[24] Alternatives to X-Galactopyranoside in Screening Recombinant Clones Based on pUC-Derived Plasmid Vectors

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Introduction

The standard repertoire of a molecular geneticist includes pUC plasmids as cloning vectors.[1] These plasmids allow detection of clones with inserts based on the color of colonies on plates containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside). This color-based selection of recombinants proved so useful over the years that it was employed in the construction of the great majority of new vectors aimed to facilitate cloning, sequencing, mutagenesis, *in vitro* transcription, and other procedures of DNA analysis and manipulation.

X-Galactopyranoside is too expensive for routine cloning experiments

[1] C. Yanisch-Perron, J. Vieira, and J. Messing, *Gene* **33,** 103 (1985).

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and the plates cannot be stored for long. There are two alternatives: The use of MacConkey plates and the examination of colony size under conditions of *lac* induction. This chapter assesses the usefulness of these methods with 17 common cloning vectors.

Principle

The principle of screening on X-Gal plates is the so-called α-complementation. A host strain carries the mutation *lacZ*ΔM15 in its β-galactosidase gene, which is complemented by a small fragment of the *lacZ* product (α peptide) coded for by the plasmid vector. Inserting a DNA fragment into the polylinker disrupts the coding region for the α peptide and results in the loss of the β-galactosidase activity, which can be monitored on indicator plates. Some host strains used for α complementation carry a superproducing allele of the *lac* repressor *lacI*^{q}. In these strains *lacZ*ΔM15 expression must be induced by the artificial inducer IPTG.

A classical means for detecting β-galactosidase, MacConkey/lactose plates,[2] was reported not to be sensitive enough in response to a weak activity resulting from α complementation.[1] In spite of this, MacConkey plates were used for screening pUC clones in different laboratories over the years, but the method did not work every time. For this reason it was not published until a recent short note appeared. The publication did not specify the plasmid used.[3] However, this seems to be a critical point because we found that MacConkey plates work with some but not all pUC plasmids and related vectors. At least some of these differences are corroborated by a report that states that most commercially available pUC18 plasmids carry a mutation in the *lacZ*α region that decreases the β-galactosidase activity up to 50 times.[4]

The last method for selection of recombinants does not use any β-galactosidase substrate at all. It is based on the observation that growth of *E. coli* colonies containing some pUC-related plasmids is inhibited under conditions of *lac* induction and that interrupting the *lacZ*α frame by cloning DNA fragments into the polylinker counteracts this inhibition.[5] As a result, the colonies with inserts appear larger than the background colonies.

[2] J. H. Miller, "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1972.

[3] M. P. Jennings and I. R. Beacham, *BioTechniques* **7,** 1082 (1989).

[4] Y. Lobet, M. G. Peacock, and W. Cieplak, *Nucleic Acids Res.* **17,** 4897 (1989).

[5] P. Karlovsky, *Nucleic Acids Res.* **15,** 6753 (1987).

TABLE I
COMPARISON OF DIFFERENT METHODS OF IDENTIFYING RECOMBINANT PLASMIDS ORIGINATING FROM pUC-LIKE VECTORS

Plasmid	X-Gal[a]	MacConkey[b]	Colony size[c]		Selected features
			37°	42°	
1. pUGA	W	−	−	−	(Control)
2. pUC19[d]	B	+	+	+	
3. pUC18[d]	B	−	−	±	
4. pUC8[e]	B	+	+	(+)	
5. pUC9[e]	B	+	−	+	
6. pUC12[e]	B	−	−	±	
7. pUC13[e]	B	+	+	(+)	
8. pUC8-1[f]	B	−	+	(+)	Expression of cloned DNA in all possible reading frames from *lac* promoter
9. pUC8-2[f]	B	−	+	(+)	
10. pUC9-1[f]	B	+	±	(±)	
11. pUC9-2[f]	B	−	±	(±)	
12. pUC*HinEco*1[g]	B	+	+	(+)	Modified polylinker
13. pK18[h]	B	+	−	(−)	Kanamycin resistance for easy insert transfer
14. pK19[h]	B	+	−	(−)	
15. pBluescript[i]	B	+	±	±	All-purpose phagemid
16. pGEM3Z[j]	B	+	±	+	SP6/T7 promoters
17. pWM528[k]	B	+	+	+	Totally synthetic plasmids
18. pWM529[k]	B	+	+	+	

[a] Colonies on X-Gal plates: B, blue; W, white.
[b] Suitability of the test on MacConkey plates at 37°: +, suitable (red colonies); −, unsuitable (white colonies).
[c] Suitability of the colony size test: +, suitable (distinct difference between plates with and without IPTG); −, method not applicable (no difference observed); ( ), poor growth.
[d] C. Yanisch-Perron, J. Vieira, and J. Messing, *Gene* **33,** 103 (1985).
[e] J. Vieira and J. Messing, *Gene* **19,** 259 (1982).
[f] Z. Hanna, C. Fregeau, G. Prefontaine, and R. Brousseau, *Gene* **30,** 247 (1984).
[g] H. J. Edenberg, L. G. Moss, and W. J. Rutter, *Gene* **58,** 297 (1987).
[h] R. D. Pridmore, *Gene* **56,** 309 (1987).
[i] pBluescript II exo/mung DNA sequencing system (Stratagene, La Jolla), 1989.
[j] Promega Catalog and Reference Guide: Riboprobe Gemini System (Promega, Madison, WI), 1987.
[k] W. Mandecki, M. A. Hayden, M. A. Shallcross, and E. Stotland, *Gene* **94,** 103 (1990).

Material and Reagents

Escherichia coli JM109 [*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ^- Δ(*lac-proAB*), (F′ *traD36 proAB lacI*^{q}*Z*ΔM15)[1]] is used throughout the study. References to plasmids are listed in Table I. pUGA is constructed by inserting a 45-bp oligonucleotide carrying UGA codons in all three

frames into the polylinker in pUC19.[1] An IPTG 100× stock solution is made 0.1 *M* in water and stored at −20°, and an X-Gal 500× stock solution is made (20 mg/ml) in dimethylformamide and stored at 4°. Both substances are added into an autoclaved L medium that has been cooled to 45–50°. MacConkey agar is bought from Difco (Detroit, MI). Solid lactose is dissolved in the autoclaved MacConkey medium (45–60°) to the concentration 10 g/liter. L medium contains 10 g tryptone (Difco), 5 g yeast extract (Difco), 5 g NaCl, and 15 g agar per liter. Ampicillin is used at a concentration of 50 μg/ml with all plasmids except pK18 and pK19, in which it was replaced by kanamycin at the same concentration.

Method I

Escherichia coli transformed with a ligation mixture is plated on MacConkey/lactose plates. Up to 300 colonies can be screened on one plate. After 1 day at 37° colonies of two types can be seen on the plates: dark red colonies contain the original vector and white colonies, sometimes with red centers, contain recombinant plasmids. Then either small liquid cultures are inoculated or colonies are streaked on plates with antibiotics in order to isolate plasmids.[6,7]

Method II

In the first variant, transformed bacteria are plated on IPTG plates in a dilution that ensures that not more than 50 colonies arise on 1 plate. The plates are incubated for 36–48 hr at 37°. Colonies containing recombinant plasmids are larger than colonies carrying the original vector; this difference becomes blurred at higher plating densities. In another variant, colonies are first plated on normal ampicillin plates without IPTG and then streaked onto IPTG plates on the second day. Heavily grown streaks are scraped with toothpicks and used to isolate plasmids by a minipreparation method.[6,7] Thinly grown streaks contain the vector without inserts. Simple L plates can be used instead of IPTG plates when working with a strain that does not overproduce *lac* repressor (HB101, DH1, etc.).

Comparison of Both Methods with Different Plasmid Cloning Vectors

To assess the practicability of both methods with various pUC-like vectors, we compared the colony phenotype of the standard laboratory

[6] H. C. Birnboim, this series, Vol. 100, p. 243.

[7] M. G. Riggs and A. McLachlan, *BioTechniques* **4**, 310 (1986).

strain *E. coli* JM109[1] transformed with 17 cloning vectors on MacConkey plates, X-Gal plates, and L plates with and without IPTG. Colony size on L plates without IPTG was used to imitate plating of *E. coli* with plasmids harboring inserts on media containing IPTG because using one specific insert would hamper the generality of the results (see Concluding Remarks, below). These experiments were performed at two temperatures because the difference in colony size generally increases with temperature, but some strains grow poorly at 42° (unpublished observations).

The results are shown in Table I. X-Galactopyranoside plates can be replaced by MacConkey plates when working with 12 plasmids. This property does not correlate with the inhibition of colony formation on *lac* induction, indicating that the cause of growth inhibition is not β-galactosidase activity. The best results regarding colony size selection were often obtained after 2 or 3 days, especially when working with vectors labeled as conditionally suitable (±) in Table I.

Concluding Remarks

With many pUC-like plasmid vectors, X-gal plates can be substituted by the cheap and stable MacConkey medium. As an alternative, a method can be used that is based on the difference in colony size between strains carrying a plasmid with an insert and those containing only a vector. This latter method demands either the presence of IPTG in the medium or the use of strains without the *lacI*^{q} allele. Both methods are useful in reducing the number of clones screened in routine cloning experiments without extra effort and expense. Simple examination of colonies before plasmid isolation can give a clue regarding the plasmid content. The methods are limited to (nonidentical) subsets of vectors tested. Other limitations are cloning of short inserts functioning as open reading frames (which preclude X-Gal selection, too) and inserts that inhibit the growth of the host strain or express β-galactosidase activity.

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