KRX Autoinduction Protocol: A Convenient Method for Protein Expression

ABSTRACT | Recombinant protein expression in bacteria is a simple, yet time-consuming process. Typically cells are grown overnight to stationary phase, diluted and grown to an optimal density, then induced. To simplify this process, we took advantage of the tight regulation of protein expression by glucose and rhamnose in the KRX strain of *E. coli*. We developed an autoinduction protocol that eliminates the need for culture density monitoring and a separate induction step. We describe early and late autoinduction protocols in LB medium and demonstrate autoinduced expression of three different proteins.

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The autoinduction protocol described here eliminates the need to monitor culture density and perform a separate induction step, simplifying the art of protein expression.

INTRODUCTION

Single Step (KRX) Competent Cells[a,b] allow efficient transformation, cloning, screening and protein expression (1), providing researchers a single strain for all their basic molecular biology needs.

KRX is an *E. coli* K strain that contains a chromosomal copy of the T7 RNA polymerase gene tightly regulated by a rhamnose promoter (*rha*P_{BAD}) (Figure 1). The *rha*P_{BAD} promoter is subject to catabolite repression by glucose via cyclic AMP (cAMP) and the cAMP receptor protein (2). The presence of rhamnose activates the promoter, but only once glucose is consumed from the culture medium. This provides dramatic control of proteins expressed via a T7 promoter. Pre-induction protein expression levels are exceptionally low (1).

Proteins critical for rhamnose metabolism [isomerase (RhaA), kinase (RhaB) and aldolase (RhaD)] are deleted in KRX. Therefore rhamnose is not metabolized by the cells or consumed during growth, allowing long culture times in the presence of rhamnose.

By titrating glucose and rhamnose concentrations, we developed an autoinduction protocol that eliminates the need to monitor culture density and perform a separate induction step. We examined the effect of medium type on this process and tested the utility of the protocol by expressing three different proteins.

GLUCOSE AND RHAMNOSE TITRATION

To test the effect of glucose and rhamnose concentrations on protein expression in KRX, we examined the timing and amount of recombinant luciferase produced under different conditions (Figure 2). For both firefly and *Renilla* luciferases, there were significant differences in protein expression at 8 hours between the highest and lowest glucose concentrations tested, 0.15% and 0.05%, respectively. The greatest difference in protein expression levels for firefly luciferase was between 8 and 16 hours (10- to 100-fold, Figure 2, Panel A) and for *Renilla* luciferase was at 8 hours (100-fold, Figure 2, Panel B). All media yielded comparable levels of luciferase activities by 24 hours.

Rhamnose titration had a minor effect on firefly luciferase expression at high glucose concentrations. In the example shown in Figure 2, Panel A, at 16 hours high glucose and rhamnose concentrations gave low firefly luciferase activity, but the magnitude of this effect varied between experiments and was not observed for *Renilla* luciferase.

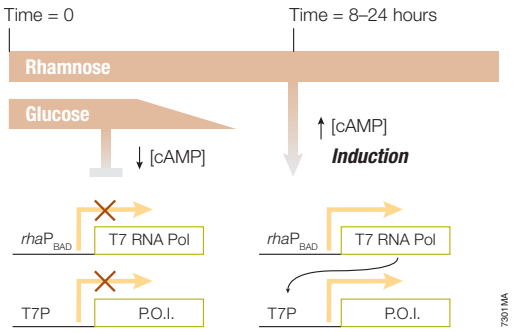
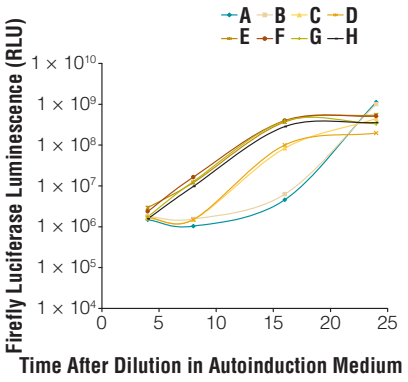


Figure 1. KRX bacteria use sugars to regulate protein expression in the autoinduction protocol. KRX contains a chromosomal copy of the T7 RNA polymerase gene driven by a rhamnose promoter (*rha*P_{BAD}). This promoter is subject to catabolite repression by glucose via cAMP and the cAMP receptor. Once glucose is depleted, the T7 promoter can be activated by rhamnose. This provides precise control of T7 RNA polymerase abundance and thereby precise control of T7 promoter (T7P)-driven production of the recombinant protein of interest (POI).

A.



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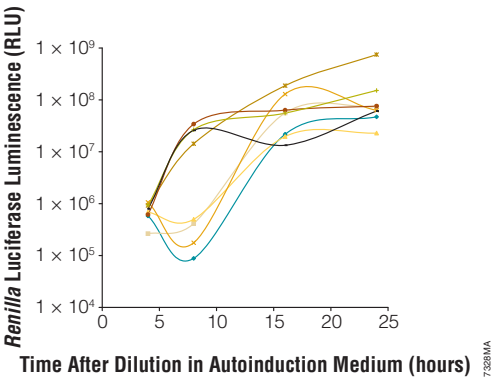

C.

Medium Supplements for Autoinduction		
Sample	Glucose (% final)	Rhamnose (% final)
A	0.15	0.2
B		0.1
C		0.05
D		0.02
E	0.05	0.2
F		0.1
G		0.05
H		0.02

Figure 2. Autoinduction of firefly and *Renilla* luciferase expression by glucose and rhamnose titration in KRX. KRX transfected with pFN6A-*luc* (**Panel A**, firefly luciferase) and KRX transfected with pFN6A-*hRluc* (**Panel B**, *Renilla* luciferase) were grown overnight in LB medium. Overnight cultures were diluted 1:100 in 3 ml of different LB induction media (LB medium with supplements indicated in **Panel C**) and incubated at 25 °C with shaking (time of dilution = 0 hours). Protein activity was measured at the indicated time points by diluting cultures 1:10 in water and assaying firefly luciferase activity with the ONE-Glo™ Luciferase Assay System (Cat.# E6110; 100 µl diluted culture + 100 µl reagent) and *Renilla* luciferase activity with the *Renilla* Luciferase Assay System (Cat.# E2810; 10 µl diluted culture + 100 µl reagent). Light output was measured on the GloMax®-Multi Detection System (Cat.# E7031). Data are the average relative light units (RLU) of replicate measurements. Data represent two independent experiments.

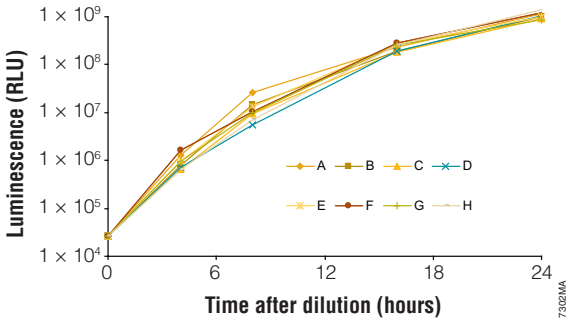

Figure 3. Titration of glucose and rhamnose did not affect firefly luciferase expression in Terrific Broth. KRX transfected with pFN6A-*luc* were grown overnight in LB medium. Overnight culture was diluted 1:100 in 3 ml of TB induction media (with supplements as indicated in Figure 2, Panel C) and incubated at 25 °C with shaking (time of dilution = 0 hours). Firefly luciferase activity was measured as described in Figure 2.

EFFECT OF MEDIA ON AUTOINDUCTION

Luria Bertani medium (LB) is most commonly used for bacterial culture and protein expression. Rich media such as Terrific Broth (TB) can be used to support higher density cultures, or minimal M9 medium can be used to restrict growth for labeling purposes (3,4). Figure 3 shows the results of autoinduction in TB medium. While TB supports autoinduction of KRX, there was no significant difference in expression levels at different glucose and rhamnose concentrations, and protein expression rates reached a plateau at 16–24 hours. *Renilla* luciferase expression was also not differentially regulated under the conditions tested (data not shown). Minimal M9 medium, which allows easy manipulation of the carbon energy source, is amenable to autoinduction using both early and late protocols (data not shown). Because autoinduction is regulated by sugars, the type of medium used will affect timing and optimal glucose and rhamnose conditions; therefore, optimization is recommended. Table 1 shows the energy sources in each of the media tested (5) and summarizes our results.

Adding glucose to KRX cultures supplies cells with a rich, consumable energy source that supports growth but delays induction by rhamnose. By titrating glucose in the induction medium, the time of protein induction can be regulated.

AUTOINDUCTION OF CRBPII

Based on glucose and rhamnose titration experiments using firefly and *Renilla* luciferases, we standardized early and late autoinduction methods in LB medium:

Early autoinduction: Dilute overnight cultures 1:100 in LB medium + 0.05% glucose + 0.1% rhamnose. Harvest cells after ~8 hours.

Table 1. Medium Composition Affects KRX Autoinduction Experiments.

Medium	Energy Source	Autoinduction Methods Supported
M9 minimal medium	20% carbon source (e.g., glucose)	Early and late autoinduction (data not shown)
Luria-Bertani (LB) medium	yeast extract 0.5% (w/v)	Early and late autoinduction (Figures 2 and 4)
Terrific Broth (TB)	yeast extract 2.4% (w/v) glycerol 0.4% (v/v)	No distinct early versus late autoinduction (Figure 3)

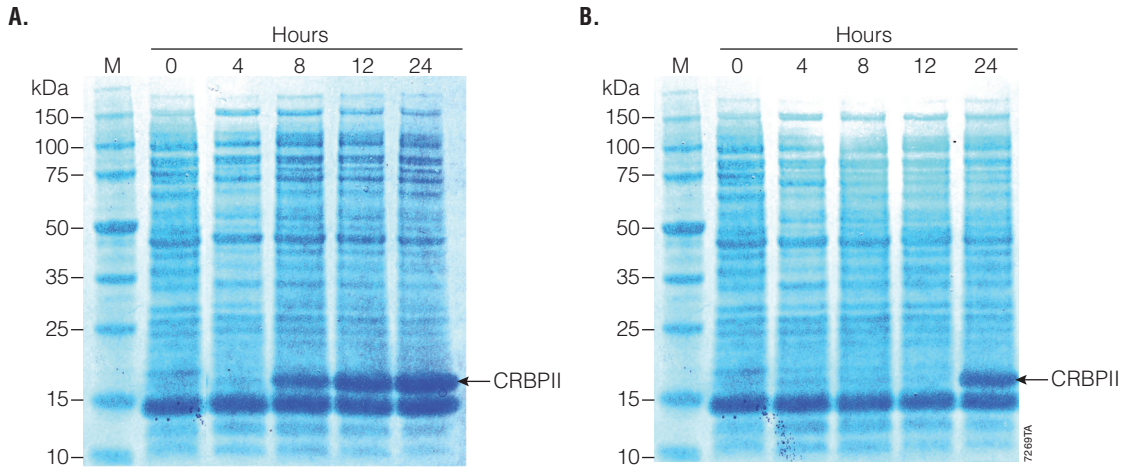

Figure 4. Autoinduction of cellular retinol binding protein II (CRBPII) expression in KRX. Autoinduction protocols for early (**Panel A**) and late (**Panel B**) induction in LB medium were tested. KRX transfected with pFN6A-CRBPII were grown overnight in LB medium with 0.2% glucose. The culture was then diluted 1:100 into 50 ml of early (LB medium with 0.05% glucose + 0.1% rhamnose) or late (LB medium with 0.15% glucose + 0.1% rhamnose) autoinduction medium (time = 0 hours). Cultures were incubated at 25 °C with shaking. At the indicated time points, 0.1 $O.D._{600}$ KRX cells were lysed in 1X FastBreak™ Reagent (Cat.# V8571). Proteins were analyzed on a 4–12% Novex NuPAGE® Bis-Tris gel and visualized using SimplyBlue™ Safestain (Invitrogen). Lanes M, Broad Range Protein Molecular Weight Markers (Cat.# V8491).

Late autoinduction: Dilute overnight cultures 1:100 in LB medium + 0.15% glucose + 0.1% rhamnose. Harvest cells after ~16–24 hours.

We used both methods to express an additional protein, human cellular retinol binding protein II (CRBPII), in the KRX strain. Figure 4, Panel A, clearly shows induction of CRBPII by 8 hours in early autoinduction medium. Figure 4, Panel B, shows the effect of late autoinduction medium on CRBPII expression, as significant protein levels are not seen until 24 hours.

Because autoinduction is regulated by sugars, the medium used will affect timing and optimal glucose and rhamnose conditions; therefore, optimization is recommended.

CONCLUSIONS

Autoinduction of protein expression is made possible by tight regulation of recombinant protein expression in KRX cells. The procedure is simple and only requires addition of glucose and rhamnose to typical *E. coli* growth media. Adding glucose to cultures supplies cells with a rich, consumable energy source that supports growth but delays induction by rhamnose. By titrating the amount of glucose in the induction medium, the time of protein induction can be regulated: low concentration for early induction, high concentration for late induction. Optimization may be required for other proteins and culture volumes, particularly with media other than those used here. These autoinduction protocols eliminate the need to monitor culture density prior to induction; overnight cultures can be diluted directly in the autoinduction medium and harvested 8–24 hours later. These protocols also highlight the tight control of protein expression in the KRX strain. This is advantageous when optimizing expression of toxic or difficult-to-express proteins.

REFERENCES

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ORDERING INFORMATION

Product	Size	Cat.#
Single Step (KRX) Competent Cells	5 × 200 µl	L3001
	20 × 50 µl	L3002
L-Rhamnose Monohydrate	10 g	L5701
	50 g	L5702

[a]Patent Pending.

[b]**Usage Restrictions for the T7 Expression System**

The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patents assigned to Brookhaven Science Associates, LLC (BSA). This technology, including bacteria, phage and plasmids that carry the gene for T7 RNA polymerase, is to be used for academic or nonprofit laboratory or licensed commercial research purposes only. By accepting or using the T7 expression technology you agree to be bound by the following conditions set forth by BSA. The initial purchaser may refuse to accept the conditions of this notice by returning this product and the enclosed materials to Promega unused.

Academic and NonProfit Laboratories

No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory unless the recipient receives a copy of this assurance notice and agrees to be bound by its terms. This limitation applies to Bacterial Strains JM109(DE3), BL21(DE3)pLysS and KRX and to any derivatives thereof.

Commercial Laboratories

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