DOE Bioenergy Technologies Office (BETO) 2017 Project Peer Review

SynTec

Synthetic Biology for Tailored Enzyme Cocktails

Biochemical Conversion
Sarah Teter
Novozymes, Inc

9 March 2017

GOAL STATEMENT

ACCELERATE INNOVATION IN ENZYME DISCOVERY

REDUCE COSTS FOR PRODUCING CELLULOSIC SUGARS

- Deliver a screening platform which can be used to reduce discovery time required for tailoring enzymes to process specific contexts
- Demonstrate use of screening tool to achieve reduction in net production costs for cellulosic sugars
- Relevance and tangible outcomes for the United States: Generalizable improvements to enzyme screening technology leads to lower biomass to sugar biochemical conversion costs

- Project Outcome: Proof of concept of achieved – Use of screening tool (to deliver improved enzyme) led to 1.4X cost reduction in sugar price in benchmark technoeconomic model (target was 1.5X)

QUAD CHART OVERVIEW

TIMELINE

- Start Aug, 2013
- End May, 2016

(R&D work done Jan 2016)

- 100% complete

BARRIERS ADDRESSED

- Bt –G (Cellulase enzyme loading)
- Bt-K (Biochemical conversion process integration)
- Others: Hemicellulase enzyme loading

BUDGET

	FY 13/14 Costs	FY 15 Costs	FY 16 Costs	Total Funding (FY 14-FY16)
DOE Funded	$ 759,333	$1,541,398	$ 199,269	$ 2,500,000
Project Cost Share Novozymes	$ 268,689	$ 994,563	$ 175,582	$ 1,438,834
Project Cost Share MBI	$ 50,919	$ 29,718	$ 11,055	$ 91,692
Project Cost Share NZNA	$ 6,349	$ 1,179	$ 2,274	$ 9,802

PARTNERS

- MBI (16.3%) technoeconomic modelling, enzyme performance validation
- Novozymes North America (NZNA) (0.5%), enzyme performance validation

PROJECT OVERVIEW

ACCELERATE ENZYME DISCOVERY-TO DELIVER LOWER COSTS FOR BIOMASS SUGAR PRODUCTION

- Develop faster, combinatorial technologies for mining natural diversity-
  - What are the best “parts” out there that can be harnessed to deconstruct biomass?
  - Past natural diversity screening at Novozymes- slow steps are **purifying and quantifying** enzymes once they’ve been cloned and expressed
  - Identify potential synergism between domains in primary screens- in past, limited combinations of domains from nature are screened for synergy
  - Investigate potential for improving synergy by “proximity” effects: cellulosome paradigm

- Rapid tailoring of enzyme cocktails for unique feedstock types and unique processes
  - AFEX-PCS (**A**mmonia **F**iber **Ex**pansion **P**retreated **C**orn **S**tover) as a test case: enzymatic conversion of hemicellulose fraction
  - “Clean sugar” concept- avoid salts from pH adjustment
  - Goal: engineer an enzyme cocktail that performs better than commercial cocktail benchmark, and without need for pH adjustment prior to hydrolysis

TECHNICAL APPROACH- OVERVIEW

Faster screening pipeline delivered through:

- Streamlining enzyme purification
- Streamlining enzyme dose normalization

} "de-bottlenecking"

Better likelihood of uncovering synergistic components:

- Exploit automation to create large numbers of combinations - look for hydrolysis that is "greater than sum of the parts"
- Test whether bringing parts together in a complex can improve performance (vs. "free" domains)

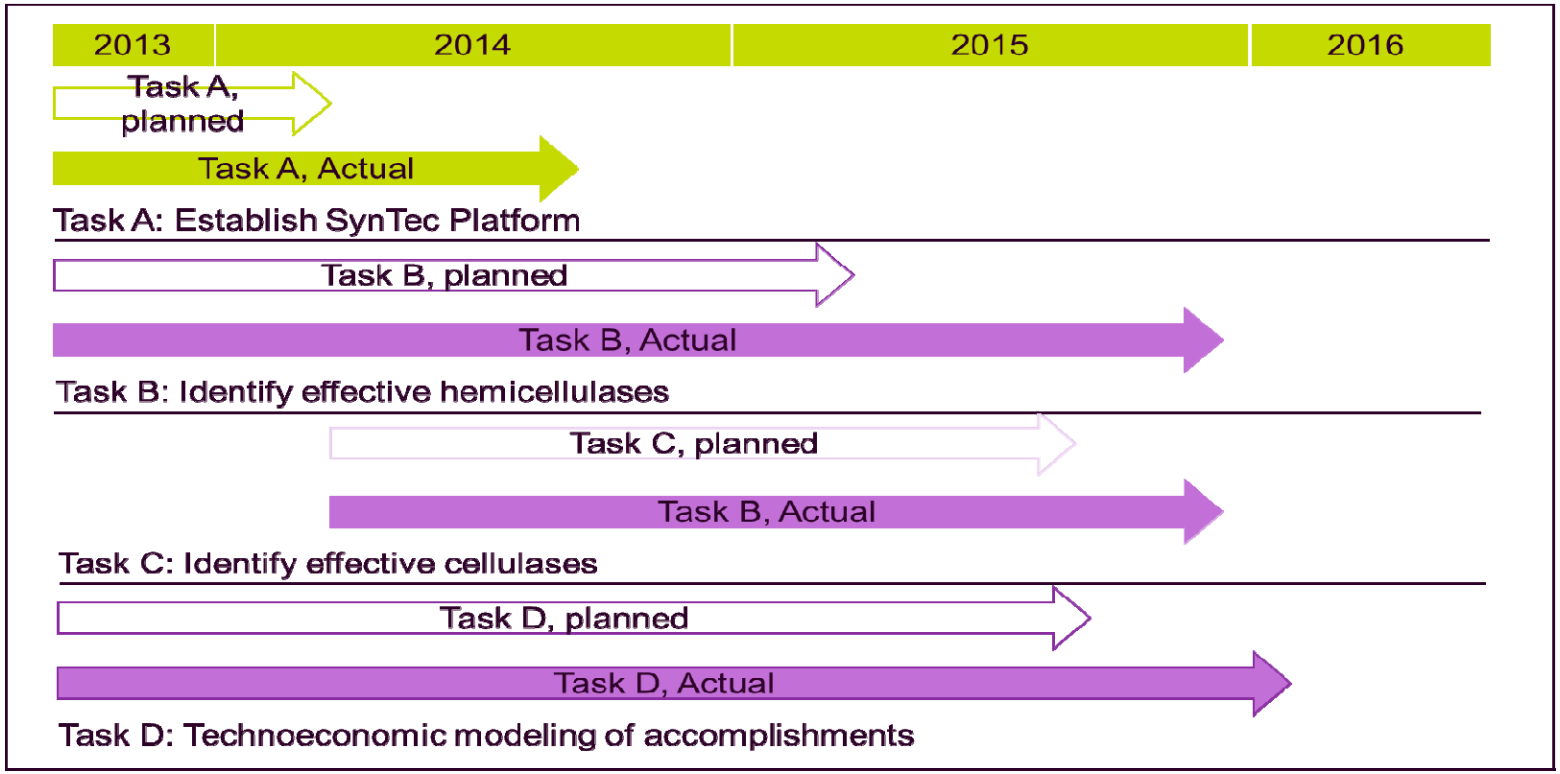
Quantify improvements through technoeconomic modeling:

- **Critical Success Factor:** Cost reduction through improved enzyme performance, assayed without pH adjustment, ~pH 6.2; corresponds to a 1.5X sugar cost reduction relative to CTec3/HTec3 measured at benchmark optimal pH of 5.0

Challenges

- Rapid construction of a novel screening platform
- Short enzyme screening time frame

MANAGEMENT APPROACH



Task A: Establish SynTec Platform

- Task A was extended through 12 mo. due to delay in engineering a thermostable scaffold

Task B: Identify hemicellulases for AFEX PCS

Task C: Identify cellulases for AFEX PCS

Task D: Technoeconomic modeling of accomplishments

TECHNICAL APPROACH

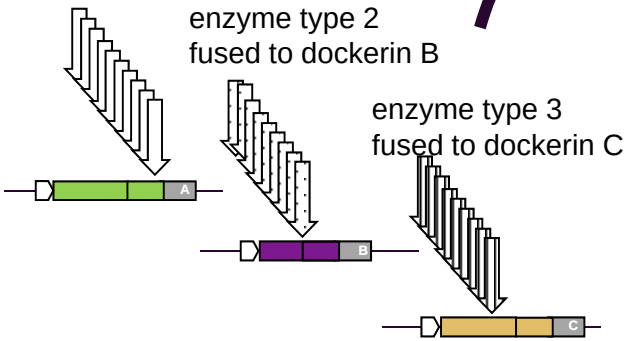
LEVERAGING PROTEIN: PROTEIN INTERACTIONS TO BUILD A STREAMLINED ENZYME SCREENING PIPELINE

1) Gene modules grouped by enzyme activity: each group with specific “tag”

enzyme type 1
fused to dockerin A

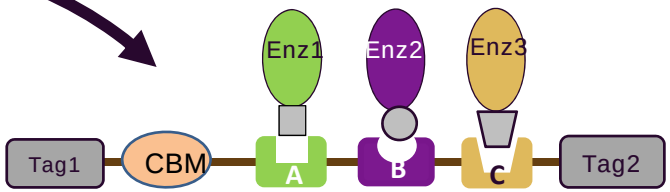
enzyme type 2
fused to dockerin B

enzyme type 3
fused to dockerin C



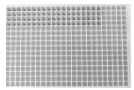
2) Expression of tagged modules

3) Automation to create enzyme mixtures (Enz 1,2,3)



4) Add Scaffold. Tags mediate *in vitro* assembly into a macromolecular complex

5) Isolate complexes (tag-based purification, dose normalization)



6) Assay various combinations of modules on a pretreated biomass feedstock (Output: glucose xylose)

7) Hits get included in next screen

TECHNICAL ACCOMPLISHMENTS- PREVIOUSLY REPORTED

2015 BETO Platform Review reported:

- Engineering a thermostable scaffoldin- Stable dockerin:cohesin interactions throughout hydrolysis allows us to screen for intramolecular synergy between domains
- Expression issues solved: Two-tag purification of scaffold gives highly pure screening reagent. Selection of dockerin tags to allow for effective expression
- Established automated screening-Optimized method for combinatorial screening of enzyme modules from nature
- Hemicellulase screening complete, estimated ~1.5X dose reduction, in low solids assay

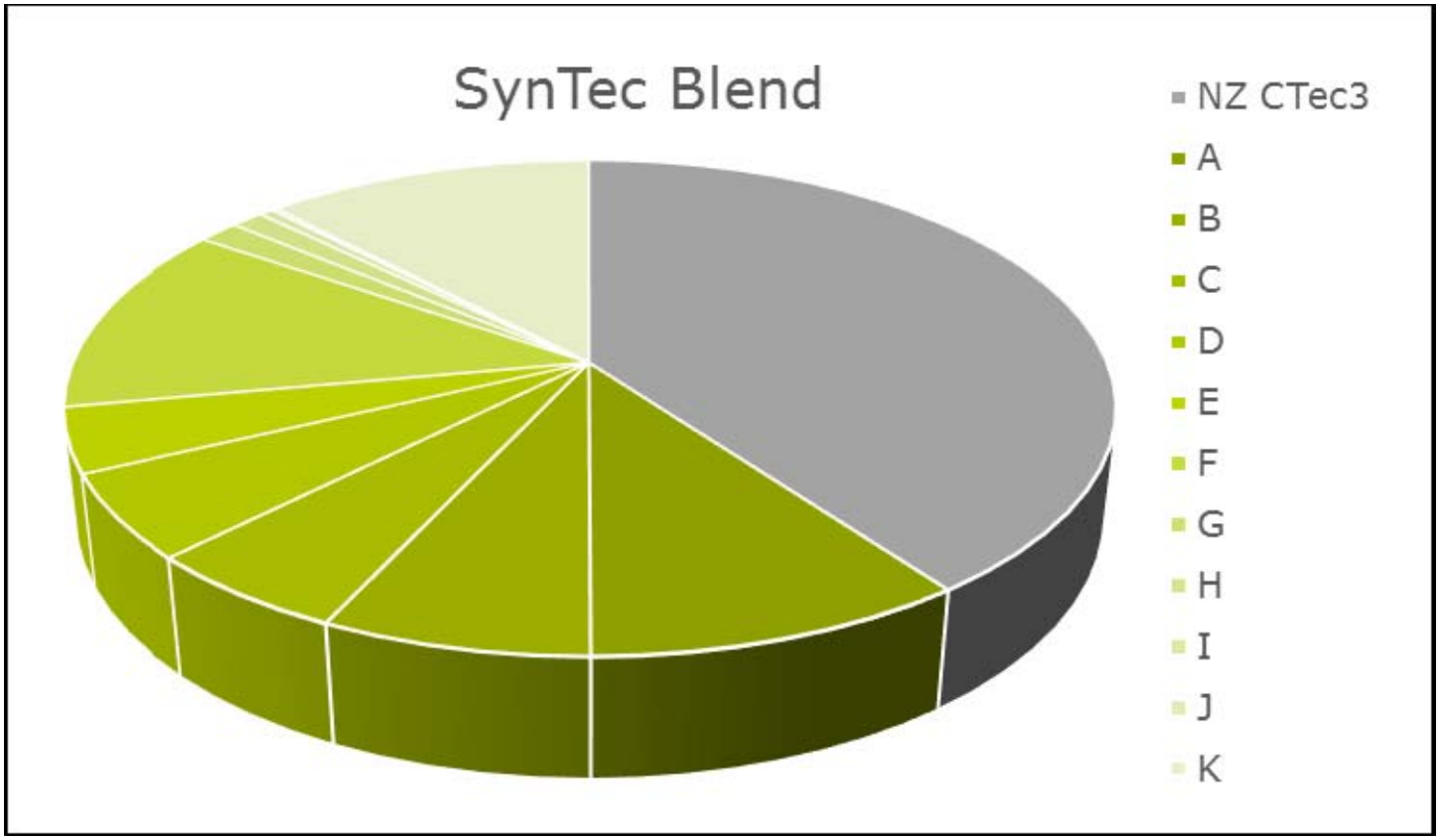
RELEVANCE

- SynTec contributes to Conversion R&D in the BETO MYPP. Overall goal: "…*develop commercially viable technologies for converting biomass feedstocks into ….chemical intermediates…*"
  - Technology will accelerate enzyme discovery
  - Reduction of enzyme dose required for production of sugars from biomass

- Application in emerging biomass industry:
  - Provides a means to quickly tailor enzyme cocktails to better match upstream and downstream unit process steps
  - AFEX example: rapid discovery of new hemicellulases that are not part of benchmark cocktail
  - ~pH 6.2 example: optimize cocktail to match process pH from pretreatment

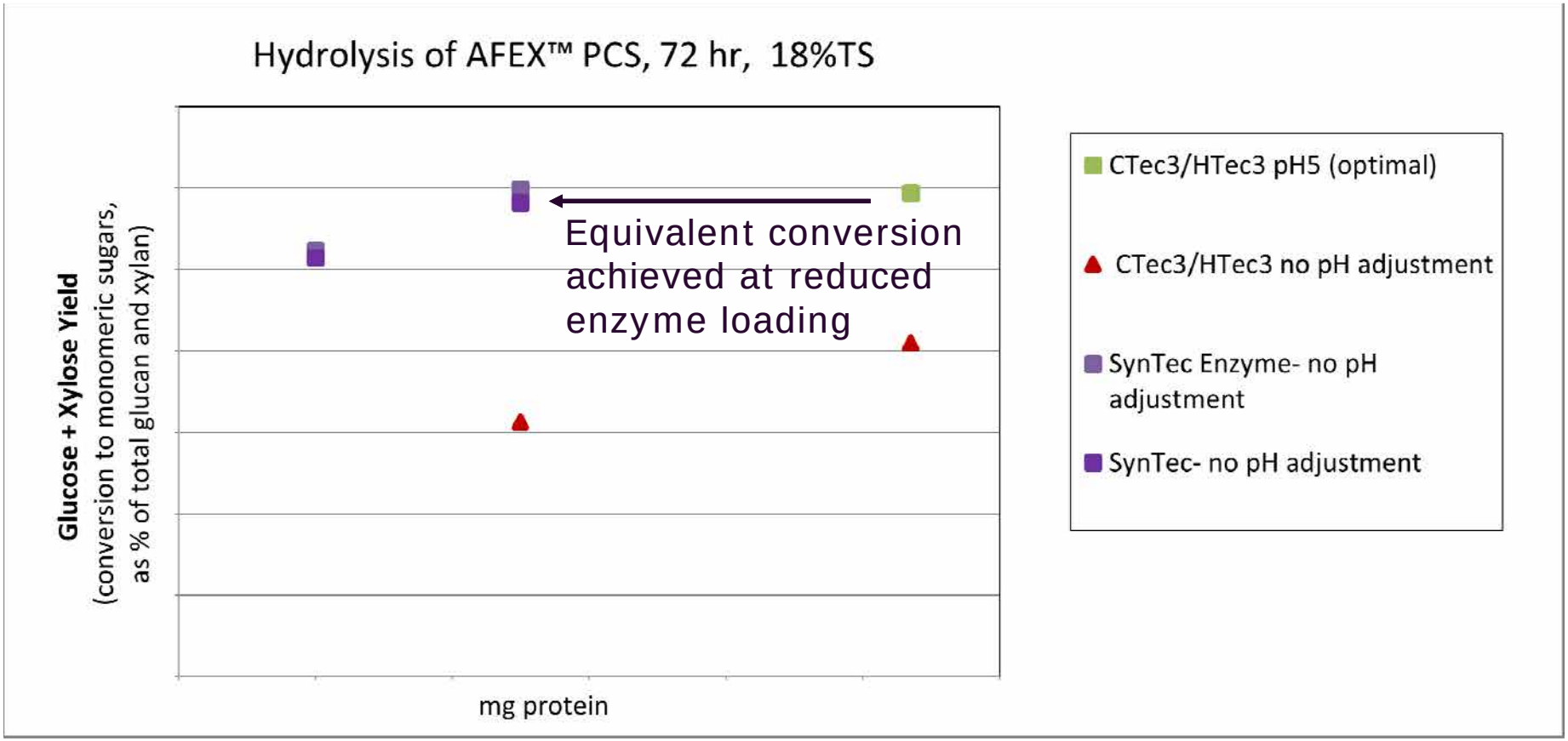
- Relevance to BETO, emerging industry, and market place: accelerating rate of enzyme cost reduction through technology innovation.

Novel enzymes identified in SynTec project were blended with CTec3



- CTec3/HTec3 - baseline enzyme cocktail (reference to which enzyme performance improvements were compared with)
- Novel enzymes (A-K) identified in project were added to CTec3, to create a “semi-synthetic blend” (product + purified components)

SynTec enzyme reaches equivalent release of sugars at reduced enzyme dose vs. Cellic CTec3



On a total sugar basis, SynTec blends achieve target level of conversion at 1.6X less enzyme *vs* CTec3 at the optimal pH

ACHIEVEMENTS

- Proof of concept of new approach- achieved 1.4X cost reduction in sugar price in benchmark technoeconomic model
  - Target was 1.5X, Cost reduction through improved enzyme performance, assayed without pH adjustment, relative to CTec3/HTec3 measured at benchmark optimal pH of 5.0
  - Relative to previous achievements, technology delivers reductions through tailoring in shorter time and with fewer resources
  - Results presented at four conferences; 5 patent applications (see extra slides for details)

No support for intramolecular synergy

- Among current top hits, we have seen benefit of including novel domains in a macromolecular complex. “Free” enzyme combinations perform similarly to same domains loaded onto the scaffold.
- Identified novel synergism between domains in primary screens- more extensive combinations of domains screened for synergy than in past

SUMMARY

KEY POINTS:

1. **Overview** Developed a faster, superior method for screening modules from nature for application in biomass conversion to sugars
2. **Approach** SynTec screening allowed us to streamline bottlenecks in purification and quantitation, and allows for broader screening of potentially synergistic modules *vs.* previous methods
3. **Technical Accomplishments**
   - 1.6X dose reduction achieved (at unadjusted pH) vs. CTec3 under its optimal conditions
   - 1.4X reduction in sugar costs in technoecnomic model vs. base case
4. **Relevance** faster discovery of enzyme cocktails tailored for specific conversion processes

Additional Slides

SynTec Team

Novozymes:
Technical project leader: Janine Lin
Johnnie Hahm, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, Fang Liu, Ronald Mullikin, Ani Tejirian, Carly Todd, William Widner, Elizabeth Znameroski, James Broering, Grace Cooley, and Kurt Creamer

MBI:
Farzaneh Teymouri
Bryan Bals

ACRONYMS

- AFEX PCS: Ammonia fiber expansion pretreated corn stover
- CBM: Carbohydrate binding module
- SynTec: Synthetic biology for tailored enzyme cocktails
- Enz: Enzyme
- CV: Coefficient of variation. Obtained by dividing the standard deviation by mean of the data
- MBI: Michigan Biotechnology Institute
- NZNA: Novozymes North America

PUBLICATIONS, PATENTS, PRESENTATIONS, AWARDS, AND COMMERCIALIZATION

Presentations

- Johnnie Hahm, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, **Janine Lin,** Fang Liu, Ronald Mullikin, Ani Tejirian, Sarah Teter, Carly Todd, William Widner, Elizabeth Znameroski, James Broering, Grace Cooley, and Kurt Creamer. **Discovering optimally tailored enzyme cocktails using a synthetic screening tool.** Invited talk**, 37th Symposium on Biotechnology for Fuels and Chemicals,** April 27-30, 2015, San Diego, CA.
- Johnnie Hahm, Paul Harris, Sumati Hasani, Ian Haydon, Tia Heu, Aubrey Jones, Michael Lamsa, Janine Lin, Fang Liu, Ronald Mullikin, Ani Tejirian, Sarah Teter, Carly Todd, William Widner, Elizabeth Znameroski. **Development and application of a synthetic cellulosome-based screening platform for enhanced enzyme discovery**. Accepted for poster presentation at **11th Carbohydrate Engineering Meeting**, May 10-13, 2015, Espoo, Finland.
- Ian Haydon, Johnnie Hahm, Elizabeth Znameroski, Fang Liu, Tia Heu, Sumati Hasani, Michael Lamsa, Aubrey Jones, William Widner, Ronald Mullikin, Paul Harris, Sarah Teter, Janine Lin. **Development of modular, self-assembling synthetic protein complexes as screening platforms for enhanced enzyme discovery**. Poster, **AIChE / SBE 2nd Synthetic Biology Conference**, June 10-13 2015, Boston, MA
- Sarah Teter and Kurt Creamer. "**R&D at Novozymes in Biomass Conversion: Advancing towards a Biobased Economy"** Oral presentations at Great Lakes Bioenergy Research Center Annual Retreat. May 21, 2015, South Bend, IN.

PATENT APPLICATIONS

1. Polypeptides having xylanase activity and polynucleotides encoding same –13145-US-PRO, 13145-WO-PCT.

2. Polypeptides having arabinofuranosidases activity and polynucleotides encoding same – 13147-US-PRO, 13147-WO-PCT.

3. Polypeptides having beta-xylosidase activity and polynucleotides encoding same –13157-US-PRO, 13157-WO-PCT.

4. Polypeptides having endoglucanase activity and polynucleotides encoding same – 14152-US-PRO.

5. Endoglucanase for neutral pH, high-temperature lignocellulose hydrolysis - 14153-US-PRO