

MRPrimerW3: an Expanded Tool for Rapid Design of Gene-Specific and Species-Specific Primers for PCR

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Polymerase chain reaction (PCR) is a standard technique for detecting and amplifying specific nucleic acid molecules. Since its discovery, PCR has expanded its range of applications in line with advancements in science and societal needs, and it is recognized as an essential technique in the fields of biology and medicine. For the best results in PCR experiments, it is essential to design gene-specific primers for experimental purposes. In recent years, as PCR-based experiments are adopted for diagnosis and metagenome profiling research, the need for designing primers that identify and target genes of each species from environmental or clinical samples in single reaction has increased. Although many primer designing tools and databases have been proposed, they often fall short for applications requiring high specificity, such as mixed or diverse sample analysis. Furthermore, these tools and databases typically support a limited number of species, making it challenging to design primers for studies involving less-researched organisms. Our previously developed web-based tool, MRPrimerW2, provides high-specific primer pairs for 9 species, but also faces these limitations. Here, we describe MRPrimerW3, the upgrade version of MRPrimerW2, to satisfy these unmet needs. MRPrimerW3 supports (i) 719 species including vertebrate, invertebrate and protozoa; (ii) designing high quality primers with consideration of filtering constraints including homology test on a huge number of off-target sequences; (iii) the best valid primers by scoring and ranking method; (iv) fast and user-friendly interfaces with upgraded architecture of database and

websites; (v) designing species-specific primers with GPU computation and multithreading; and (vi) expandability, as it allows for independent database construction for each species. We aim to provide primers for an even broader range of species in the future. Free access: <https://primer.kaist.ac.kr/>