Online Resource 2 - PCR and identification of fungal isolates

DNA from mycelia growing on agar was extracted from 1-2 plates with ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research) according to the manufacturer's instruction with β-mercaptoethanol 0.5% (v/v) supplemented to the DNA Binding Buffer. Extracted DNA concentration was measured on a NanoDrop 2000 (Thermo Scientific) with DNA 50 standard settings. Yields ranged from 13.1-120 ng/µl extracted from 30-255 mg fungi material – wet weight. For 50 µl PCR reactions, the kit and primer pair used were TopTaq Master Mix (Qiagen) and primer ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') paired with ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Eurofin). At least 2 ng DNA/reaction were used for each amplification reaction. Touchdown PCR reaction program was: 95°C for 5 min followed by 40x(95°-20s, 58°C-20s: -0.5°C▼, 72°C-30s) with 1 minute final extension at 72°C and 4°C hold. ITS of *A. serialis, A. sinuosa, A. xantha, G. sepiarium, S. himantoides* and *S. lacrymans,* were successfully amplified with this program as confirmed from 10 µl of PCR reaction mixture developed on a 2% TBE agarose gel post-stained with ethidium bromide. ITS of *C. puteana* was amplified with a gradient program 46-58°C for 35 cycles with otherwise the same conditions as the touchdown program without a temperature descent per cycle. Amplification at the relatively low temperature of 52.6 °C was selected for PCR product purification. *Heterobasidion parviporum* was re-inoculated onto 5 agar-plates and extracted in the same way as above. ITSOF-T (5'-ACT TGG TCA TTT AGA GGA AGT-3') and ITS4-B1 (5'-CAA GRG ACT TRT ACA CGG TCC A-3') (Metabion) and TopTaq Master Mix was used to amplify the ITS-region of *C. puteana* with the touchdown program above. All amplicons were purified with QIAquick PCR purification kit (Qiagen). Purified PCR products were sent to GATC for both forward and reverse sequencing. Sequencing primers were shortened to be in accord with GATC optimal requirements with forward primer Li-ITSOF-T (5'-CTT GGT CAT TTA GAG GAA G-3') and reverse primer Li-ITS4-B1 (5'-AAG RGA CTT RTA CAC GGT C-3') (Metabion). Sequences were assembled and their respective contigs were manually curated with CodonCode Aligner (v 4.0.4). Contigs were searched with nucleotide (nr/nt) Megablast which unambiguously confirmed their identity with the exception of *A. serialis*. The highest score was indeed another *A. serialis* isolate (MK454923.1) with an identity of 99,74 % and two gaps, however, the two following specimens with the highest scores were found from an *A. leucaena* (JQ700278.1) sequence with 98% identity (1 gap) and the second was *A. infirma* (JQ700287.1), 98% identity with 2 gaps. A distance tree of results using multiple sequence alignment was performed with the first 100 blastn results with a max sequence difference of 0.75. The phylogram showed that *A. serialis* clustered well with other *A. serialis* sequences and not in the same group as *A. leucaena* and *A. infirma*. In addition, the morphology of the culture was consistent with reference cultures of *A. serialis*.