The Jobs of the Future:

Experts say these careers will be in high demand over the next decade. Pick your personality type and find a great gig you may never have thought of!

- **Robot Personalizer:**
  - what you'll do: give home robots personalities so they will interact well with people.
  - why it'll be big: By 2025, sales of home robots will jump to $65 billion a year—and those robots will need to be friendly.

- **Energy Engineer:**
  - what you'll do: develop new energy sources, so people aren't as dependent on oil.
  - why it'll be big: By 2020, half of the world's oil supply may be gone—we'll need new ways to power homes and cars.

- **Genetic Engineer:**
  - what you'll do: study people's genes to determine if they are predisposed to certain diseases.
  - why it'll be big: The technology to read a person's genetic makeup will soon exist—so we can use it to prevent illnesses.
Herb Boyer and Stanley Cohen brainstorm at a conference in Hawaii, November 1972
Construction of Biologically Functional Bacterial Plasmids In Vitro

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ABSTRACT The construction of new plasmid DNA species by in vitro joining of restriction endonuclease-generated fragments of separate plasmids is described. Newly constructed plasmids that are inserted into Escherichia coli by transformation are shown to be biologically functional replicons that possess genetic properties and nucleotide base sequences from both of the parent DNA molecules. Functional plasmids can be obtained by reassociation of endonuclease-generated fragments of larger replicons, as well as by joining of plasmid DNA molecules of entirely different origin.

Controlled sharing of antibiotic resistance (R factor) DNA leads to formation of plasmid DNA segments that can be taken up by appropriately treated Escherichia coli cells and that recombine to form new, autonomously replicating plasmids (1). One such plasmid is that formed after transformation of E. coli by a fragment of chromosomal R6K DNA, pSC101 (previously referred to as Tn5-5), has a molecular weight of 3.8 x 10^8, which represents about 10% of the genome of the parent R factor. This plasmid carries genetic information necessary for its own replication and for expression of resistance to tetracycline, but lacks the other drug resistance determinants and the fertility functions carried by R6K (5).

Two recently described restriction endonucleases, EcoRI and EcoRRI, leave double-stranded DNA so as to produce short overhangs at single-stranded ends. The nucleotide sequence cleaved are unique and self-complementary (2-4). Thus, DNA fragments produced by one of these enzymes can associate by hydrogen-bonding with other fragments produced by the same enzyme. After hydrogen-bonding, the 5'-hydroxyl and 3'-phosphate ends can be joined by DNA ligase (6). Thus, these restriction endonucleases appeared to have great potential value for the construction of new plasmid species by joining DNA molecules from different sources. The EcoRI endonuclease seemed especially useful for this purpose, because on a random basis the sequence cleaved is expected to occur only about once for every 4,000 to 10,000 nucleotide pairs (2); thus, most EcoRI-generated DNA fragments should contain one or more intact genes.

We describe here the construction of new plasmid DNA species by in vitro association of the EcoRI-derived DNA fragments from separate plasmids. In one instance a new plasmid has been constructed from two DNA species of entirely different origin, while in another, a plasmid which has itself been derived from EcoRI-generated DNA fragments of a larger parent plasmid genome has been linked to another replicon derived independently from the same parent plasmid. Plasmids that have been constructed by the in vitro joining of EcoRI-generated fragments have been inserted into appropriately-treated E. coli by transformation (7) and have been shown to form biologically functional replicons that possess genetic properties and nucleotide base sequences of both parent DNA species.

MATERIALS AND METHODS

E. coli strain W1485 containing the R6K101 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R factors and procedures for DNA isolation, electron microscopy, and transformation of E. coli by plasmid DNA have been described (7, 8). Purification and use of the EcoRI restriction endonuclease have been described (5). Plasmid heteroduplex studies were performed as previously described (9, 10). E. coli DNA ligase was a gift from P. Mootz and R. L. Lehman and was used as described (11). The detailed procedures for gel electrophoresis of DNA will be described elsewhere (Helling, Goodman, and Boyer, in preparation); in brief, duplex DNA was subjected to electrophoresis in a tube-type apparatus ( Hoefer Scientific Instruments) (0.6 X 15 cm gel) at about 200 volts for 3-4 hours. The gels were then stained with ethidium bromide (0.5 μg/ml) and the DNA was visualized by fluorescence under long wavelength ultraviolet light (365 light). The molecular weight of each fragment in the range of 1 to 300 X 10^6 was determined from its mobility relative to the mobilities of DNA standards of known molecular weight included in the same gel (Helling, Goodman, and Boyer, in preparation).

RESULTS

R6K and pSC101 plasmid DNA preparations were treated with the EcoRI restriction endonuclease, and the resulting DNA products were analyzed by electrophoresis in agarose gels. Photographs of the fluorescent DNA bands derived from these plasmids are presented in Fig. 1 and 2. Only one band is observed after EcoRI endonucleolytic digestion of pSC101 DNA (Fig. 1), suggesting that this plasmid has a single site susceptible to cleavage by the enzyme. In addition, endonuclease-treated pSC101 DNA is located at the position in the gel that would be expected if the covalently closed circular plasmid were cleaved once to form noncircular DNA of the same molecular weight. The molecular weight of the linear fragment estimated from its mobility in the gel is 3.8 X 10^6, in agreement with independent measurements of the size of the intact molecule (1). Because pSC101 has a single EcoRI cleavage site and is derived from R6K, the equivalent DNA sequences of
The African Clawed Frog
*Xenopus laevis*

**Replication and Transcription of Eukaryotic DNA in Escherichia coli**


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**Abstract**

Fragments of amplified *Xenopus laevis* DNA, coding for 16S and 23S ribosomal RNA and generated by EcoRI restriction endonuclease, have been ligated into *E. coli* by transformation. These recombinant plasmids, containing both eukaryotic and prokaryotic DNA, replicate stably in *E. coli*. RNA isolated from *E. coli* minicells harboring the plasmids hybridizes to amplified *X. laevis* DNA.

Recombinant DNA molecules constructed in *E. coli* from separate plasmids (1, 2) by the joining of DNA fragments having cohesive termini (3, 4) generated by the EcoRI restriction endonuclease (5, 6) can form biologically functional replicates when introduced into *E. coli* by transformation (7). The *E. coli* tetracycline resistance plasmid, pSC101 (6, 7), with molecular weight 5.8 × 10^6, is useful for selection of recombinant plasmids in *E. coli* transformants, since insertion of a DNA segment at the single EcoRI cleavage site does not interfere with expression of the tetracycline resistance gene(s) or with the replication functions of the plasmid (1, 2).

This report describes the isolation and use of *E. coli* and *X. laevis* DNA cleaved by EcoRI endonuclease, and subsequent recovery of recombinant DNA molecules from transformed *E. coli* in the absence of selection for genetic properties expressed by the eukaryotic DNA. The amplified rDNA (coding for 16S and 23S ribosomal RNA) of *X. laevis* was used as a source of eukaryotic DNA, since it has been well characterized and can be isolated in quantity (9, 10). Recombinant plasmids containing both *X. laevis* and pSC101 DNA replicate stably in *E. coli*, where they are capable of synthesizing RNA complementary to *X. laevis* DNA.

**Materials and Methods**

DNA coding for ribosomal RNA of *X. laevis* isolated by CaCO_3-gradient centrifugation, and 5'-labeled 180 and 28S rDNA of *X. laevis* ribosomal RNA were the generous gifts of Dr. D. D. Brown. Bacterial strains and the tetracycline resistance plasmid pSC101 have been described (6, 7). Covalently-closed circular plasmid DNA was isolated as described (8, 11), or

**Abbreviations:** rRNA, ribosomal RNA; rDNA, amplified DNA; RNA: rDNA, amplified DNA containing the genes for 16S and 23S rRNA; EcoRI, the restriction and modification host specificity of *E. coli* controlled by the 6′ plasmid, pBR322;

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**Results**

Cloning of rDNA of *X. laevis*. Linear molecules of *X. laevis* rDNA (molecular weight about 3.1 × 10^6, as determined by electron microscopy) were treated with excess EcoRI endonuclease. After complete digestion, about 44% of the molecules had a molecular weight of 3.1 × 10^6 (Fig. 1) and a second major class (25%) had a molecular weight of 4.3
Such was the speculative fever ...

The whole affair left Wall Street slightly dazed. Within minutes, the new stock leaped from its offering price of $35 a share to $89. As thousands of buyers bid for a piece of the action, brokerage houses had to resort to strict rationing. When a Beverly Hills marine demanded 100 shares, the broker apologetically explained that he could give her only two.

Such was the speculative fever when an obscure company named Genentech came to the over-the-counter market with a $56 million stock offering last fall. Veteran traders had never seen such commotion over an embryonic company, which had only 140 employees, sold no product to the public and showed a profit for just one year, at a rate of 2c per share. In fact, Genentech is only one of a growing number of similar companies just coming into existence that offer little more than vague promises of scientific things to come.

But what promises, what dazzling things to come—a new alphabet that may one day turn the beasts of creation into genetic gold. That alphabet is already capable of making new drugs like the antiviral agent interferon, a possible weapon with which to attack cancer. In the future, it may produce vaccines against hepatitis and malaria; miracle products like low-calorie sugar; hardy self-fertilizing food crops that could usher in a new “green revolution”; fuels, plastics and other industrial chemicals, out of civilization’s wastes; mining and refining processes to relieve Malayan anxieties about a future without sufficient raw materials.

Such things now seem within man’s reach through the commercial adaptation of gene splicing, or recombinant DNA (for deoxyribonucleic acid). It is the genetic equivalent of transforming the world—a genie that, scientists hope, the world will never want to put back into the bottle.

In recent years, scientists have also developed other techniques in genetic engineering. Most aim at modifying the hereditary mechanisms of microorganisms or cells for purposes of research or commerce. Others include the fusion of cells, DNA synthesis and the creation of hybridomas, long-lived cells that are designed to produce pure antibodies for use against disease. But of all these marvels, it is gene splicing that scientists consider the most exciting.

Says the University of Zurich’s Charles Weissmann, 60, who last year became the first scientist to make bacteria produce a facsimile of human interferon: “Biologists have never had this tool in the laboratory before. It’s like building a tunnel into the jungle with a compass.”

Gene splicing is the most powerful and awesome skill acquired by man since the splitting of the atom. It is an unparalleled exploratory tool for examining, and in the process changing, the complicated machinery of heredity. If a gene of unknown function is inserted into bacteria, it can act as a probe that lets scientists see precisely what it does. By such techniques, researches hope to learn more about human genes and what they do. In a sense, gene splicing is the first attempt to make bacteria do the thinking of people—something only a genie might do.
Monsanto conducts the first field trial of genetically-modified plants in 1987—attracting the attention of a single (part-time) television reporter.
Protest Against Genetically Modified Organisms
Vienna, Austria, 5 April 2006
The Iconic Discovery of Twentieth-Century Biology:
James Watson and Francis Crick Uncover the Double Helical Structure of DNA in 1953
“Equally strong opposition to programs aimed at preventing the birth of severely genetically impaired children comes from individuals who believe that all human life is a reflection of God’s existence and should be cherished and supported with all the resources at human disposal. ... But such arguments present no validity to those of us who see no evidence for the sanctity (holiness) of life, believing instead that human as well as all other forms of life are products not of God’s hand but of an evolutionary process operating under Darwinian principles of natural selection.”—Jim Watson (1994)
“God did extraordinary miracles through Paul, so that even handkerchiefs and aprons that had touched him were taken to the sick, and their illnesses were cured and the evil spirits left them.”
Acts 19:11-12.
“Scientists and doctors are the people who are going to do the miracles of the future, let the lame walk, stop the cancers, restore sight. It’s all sort of within our vision. So I don’t worry, you know, that some people say we are ‘playing God’. But that’s an awfully good thing to do because people are looking for gods to do good things.”

St. James of Cold Spring Harbor?
In his first televised address to the nation as president, George W. Bush announces that no federal research funding would be permitted on embryonic stem-cell lines created after August 9th, 2001.

President Bush: “I will also name a President's council to monitor stem cell research, to recommend appropriate guidelines and regulations, and to consider all of the medical and ethical ramifications of biomedical innovation. This council will consist of leading scientists, doctors, ethicists, lawyers, theologians and others, and will be chaired by Dr. Leon Kass, a leading biomedical ethicist from the University of Chicago.”
Leon R. Kass
The Addie Clark Harding Professor in the Committee on Social Thought and the College at the University of Chicago
“Technology, in its fullest meaning, is the disposition rationally to order and predict and control everything feasible in order to master fortune and spontaneity, violence and wildness, and leave nothing to chance, all for human benefit. ... Modern science seeks knowledge of how things work, to be used as a means for the relief of all humanity, knowers and non-knowers alike. ... Modern science is practical and artful not only in its ends. Its very notions and ways manifest a conception of the interrelation of knowledge and power. Nature herself is conceived energetically and mechanistically.”

"The project for the mastery of nature, even as it provides limitless power, leaves the ‘master’ lost at sea. Lacking knowledge of ends and goals, lacking standards of good and bad, right and wrong, we know not who we are nor where we are going. Yet we travel fast and freely, progressively achieving our own estrangement—from our communities, from our nature, from our very selves. ... Man does not live by rationality alone. Indeed, the foundations of our humanity—our sentiments, loves, attitudes, mores and character, as well as the familial, social, religious and political institutions that nourish and are nourished by them—are not laid by scientific reason or rational technique, and may, in truth, be undermined by them, especially if our much-vaunted scientific rationality is ... [as I believe] philosophically unsound and finally unreasonable."

“Man is the watershed that divides the world of the familiar into those things which belong to nature and those which are made by men. To lay one’s hands on human generation is to take a major step towards making man himself simply another of the man-made things. Thus, human nature becomes simply the last part of nature which is to succumb to the modern technological project, a project which has already turned the rest of nature into a raw material at human disposal.”

Leon Kass and the Persistence Power of “Blind Chance”

“Our conquest of Nature has made us slaves of blind chance. We triumph over nature’s unpredictabilities only to subject ourselves to the still greater unpredictability of our capricious wills and fickle opinions.”

“Just as we must do battle with antimodern fanaticism and barbaric disregard for human life, we must avoid runaway scientism and the utopian project to remake humankind in the image of our choosing. To safeguard the human future rests on our ability to steer a prudent middle course, avoiding the inhuman Osama bin Ladens on the one side and the posthuman Brave New Worlders on the other. Unfortunately, we are not yet aware of the gravity of our situation.”

Leon Kass Identifies Dire Threats to the Future of Humanity:

“The inhuman Osama bin Ladens”

“The posthuman Brave New Worlders” (like James Watson)
Ancient Greece

Thomas Cole, *Dream of Arcadia* (1838)