GCTCAG-3′) and 530r (5′-GA/TATTACCGCGGCG-GCTG-3′). PCR amplification was performed in a total volume of 100 µl, containing 0.5 µg of each primer, 10 µl of 10 × reaction buffer (Boehringer Mannheim), 1 mM (each) dATP, dGTP, dTTP and dCTP, and 100 ng of each bacterial DNA. The reaction mixtures were incubated in a thermal cycler for 3 min at 98 °C. Two units of Taq Polymerase (Boehringer Mannheim, Germany) were added and the reaction was performed for 28 cycles at 55 °C for 1 min, 72 °C for 2 min, and 93 °C for 1 min. The final cycle was an elongation step at 72 °C for 5 min.

**Image analysis.** 5 µl of the PCR solutions were loaded onto a vertical gel, consisting of 3% Metaphor agarose (FMC, Rockland, Maine). Gels were run at 5 V/cm for 14–16 h at 4 °C, and stained with SYBR green (Molecular Probes, Ca.). Gel images were converted to digitized files and analysed by Image Quant 3.3 (Molecular Dynamics).

**Results and conclusion**

As determined by image analysis of SYBR green-stained amplification products the experimentally determined ratio corresponded well with the expected ratio calculated from the number of *rrn* genes per equimolar amount of DNA in mixtures containing DNA of *Escherichia coli* and '*Thermus thermophilus*' and DNA of *Pseudomonas aeruginosa* and '*T. thermophilus*'. The values for the pair *Bacillus subtilis* and '*T. thermophilus*' showed higher deviation from the predicted value. The dependence of the amount of 16S rDNA amplification products on these two parameters makes it impossible to quantify the number of species present in 16S rDNA clone library of an environmental sample, as long as these two parameters are unknown for these species.

1 Farrelly, V., Rainey, F. A., and Stackebrandt, E., Environ. appl. Microbiol. *61* (1995) 2798.

## Simple 'universal' DNA extraction procedure compatible with direct PCR amplification

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Large scale use of amplification methods in diagnostic laboratories requires efficient but simple DNA extraction procedures compatible with various types of clinical specimens. A combination of SDS and proteinase K has been widely used for solubilizing tissues as well as for the extraction of DNA from microorganisms. However, SDS is inhibitory for *Taq* polymerase at any useful concentration[1] and, therefore, does not allow direct amplification without extraction with organic solvents and ethanol precipitation. We have used Tween 20 to neutralize the activity of SDS on *Taq* polymerase in the amplification mix, resulting in a simple and rapid extraction procedure applicable to a variety of clinical specimens which does not require further purification steps.

Tissues (disintegrated mechanically), sputum (liquefied and centrifuged), liquid specimens (centrifuged) or bacterial cultures were incubated with digestion buffer (50 mM Tris-HCl, pH 8.5, 1 mM EDTA, 0.5% SDS, 200 µg/ml proteinase K) at 55 °C for 3 h or overnight (tissue) followed by heating at 95 °C for 10 min and transferring a maximum of 10 µl directly into the amplification tube. Amplification was in a final volume of 100 µl containing 2% (v/v) Tween 20. This extraction procedure has successfully been used in conjunction with various PCR systems including amplification of *Rochalimaea (Bartonella) henselae* and *R. quintana*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*.

The sensitivity of the procedure was determined using a eubacterial amplification system[2]. Ten µl of the amplified material were analyzed by gel electrophoresis and ethidium bromide staining without hybridization: the equivalent of between 7 (gram-negative bacteria) and 600 (gram-positive bacteria, *Mycobacterium fortuitum*) organisms per amplification was reproducibly visible on the agarose gels. We conclude that DNA extraction with SDS and proteinase K is simple, rapid, applicable to large numbers of specimens, and allows direct amplification without further purification if Tween 20 is included in the amplification mix. It is also compatible with the use of uracil-N-glycosylase (UNG) for the prevention of false positives due to amplicon carry-over (contamination control).

1 Kawasaki, E. S., in: PCR Protocols: A guide to Methods and Applications, pp. 146–152. Eds M. A. Innis, D. H. Gelfand, J. J. Sninsky and Th. J. White. Academic Press, San Diego 1990.
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## Detection of *Aspergillus fumigatus* by polymerase chain reaction (PCR)

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Invasive aspergilloses (IA) have a poor prognosis, but it is known that early diagnosis and treatment result in a more favourable outcome. PCR could contribute to a rapid diagnosis. In this study we developed a PCR to detect *Aspergillus fumigatus* and assessed the application to clinical specimens.

The primers were designed based on the published cDNA sequences of the cytotoxin Asp f1, because