Figure S2 A

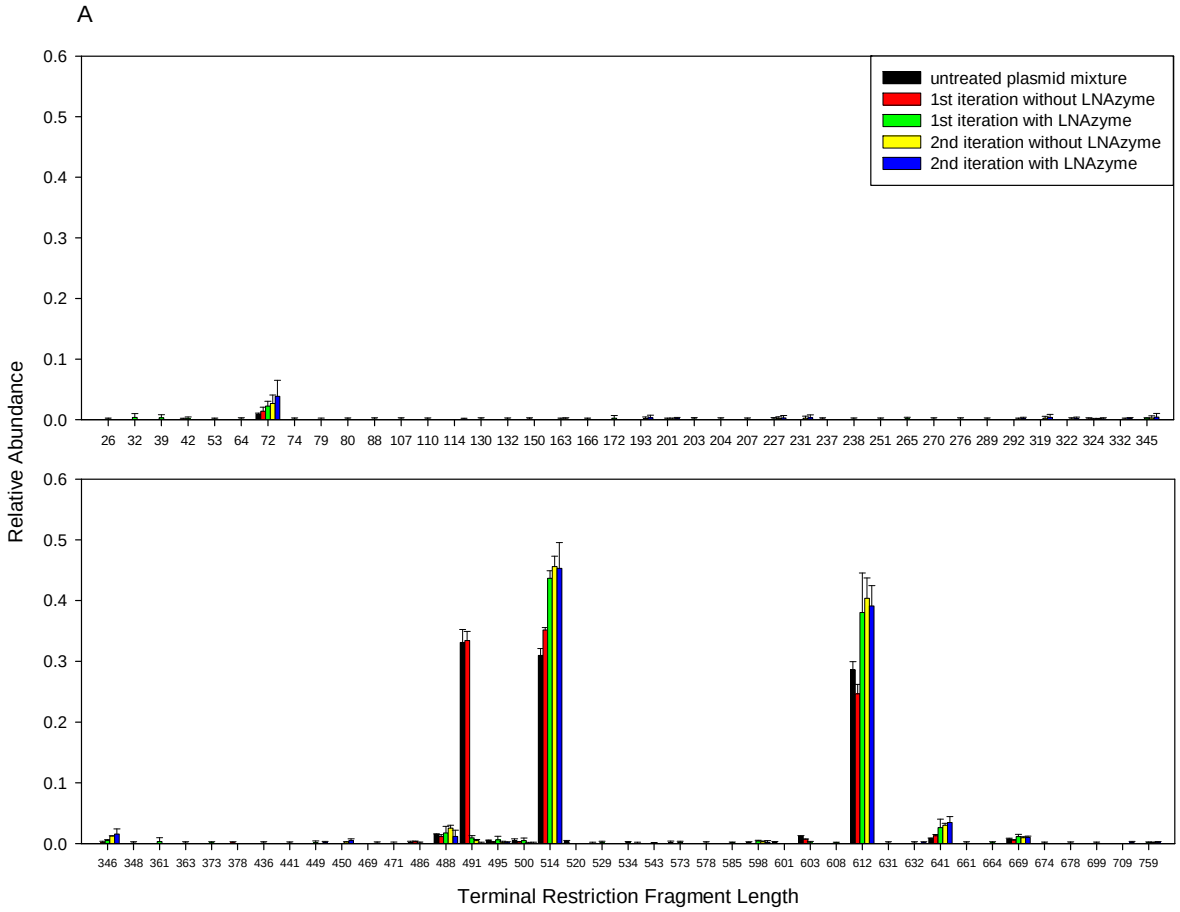
A
untreated plasmid mixture
1st iteration without LNAzyme
1st iteration with LNAzyme
2nd iteration without LNAzyme
2nd iteration with LNAzyme
Relative Abundance
0.0
0.1
0.2
0.3
0.4
0.5
0.6
26 32 39 42 53 64 72 74 79 80 88 107 110 114 130 132 150 163 166 172 193 201 203 204 207 227 231 237 238 251 265 270 276 289 292 319 322 324 332 345
346 348 361 363 373 378 436 441 449 450 469 471 486 488 491 495 500 514 520 529 534 543 573 578 585 598 601 603 608 612 631 632 641 661 664 669 674 678 699 709 759
Terminal Restriction Fragment Length

Figure S2 B

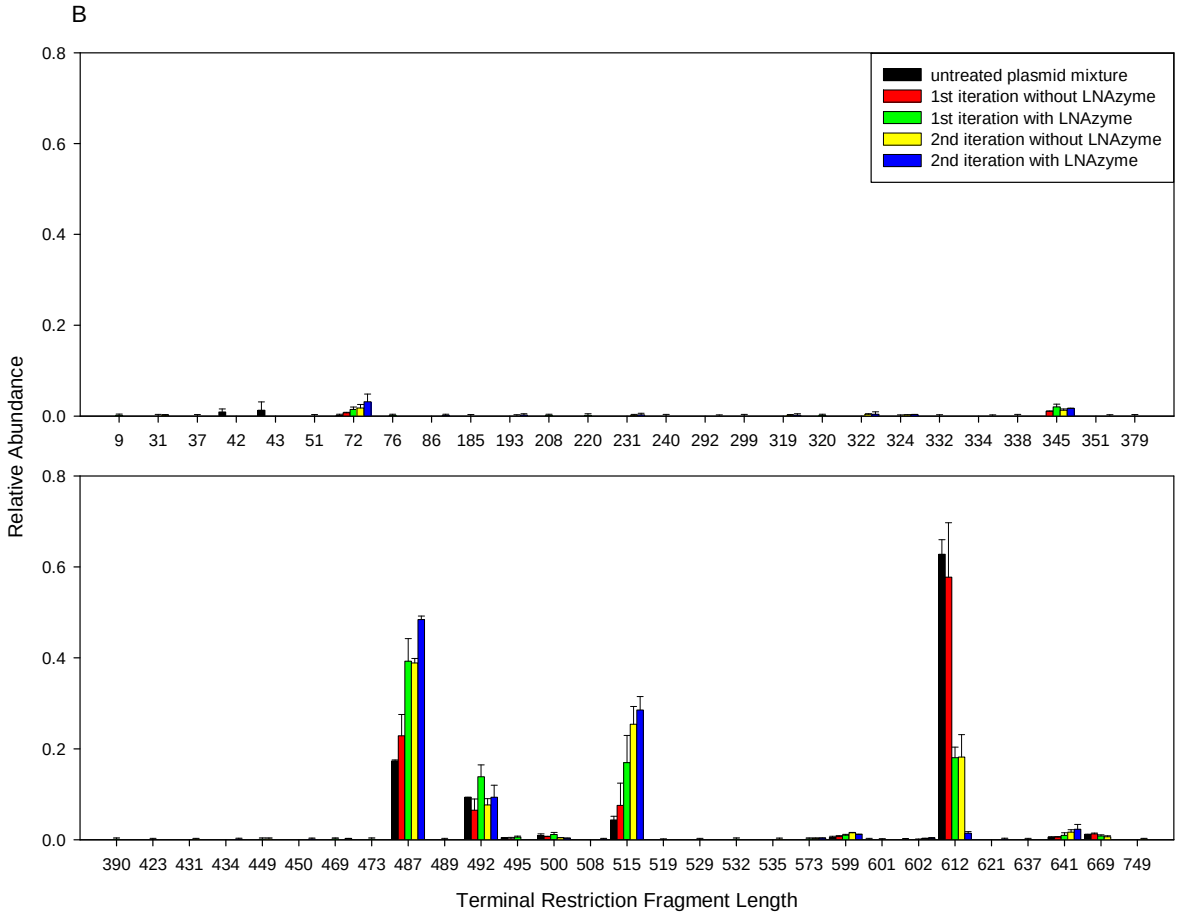
B
untreated plasmid mixture
1st iteration without LNAzyme
1st iteration with LNAzyme
2nd iteration without LNAzyme
2nd iteration with LNAzyme
Relative Abundance
0.0
0.2
0.4
0.6
0.8
9 31 37 42 43 51 72 76 86 185 193 208 220 231 240 292 299 319 320 322 324 332 334 338 345 351 379
390 423 431 434 449 450 469 473 487 489 492 495 500 508 515 519 529 532 535 573 599 601 602 612 621 637 641 669 749
Terminal Restriction Fragment Length

Figure S2 C

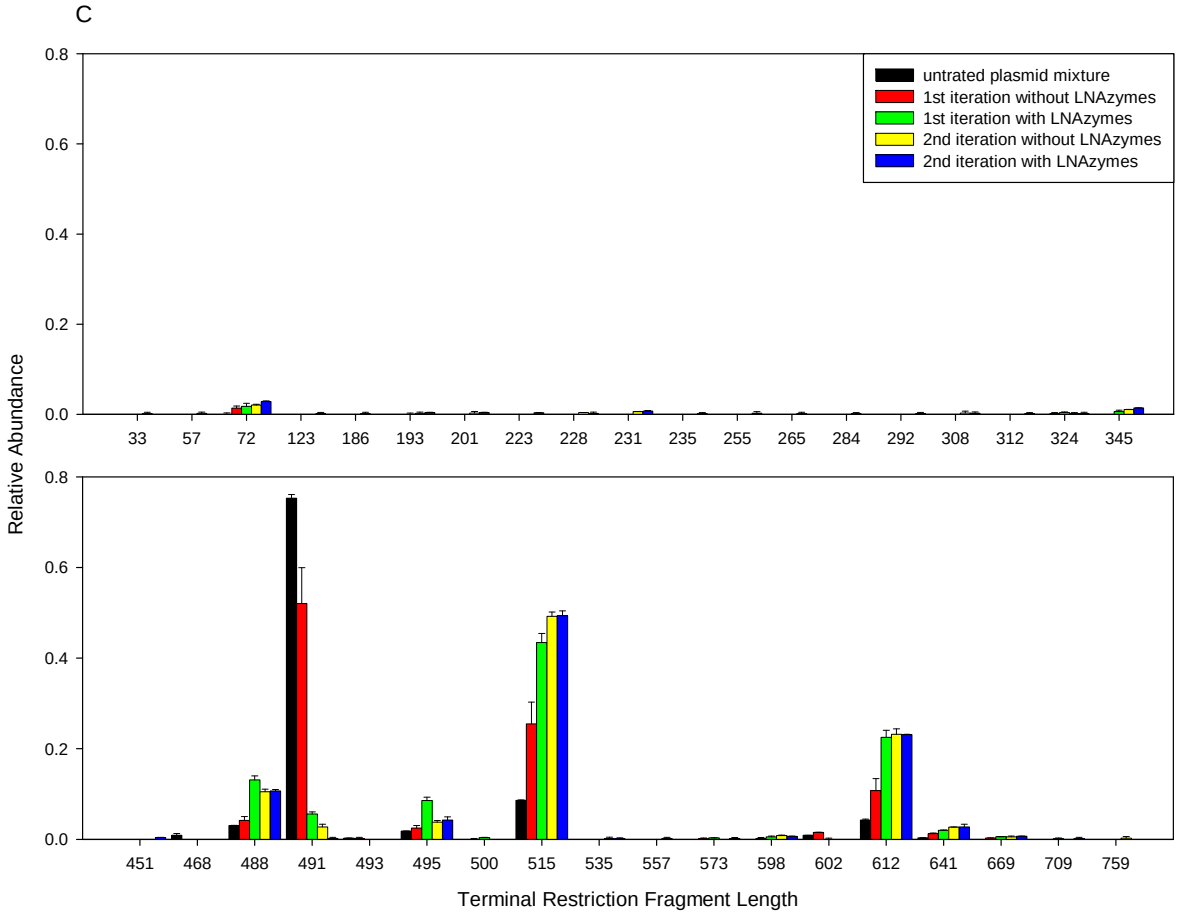
C
untrated plasmid mixture
1st iteration without LNAzymes
1st iteration with LNAzymes
2nd iteration without LNAzymes
2nd iteration with LNAzymes
Relative Abundance
0.0
0.2
0.4
0.6
0.8
33 57 72 123 186 193 201 223 228 231 235 255 265 284 292 308 312 324 345
451 468 488 491 493 495 500 515 535 557 573 598 602 612 641 669 709 759
Terminal Restriction Fragment Length

Figure S2 D

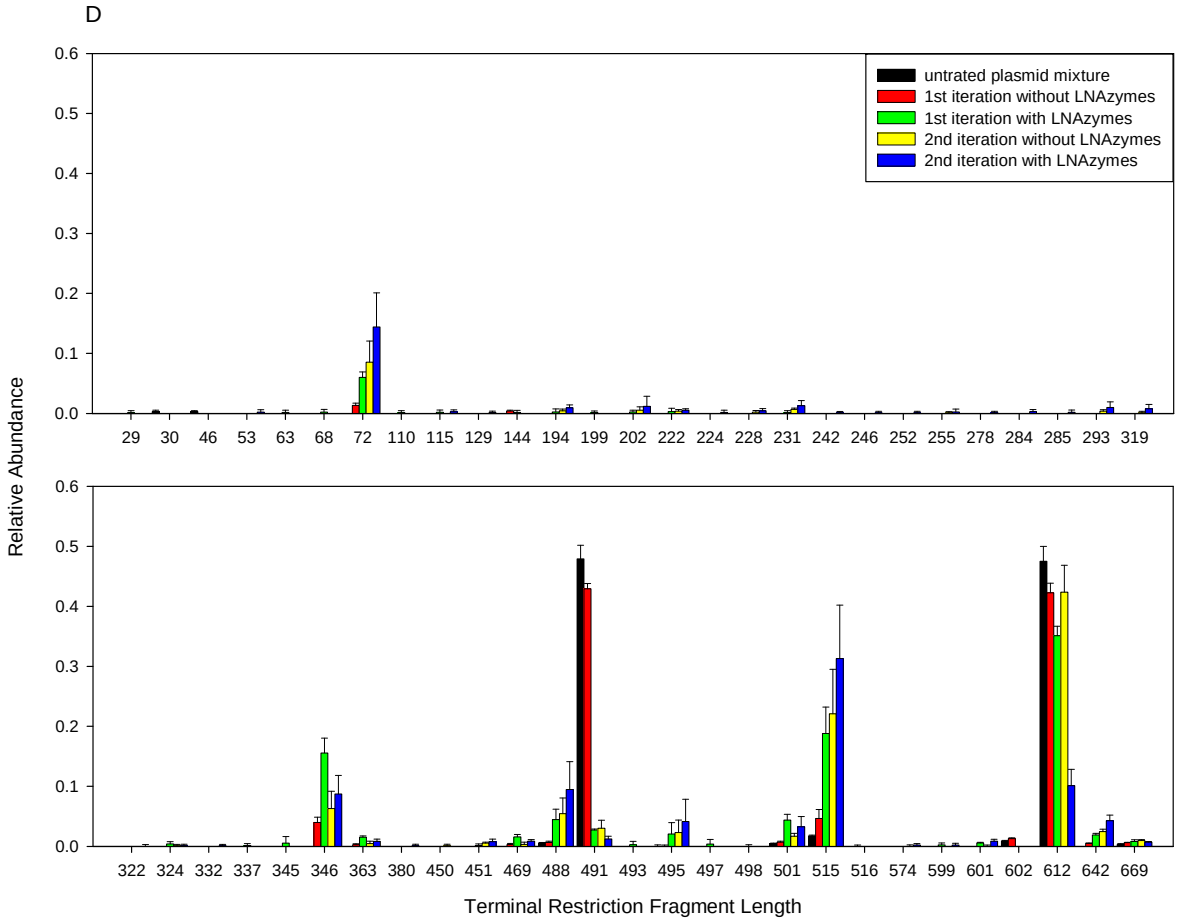

Fig. S2. Evaluation of the LNAzyme cleavage protocol with artificial mixtures of cloned partial 16S rRNA genes (see Table 1 in the main text). The bars show the relative abundances of the rRNA genes in the mixtures before and after one or two iterations of the LNAzyme-mediated depletion of the *Nitrosomonas* and/or *Nitrospira* rRNA. Control experiments without added LNAzymes are also shown (the second iteration without LNAzyme was performed after a first iteration with LNAzyme). The relative abundances were determined by T-RFLP analysis. This figure shows the complete T-RFLP profiles, whereas Fig. 3 (main text) contains the relevant peaks only. Terminal restriction fragment lengths were determined to be 488 bases for *Commamonas*, 492 bases for *Nitrosomonas*, 515 bases for the *Chloroflexi*-related organism, and 612 bases for *Nitrospira*. Panels (A)-(D) show the same experiments as the respective panels of Fig. 3 (main text). The non-target peaks represent artifacts, which are common in T-RFLP analyses of mixed templates and may be caused by the presence of single-stranded PCR amplicons (1), the formation of heteroduplexes or chimeras (2), or by

incomplete or unspecific cleavage of the targets by the restriction enzyme (3). Error bars depict standard deviations of three replicate experiments.

References:

1. **Egert, M., and M. W. Friedrich.** 2003. Formation of pseudo-terminal restriction fragments, a PCR-related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. Appl. Environ. Microbiol. **69:**2555-2562.

2. **Hartmann, M., and F. Widmer.** 2008. Reliability for detecting composition and changes of microbial communities by T-RFLP genetic profiling. FEMS Microbiol. Ecol. **63:**249-260.

3. **Osborn, A. M., E. R. B. Moore, and K. N. Timmis.** 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ. Microbiol. **2:**39-50.