliquot of **PCR-grade water**.
- ☐ 02 Thaw an aliquot of **Platinum II polymerase**, keep it on ice.
- ☐ 03 Thaw **10 µM primer** aliquots, keep them on ice.
- ☐ 04 Dilute reasonable volumes of primers you will need to **2.5 µM**
  i.e. 2 µL of primer per sample plus excess
- ☐ 05 **Calculate** the master mix composition you will need for all the samples together.
- ☐ 06 Compose the **master mix** for the whole PCR reaction. Don't forget to include negative controls, where instead of DNA you add pure water.

Master mix	1×	×
Polymerase 2×	10,0 µL	
FW primer 2.5 µM	2,0 µL	–
REV primer 2.5 µM	2,0 µL	–
DEPC-H_2O	9,0 µL	
DNA sample	2,0 µL	–

- ☐ 07 Pipette **19 µL** of the master mix into the wells.
- ☐ 08 Pipette **2 µL** of the **2.5 µM forward** primer. One row, one primer.
- ☐ 09 Pipette **2 µL** of the **2.5 µM reverse** primer. One column, one primer.
- ☐ 10 Pipette **2 µL** of your **DNA** sample into the well
- ☐ 10 Cover the wells with strong adhesive foil. Make sure it **adhered properly**.
- ☐ 11 Do a quick vortex to mix the reactions and **quick spin**.
- ☐ 12 Put the plate into the cycler and run the following programme. You need to change the **ramp rate of the annealing to 60 %.**

Amplification programme	Temperature	Time	
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	45 s	**35×**
Annealing	52 °C	60 s	
Extension	72 °C	90 s	
Final extension	72 °C	5 min	
	12 °C	Hold	