Using chromosomal $lacI^{Q1}$ to control expression of genes on high-copy-number-number plasmids in *Escherichia coli*

Christopher B. Glascock, Michael J. Weickert; Gene 223; 1998 : 221-231
Outline

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1. Introduction

LacI-mediated repression of a promoter

- Repressed by lac repressor
- Induction by lactose or non-metabolizable analogues (IPTG)
Ratio of repressor protein to the operator sites determines the degree of repression.
\( \text{lacr}^Q \)

- \( \text{lacr}^Q \) Promotor on the same plasmid \( \rightarrow \) overproduction of LacI
- \( \text{lacr}^Q \) mutation: C\(\rightarrow\)T at -35 of the promotor region of lacI

\[
\begin{array}{ccc}
\text{-35 region} & \text{-10 region} \\
\text{P}_{\text{lacr}} & \text{GCGCAA 17 bp CATGAT} & 10 \text{ molecules} \\
\text{P}_{\text{lacr}}^Q & \text{GTGCAA 17 bp CATGAT} & 100 \text{ molecules} \\
\text{P}_{\text{lacr}}^{Q1} & \text{TTGACA 18 bp CATGAT} & 1700 \text{ molecules} \\
\end{array}
\]

Vorlesung Gentechnik 2011, Schumann, Kapitel 10
Important plasmids

- pSGE712: with $lacI$
- pSGE714: without $lacI$, pBR ori
- pSGE721: without $lacI$, pUC ori

- pBR ori: 39-55 copies/cell -> can be repressed by LacI from a chromosomal $lacI^Q$
- pUC ori: 500-700 copies/cell -> not effectively controlled by $lacI^Q$
2. Objectives

- The $lacI^{Q1}$ allele should improve cloning of potentially detrimental genes by better controlling expression from $lac$-based promoters on hcn plasmids.
- The use of $lacI^{Q1}$ induces higher expression of genes on hcn plasmids at low IPTG concentrations.
- Using $lacI^{Q1}$ hosts eliminates the requirement to maintain $lacI$ on the plasmid to regulate gene expression on hcn expression plasmids.
3. Results

• P1 transduction to introduce $\textit{lacI}^Q$ allele into SGE1661 ($\textit{lacI}^+$)
• Identification of Km$^R$ candidates
• Introduction of pSGE712 and pSGE714 into all candidates and SGE1661 as a control
• Growth in absence and presence of IPTG
Plasmid β-LacZ production from lacI\(^Q\) transduction candidates

<table>
<thead>
<tr>
<th>lacI genotype</th>
<th>β-LacZ (u)(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pSGE712 (lacI(^t))</td>
</tr>
<tr>
<td></td>
<td>− IPTG</td>
</tr>
<tr>
<td>SGE1661(^b)</td>
<td></td>
</tr>
<tr>
<td>lacI(^t) (control SGE1661)</td>
<td>1439</td>
</tr>
<tr>
<td>Four lacI(^t) transduction candidates</td>
<td>1068 ± 119</td>
</tr>
<tr>
<td>Two lacI(^Q) transduction candidates (SGE1669 and SGE1670)</td>
<td>1057 ± 326</td>
</tr>
</tbody>
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SDS-PAGE of soluble and insoluble fractions

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• Many copies of the \textit{lac} operator -> alternative binding sites for \textit{Lacl} -> inducing transcription
• Introduction of pUC19 into SGE1661 (\textit{lacI}+)
  -> induction of \textit{lacZ} without IPTG from 0,3 u to 1815 u (u: units of \textit{βGal})
• Introduction of pUC19 into SGE1675 (\textit{lacI}Q)
  -> no induction of the chromosomal \textit{lac} operon
=> level of \textit{Lacl} in this strain was sufficient to bind 500 copies of pUC19/cell and repress the \textit{lac} operon on the chromosome
• level of \textit{LacZ} before induction was 60fold higher in SGE1675 than in SGE1661
Authentic $lacI^Q$ did not repress transcription from hcn plasmids

Authentic $lacI^Q$ strains were transfomed with:

• pSGE712 (medium-copy vector, with $lacI$)
• pSGE714 (medium-copy vector, without $lacI$)
• pSGE721 (high-copy-number vector, without $lacI$)
Measuring of β-LacZ fusion protein activity with and without IPTG

<table>
<thead>
<tr>
<th>Strain/repressor(^a)</th>
<th>Plasmid(^b)</th>
<th>(\beta)-LacZ (u)(^c)</th>
<th>– IPTG</th>
<th>+ IPTG</th>
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<tr>
<td>D1210/lacI(^Q)</td>
<td>pSGE712</td>
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<td>52 482</td>
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<td>25 141</td>
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<td>39 770</td>
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<td>SGE1675/lacI(^Q1)</td>
<td>pSGE721</td>
<td>1778</td>
<td>27 124</td>
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</tr>
</tbody>
</table>

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Identification and characterization of $lacI^{Q1}$

• 600 additional bp of the region upstream of $lacI$ were amplified by PCR, subcloned and sequenced from strain C600 (wt $lacI$)

• Oligos were designed and used to amplify the $PlacI$ region

• Gelelektrophoreosis
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Sequences of $lacI$, $lacI^Q$, $lacI^{Q1}$

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$PlacI$ sequence in SGE1661  ->  wt $lacI$
In SGE1670 and SGE1675  ->  $lacI^{Q1}$
Measuring the induction

- Activity of the β-LacZ fusion expressed from strains with medium or high copy number plasmids -> induction of *Ptac* on plasmids
- Strains carrying plasmids without the β-LacZ fusion -> induction of chromosomal *lacZ*
- Amount of LacZ induced from the chromosomal gene was inversely proportional to the plasmid copy number and therefore, the LacI molar concentration in the cell
Synthesis of plasmid $\beta$-LacZ and chromosomal LacZ

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Synthesis of plasmid and chromosomal β-LacZ

• The induction of plasmid β-LacZ occurred at lower IPTG concentrations and appeared less sensitive to the LacI concentration
4. Conclusions

- The $lacI^Q$ allele was not sufficient to control transcription from the $tac$ promoter on hcn plasmids because the $lac$ operator sites on the plasmid exceed the LacI molecules in the cell.
- Forming “sandwich” associations (1 LacI tetramer binds to 2 operators) reduces the excess of operator sites => partial repression of plasmid promoters in $lacI^Q$ strains.
- Operator titration affects $Plac$ induction from the chromosome less than $Ptac$ induction on the plasmid because the native $lac$ promoter with three operator sites is more effective at capturing LacI than the single operator site on the plasmid.
• Transcription of $lacI^{Q1}$ is 17-fold stronger than that of $lacI^Q$ enough to repress $Ptac$ transcripton from a hcn plasmid (∼500 copies)

• The increased promoter strength accounts for the unexpected higher uninduced levels of βGal in SGE1675. Since the $lacI$ gene is upstream of and colinear with the $lacZ$ gene, the increased transcription from the $lacI^{Q1}$ promotor continues into the $lac$ operon -> higher levels of $lacZ$ expression

• The magnitude of induction of the $lac$ operon on the bacterial chromosome is proportional to the molar concentration of Lacl in the cell

• As the concentration of Lacl increases, the LacZ activity from the chromosomal $lac$ operon induced by 1-10mM IPTG decline, even in the presence of plasmids containing $lac$ operators
A new promotor was identified allowing high level expression of the *lacI* gene
6. Discussion

1. Was versteht man unter einer Promotor-up Mutation?

2. Der \textit{lac}-Promotor kann mit IPTG oder Allo-lactose induziert werden. Welche Vorteile bietet IPTG?