## United States Patent [19]

Uhlin et al.

[11] Patent Number:

4,487,835

[45] Date of Patent:

Dec. 11, 1984

### [54] PLASMID HAVING TEMPERATURE DEPENDENT PLASMID COPY NUMBER

[75] Inventors: Bernt E. Uhlin; Kurt Nordström; Soeren Molin, Petter Gustaffson,

all of Odense Denmark

[73] Assignee: A/S Alfred Benzon, Copenhagen,

Denmark

[21] Appl. No.: 214,423

[22] Filed: Dec. 8, 1980

#### Related U.S. Application Data

[62] Division of Ser. No. 908,108, May 22, 1978.

## [30] Foreign Application Priority Data

Dec. 30, 1977 [DE] Fed. Rep. of Germany ...... 2759053

[58] Field of Search ...... 435/317, 172, 68

[56] References Cited
PUBLICATIONS

Goebel, Eur. J. Biochem., 43, pp. 126-130, (1974). Journal of Bacteriology, Jan. 1975, pp. 354-362.

"Construction of a Colicin E1-R Factor Composite Plasmid in Vitro Means for Amplification of Deoxyribonucleic Acid", by Tanaka et al., Journal of Bacteriology, May 1972, pp. 529-537.

"Properties of an R Factor Which Originated in *Pseudomonas aeruginosa* 1822", by John Grinsted et al. Genet. Res., Camb. (1968), 12, pp. 169-185, "Resistance

Genet. Res., Camb. (1968), 12, pp. 169–185, "Resistance of *Escherichia coli* to penicillins", by Hans G. Boman et al.

Genetics 39, Jul. 1954, "Segregation of New Lysogenic Types During Growth of a Doubly Lysogenic Strain Derived from *Escherichia coli*", by R. K. Appleyard. Journal of Bacteriology, Apr. 1976, pp. 1-6, "Translocation of a Discrete Piece of Deoxyribonucleic Acid Carrying an amp Gene Between Replicons in *Escherichia coli*", by P. M. Bennett et al.

Nature, vol. 214, May 27, 1967, "Mutant Drug Resistant

Factors of High Transmissibility", by Elinor Meynell et al.

Molec., gen. Genet., 142, 239-249, (1975), "The Generation of a CoIE1-Ap Cloning Vehicle which Allows Detection of Inserted DNA", by Magdalene So et al. Journal of Bacteriology, May 1972, (pp. 562-569), "Mutations in R Factors of *Escherichia coli* Causing an Increased Number of R-Factor Copies per Chromosome", by Kurt Nordstrom et al.

Journal of Bacteriology, Aug. 1975, pp. 658-665, "Class of Small Multicopy Plasmids Originating From the Mutant Antibiotic Resistance Factor R1drd-19B2", by Werner Goebel et al.

Cell, vol. 11, 11–23, May 1977, "Translocatable Elements in Procaryotes", Review by Nancy Kleckner. Abstract entitled, "A Mutant Plasmid with Altered Replication Control", by Grindley et al., Distributed at Second ASM Meeting on Extrachromosomal Elements, Jackson, Wyoming, 2/20–25/77, No. 5.

Microbiology—1978, Edited by David Schlesinger, 1978, pp. 71-73.

Abstract Distributed at Workshop on Plasmids and Genetic E Change between Bacteria, Berlin, May 22-25, 1976.

Abstract entitled, "Control of Plasmid R1 Replication in *Escherichia coli*: Genetics, Selection, Timing and Control Parameters", by Nordstrom et al., distributed a Second ASM Meeting on Extrachromosomal Elements, Jackson, Wyoming, 2/20-25, 1977, No. 1.

(List continued on next page.)

Primary Examiner—Alvin E. Tanenholtz Attorney, Agent, or Firm—Hubbell, Cohen, Stiefel & Gross

## [57] ABSTRACT

Gene products of plasmid DNA, such as proteins, are prepared in high yields by cultivating bacteria carrying a plasmid which shows a controlled constant plasmid copy number at one temperature and a much higher or totally uncontrolled copy number at a different temperature. The plasmid may be prepared by recombinant DNA technique using a cloning vector showing the temperature dependent plasmid copy number pattern.

9 Claims, 4 Drawing Figures



Sanofi/Regeneron Ex. 1033, pg 938

#### OTHER PUBLICATIONS

Topics in Infectious Diseases, vol. 2, R-Factors: Their Properties and Possible Control, "Copy Mutants of the Plasmid R1 as a Tool in Studies of Control of Plasmid Replication", Nordstrom et al., pp. 299-332.

Excerpts from Petter Gustafsson's doctoral thesis, May

27, 1977, pp. 29, 31, 8 and 9.

Abstract distributed at FEMS Meeting in Paris, Dec. 1-2, 1976, entitled "Replication Control: Copy Mutants of Plasmids", Nordstrom et al.

Abstract distributed at Symposium about R factors in Tokyo, Japan, Oct. 26-28, 1977, entitled "Replication and Copy Number Control of The R Plasmid R1 in E. coli", Uhlin et al.

Journal of Bacteriology, "Cold-sensitive Mutations in Salmonella typhimurium Which Affect Ribosome Synthesis", by Tai et al., Mar. 1969, pp. 1298-1304.

Journal of Bacteriology, "Characteristics of Cold-Sensitive Mutants of Escherichia coli K-12 Defective in Deoxyribonucleic Acid Replication", by Wehr et al., Jul. 1974, pp. 99-107.

Journal of Bacteriology, Mutants of Escherichia coli with Cold-Sensitive Deoxyribonucleic Acid Synthesis, by

Waskell et al., Jun. 1974, pp. 1027-1040.

Temperature and Life, "II Genetic Regulation of Temperature Responses", Ingraham et al., 1973, pp. 60-85. Proc. Soc. Gen. Microbiol., vol. 2, 1975, K. Nord-stroem et al.: "Replication of the Plasmid R1".

Microbiology 1978; K. Nordstroem et al.: "Control of Replication of Plasmid R1 is Escherichia coli".

Chemical Abstracts, vol. 88, 1978, Ref. 133092v, No. 19; N. D. F. Grindley et al.: "Mutant Plasmid with Altered Replication Control"

Chemical Abstracts, vol. 89, 6/11/1978, Ref. 159 981e, P. M. Andreoli et al.: "Isolation and Characterization of a Clo DF1 3xTn901 Plasmid Mutant with Thermosensitive Control of DNA Replication".

Chemical Abstracts, vol. 83, No. 17, 27/10/1975, Ref. 144 393s; W. Goebel et al. "Class of Small Multicopy Plasmids Originating From the Mutant Antibiotic Resistance Factor R1drd-19B2"

Chemical Abstracts, vol. 84, No. 25, 21/6/1976, ref. 176 581p; H. Hashimoto et al.: "Isolation of a Miniplasmid From an R Factor".

Journal of Bacteriology, vol. 131, No. 2, Aug. 1977, T. Hashimoto et al.: "Mutations to Temperature Sensitivity in R Plasmid p SC 101".

Molec. Gen. Genet. 165, 1978; B. E. Uhlin et al.: "A Runaway-Replication Mutant of Plasmid R1drd-19; Temperature Dependent Loss of Copy Number Con-

Plasmid, 1, 1977, B. E. Uhlin and K. Nordstroem: "R Plasmid Gene Dosage Effects in Escherichia coli K-12: Copy Mutants of the R Plasmid R1drd-19".

Plasmid, 1, 1977, K. Nordstroem et al.: "Phenotypic and Genetic Symbols for Plasmid Copy Number Mutants".

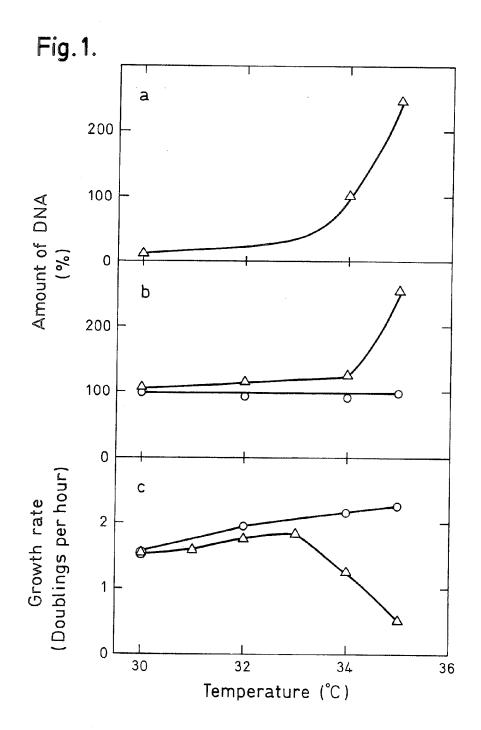
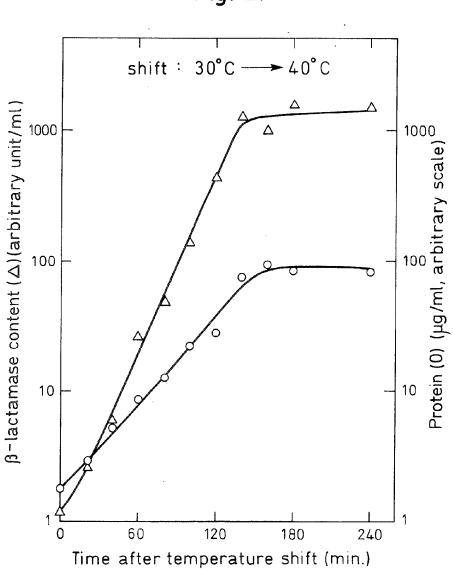
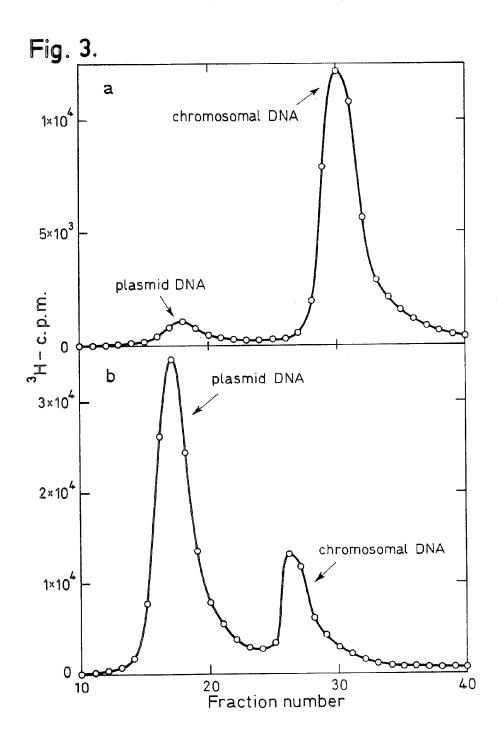


Fig. 2.





# DOCKET A L A R M

# Explore Litigation Insights



Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

# **Real-Time Litigation Alerts**



Keep your litigation team up-to-date with **real-time** alerts and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## **Advanced Docket Research**



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

# **Analytics At Your Fingertips**



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## **LAW FIRMS**

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## **FINANCIAL INSTITUTIONS**

Litigation and bankruptcy checks for companies and debtors.

## **E-DISCOVERY AND LEGAL VENDORS**

Sync your system to PACER to automate legal marketing.

