Introduction to Directed Evolution, a Powerful Method for Protein Engineering

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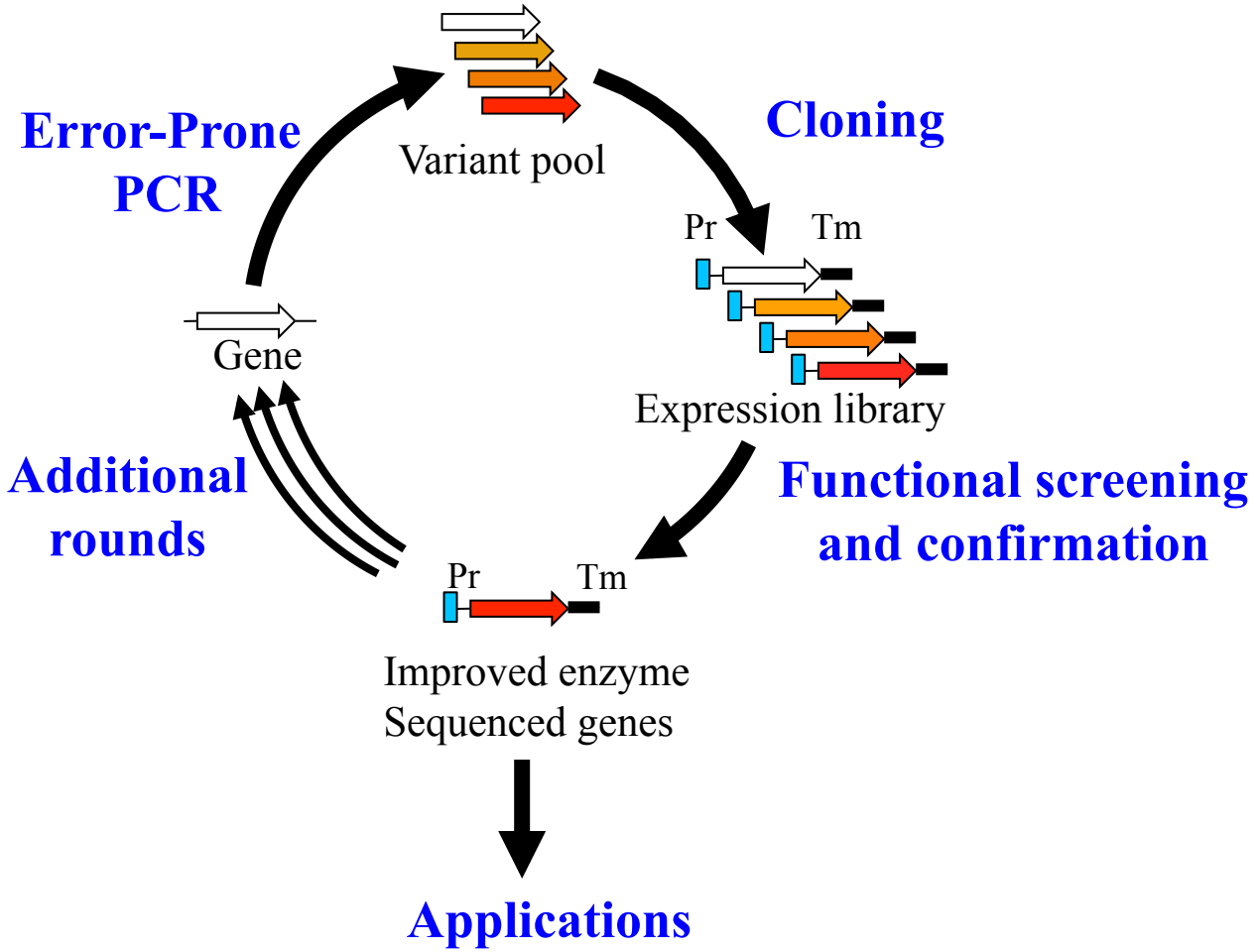
What is Directed Evolution?

- Directed Evolution (DE): a method used in protein engineering that mimics the process of natural selection to evolve proteins or nucleic acids toward a user-defined goal

- Introducing random mutations through mutagenic PCR (or error-prone PCR), and screening for variants that display new or improved characteristics

Directed Evolution Workflow



Screening for Improved Variants

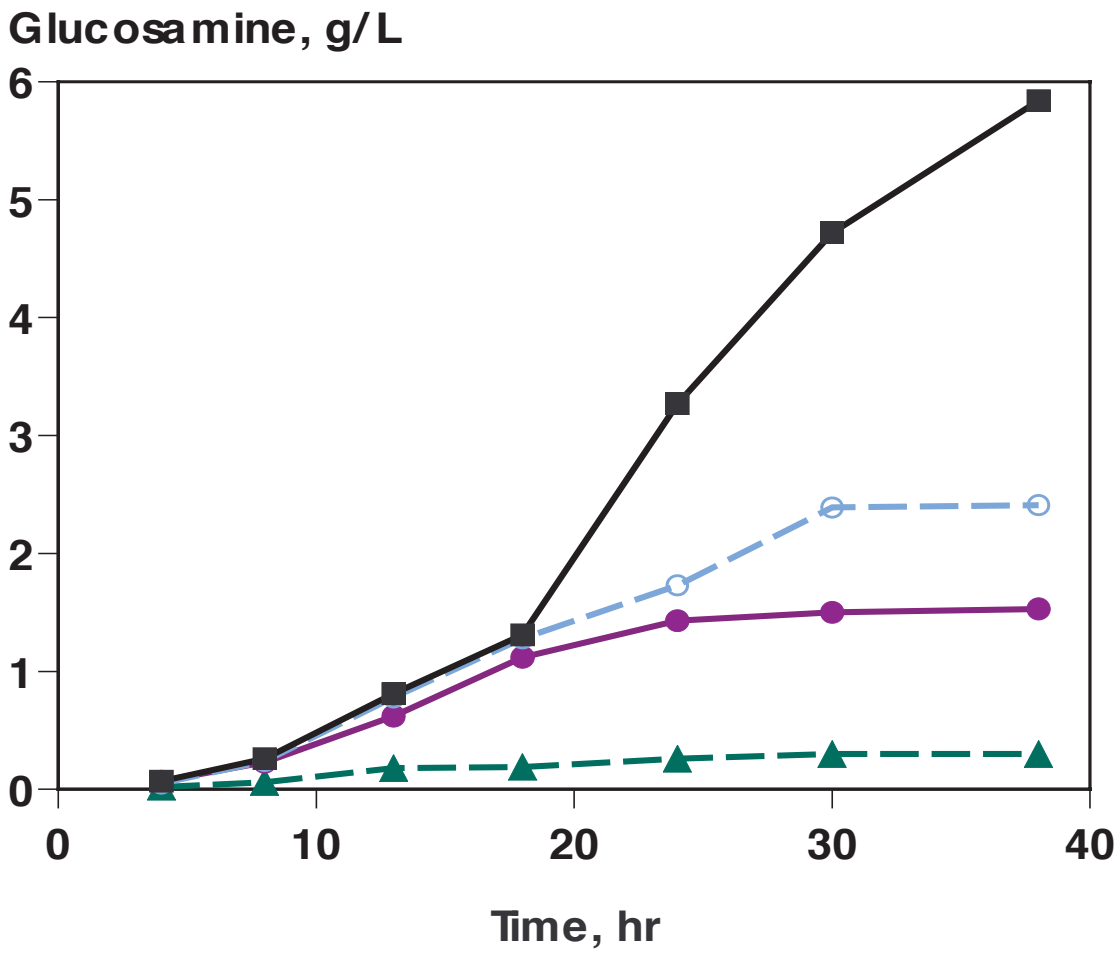
- Screening on agar plates or in liquid medium in microtiter plates
- Activity detection (direct or indirect) preferably medium- or high-through put.
- Screening/selection methods:
  - Selection: relying on a direct link between cell growth and improved or acquired enzyme function (enhanced stress tolerances, nutrient utilization...)
  - Formation of colorogenic or fluorogenic products
  - Halo size: cellulase screening

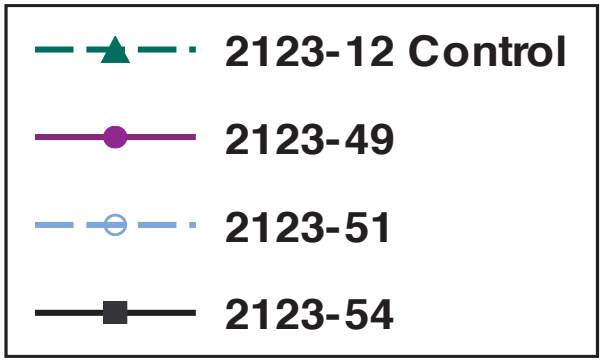
A sensitive, reliable and medium- or high-through-put screening method is key to success in Directed Evolution

BTR Example:
Directed Evolution of Glucosamine Synthase (GlmS)

- GlmS catalyzes the synthesis of glucosamine-6-P from fructose-6-P and glutamine
- Screening by cross-feeding plate assay
  - A glucosamine auxotroph *E. coli* mutant used as an indicator in screening
  - Colonies replicated on plates overlaid with cells of the auxotrophic mutant
  - Halo size: proportional to levels of glucosamine produced by individual clones
- Two screening:
  - 1st: Screened 4368 colonies, 96 candidates, 30 clones confirmed
  - 2nd: screened 6344 colonies, 54 clones confirmed

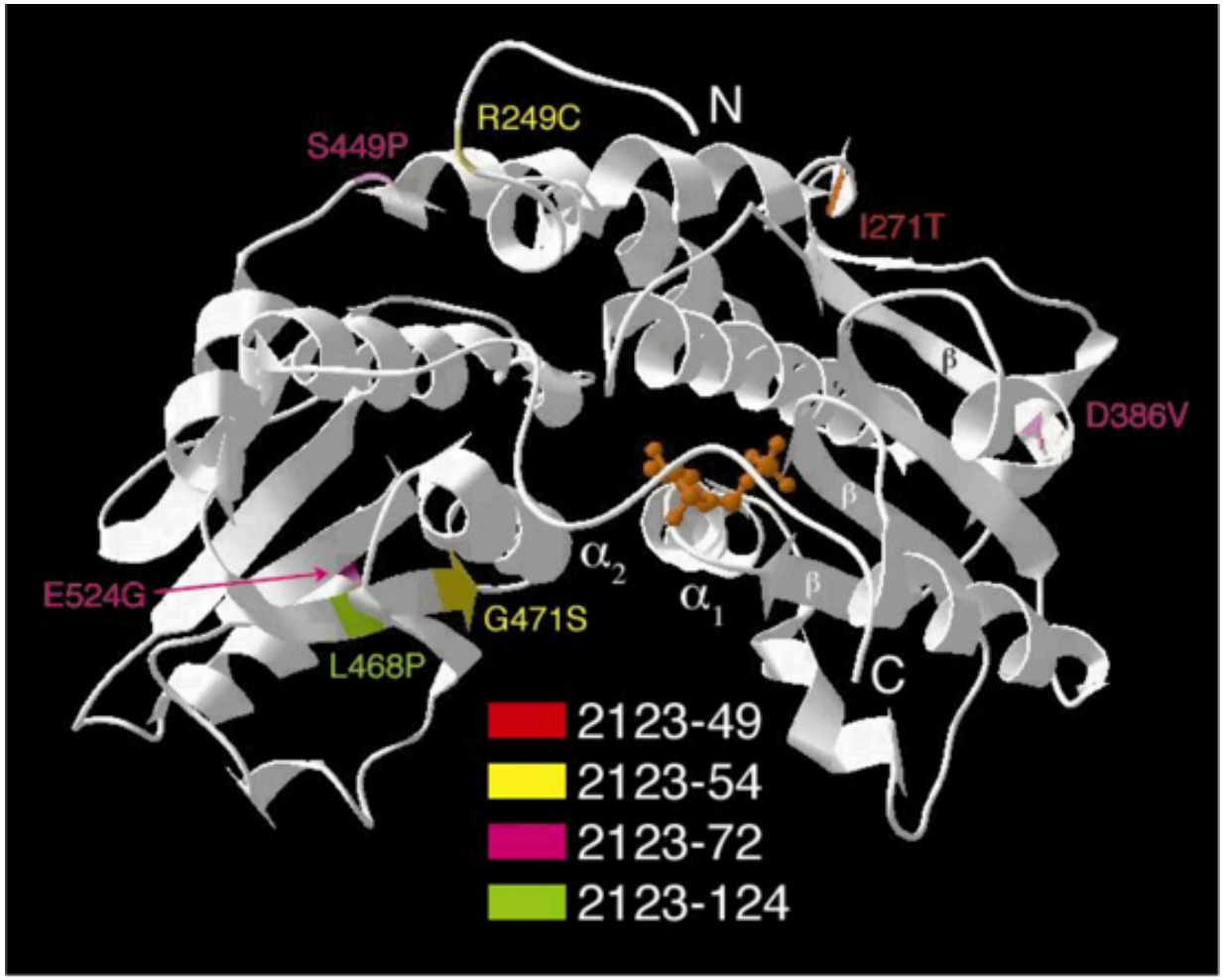
Shake Flask Confirmation of Isolated GlmS Mutants





- Control: WT GlmS, sensitive to product inhibition
- Variants: reduced sensitivity to the inhibition, increased production

Mutations in the GlmS Isomerase Domian



Contract Research Service at Bio-Technical Resources

Bio-Technical Resources (BTR) has the capacity and experiences in designing and applying Directed Evolution for enzyme and strain improvement. Please contact us for further discussion for your needs and we look forward to working with you.

Directed Evolution References:

- Moore J and Arnold F. Directed evolution of a *para*-nitrobenzyl esterase for aqueous-organic solvents. Nat Biotechnol, 1996, **14**:458-467
- Deng MD, Grund AD, Wassink SL, Peng SS, Nielsen KL, Huckins BD, Walsh BL and Burlingame RP. Directed evolution and characterization of *Escherichia coli* glucosamine synthase. Biochimie, 2006, **88:**419-429