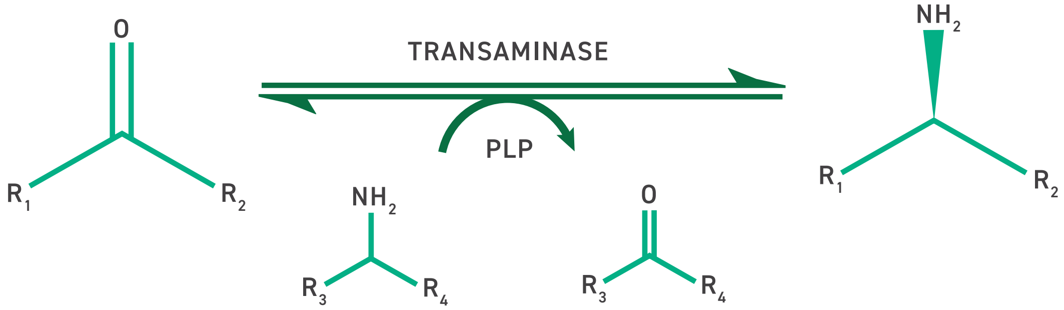
# bitBiome and Twist Bioscience Transaminase Enzyme Engineering

## Incorporating Biocatalysis in Synthetic Chemistry Through Next-Gen Enzyme-Kit Screening

Small molecule drug innovation has created new synthetic challenges, often requiring advanced catalysts to achieve precise chemical transformations. Enzymes, or “biocatalysts,” are particularly effective due to their ability to be highly specific and selective, providing a crucial advantage in installing chiral centers into drugs. The stereoselective amination of prochiral ketones is essential to pharmaceutical manufacturing as chiral amines are vital building blocks for many active pharmaceutical ingredients.

Transaminases (TAs) are the preferred biocatalyst for synthesizing these chiral amines. They catalyze two key reactions: the oxidative deamination of amine donors and the reductive amination of amine acceptors, relying on pyridoxal 5'-phosphate (PLP) as a cofactor.



Traditionally, when faced with optimizing a new reaction, biocatalysis teams must select a starting background from a limited set of known “wild-type” enzymes or from commercial kits with very limited diversity. This starting background is then engineered to meet the specific needs of the reaction. However, low diversity in the set of starting points can hinder the success of the project. For that reason, off-the-shelf kits featuring many diverse, pre-validated enzymes provide an efficient solution for the selection of strong starting point enzymes.

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### UNLOCKING THE POWER OF OUR PLANET’S MICROBES

Environmental microbiomes represent a vast and largely untapped resource for discovering novel biocatalytic enzymes. At the heart of bitBiome’s technology is bit-MAP®, a patented microbial single-cell sequencing method that isolates individual bacteria and amplifies their genomes within microfluidic gel capsules. This approach provides a 100-fold improvement in gene recovery compared to conventional shotgun metagenomics.

Leveraging bit-MAP, bitBiome has built the bit-GEM database—a proprietary repository containing over 2 billion microbial genes and growing by 1 billion genes annually. bit-GEM is thus one of the largest repositories for mining microbial genetic diversity. bit-QED, bitBiome’s custom bioinformatics and AI-driven enzyme discovery and engineering platform can be used to mine bit-GEM for candidate enzymes and then further optimize them.

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## AN EFFICIENT SOLUTION FOR BIOCATALYST DISCOVERY

bitBiome, in collaboration with Twist Bioscience, is excited to introduce the Twist-bitBiome Transaminase Kit, developed to address the challenges of screening complex prochiral ketone substrates in pharmaceutical manufacturing.

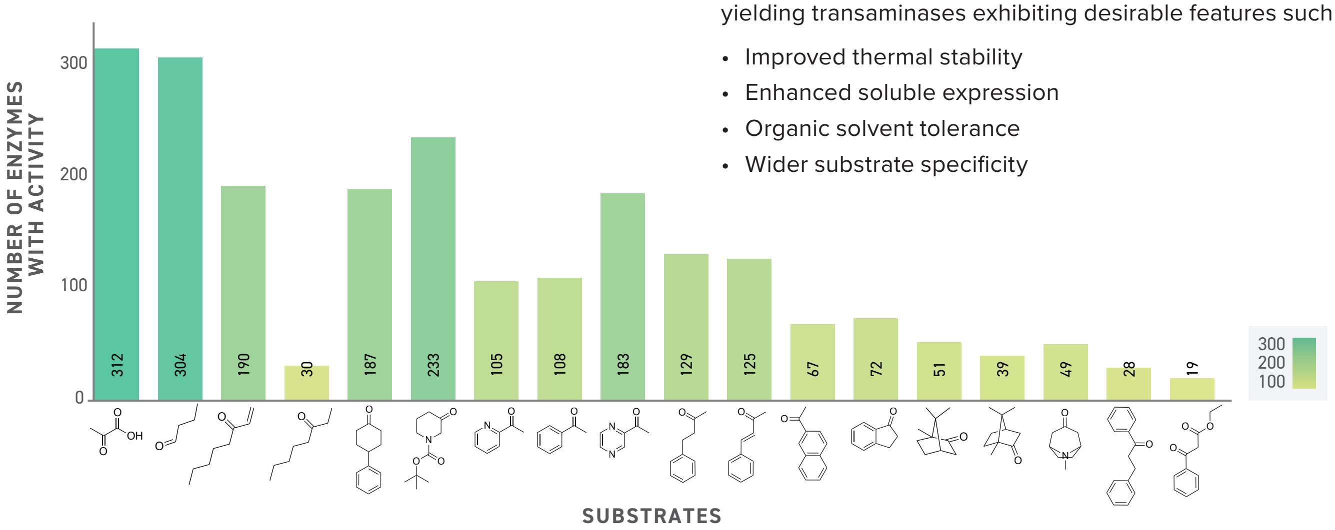
By integrating Twist Bioscience's precision DNA synthesis technology with bitBiome's advanced enzyme discovery and engineering platform (bit-QED), the partnership has created an ideal solution for rapidly identifying and optimizing any desired biocatalyst.

The Twist-bitBiome Transaminase Kit comprises 48 transaminases, selected from a pool of over 1200 candidates identified in bitBiome's proprietary bit-GEM database and further optimized using in silico design with bit-QED. Each transaminase in the kit has been experimentally validated for its broad substrate reactivity, high stereoselectivity, and stability, making it particularly suitable for identifying a strong starting point during the initial catalyst screening steps, even with sterically challenging ketones and reaction conditions.

### Key Features of Twist-bitBiome Transaminase Kit

- Enzymes are sourced from bit-GEM and optimized with bit-QED and show low sequence homology to transaminases found in public databases.
- A set of enzymes experimentally validated against 18 substrates, representing a variety of structural skeletons.
- Most enzymes are solvent stable at 10% DMSO and up to 60 °C.
- Facile screening methodology to identify hits against target ketones.
- All enzymes can be further optimized by bitBiome towards specific process conditions.

Transaminase Activity Against 18 Substrates



## ADVANCED IN SILICO APPROACHES IN BIT-QED FOR MINING BIT-GEM

### Surface Feature Analysis

The bit-GEM database contains millions of novel transaminase sequences. The identification of sequences with the most promise for biocatalysis requires sophisticated in silico search techniques, such as bitBiome's proprietary Surface Feature Analysis (SFA) methodology. SFA moves beyond conventional approaches that rely solely on sequence homology or shape complementarity and characterizes transaminase candidates based on the physicochemical properties of their active sites.

SFA analyzes critical active site features such as:

- Charge Distribution
- Hydrophobicity
- Hydrogen Bonding Networks
- Binding Pocket Architecture

These attributes are derived from high-quality structure models of the enzyme sequences and enable SFA to detect subtle structural differences that can dramatically influence substrate preference and catalytic efficiency.

### Ancestral Sequence Reconstruction

While bit-GEM yielded thousands of hits for S-selective transaminase sequences, R-selective transaminases were primarily discovered in fungal genomes. To optimize these natural sequences for broader substrate selectivity and robustness, bitBiome employed Ancestral Sequence Reconstruction (ASR).

ASR uses the evolutionary history of enzymes to predict and reconstruct ancestral protein sequences. The hypothesis driving ASR is that ancestral enzymes, having existed before specialization to specific substrates, evolved to function under primeval and extreme conditions. These enzymes are thought to exhibit broad substrate specificity, enhanced robustness, and adaptability.

With bitBiome's proprietary and extensive bit-GEM database, ancestral sequences can be reconstructed with high confidence, yielding transaminases exhibiting desirable features such as:

- Improved thermal stability
- Enhanced soluble expression
- Organic solvent tolerance
- Wider substrate specificity

## OPTIMIZING ENZYMES FOR PROCESS CHEMISTRY

The Twist-bitBiome Transaminase Kit not only accelerates the discovery of initial enzyme candidates with desired chemistry but also provides a pathway for further optimization using bitBiome's and Twist Bioscience's advanced platforms. Upon finding candidate enzymes with the transaminase kit, bitBiome can rapidly optimize these starting point enzymes to generate enzymes uniquely suited to your specific process conditions.

**Further Genome Mining of the bit-GEM Database:**
Multiple close homologs of the initial enzyme candidate exist in the bit-GEM database with improved features for any particular process condition. After identifying candidate sequences, bitBiome can rapidly identify alternative sequences with improved qualities.

**Twist Bioscience Gene Synthesis:**
Leveraging a high-precision, silicon-based DNA synthesis platform, Twist Bioscience produces Multiplex Gene Fragments with high uniformity and representation. This technology supports high-throughput synthesis of at least 1000 gene fragments per pool, enabling high-throughput screening of candidate enzymes identified from bit-GEM and optimized via bit-QED.

**bit-QED Protein Engineering Platform:**
bit-QED is a state-of-the-art workflow for tailoring enzyme performance to specific process needs. It leverages established in silico techniques such as SFA, ASR, and the latest advances in protein large language models. Clusters of transaminase sequences generated from bit-QED's advanced techniques are then synthesized and cloned using Twist Bioscience's gene synthesis capabilities. bitBiome will then express these genes to generate the enzymes of interest, which are then evaluated against many challenging substrates to confirm their capabilities.

**QUESTIONS?** Get in touch at **sales@twistbioscience.com** or learn more at **twistbioscience.com**

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