**Standard Operating Procedure for Nodule Bacteria Isolates: DNA Extraction & PCR**

1. Grow bacterial culture in YMB o/n for not more than 12 h
2. Aliquot 0.5 mL of the liquid culture at log phase in 1.5 or 2 mL microfuge tube
3. Centrifuge 14,000 rpm, 10 min
4. Remove the supernatant by decanting, and all residual fluid with a pipette.
5. Add in 400 μL TE[[1]](#footnote-1) buffer (by drawing with 1 mL pipette), to re-suspend pellet
6. Add 10 μL 20% [w/v] SDS[[2]](#footnote-2), (final conc. 0.5 %), vortex to lyse
7. Add 10 μL (>800 u mL-1 proteinase K (Sigma #P4850), mix well/vortex
8. Incubate 37 oC 1 h
9. Add 420 μL Phenol:Chloroform:IAA[[3]](#footnote-3) (Sigma #P2069 (100 mL)) and vortex
10. Centrifuge 14,000 rpm, 15 min.
11. Recover **CAREFULLY** the aqueous phase (175 or 300 uL[[4]](#footnote-4))
12. Add 15 or 30 μL (1/10th vol.) 3M Sodium Acetate[[5]](#footnote-5) and mix well
13. Add 570 or 990 μL (3x vol.) isopropanol (Sigma #I9030 (500 mL)), mix well
14. Incubate -80 oC 15 min. or -20 oC over-night
15. Centrifuge ~14,000 rpm for 15 min., to pellet DNA
16. Remove liquid by decanting then remove residual liquid with pipette tip
17. Wash 200 μL 70% [v/v] EtOH
18. Centrifuge ~14,000 rpm for 1 min., to re-pelleted
19. Remove all liquid with pipette
20. Dry 15 min. in 37 oC oven
21. Add 25 or 50 μL TE buffer to re-suspended pellet[[6]](#footnote-6)
22. Store at -20 oC

**PCR Reaction[[7]](#footnote-7) (50 µL, using Promega GoTaq)**

1. MilliQ-Water 32.5 μL
2. 5x Green GoTaq® Flexi Buffer (Promega) 10 μL
3. 25mM dNTPs (Invitrogen) 0.5 μL
4. 25mM MgCl2 (Promega) 3 μL
5. 10 uM Forward primer 1 μL
6. 10 uM Reverse primer 1 μL
7. GoTaq polymerase(Promega) 1 μL
8. DNA Template 1 μL

**Thermal Profile [[8]](#footnote-8)**

1x Denaturation 95 oC 2 min.

see table below Denaturation 95 oC 1.5 min.

Annealing see table below 30 s

Elongation 72 oC 1.5 min.

1x Elongation 72 o 15 min.

1x Stand 10 oC

* Use AbGene Thermofast (non-skirted) 0.2 mL \_PCR plates (#AB-0600)
* Sealed with an AbGene ‘adhesive PCR foil’ (#AB-0626)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Primer** | **Primer****Code** | **oC** | **Cycles** | **Product-Size****(~bp)** | **Gel %** **[w/v] Agarose** | **ladder** |
| **16S RNA[[9]](#footnote-9)** | 8-27F & rD1 | 56 | 35 | 1500 | 1 | 1 kb |
| ***nodA[[10]](#footnote-10)*** | nodA-1 & -2 | 50  | 40 | 600 | 2 | 100 bp |
| ***nodD[[11]](#footnote-11)*** | NBA12 & Y6 | 55  | 35 | ~750 | 2 | 100 bp |

**Agarose gel electrophoresis:** Run (80 V for 20 min) 5μl of PCR product in an agarose gel (50 mL) with 1 μl SYBR-SAFE (Invitrogen). Use 5μl of ladder alongside the PCR products.

1. 10 mM Tris-Cl, pH 7.5 and 1 mM EDTA. Make from sterile stocks. **1M Tris-HCl,** FW 121.14 g :. Add 12.114 g to 90 mL-1, pH 8.0 with 1N HCl - top-up to 100 mL. **500 mM EDTA**, FW 372.24 g :. Add 18.612 g to 90 mL-1; pH 8.0 with NaOH pellets - to 100 mL [↑](#footnote-ref-1)
2. May need warmed to ensure all the SDS dissolves. [↑](#footnote-ref-2)
3. The stock should be supplemented (buffered), using all of the alkaline-buffer aliquot provided from the manufacturer, and shaken well and allowed to settle (over-night) before use. Also, **NB** DO NOT take the upper layer, this is liquid-buffer. Take the solvent (lower) layer. [↑](#footnote-ref-3)
4. Avoid removing the white cloudy interface: taking 175 or 350 μL depending on the sample. [↑](#footnote-ref-4)
5. 3M NaOAc, FW 136.08 g :. Add 40.824 g to 90 mL, pH 5.2 with acetic acid - top-up to 100 mL. [↑](#footnote-ref-5)
6. The DNA suspension volume should be apportioned to the volume of aqueous phase removed (step 9). [↑](#footnote-ref-6)
7. This is for 1 sample. Prepare a Master Mix (MM) of a volume that in sufficient for the total number of samples you will PCR, **plus extra** (virtual) samples to compensate for lost liquid adhering to the tube side or small pipetting errors. Generally prepare one extra sample for every ten you will actually process. To the MM add all the ingredients **except DNA template,** dispensing 48 $µ$L per plate well with a single clean pipette tip. After this add 2 $µ$L DNA, using a fresh tip each time. [↑](#footnote-ref-7)
8. Before PCR: if using AbGene 8-well tube strips seal using the caps. If using AbGene 96 well PCR plate, seal the plate with a AbGene PCR-plate foil-seal. [↑](#footnote-ref-8)
9. 16S rRNA Weisburg et al., (1991) *Bacteriol.* **173**: 697-703.

For.-AGA GTT TGA TCC TGG CTC AG; Rev.-AAG GAG GTG ATC CAG CC [↑](#footnote-ref-9)
10. *nodA -* Haukka *et al.*, (1998) *App Env Microbiol* **64**: 419-426.

For.-TGC RGT GGA ARN TRN NCT GGG AAA;

Rev.-GGN CCG TCR TCR AAW GTC ARG TA [↑](#footnote-ref-10)
11. *NodD –* Laguerre et al., (1996) *App Env Microbiol* **62**:2029 - NBA12 – GGA TSG CAA TCA TCT AYR GMR TC

Mutch & Young JPW (2004) *Mol Ecol* **13**:2435-2444 - Y6- CGC AWC CAN ATR TTY CCN GGR TC [↑](#footnote-ref-11)