CC/NUMBER 47
® NOVEMBER 19,1990

This Week's Citation Classic

Bolivar F, Rodriguez R L, Greene P J, Betlach M C, Heyneker H L, Boyer H W, Crosa J H & Falkow S. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* 2:95-113, 1977.
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The plasmid pBR322 was one of the first certified EK2 multipurpose cloning vectors to be available for the efficient cloning and propagation of recombinant molecules in *Escherichia coli*. This DNA molecule has been extensively used because of its simplicity and the availability of its nucleotide sequence since the early days. Today, pBR 322 is still used as a molecular cloning vehicle, although more advanced vectors have been developed from it.[1] [The *SCI*® indicates that this paper has been cited in more than 3,395 publications, making it the most-cited paper from this journal.]

Multipurpose Tools in Molecular Biology

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June 13, 1990

Significant advances in scientific endeavors are always accomplished faster if work is supported by efficient tools and methodologies. The inability to concretize ideas in scientific research, due to methodology limitations, may be quite frustrating. Therefore, methods and research tools are usually subject to evolution themselves, so their refinement becomes a strategic aspect of scientific progress. The story of the design, construction, and characterization of the multipurpose cloning vehicle, the plasmid pBR322, is an example of this notion.

In the early days of molecular cloning, plasmid vectors were poorly characterized: They exhibited high molecular weights and were devoid of convenient cloning sites. Little was known about their intrinsic features, such as stability, coding properties, and functions. This was the scenario when I joined Herbert Boyer's group at the University of California, San Francisco, in 1975. This group was composed of several bright scientists from different countries working hard on the isolation and manipulation of specific genes. However, progress in the work was slow, I believe mainly because of limited tools, particularly the cloning vectors and low purity restriction endonucleases and T4 DNA ligase. Inevitably, some of the members of the laboratory decided to work towards the development of new and more efficient tools.

Initially, Herb Boyer was not very keen on the idea of constructing a new vector because we already had pMB9, so most of this work was done during our "spare time." Nevertheless, when pBR322 was constructed, he became a strong supporter of the new cloning vehicle and made it readily available to the scientific community. The plasmid was distributed to over 300 laboratories all over the world during those early days. I strongly believe that this was one of the reasons scientists adopted pBR322 as a member of their labs.[2] Convenience of cloning pBR322 by inactivation of antibiotic resistance genes, and various unique restriction sites, offered a simple way to design experiments and a rapid analysis of results, rendering pBR322 quite superior to its parental plasmid, pMB9. Moreover, safety of the cloning procedures was then in the minds of millions, and pBR322 was built with a highly diminished ability to propagate outside laboratory cells. In fact, this was the first example of an EK2 system to be certified as safe according to the National Institutes of Health recombinant DNA guidelines. Finally, the elucidation of the complete nucleotide sequence of pBR322 two years later by Greg Sutcliffe at Wally Gilbert's lab[3] strongly contributed to its popularity, yielding experimental design more versatile and precise. The following quotation summarizes the impact of pBR322 in the late 1970s and early 1980s: "It was, in short, a compact dream machine of a plasmid."[4]

After more than a decade, pBR322 is still being used in a large variety of ways. Most importantly, this multipurpose cloning vector has been used as the parental plasmid of many specialized vectors utilized today, not only for *Escherichia coli*, but also for interspecies shuttle vectors. Its origin of replication, coding regions, structural features, and functional capabilities have been so extensively studied that parts of pBR322 continue to be used as components for the development of new plasmids.[1] The paper describing the construction of pBR322 became a *Citation Classic* long ago.[5] However, it is more meaningful to me that the original paper is not referred to anymore in a large number of papers, perhaps having achieved wide recognition.

Finally, as my good friend Pierre Prentki quoted to me once, "...a myth starts when the same character belongs to more than one story." pBR322 has now transcended the walls of molecular biology laboratories. Its restriction map is exhibited in watches distributed by Boehringer Mannheim, and even a love story has been published regarding one of the derivatives of pBR322: "...One day, a messenger of King Pebearius CCCXXII came through Coliborough upon Tween and told the villagers about the great misfortune that had befallen the king. A strange blue light had been seen in the palace one night, and the next morning Princess Clonia had not appeared for lunch."[6]

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2. **Bolivar F.** Plasmid pBR322: the multipurpose cloning vector. *Focus* 10:60-4, 1988.
3. **Sutcliffe J G.** Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. *Cold Spring Harbor Symp. Quant. Biol.* 43:77-80, 1979. (Cited 1,020 times.)
4. **Hall S S.** *Invisible frontiers. The race to synthesize a human gene.* Washington, DC: Tempus Books, 1987.
5. **Garfield E.** The articles most cited in 1961-1982, 4. 100 additional *Citation Classics*. *Current Contents* (40):3-9, 1 October 1984.
6. **Ibelgaufts H.** How little Tom Plasmid won the hands of Princess Clonia. *Trends Biochem. Sci.* 6:III-IV, 1981.

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