USER cloning is a fast and versatile method for engineering of plasmid DNA. We have developed a user friendly Web server tool that automates the design of optimal PCR primers for several distinct USER cloning-based applications. Our Web server, named AMUSER (Automated DNA Modifications with USER cloning), facilitates DNA assembly and introduction of virtually any type of site-directed mutagenesis by designing optimal PCR primers for the desired genetic changes. To demonstrate the utility, we designed primers for a simultaneous two-position site-directed mutagenesis of green fluorescent protein (GFP) to yellow fluorescent protein (YFP), which in a single step reaction resulted in a 94% cloning efficiency. AMUSER also supports degenerate nucleotide primers, single insert combinatorial assembly, and flexible parameters for PCR amplification. AMUSER is freely available online at .