ELECTROPHORETIC ASSESSMENT OF RECOMBINANT Λ-EXONUCLEASE PRODUCTION IN DIFFERENT *E. COLI* STRAINS

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INTRODUCTION

- **Lambda exonuclease (λ-exo),** isolated from lambda bacteriophage, hydrolases double-stranded DNA (dsDNA) in the highly processive manner in 5'→3' direction, yielding mononucleotides and single-stranded DNA (ssDNA). These unique enzymatic properties offer several promising biotechnological applications, such as highly sensitive quantification of DNA modifications and single-molecule sequencing.
- Electrophoretic techniques provide a valuable set of tools for monitoring protein levels at any stage of recombinant production.
- **The aim of this study** was to optimize soluble expression of λ-exo in *E. coli* strains and elucidate the potential of densitometric analysis as a screening platform for maximizing recombinant protein production.

MATERIALS AND METHODS

- N-terminally His-tagged λ-exo thrombin fusion

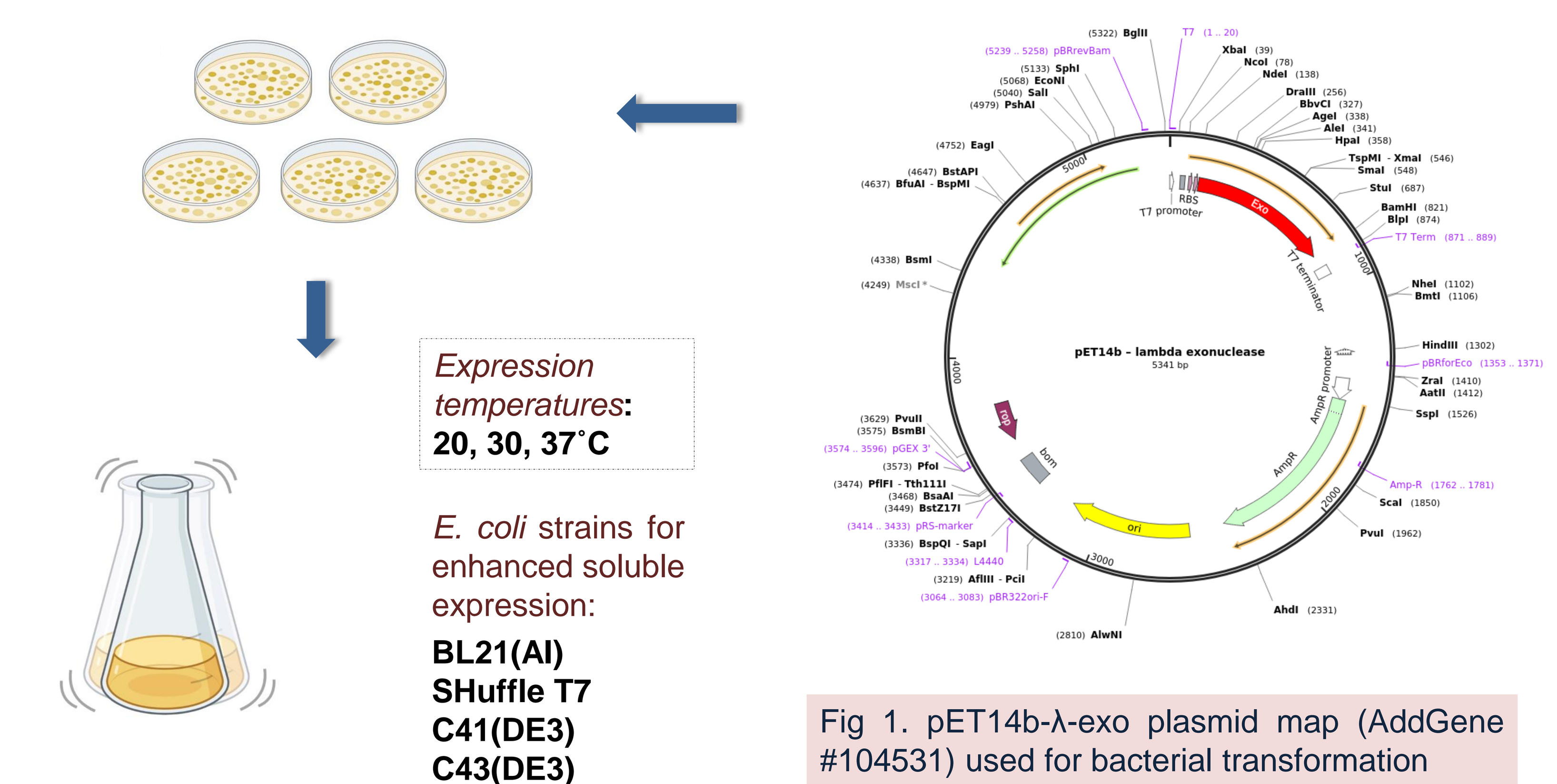
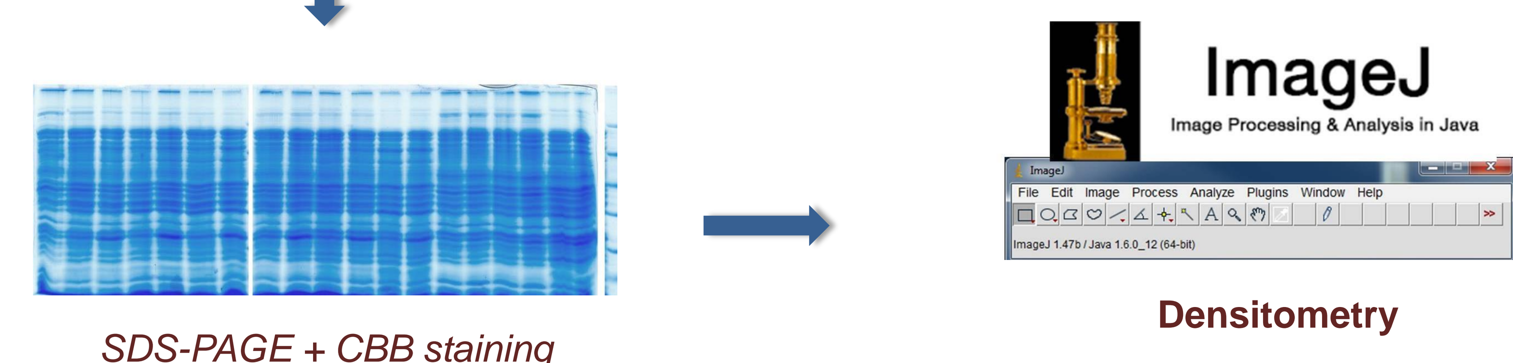
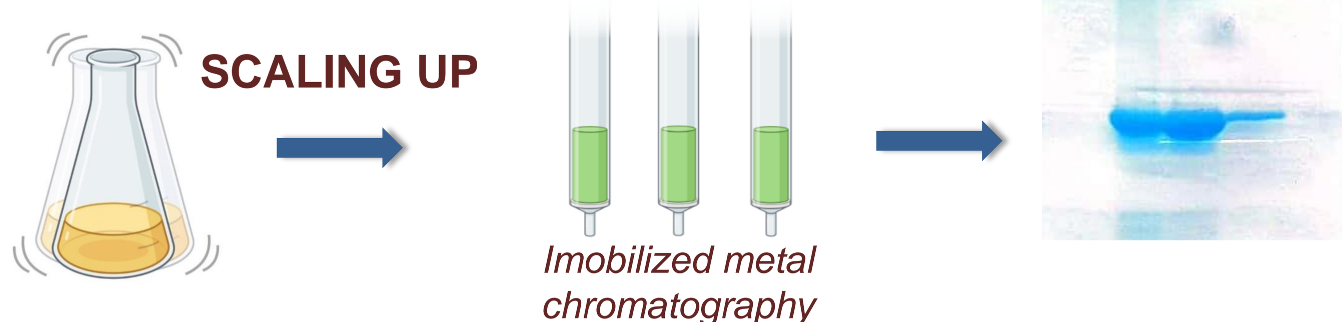


Fig 1. pET14b-λ-exo plasmid map (AddGene #104531) used for bacterial transformation

- Bacterial suspension aliquots were taken at **three time points (4, 6, 20 h post-induction)** and analysed on SDS-PA gels for total protein expression, soluble and insoluble cytoplasmatic fractions



- **NIH ImageJ –** a freely available java-based image processing and analysis program was used to determine the relative intensity of λ-exo signal (28 kDa) over lane intensity and compared with negative control
- Results of the densitometric analysis were used to estimate expression levels of λ-exo and determine optimal conditions for high-yield production



RESULTS

- We identified *E.coli* BL21(AI), SHuffle T7 and C41(DE3) as good producers of recombinant λ-exo

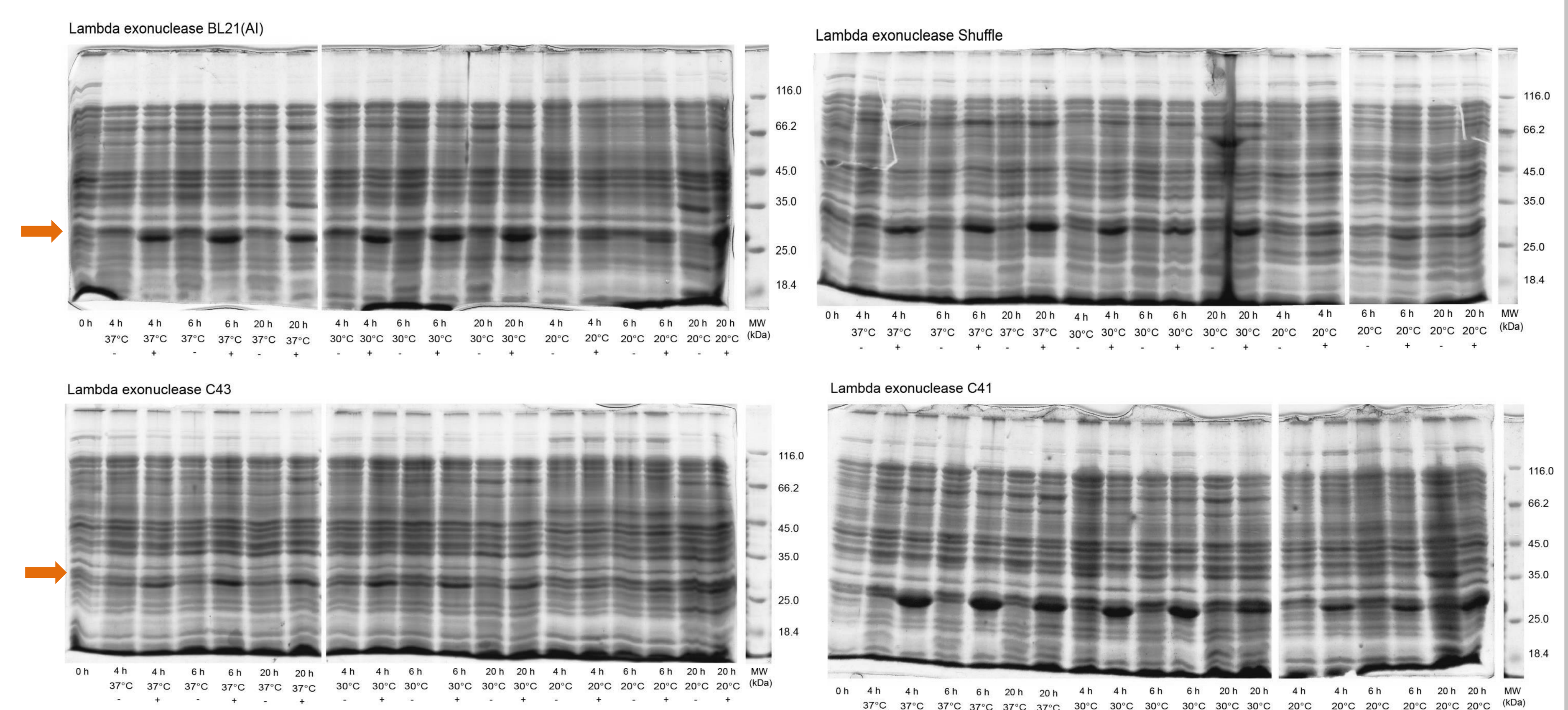


Fig 2. Results of electrophoretic analysis of the λ-exo expression

Optimal expression conditions: **30˚C, 6 h post-induction**

Table 1. Results of densitometric analysis of the λ-exo expression levels

Temperature	Time points	Lambda exonuclease band intensity: lane intensity (%)			
		BL21(AI)	Shuffle T7	C41(DE3)	C43(DE3)
	0 h	2.62%	6.07%	3.48%	3.02%
37˚C	4 h	6.91%	10.29%	8.65%	4.95%
	6 h	6.36%	10.96%	9.22%	4.75%
	20 h	5.22%	12.34%	7.15%	5.88%
30˚C	4 h,	6.20%	11.30%	7.43%	5.34%
	6 h	6.35%	11.69%	7.58%	5.52%
	20 h	6.49%	11.21%	4.57%	4.74%
20˚C	4 h	3.33%	6.44%	6.13%	3.22%
	6 h	5.63%	7.46%	5.86%	3.46%
	20 h	6.70%	8.87%	6.89%	5.87%

- Soluble recombinant λ-exo was successfully purified from crude cell lysates in satisfactory yield

ACKNOWLEDGEMENTS

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CONCLUSION

Our data suggest that densitometric analysis could serve as a powerful low-cost screening platform for improving recombinant protein expression strategies.