HTS Cellulase F Mutants

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Abstract

Background: The field of plasmid-based functional proteomics requires the rapid assay of proteins expressed from plasmid libraries. Automation is essential since large sets of mutant open reading frames are being cloned for evaluation. To date no integrated automated platform is available to carry out the entire process including production of plasmid libraries, expression of cloned genes, and functional testing of expressed proteins.

Results: We used a functional proteomic assay in a multiplexed setting on an integrated plasmid-based robotic workcell for high-throughput screening of mutants of cellulase F, an endoglucanase from the anaerobic fungus Orpinomyces PC-2. This allowed us to identify plasmids containing optimized clones expressing mutants with improved activity at lower pH. A plasmid library of mutagenized clones of the celF gene with targeted variations in the last four codons was constructed by site-directed PCR mutagenesis and transformed into Escherichia coli. A robotic picker integrated into the workcell was used to inoculate medium in a 96-well deep well plate, combining the transformants into a multiplexed set in each well, and the plate was incubated on the workcell. Plasmids were prepared from the multiplexed culture on the liquid handler component of the workcell and used for in vitro transcription/translation. The multiplexed expressed recombinant proteins were screened for improved activity and stability in an azo-carboxymethylcellulose plate assay. The multiplexed wells containing mutants with improved activity were identified and linked back to the corresponding multiplexed cultures stored in glycerol. Spread plates were prepared from the glycerol stocks and the workcell was used to pick single colonies from the spread plates, prepare plasmid, produce recombinant protein, and assay for activity. The screening assay and subsequent deconvolution of the multiplexed wells resulted in identification of improved CelF mutants and corresponding optimized clones in expression-ready plasmids.

Conclusion: The multiplex method using an integrated automated platform for high-throughput screening in a functional proteomic assay allows rapid identification of plasmids containing optimized clones ready for use in subsequent applications including transformations to produce improved strains or cell lines.