

Common 5S rRNA variants are likely to be accepted in many sequence contexts.

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Over evolutionary time RNA sequences which are successfully fixed in a population are selected from among those that satisfy the structural and chemical requirements imposed by the function of the RNA. These sequences together comprise the structure space of the RNA. In principle, a comprehensive understanding of RNA structure and function would make it possible to enumerate which specific RNA sequences belong to a particular structure space and which do not. We are using bacterial 5S rRNA as a model system to attempt to identify principles that can be used to predict which sequences do or do not belong to the 5S rRNA structure space. One promising idea is the very intuitive notion that frequently seen sequence changes in an aligned data set of naturally occurring 5S rRNAs would be widely accepted in many other 5S rRNA sequence contexts. To test this hypothesis, we first developed well-defined operational definitions for a *Vibrio* region of the 5S rRNA structure space and what is meant by a highly variable position. Fourteen sequence variants (10 point changes and 4 base-pair changes) were identified in this way, which, by the hypothesis, would be expected to incorporate successfully in any of the known sequences in the *Vibrio* region. All 14 of these changes were constructed and separately introduced into the *Vibrio proteolyticus* 5S rRNA sequence where they are not normally found. Each variant was evaluated for its ability to function as a valid 5S rRNA in an *E. coli* cellular context. It was found that 93% (13/14) of the variants tested are likely valid 5S rRNAs in this context. In addition, seven variants were constructed that, although present in the *Vibrio* region, did not meet the stringent criteria for a highly variable position. In this case, 86% (6/7) are likely valid. As a control we also examined seven variants that are seldom or never seen in the *Vibrio* region of 5S rRNA sequence space. In this case only two of seven were found to be potentially valid. The results demonstrate that changes that occur multiple times in a local region of RNA sequence space in fact usually will be accepted in any sequence context in that same local region.

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