The study of fungal diversity may lead to many fundamental discoveries and conclusions. Molecular genetics, and particularly high throughput sequencing methods using short DNA fragments as barcodes, has recently experienced a boom. The most frequently used marker for fungal research is the partial region of nuclear ribosomal DNA called ITS (Internal Transcribed Spacer). It occurs in the form of tandem repetitions of up to 200 copies. This fact greatly simplifies its amplification from the environment but also introduces some negatives. One of them can be an existence of intragenomic and intraspecific variability which confounds diversity estimates by exaggerating the real number of species. Using alternative low-copy markers can easily prevent these problems. In this study *EF-1α* and *RPB2* protein-coding genes were compared with traditionally used ITS1 and ITS2 markers. An artificial mock community was created by blending genomic DNA of different fungal lineages. The community was sequenced for all markers and the data were processed according to guidelines commonly used in environmental studies. The results show that ITS2 is unequivocally a more suitable marker for environmental studies than other compared markers. The average coefficient of overestimation was deemed to be approximately two for ITS1, ITS2, but also for *RPB2*. *EF-1α* showed largely increased polymorphism within species and therefore this region is not recommended for environmental studies.