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Development of Vitamin B12 Biosensor for High Throughput Screening

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1. Introduction

Cobalamin or Vitamin B_{12} (hereafter referred to as B_{12}), is an essential micronutrient needed for the development and function of the central nervous system, red blood cell formation and DNA synthesis. The inability of humans to synthesize B_{12}, coupled with excess B_{12} being excreted through urine means that a consistent supply of B_{12} is physiologically necessary.

However, **B_{12} is synthesized exclusively by microorganisms and is thus often absent in plant-based foods**. With increasing adoption of plant-based diets driven by health, environmental, and animal welfare concerns, B_{12} deficiency is becoming increasingly prevalent

2. Motivation

Biofortification using B_{12} -producing bacteria offers a promising strategy to combat rising vitamin B_{12} deficiency. However, the complex B_{12} synthesis pathway complicates rational engineering, necessitating random mutagenesis that produces extensive strain libraries requiring high-throughput screening.

Current quantification methods present a major limitation: they are either too laborious for screening large libraries due to tedious sample preparation or lack specificity for biologically active B_{12} forms. Our project aims to use a B_{12} biosensor capable of direct detection, eliminating sample preparation requirements and enabling high-throughput screening of mutant libraries.

3. Approach

We propose using a B_{12} -responsive riboswitch derived from *Escherichia coli* as the basis for our biosensor. Riboswitches are cis-acting, structured regulatory RNA sequences which regulate gene expression in response to a target ligand**. The *E. coli* B_{12} riboswitch is an ideal candidate because it exhibits specificity for biologically active forms of B_{12}, particularly adenosyl-cobalamin, and can detect B12 even in complex media such as cell lysates, significantly reducing sample preparation requirements**. Since the biosensor cannot be introduced into B_{12} -producing strains destined for food applications, we will use *E. coli* as the chassis cell for biosensor construction, with B_{12} production strains screened externally.

4. Results

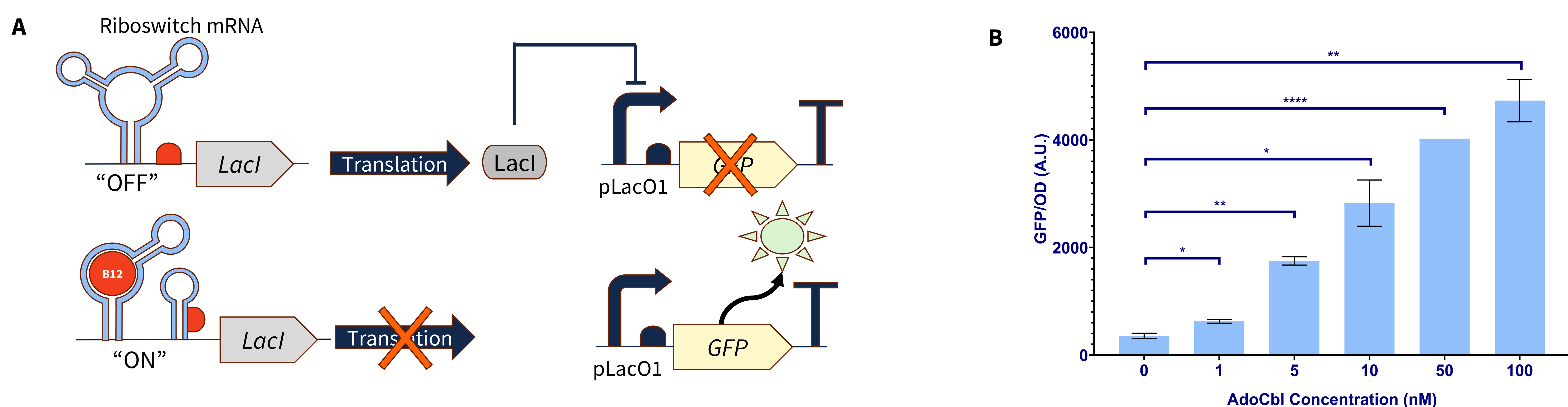

Figure 1. Vitamin B_{12} biosensor development. A) Illustration of Vitamin B_{12} biosensor sensing mechanism. The B_{12} riboswitch is used to down regulate the expression of the transcription factor, LacI in response to B_{12} via conformational changes to the mRNA structure which favour occlusion of the ribosome binding site (RBS). The LacI transcription factor was used to negatively control the expression of our green fluorescent protein (GFP) reporter gene, inverting the signal from the B_{12} riboswitch to positively correlate B_{12} concentration with sample fluorescence. B) Dose Response of riboswitch-based biosensor when induced with increasing concentrations of B_{12}. Biosensor fluorescence readings were taken at 24 hours post induction, and show significantly increased GFP expression as B_{12} concentration is increased from 1nM to 100nM, successfully demonstrating the ability of our B_{12} biosensor to differentiate between different concentrations of B_{12}.

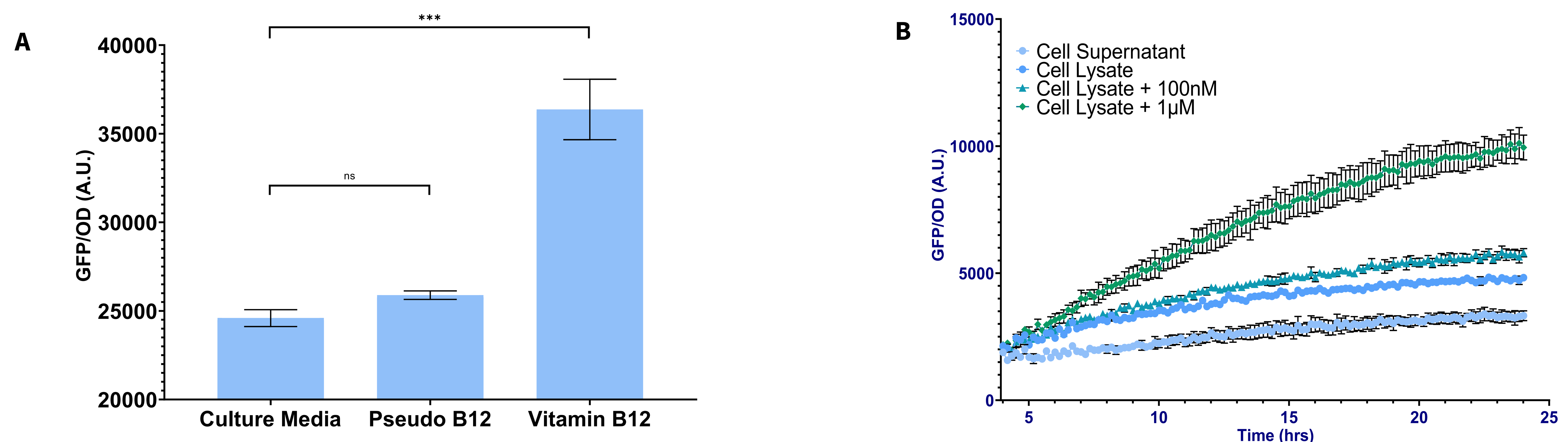

Figure 2. Response from B_{12} biosensors when tested with cell lysates from B_{12} -producing bacteria. Cell lysates were prepared by heating samples to 95°C release intracellular B_{12} and denature B_{12} binding proteins, followed by centrifugation remove cellular debris. A) Specificity of B_{12} biosensors to active forms of B_{12} was assessed using cell lysates from *Limosilactobacillus reuteri DSM 20016*, and *Propionibacterium freudenreichii subsp. Shermanii DSM20271* known pseudo B_{12} and active B_{12} producing bacteria respectively. We demonstrated that induction with pseudo B_{12} illicited minimal response from the biosensor, as compared to induction with active B_{12}. B) The ability of the biosensor to screen for higher-yielding B_{12} producers was also demonstrated through the addition of exogenous B12 to *P. Shermanii* cell lysate samples. We observed that the B_{12} biosensors were able to identify samples with increased B_{12} concentration even with minimal sample preparation. Taken together, these results established our confidence in our B_{12} biosensor's ability to screen for high producers of active forms of B_{12}.

5. Conclusion

We have successfully demonstrated that a riboswitch-based B_{12} biosensor is able to differentiate between a wide range of concentrations of B_{12}, whilst being able to distinguish between active and inactive forms of B_{12}. Additionally, we demonstrated that a **biosensor-based screening approach can significantly reduce the need for sample preparation, allowing for high throughput screening** of B_{12} producing strains.

6. Future Work

- Developing alternative B_{12} biosensors with different dynamic range to allow for more targeted screening of B12 producing strains
- Random mutagenesis of B_{12} producing strains to create strain libraries for screening of B_{12} high-producers
- Optimization of the screening workflow to increase throughput of screening
- Validation of top producing strains in food biofortification contexts

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