Journal of Libyan Academy Bani Walid 2026
مجلة الأكاديمية الليبية بني وليد
e-ISSN: 3104-3860
Volume 2, Issue 1, 2026, Pages: 46-65
Website: https://journals.labjournal.ly/index.php/Jlabw/index

Segregational Stability of the High-Copy Plasmid pUC19 in Escherichia coli Trans10 During Serial Passaging

Salem Ebraiek [*1] , **Saad Aboualkasem** [2]
[1] Public Health Department, Faculty of Health Sciences, Misurata University, Libya
[2] Department of Biotechnology, Faculty of Science, Misurata University, Libya
*Email: saad_libya@ymail.com

الاستقرار الانفصالي للبلازميد عالي النسخ *pUC19* في بكتيريا *Escherichia coli* *Trans10* أثناء التمرير المتسلسل

سالم إبريك [1*]، **سعد أبو القاسم** [2]
[1] قسم الصحة العامة، كلية العلوم الصحية، جامعة مصراتة، ليبيا.
[2] قسم التقنيات الحيوية، كلية العلوم، جامعة مصراتة، ليبيا.

Received: 21-10-2025	**Accepted:** 26-12-2025	**Published:** 08-01-2026

Abstract

The stability of plasmids is an important aspect of molecular cloning and recombinant DNA technology, directly impacting the reliability and reproducibility of plasmid-based experiments. In this work, we have studied the stability of the high-copy plasmid pUC19 in E.\ coli Trans10 during seven consecutive days of continuous serial passaging in selective (ampicillin containing) and non-selective conditions. After chemical transformation with heat shock in $CaCl_2$, 100 random colonies were propagated daily and the excess time of the experiment confirmed maintenance of the plasmid. All colonies were positive for pUC19 protection, regardless of antibiotic. These results suggest that the multicopy replication of pUC19 along with the genetic background of E. coli Trans10 is reliable to carry out plasmid inheritance through several generations. This work has demonstrated the stability of pUC19 as a cloning vector in Trans10 and presented an evidence that strong plasmid maintenance of it is ensured even without consistent antibiotic selection, justifying its usefulness for basic molecular biology experiments.

Keywords: pUC19, stability plasmid, Escherichia coli Trans10, high copy construction, serial passaging .

الملخص

يُعدّ استقرار البلازميدات جانبًا مهمًا في تقنيات الاستنساخ الجزيئي والحمض النووي المعاد التركيب، إذ يؤثر مباشرة في موثوقية وقابلية تكرار التجارب المعتمدة على البلازميدات. في هذه الدراسة، تم فحص استقرار البلازميد عالي النسخ pUC19 في بكتيريا Escherichia coli Trans10 خلال سبعة أيام متتالية من التمرير المتسلسل المستمر في ظروف

انتقائية (بوجود الأمبيسيلين) وغير انتقائية. بعد التحويل الكيميائي بطريقة الصدمة الحرارية باستخدام $CaCl_2$، جرى استزراع 100 مستعمرة عشوائية يوميًا، وأكّد طول مدة التجربة المحافظة على البلازميد. أظهرت جميع المستعمرات نتيجة إيجابية لوجود بلازميد pUC19 بغضّ النظر عن وجود المضاد الحيوي. تشير هذه النتائج إلى أن تضاعف النسخ المتعدد لبلازميد pUC19 إلى جانب الخلفية الوراثية لبكتيريا E. coli Trans10 يُعدّ موثوقًا في ضمان توريث البلازميد عبر عدة أجيال. وقد برهنت هذه الدراسة على استقرار pUC19 بوصفه ناقل استنساخ في سلالة Trans10، وقدّمت دليلًا على أن المحافظة القوية على البلازميد تتحقق حتى في غياب الانتقاء المستمر بالمضاد الحيوي، مما يبرر فائدته في تجارب البيولوجيا الجزيئية الأساسية.

الكلمات المفتاحية: pUC19، استقرار البلازميد، بلازميد عالي النسخ، التمرير المتسلسل.

Introduction

Plasmid stability is one of the most important parameters in the field of molecular biology and biotechnology as it affects directly to plasmid-based systems reliability, productivity and reproducibility. Plasmids are nonchromosomal replicons that can be used as vectors in cloning, gene expression, metabolic engineering and vaccine development. However, plasmid stabilities are not easily guaranteed over many generations in host cells particularly without selective pressure and thus stability of plasmids is a major issue both academically and industrially [1] [2].

Plasmid stability consists of two related components: structural stability, which implies that the plasmid structure is not altered by deletion, insertion or rearrangement; and segregational stability, characterized by retention of the plasmid in its host cell during division. Instability can result from a number of causes, among which are plasmid copy number, size, origin of replication (ORI), metabolic load on the host and interactions between plasmid-encoded functions and components in the host cell [3]. In addition, the plasmid maintenance could be influenced by environmental status and growing conditions, particularly on large-scale or long-time culture scale [4].

Naturally and artificially stabilizing mechanisms have been found that can attenuate plasmid loss. Among these are active segregation systems, multimers resolvase systems and (toxin — antitoxin) modules which together act to increase plasmid maintenance in a population of bacteria [5]. Molecular insights into these mechanisms are useful for the rational design of stable plasmid vectors and development of expression systems that rely on constant gene dosage and phenotypic stability [6].

In biotechnological / biomedical research the plasmid stability is especially relevant to maintain continuous and stable expression of recombinant genes, to lower batch-to-batch variance and in order to minimize or eliminate antibiotic selection not only because it becomes increasingly impossible due to regulatorial and biosafety constraints. Consequently, a thorough study of plasmid stability and its underlying determinants is still the basis for the establishment of stable plasmid-based platforms as well as high performing genetic and metabolic engineering tools [7].

pUC19 is one of the most commonly selected high-copy plasmid vectors for cloning in E. coli. It is a small (about 2.7 kb) plasmid from the pUC series with a modified pMB1 origin of replication that supports high copy number. Although this feature enables rapid plasmid amplification and a high DNA production, it could also represent a metabolic load to host cell being related to plasmid stability during long culture process [8][9].

The vector has the bla gene which provides resistance to ampicillin, a commonly utilized antibiotic for plasmid selection and maintenance [10]. pUC19 also carries a multiple cloning site surrounded by lacZα sequence that allows recombinant clones to be identified by blue–white screening. Due to the presence of a large number of copies and an absence of active

partitioning systems, pUC19 typically depends on antibiotics for stable maintenance in bacterial populations [11].

Due to these features, pUC19 is widely used in routine molecular cloning, subcloning and DNA propagation. However, its poor segregational stability in the absence of selection renders it an ideal model vector for investigating parameters that contribute to plasmid maintenance especially in terms of host-plasmid interactions and metabolic load [8].

The objective of this study is to determine the stability of a high-copy number plasmid pUC19 in *E.coli* Trans10 and to identify variables which affect its maintenance as bacteria multiply. In particular, we aim to determine the percentage of plasmid retention in both selective and non-selective conditions, interrogate the influence of serial subculturing (single colonies or liquid culture) as well as growth parameters on plasmid stability, and present experimental data pertinent for validity and reproducibility of a pUC19-based cloning system . The results of this study will help the unraveling of the host–plasmid interaction and the design of strategies to enhance plasmid maintenance, thereby decreasing reliance on antibiotic selection.

Materials and methods

Luria–Bertani medium (LB), calcium chloride ($CaCl_2$), *Escherichia coli* Trans 10 (E. coli Trans10), PUC19 cloning vector.

Chemical Transformation of E. coli Trans10

The $CaCl_2$ chemical transformation complemented with heat shock was used to transform plasmids. Basically, the competent *E.coli* Trans10 cells were made by cold $CaCl_2$ buffer. Competent cells were mixed with pUC19 plasmid DNA and the sample was put on ice then exposed to a brief heat shock at 42 °C. The cells were resusupended in ice and subsequently salvaged in LB broth without antibiotics and cultured at 37 °C with shaking. The transformed cells were plated onto LB agar plates supplemented with ampicillin and incubated at 37 °C for 24 h.

Plasmid Stability Assay

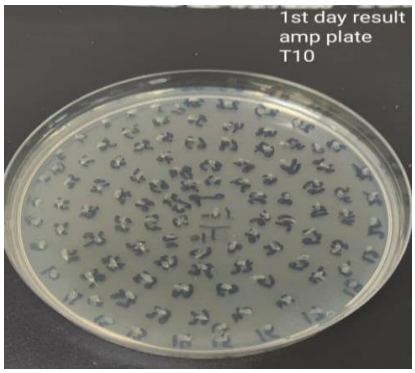
A single transformed colony was inoculated into 4 mL LB broth and incubated at 37 °C with shaking at 150 rpm for 2 h. Serial dilutions were prepared, and 100 µL of the 10^{-3} dilution was plated on antibiotic-free LB agar to obtain more than 100 colonies (Day 1) (Fig 1). One hundred colonies were randomly selected and transferred daily into three LB broths with ampicillin and three without antibiotics. This serial passaging was continued for seven days at 37 °C. Plasmid stability was evaluated by comparing growth under selective and non-selective conditions.

Results

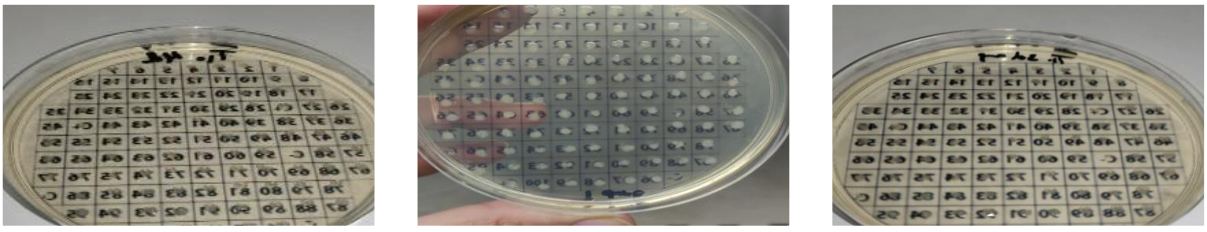
Plasmid stability analysis showed that pUC19 was maintained stably in *Escherichia coli* Trans10 during the whole experimental period. After chemical transformation and the first selection step, all 100 randomly selected colonies did not lose its plasmid after seven days continuous serial sub-culturing. Significantly, no plasmid loss was detected in selective or non-selective growth conditions, showing the plasmid to be 100 % stable during bacterial repeat replication.

During the experiment, pUC19 was stably maintained throughout several passages of colonies grown in absence of LB antibiotic selection as indicated by continued ability to grow on amp-containing media. This stability remained unchanged even upon daily transfer of a defined number of colonies, which otherwise represents an environment favoring segregational loss of plasmid, mainly if it is a high copy number plasmid without active partition. Since the plasmid

curing could not be detected, this suggests that “the high copy number in combination with the genetic background of E. coli Trans10 was enough to secure perfect maintenance at multiple generations”.



In addition, similar growth patterns were found between the selected cultures and those grown in absence of antibiotics, which suggest that plasmid retention was not strictly dependent on selection pressure of ampicillin. This result also indicates a good host–plasmid compatibility and low burden of metabolism under the conditions used. All together, these findings indicate that pUC19 shows a remarkable segregationally stability in E. coli Trans10 after long term serial passaging, which underlines the reliability of this vector in routine cloning experiments and validates it as a stabilized plasmid system in laboratory short/medium term cultures (Fig 2: A, B, C).



A B C

Figure 1. first 100 colonies replicaplating in LB agar plate mediume without ampicillin

Figure 2: A, B, and C showed LB agar plates with the results of 100 colonies replicatin with different days, 2nd, 4th, and 7th days respectively. all plates showed stability 100% of PUC19 in E. Coli Trans10

Discussion

The findings of the current study indicate that the high copy plasmid pUC19 possessed absolute (100%) segregational stability in *Escherichia coli* Trans10 following serial passaging for seven consecutive days, even when grown in the absence of antibiotic selection. These results suggest that, in the frames of experiments performed, transformed bacteria kept plasmid during all consecutive generations and no loss could be observed. This sustained pUC19 maintenance is particularly impressive as high copy plasmids of the ColE1/pMB1 class such as pUC19 are generally believed to segregate at random during cell division and are presumed to be

susceptible to loss in the absence of selective pressure, because they encode no active partitioning systems on their vector backbone. ColE1 type plasmids tend to depend on high copy number for low (but control) levels of plasmid free segregants, with antibiotic selection being used in experimental systems as the most general form of external modulator for enrichment enhancement [11].

In circadian with other results demonstrating that plasmid is not always unstable in the absence of selective pressure,7,64 our findings on the complete percentage retention suggest that a combination of high-copy number and host strain background used in E. coli Trans10 may lead to stable maintenance of plasmids. Some of the previous studies evaluating pUC19 retention in other E. coli hosts have displayed varying results, frequently observing substantial loss of plasmid over time when cultures are grown in antibiotic-free conditions, including especially continuous culture or conditions (e.g., a slower growth rate) that place stress upon metabolism. For instance, in experiments in which the stability of pUC19 was compared to that of engineered vectors under typical laboratory selection and growth conditions, the parental form pUC19 displayed fairly rapid loss over time in strains such as DH5α or BL21(DE3), with half-life times to reach 50% plasmid retention on a scale of tens of hours under non-selective conditions, and appreciable improvement observed only when adding stabilizing functions such as toxin–antitoxin modules or partition systems [12] [6].

The relative destabilization of those structures versus the general stability seen here could be due to a number of differences. First, there may be differences between the strains in plasmid replication and partitioning kinetics that could affect inheritance; it is possible that the Trans10 host carries specific genomic or regulatory elements that reduce plasmid loss under our experimental conditions. To the second point, differences in experimental design (e.g. discrete serial passaging with daily plating of a large number of colonies) may simply bias against observing already more rare plasmid free segregants that would be better assayed using continuous culture or high resolution tracking methods. Third, the seven day period is rather short, possibly not enough to show long term instability that only shows after many rounds of propagation. This is in agreement with general literature indicating that plasmid stability depends on growth environment, metabolic load and genetic background and can show huge differences even the origin of replication between those vectors is unique [13].

These finding have important inferences for the design and application of pUC19 in standard cloning experiments. Stable maintenance in E. coli Trans10 demonstrates that, under specific conditions, longer term plasmid retention can be maintained without reliance upon antibiotic supplementation—an attractive result for situations where plasmids are not reproducibly transferred (i.e., “plasming”) from master clones and use of an antibiotic is prohibitive for biosafety or economic reasons. However, further analysis with quantitative determination of the plasmid copy number as well as additional rounds of propagation and comparisons to different vectors and host strains may be valuable for describing the limits of this stability and generalizing these studies. Furthermore, adding the molecular tools to quantitatively assess the dynamics of plasmid loss or retention at the population level (e.g., FCM or reporter gene assay) could contribute to a more detailed description of the associated stability [11], [12], [6].

Conclusion

In the present work, we show that pUC19 high copy number plasmid is fully segregationally stable for 7 successive days in *Escherichia coli* Trans10 host strain without antibiotic pressure. The results seemed to suggest the Trans10 host was well-suited for pUC19, presumably due to plasmid's high copy number and host bacteria's genetic background, which guarantees normal inheritance of the plasmid through multiple generations. These results indicate that pUC19 can serve as a robust tool for regular cloning and plasmid preps in E. coli Trans10 without the need of selective pressure, improving its usefulness for molecular biology methods where consistent maintenance of plasmids is essential. This study establishes a basis for aiming future research specifically on host–plasmid interplay and conditions driving plasmid stability in various growth situations and following prolonged propagation.

References

[1] M. Levisson, R. B. Spruijt, I. N. Winkel, S. W. M. Kengen, J. Van Der Oost, and H. Schlüter, Molecular biotechnology : principles and applications of recombinant DNA. 2010. [Online]. Available: https://www.ptonline.com/articles/how-to-get-better-mfi-results

[2] L. Snyder, T. M. Henkin, J. E. Peters, and W. Champness, Molecular Genetics of Bacteria, 4th Edition, vol. 1. 2013. doi: 10.1128/9781555817169.

[3] "Segregational Instability of pUBl1 O-Derived Recombinant," vol. 244, pp. 235–244, 1985.

[4] K. Friehs, "Plasmid copy number and plasmid stability.," Adv. Biochem. Eng. Biotechnol., vol. 86, pp. 47–82, 2004, doi: 10.1007/b12440.

[5] L. Sevillano, M. Díaz, and R. I. Santamaría, "Stable expression plasmids for Streptomyces based on a toxin-antitoxin system," pp. 1–10, 2013.

[6] S. Aboualkasem, D. S. Retnoningrum, A. Refiani, and C. Riani, "Separate Toxin-Antidote Stabilization System in Two Plasmids for Recombinant Protein Production," Curr. Pharm. Biotechnol., vol. 19, Feb. 2018, doi: 10.2174/1389201019666180226154342.

[7] G. Vandermeulen, C. Marie, D. Scherman, and V. Préat, "New generation of plasmid backbones devoid of antibiotic resistance marker for gene therapy trials.," Mol. Ther., vol. 19, no. 11, pp. 1942–9, 2011, doi: 10.1038/mt.2011.182.

[8] S. Lin-Chao, W. -T Chen, and T. -T Wong, "High copy number of the pUC plasmid results from a Rom/Rop-suppressible point mutation in RNA II," Mol. Microbiol., vol. 6, no. 22, pp. 3385–3393, Nov. 1992, doi: 10.1111/J.1365-2958.1992.TB02206.X;WGROUP:STRING:PUBLICATION.

[9] A. D. A. C. M. Peijnenburg, S. Bron, and G. Venema, "Structural Plasmid Instability in Recombination- and Repair-Deficient Strains of Bacillus subtilis," 1987.

[10] M. Genes, "Guidance for Industry Use of Antibiotic Resistance," 1997.

[11] M. S. Standley, S. Million-Weaver, D. L. Alexander, S. Hu, and M. Camps, "Genetic control of ColE1 plasmid stability that is independent of plasmid copy number regulation," Curr. Genet., vol. 65, no. 1, p. 179, Feb. 2018, doi: 10.1007/S00294-018-0858-0.

[12] Y. C. Liao, B. Saengsawang, J. W. Chen, X. Z. Zhuo, and S. Y. Li, "Construction of an Antibiotic-Free Vector and its Application in the Metabolic Engineering of Escherichia Coli for Polyhydroxybutyrate Production," Front. Bioeng. Biotechnol., vol. 10, p. 837944, Jun. 2022, doi: 10.3389/FBIOE.2022.837944/BIBTEX.

[13] T. Wein, N. F. Hülter, I. Mizrahi, and T. Dagan, "Emergence of plasmid stability under non-selective conditions maintains antibiotic resistance," Nat. Commun., vol. 10, no. 1, Dec. 2019, doi: 10.1038/S41467-019-10600-7.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.