Title: High-Yield Production and Purification of a Recombinant *Serratia marcescens* Nuclease in *Komagataella phaffii*

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1. Introduction

Nucleases are critical tools for removing contaminating nucleic acids during recombinant protein purification, reducing viscosity, and improving downstream processing. The nuclease from *S. marcescens* is a highly efficient, dimeric protein with two essential disulfide bonds. It offers superior catalytic efficiency. Approximately **4x greater than staphylococcal nuclease** and **34x greater than bovine pancreatic Dnase**[1].

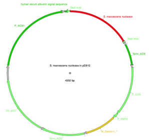
Objective: To develop a robust fermentation and purification process for the high-yield production of recombinant *S. marcescens* nuclease using an animal-origin free (AF) system in *K. phaffii.*

2. Methods

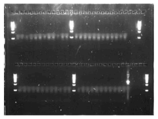
Strain & Clone Construction

Host Strain: *Komagataella phaffii* PPS-9011 (BG11, *aox1*Δ (*MutS*)) from ATUM.

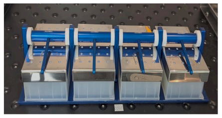
Clone Design: A codon-optimized transformation clone was constructed by ATUM.



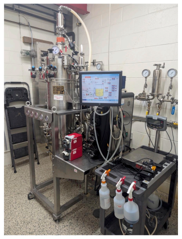
Secretion Sequence Screening: 11 different secretion sequences were screened in a 4 x 24 well plate format (96 samples per sequence). Lead candidates were identified via DNA/RNA digestion assay of culture supernatants.



Gel of typical DNA/RNA digest with culture supernatants.



4 by 24 well 2 ml shake plates



Bioflo 610 production scale fermenter

Fermentation Optimization

Initial fermentation scale-up revealed methanol intolerance in selected clones.

In collaboration with **Validogen** an optimized feeding strategy was developed:

First, generation of high cell mass, followed by induction with minimal necessary methanol to achieve induction.

Purification Process

Concentration & Diafiltration: Cell removal was achieved via depth filtration. The cell-free filtrate was concentrated and diafiltered into 10mM Tris-HCl, 2mM $MgCl_2$, pH 8.0 via tangential flow filtration.

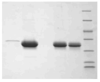
Protease Inhibition: The concentrate was treated with PMSF, then diafiltered again to remove the excess inhibitor and degradation products.

Ion-Exchange Chromatography: Final purification was achieved using an optimized gradient elution on a Q Sepharose column leading to ≥ 95% purity.

Formulation: The final product was dialyzed into a storage buffer (20mM Tris-HCl, 2mM $MgCl_2$, 20mM NaCl, 50% Glycerol) and diluted down to a working concentration of ≥250ku/ml.

3. Results & Product Profile

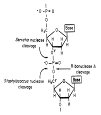
Purity: >95% as determined by SDS-PAGE. Following PNGase F treatment to remove N-glycosylation from expression host.

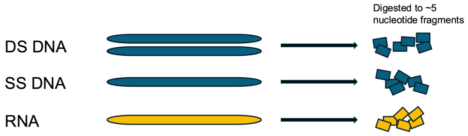


Endotoxin: <0.001EU / Ku enzyme due to non-bacterial expression host and purification process.

Versatility: Efficiently degrades all forms of DNA and RNA (single/double-stranded, linear/circular).[3]

Digestion Profile: Produces 5'-monophosphate terminated oligonucleotides of 3-5 bases. Ineffective on fragments <5 bp. [2]





Compatibility:

Effective on both native and heat-denatured nucleic acids.

Compatible with Triton X-100.

pH Optimum: 8.2 - 9.2. Active between 6.0 and 10.0.[3]

4. Key Applications

- **Nucleic Acid Removal:** Effectively removes DNA and RNA from recombinant protein preps.
- **Viscosity Reduction:** Significantly lowers sample viscosity for easier handling.
- **Improved Analysis:** Prevents cell clumping during dissociations. Enhances protein resolution on gels.
- Worthington *S. marcescens* nuclease has found significant utility internally. Several processes rely on large-scale lysis of bacterial cells. Historically, nucleic acid contamination has been managed with comparatively large quantities of pancreatic DNase I or mechanical shearing. Fractionation steps following lysis such as clarification or ammonium sulfate precipitation were often inefficient, requiring long centrifugation times due to residual viscosity. A standard addition of at 50 units/ml (only 200ku per kg of cell paste) leads to extremely rapid fluidization of the lysate and has shortened processing times and increased recovery.

5. Conclusion

We successfully established a scalable, animal free production process for a highly active *S. marcescens* nuclease in *K. phaffii*. By overcoming initial challenges in clone selection and fermentation, we now produce a chromatographically pure, versatile, and highly efficient nuclease ideal for downstream bioprocessing applications.

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