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Rapid Microbe-independent Plasmid Cloning and Analysis in Mammalian Cells

Stanford researchers have developed Microbe-Independent Deep Assembly and Screening (MIDAS-M), a novel platform that dramatically accelerates the cloning of protein variants and its analysis in mammalian cells.

Characterizing how amino acid changes affect protein function is fundamental to biotechnology and biomedical research. However, traditional methods are slow and laborious, involving cloning variant genes in microbes, purifying DNA, and then transferring them to mammalian cells for testing. This multi-step process creates a significant bottleneck for developing new protein-based therapeutics and for generating the large datasets needed to train machine-learning (ML) algorithms for protein function prediction.

MIDAS replaces this entirely with two rounds of PCR followed by transfection of the completed reactions directly into mammalian cells. All procedures from receipt of PCR primers to protein functional assays are carried out in standard microplate arrays, and can be completed within a time frame as short as 24 hours.

Specifically, full transcription units are synthesized from a single parental plasmid template using a nested primer design that prevents re-amplification of non-mutated template, then transfected directly into mammalian cells in multi-well plates for next-day functional assays. MIDAS-MM enables single-site mutagenesis; MIDAS-MP extends this to simultaneous mutagenesis at multiple sites.

Overall, MIDAS allows for faster cloning and same day functional screening of hundreds to thousands of variants in 48-fold less time and for 10-fold lower cost compared to plasmid cloning and screening.

Stage of Development
Method is fully realized and demonstrated in cells.

Applications

- High-throughput protein engineering and variant screening in mammalian cells
- Sequence-fitness dataset generation for machine learning model training

Advantages

- Eliminates all bacterial cloning steps
- Fully compatible with high-quality multiwell plate assays
- At least 48 times faster and 10 times less expensive than traditional plasmid cloning workflows

Publications

- Wu Y, Wang P, Liu LX, Song D, Qin Q, Gao C, Hageman M, Kirkland TA, Su Y, Lin MZ. Fast analysis and engineering of protein function by microbe-independent deep assembly and screening. *Mol Syst Biol*. 2026 Apr 23. doi: 10.1038/s44320-026-00210-z. PMID: 42026235.

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