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A method to convert mRNA into a gRNA library for CRISPR/Cas9 editing of any organism

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The clustered regularly interspersed palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein 9) system is a powerful tool for genome editing that can be used to construct a guide RNA (gRNA) library for genetic screening. For gRNA design, one must know the sequence of the 20-mer flanking the protospacer adjacent motif (PAM), which seriously impedes experimentally making gRNA. I have described a method to construct a gRNA library via molecular biology techniques without relying on bioinformatics. Briefly, one synthesizes complementary DNA from the mRNA sequence using a semi-random primer containing a PAM complementary sequence and then cuts out the 20-mer adjacent to the PAM using type IIS and type III restriction enzymes to create a gRNA library. The described approach does not require prior knowledge about the target DNA sequences, making it applicable to any species.

Biography

Hiroshi Arakawa studied at Kyoto University (Kyoto, Japan), where he obtained his diploma and Ph.D in Molecular Biology in Hideo Yamagishi's laboratory. Following post-doctoral studies in Jean-Marie Buerstedde's laboratory in Heinrich-Pette-Institut (Hamburg, Germany), he worked as a Senior Research Fellow in Jean-Marie Buerstedde's laboratory in Helmholtz Center Munich (Munich, Germany). He moved to IFOM (Milan, Italy) as a staff scientist in 2011. He has so far studied the molecular mechanism of immunoglobulin gene conversion and somatic hypermutation, and their application to artificial evolution system. He has recently invented a method to convert mRNA into a gRNA library, which can be applied to forward genetic screening in any species.

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