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A Primer on Genetic Engineering III: Identification and Manipulation of Genes in Humans

Mary K. Howett Ph.D.

A PRIMER OF GENETIC ENGINEERING III: IDENTIFICATION AND MANIPULATION OF GENES IN HUMANS.

*Mary K. Howett, Ph.D.**

I'd like to spend some time now trying to transfer what we've just heard about DNA and DNA cloning into ways in which people are beginning to use this technology in relationship to humans. Specifically, we'll talk about gene therapy, the delivery of genes actually to human cells, and possible applications in adults and in newborns.

Where do we get DNA if we want to analyze it? I'm sure many of you in this room have seen the popular movie *Jurassic Park* in which DNA was isolated from a specimen embedded in amber. It's now possible to get sufficient DNA for analysis from a very large variety of different places, including a single drop of blood.

The Armed Services are now instituting a plan to actually store a single drop of blood on a file card for every enlisted man. There will never again be an addition to the Tomb of the Unknown Soldier, because this will essentially create a DNA database for at least that very large segment of our population.

It's possible to analyze three individual hair follicles, you can recover enough DNA from this, tissue fragments from both intact or pieces of decayed corpses, or specimens that have embedded in paraffin in hospitals and kept for long periods of time. Other bodily fluids, the most common of which probably to be analyzed is semen from rape scenes or crime scenes can also be examined.

We were having a discussion when we were preparing these slides. We know for a fact that DNA has been recovered from some fossilized plants and animals that are at least 500,000 years old, and someone was saying during our discussion that perhaps back as far as a million years. It should be clear that in fossils, the DNA is very decayed and fragmented, but because you're working with a very large number of molecules, millions of copies of a single molecule, it may still be possible to get sequence information. If you have even one intact molecule out of these millions of decayed molecules and you use PCR technology, you can recover even very low numbers of DNA from a massively decayed DNA.

We should point out that for all of these samples, and because of the sensitivity of PCR technology, it's very important that you know that the DNA that you're recovering is, in fact, the DNA that you think it is. That's been one of the drawbacks of PCR technology, which is that often times the samples can be contaminated with exogenous DNA. Organisms on the bench top, dropped pieces of skin, or other contaminating DNA in our environment can get into the PCR reaction and cause problems in the analyses. (See: Fig. 1 - Page 31).

When the PCR product is being analyzed for identity with a previously known DNA sample, the most common technique that is used is referred to as Restriction Fragment Length Polymorphism or RFLP analysis. This is the technique now used for identification in forensic cases and for identification in paternity cases. It is essentially the technique which is used to establish identity between sample A and sample B.

In this technique a defined DNA fragment is examined. Of the very long DNA which

*B.S., Philadelphia College of Pharmacy and Science, 1969; Ph.D. University of Pennsylvania, 1976.

is present in the cell, only a very short piece is analyzed. The way that piece is picked out for analysis is that specific primers are used in the DNA analysis, which are known to only hybridize to one particular region of the DNA. So this DNA actually extends very long on the right and very long on the left, but when you do the PCR amplification, you only look at the DNA which is made after the PCR amplification between these two primers.

Once the PCR product is isolated, it can be cut and compared to standard DNA. Bands generated by the cutting can be isolated by electrophoresis on a gelatin matrix. They separate on the basis of size. Two DNA samples that are identical, can be seen in lanes one and two. If you compared lane two to lane three, these two DNA samples are non-identical.

What the scientist or the technician sees after the RFLP analysis is the presence of these bands on the gelatin matrix and the comparison of these bands between the samples. A practical example of this would be to look at a specific segment of DNA in a forensic scene where you had a semen sample taken from the scene of the crime and you compared it with a fresh DNA sample taken from a blood test from the suspected perpetrator. If the two samples match one another, there's a very high instance of certainty that the individuals are identical. (See: Fig. 2 - Page 32).

Now, once you have cloned a gene into a plasmid, or amplified it by PCR and subsequently cloned it, then you can use this DNA for gene replacement therapy. I've listed here possible ways in which replacements are done. Some of these are currently in practice. Some of these are still theoretical in regard to humans.

The first method is to draw blood from the patient, isolate the white blood cells, and grow them in culture, those techniques are now available. The cloned DNA is introduced into the white blood cells growing in culture. After making sure the cells contain the gene of interest and are expressing the protein product of that gene, they can be reintroduced into the patient. The white blood cells will now be expressing your new protein.

This technique is in practice. It has been used for a condition called ADA deficiency. ADA stands for a protein called adenosine deaminase. Children who are born lacking this gene have the boy in the bubble syndrome. They're globally immunodeficient and can't mount an immune response to any infectious organisms. They have to be kept in a totally protected environment.

This technique has been used to replace the ADA back into several of these patients and they are expressing the ADA proteins. It has been considered so successful that now white blood cells have been readministered back into newborn children lacking this gene. It is still unknown whether this will correct their immunodeficiency, but we do know that these patients are expressing ADA.

A second technique is to clone a replacement gene into a disabled virus. By disabled, we mean a virus that's no longer capable of causing disease. An example of this technique is that people have now cloned the gene which corrects the deficit for cystic fibrosis into a rhinovirus, a common cold virus. This disabled rhinovirus is now being administered to humans by aerosolizing it into the lungs. Rhinovirus normally grows in the lungs.

In cystic fibrosis, mucus accumulates in the lungs of these children and their deficit is a lack of respiratory capability. This delivery system takes the gene, puts it back into the lungs, and allows it to be expressed at the site of the deficit.

A third possibility is to inject the gene directly into muscle cells. Such experiments

have been done only in animals to date. It's a particular characteristic of muscle cells that they share their borders. So if you look at a cross section of muscle, instead of seeing nice individual cells, what you see is a fusion between the cells.

If a gene is injected into one muscle cell, it actually will diffuse among cells and the protein product will also diffuse. This technique is being used experimentally in boys suffering from Duchenne's muscular dystrophy. In that disease, the boys are missing a protein called dystrophin, which is essential to their muscle organization, and they die from muscle degeneration. There is an investigator in Memphis, who has reported some success, replacing the dystrophin gene into muscles of these patients.

Eventually we will be able to deliver genes directly into embryos. This has been done in animals and it has produced types of animals that we refer to as transgenic animals. The next slide will show you how this works. By being able to replace a gene in a newborn child or possibly even in a fetus, it would be possible to correct the deficit before the development of disease. (See: Fig. 3 - Page 33).

In summary, the presence of the DNA actually directs the protein-product production. The recombinant DNA which carries the gene of interest can be put into either a bacterial cell, a prokaryotic cell, or into a eukaryotic cell, a human white blood cell or a human embryonic cell. The plasmid or the recombinant DNA will amplify itself. Copies will be made. Sometimes, the protein is automatically made, however, sometimes it's undesirable for the protein to be made continuously.

It is possible to install switches for the protein, amplify the plasmid in the absence of the protein, and then turn the protein production on at the desired time. The protein, once it is made, acts in the cells which have now been delivered back to the body. It is also possible to take the protein product, purify it and use it. There is now a revolution caused by biotechnology in the pharmaceutical industry where we have protein products which have been made in this way which are specifically being purified and used to produce pharmaceuticals.

An outstanding example of this is the current vaccine against hepatitis B virus. The hepatitis B virus vaccine uses a recombinant plasmid to make one of the virus proteins. The virus protein is subsequently purified and used as a vaccine.

This vaccine is extremely safe. It doesn't contain any live hepatitis B virus. It doesn't contain any HIV virus, which was a concern in the original hepatitis vaccine, because it was made from human serum. And so the hepatavax or recombinant hepatavax vaccine is extremely safe and a good example of this technology. (See: Fig. 4 - Page 34).

This illustrates one of the things that we've already talked about, the concept that one can take white blood cells in culture, amplify the plasmid or introduce the plasmid directly into the white blood cells and return them to the patient. (See: Fig. 5 - Page 35).

This slide will illustrate the concept of introducing a gene into an embryo. This approach, the production of transgenic animals, has been used both at a research level, many of us use transgenic mice in our work, and also very extensively at an agricultural level to produce various new animals and new plants.

If I could digress for a minute, people will talk about controversy over genetically engineered tomatoes. I think it's important for people considering molecular biology to realize that many, many plants and animals in the last 200 years have been genetically

altered. Just about every fruit and vegetable that we eat is a genetic variant of the original wild type specimen. It just wasn't done using molecular biology techniques. It was done using normal agricultural fertilization techniques, grafting, and cross-pollination.

Many of those very large pears, very large apples that we eat are genetically abnormal compared to their parent. Most of them possess double copies of their normal genome. Instead of having one copy of the genome, they have two. Most of them are genetically sterile. They can't reproduce themselves. They can only be reproduced by grafting. I don't think the general public has an appreciation that we're constantly being exposed to genetically altered animals and genetically altered plants. It just hasn't been done by cloning methods.

In transgenic animal production, the normal method is to take a very small eight-cell embryo. The embryo either can be produced in culture by in vitro fertilization or can be extracted from an animal after fertilization occurs normally. Each one of the cells of these eight-cell embryos is totipotent. Each cell can actually make a whole animal.

We take a single cell from those embryos using a very fine glass capillary, glass needle, we inject DNA directly into the cell. After that, the cell is reintroduced into this mass and the embryo is implanted into a foster mother and the offspring are allowed to develop.

In this case, I've illustrated this as the gene for black fur appearing in the offspring of the mother who has white fur. This has produced pin-striped mice; these animals express the gene for black fur and for white fur. We can then mate these animals and you can establish carrier lines which are permanently genetically altered and carry this gene.

Member of the Audience: What about those other seven cells that didn't get the new gene?

Dr. Howett: That's why I've indicated here that not all the cells will express the black fur. In fact, this animal will be what we call a genetic chimera. It's a mixture of cells expressing white fur and cells expressing black fur.

If you're trying to replace a gene in an organism, it's not necessary that the product be expressed in every cell. In fact, sometimes you want the product to only be expressed in certain cells.

So remember earlier when we were talking about switches, how liver cells know they're liver cells and skin cells know they're skin cells? You can install your replacement gene under the control of individual switches so that it will only be expressed in skin, for example, or it will only be expressed in liver. We call these switches, promoters. When people talk about skin-specific promoters, it means genes will only be turned on in skin. Liver-specific promoters, genes will only be turned on in liver. (See: Fig. 6 - Page 36).

The existence of transgenic animals tells us that we can eventually do this in humans. Of course, this has been a subject of much controversy in the popular press. One would hope that only beneficial things such as using these as gene therapy targets would come from this technology, but many other things have been discussed.

So we have a situation where we have the egg and the sperm uniting, again, in this case by in vitro fertilization, leading to a fertilized embryo with a genome in the nucleus. We can grow this in a test tube or in a Petri dish to an eight-cell embryo. A single cell can

be taken from this embryo, and we can use this single cell as a target for a number of different techniques. Using the same methodology that I just talked about for transgenic animals, this can be gene therapy target. We can introduce DNA into this cell, or use this cell for genetic testing.

One thing I didn't tell you is that when you take this one cell away, the seven cells still can go on and produce a whole embryo. There have now been experimental reports where a single cell can be tested for a particular genetic deficit.

One aspect that has been very much in the press in the last three months is that you can use these cells for twin generation. It has been routinely done in certain agricultural animals that you can use these cells for twin generation. Theoretically, if all eight of these cells are totipotent, each one of these eight cells could produce an individual that would be a twin of the other seven.

This is an important ethical consideration. This has not yet been done with humans. What was done and what was reported as "human cloning" was where embryos were taken in the laboratory, embryos that already had a lethal genetic deficit and could not develop into fetuses, and it was shown that you could separate these eight cells and that you could get them to develop further in the test tube. That was the experiment.

As a biologist, the result of that experiment is not very surprising. In cattle you can take the eight-celled embryo, flush it out of the fallopian tube of the fertilized cow, divide it into eight single cells, transplant it into eight foster mothers and produce eight identical cows. So the totipotency of these cells is already established. What was spectacular about this was the fact that it was done in humans. (See: Fig. 7 - Page 37).

I've listed here examples of human genetic diseases that are targets for gene therapy. We've already talked about one, global immunodeficiency, and we've talked about Duchenne's muscular dystrophy and cystic fibrosis. Huntington disease is a degenerative nervous system disease resulting in death. The gene for this has also been cloned. A number of genes increasing susceptibility to colon cancer have been identified and cloned, mainly for patients who have a hereditary or familial colon cancer syndrome. And recently genes for breast cancer have also been cloned.

This is a very small list. I would estimate now that there are hundreds of human defects where genes have been identified, either errors or missing genes, where possible gene therapy could be used.

Member of Audience: Of those hundreds of genetically-mediated diseases for which we have now identified genes, how many of those are going to be preventable or are presently preventable, or is this essentially predictive technology?

Dr. Howett: I don't think we really know the answer to that question at this time because there has not yet been a case where replacement of a gene has definitively indicated that the gene can be replaced and cure the deficit.

The only cases where genes have actually gone into humans' ADA deficiency, where they know the protein is being made, but don't yet know the long-term effect on the immune system of those children; Duchenne's, where they know that dystrophine is made and there is some improvement in the flexibility and muscular movement; but those are very limited studies involving very small groups of muscles.

Member of the Audience: There were two or three, that have been treated with the adenosine diaminase gene; is that correct?

Dr. Howett: I think it's more than that. I think it's been delivered to three newborns and about four or six older children.

Member of the Audience: Muscular dystrophy is what, fifty or sixty?

Dr. Howett: Muscular dystrophy, fifty or sixty boys have been treated, and there's a controversy in the literature because not everybody agrees with the results. You know, very small numbers.

There are many examples in experimental situations, however not in humans, where you can correct a deficit merely by replacing the gene. So it's predictive.

Member of the Audience: So I want to be sure I understand this, okay.

Dr. Howett: Yes, okay.

Member of the Audience: There are several hundred diseases –

Dr. Howett: Correct.

Member of the Audience: We can now basically take a drop of blood or a sputum sample or something, and we can predict whether these people are going to get this condition.

Dr. Howett: Let me make it clear that depending on what the disease is, sometimes that's a one hundred percent correlation and sometimes it's not.

Member of the Audience: Right.

Dr. Howett: All right. In Duchenne's, for example, you can say every boy who carries this gene will get Duchenne's. It's one hundred percent.

Member of the Audience: The same with Huntington's?

Dr. Howett: Yes. But with some diseases, particularly with cancers, it's not. It's a risk factor.

Member of the Audience: For the breast cancer, isn't there one now that's like a ninety percent chance of developing breast cancer?

Dr. Howett: That analysis has only been done in women who have had a familial breast cancer, where several members of a single family have the disease. So the gene that's been identified is in familial breast cancer.

Ms. Weber: I read yesterday in the paper that a new tumor-suppressing gene has been found, which is supposed to be more exciting and more predictive than the actual discovery of breast cancer or colon cancer genes. Could you just elaborate?

Dr. Howett: I don't know very much about it. It's called P16, which refers to the size, as opposed to P53 which is 53,000 and P16 is 16,000 moleculur weight. It's published in this week's *Science.* I haven't read the paper yet.

Member of the Audience: I believe you said you can tell when you're going to contract -- excuse me, that you will contract this disease. How about the variations of these different diseases?

Dr. Howett: In terms of time, that's usually attributed to the clinical syndrome associated with it. For example, in Duchenne's muscular dystrophy, boys start showing symptoms on an average at the age of six. They're usually in wheelchairs by the age of twelve and they usually die in their teens or early twenties. That's an average. You can't predict it in a single patient. Some will do worse, some will do better.

Then my last slide, (Fig. 8 - Page 38) will summarize all the things that we've been talking about, which are the various applications of molecular biology. Obviously, you can use these techniques to do research on basic aspects of life and of disease. You can use it for genetic testing, to predict disease and to identify individuals. You can use it to develop new drugs, drugs that are made from cloned genes.

You can also use these methods to establish gene therapy, put genes back into patients and correct deficits. Genes can also be used for identification and, although we haven't talked about it in detail, genes can be used to change animals and plants for agricultural improvements.

Dr. Bardales and Dr. Roberts why don't you come up as well and we can all take questions.

Member of the Audience: Can you transplant across species?

Dr. Howett: Genes — yes, it is possible. We talk about DNA sequences as being conserved or non-conserved. Some genes are very highly conserved, so that if you look back through humans, primates, mammals, birds, all the way back to bacteria, the organization of the gene, the structure of the gene, the way in which it's controlled is very, very similar. And if you look at the sequence of the gene, it's very, very similar.

You can certainly show hybridization across species of those genes, and in some cases you can make the product from one species in the cell of another species and you can show that it will function. It depends on which gene you're talking about.

Dr. Roberts: I think one of the well known examples of that was the introduction of the human growth hormone gene into mice, where the result is a larger mouse. Of course, in the popular press the question was, do we really need larger mice?

It was the establishment of the technique that was important in that experiment. It is

possible to take genes of one species and introduce them into another.

Dr. Howett: That's a very good example of what you were asking. Recombinant human growth hormone, the product of that gene cloning is now being given electively to some individuals of short stature, children of short stature. That's an example where they do have data that it corrects the deficit.

Member of the Audience: A little follow-up on what I said earlier. These various defects that we were talking about, most of them can now be identified in utero; correct?

Dr. Howett: Correct.

Member of the Audience: The technology is now available for parents to determine while this infant is in utero which of these defects the child is likely to have; is that correct?

Dr. Howett: That is correct. The most common prenatal testing right now is done by the method called amniocentesis, which is performed at sixteen weeks of gestation. A new technology called chorionic villus biopsy was introduced which can be done at eight weeks, but my belief is that this technique will soon be discontinued, because a number of children resulting from these pregnancies have had deficits in digit formation. They have missing toes or missing fingers. Testing a single embryonic cell from the eight-cell embryo is not currently in clinical practice.

In summary, for these deficits where genes have been identified, it is possible to identify the deficits by DNA technology. Only a handful of these genes, however, are currently being used in routine clinical screening.

Dr. Bardales: It has been attempted at the eight-cell stage to identify the cystic fibrosis defect. It's possible to take one of those eight cells, identify the defect, and then *in vitro* fertilization techniques have allowed a choice for couples.

Member of the Audience: Did you have any thoughts on that PCR question that I asked earlier?

Dr. Howett: You want to know what is the advantage of having a PCR product?

Member of the Audience: I always thought of the PCR product as a convenience of amplifying and getting your result faster, but I've been hearing people talk about the PCR product as though there was additional information more than from the Southern Blot.

Dr. Howett: Yes, there is. There is a gene you want to examine and you want to find out if there's a particular deficit. The deficit is a very small deficit, not small in terms of its consequence necessarily, but small in terms of its actual change in sequence.

It's a single base pair change or single base pair deletion; it doesn't really change the size of the gene and it doesn't really change the overall composition of the gene. The

normal probe would still hybridize on a Southern Blot. Southern Blot, for those of you who don't know, is when you put the DNA on the filter paper and hybridize it with a radioactive probe, okay, as discussed by Dr. Bardales.

In a PCR product, you can see that change. There are two ways you can do it. First, you can design a specific primer which will only pick up the mutated gene or because you can get the PCR product from in a large copy number, you can sequence it and then you see the base pair change. So there are some advantages to having the PCR product compared to just doing the Southern Blot.

Member of the Audience: I think in at least one instance it was related to fragile X.

Dr. Howett: I'm not sure.

Member of the Audience: For the genetic identity test, the RFLP test, are there standard sequences that are always cut out of the same chromosomes and compared for the same people?

Dr. Howett: Yes. There are a standard set of primers that are used. So if the DNA is very long, only certain regions of the DNA are examined. I don't know specifically what they are. I do know that it's more than one set of primers.

Member of the Audience: I was going to ask you how many.

Dr. Howett: It's multiple sets of primers, and a certain percentage of correspondence has been calculated. I don't know the exact percentage of correspondence, but it's very high.

Member of the Audience: Just a technical question. It's always been my impression that the number of base pairs is because the genes are laid out in linear fashion and that there's no instance where a single base pair could be used to define more than one gene. Is that correct?

Dr. Howett: No. He's asking if genes can overlap.

Member of the Audience: Yes.

Dr. Roberts: Yes, it's possible. I made the statement that the actual coding information for a gene is contained within just one of the two strands. It's also possible to have genes encoded in the DNA in an overlapping fashion on different strands. That's true of many viruses which have compact genomes where all their genetic information is contained within a fairly small DNA molecule.

Dr. Howett: The other thing I think is that even a single gene or what we call a single open reading frame can somehow be processed in different ways so that it makes different

proteins. The proteins may be subsets of one another, but they do different things.

Member of the Audience: So we have to deal with compression of logarithms here?

Dr. Howett: Definitely.

Member of the Audience: My question is with regard to cross species organ transplant, like from apes to humans, is it possible to do some type of gene therapy treatment on animal embryos to make them more suitable for transplant in humans?

Dr. Howett: Actually, those very experiments are now being done. The rejection antigens on the surface of your cells are called HLA antigens, and people are actually now trying to transplant HLA antigens from humans into pigs, into swine. This process would make transgenic pigs that would be expressing human proteins. Such organs would be hypothetically more suitable for transplants.

Dr. Bardales: It's interesting, one of the things that you mentioned about cross species. Dr. Howett talked about genetic sequences that were conserved between species. There have been experiments, done in the laboratory, in which researchers are studying cell division in yeast cells and they've located a gene that's involved in telling the yeast cell when it should divide.

One of the things these researchers have found is that you can take the same gene – it's highly conserved – from humans, put it back in the yeast that are missing this gene, and the human gene will tell the yeast cell to divide.

Dr. Howett: There's a thing called the Gene Bank and you can enter DNA sequence. It's a computerized database. You can enter sequence data for your gene which is unknown and you can say, give me all the genes with one hundred percent correlation to this sequence, all the genes with a ninety percent correlation, eighty percent, et cetera, and keep lowering the correlation.

There's a matching between those sequences and then the computer will tell you that your human muscle gene is the same as a gene in fruit fly wings, and then you'll be pulling your hair out trying to figure out why. Those data are available.

In addition, now a venture capital firm has started automated DNA sequencing and has now sequenced more than 100,000 open reading frames. They're hoping to eventually patent and capitalize on this sequence information from the human genome.

To return to the concept of size, the places where we think there are potential genes represent only three percent, approximately, of the DNA. So there may be yet other things encoded in the DNA that we don't know about or the rest of the DNA may be serving an editing function for rearranging and reorganizing this three percent.

Member of the Audience: So far you've dealt primarily with diseases, what are commonly known as diseases. As I understand it, there's at least one Pennsylvania researcher who is working on genes related to intelligence, and there is one fellow out in California

who has now announced that he has found a gene that mediates in the direction of homosexuality. What other work is being done on behavioral genes from which one could theoretically diagnose a tendency towards certain behaviors?

Dr. Howett: You have to think about how people do this, how they make this correlation, okay. There's been data published suggesting that getting divorced is a familial trait and people are trying to identify a gene associated with getting divorced. Since my husband is a divorce lawyer, he would like to know about it.

In the case of the homosexuality study, they took males who self identified as homosexuals and also self identified as either having a sibling, a maternal cousin or a maternal uncle as also being homosexual, and they did a genetic analysis. They identified a particular gene sequence that was present in something like forty-six percent of these individuals, but not in all of them.

I don't know the answer to the question: "Is behavior mediated by genetic inheritance?" Even if it is, is all behavior mediated by genetic inheritance? I think that's a total black box and that we're not going to know the answer to that for some time. In terms of whether people should be working on it or not, that's an ethical and a societal issue.

Member of the Audience: This question falls between the behavior research and the genetic diseases that are identifiable. There's something else called a genetic predisposition to a physical disease or a neurological disease.

Can you identify those diseases where there may be a genetic predisposition that is not the cause of the disease, but that, taken together with some unknown environmental cause possibly, the two together produce this disease?

Dr. Howett: That's correct.

Member of the Audience: I think, for example, of crippling polio, where if you've inherited a certain receptor the polio virus invades the body, and cripples that person. If you have not inherited that receptor, you will not be crippled by the virus. Are there other diseases where there's a genetic predisposition, taken in conjunction with something else, that then produces the disease?

Dr. Howett: We talked earlier about the concept that not all genetic markers are one hundred percent correlation. So let's talk about colon cancer, for example. Let's say that in order to get colon cancer, you have to have a mutational event in your colon which changes your normal cell to an abnormal cell, and that mutational event can be caused by any number of things. It can be caused by cigarette smoke. It can be caused by the amount of alcohol that you drink. It can be caused by the increased soy sauce in your diet. We don't know exactly what causes it.

Let's say you introduce some mutation into your colon, and as a consequence of that mutation, you have the bad luck of holding onto that mutation and you get a cancer from it. Most mutations in your colon go away. Every time you go out in the sun, you get UV radiation of your skin, you get mutations. For most of them your body says, there's a

mutation there, and it repairs it. It's only in the unfortunate case where you keep your mutation and the mutation is established that you actually wind up getting the cancer.

Now, the gene which indicates that there's a familial form of colon cancer may not – it's probably not the gene causing the mutation. It's probably some other ancillary gene involved in the whole process. It might be a gene involved in DNA repair. If you have that gene, you can't repair the DNA as well; but if you don't have it, you can repair your DNA at a normal rate. So your overall risk of keeping your mutation is lower if you don't have the gene and your overall risk of keeping the mutation is higher if you do have the gene.

So when you're talking about predisposition, you're talking about multi-factorial events, and we don't in many cases, even when we identify these genes, we don't exactly know the molecular mechanism by which they work. But it's not one hundred percent correlation.

Dr. Bardales: I'd like to interject at this point that we've been talking about mutations as mainly being bad events that occur to us. Everyone should realize, that mutations don't necessarily cause disease. Over millions of years of evolution, it's been mutations that have been responsible for us evolving into who we are today. All different species have evolved by mutation.

So sometimes mutations or changes in the base sequence cause advantageous changes in the organism, allow them better adaptation to their environment and increased ability for survival. So when you hear the word "mutation", don't necessarily assume that it's going to have some deleterious effect on the organism.

Member of the Audience: Does that suggest something regarding the survival rate of the species when a mutation is artificially introduced, though? For example, the ability of a hybrid plant to reproduce and survive, given the variety of climatic conditions.

Dr. Bardales: There's been some debate in the popular press about introducing a mutation into a plant and having that plant overgrowing all other species. I think, for example, last summer during the flooding in the Midwest, there was actually a plot of genetic corn. When agricultural products are genetically altered, there's a strict guideline for where these plants can be grown and different types of fences must be erected. During the flooding, it overcame all those fences and the corn actually flooded downstream. There was some concern in the popular press that this corn was going to take over.

But the thing to remember is when people genetically engineer plants, they also genetically engineer controls so that those things don't happen. For example, in this particular corn, the flooding occurred at a time in which the plants were not able to develop. After they had been uprooted, they were not able to reseed. So genetic engineering should build in the ability to control when and how things grow.

Member of the Audience: Actually, my concern is just the opposite, that by essentially developing genetic hybrids, we may be breeding out a variety that allows species to flourish.

Dr. Bardales: Mother Nature is going to allow environmental mutation to occur at its own rate, and I don't believe that we as scientists are going to affect that normal rate of mutation.

Dr. Howett: If you engineered every boy who had muscular dystrophy so that he would be cured and he would now live and reproduce, there's no doubt about the fact that you would be altering the evolution of the human species.

But that's also true for penicillin. Every time you save somebody who would have otherwise died from Strep throat, you're altering evolution. That's true for all virus vaccines. Virtually every medicine that is used to change the course of disease alters the course of evolution, because it allows individuals who would normally not survive and who would normally not reproduce in the gene pool and allows them to do so.

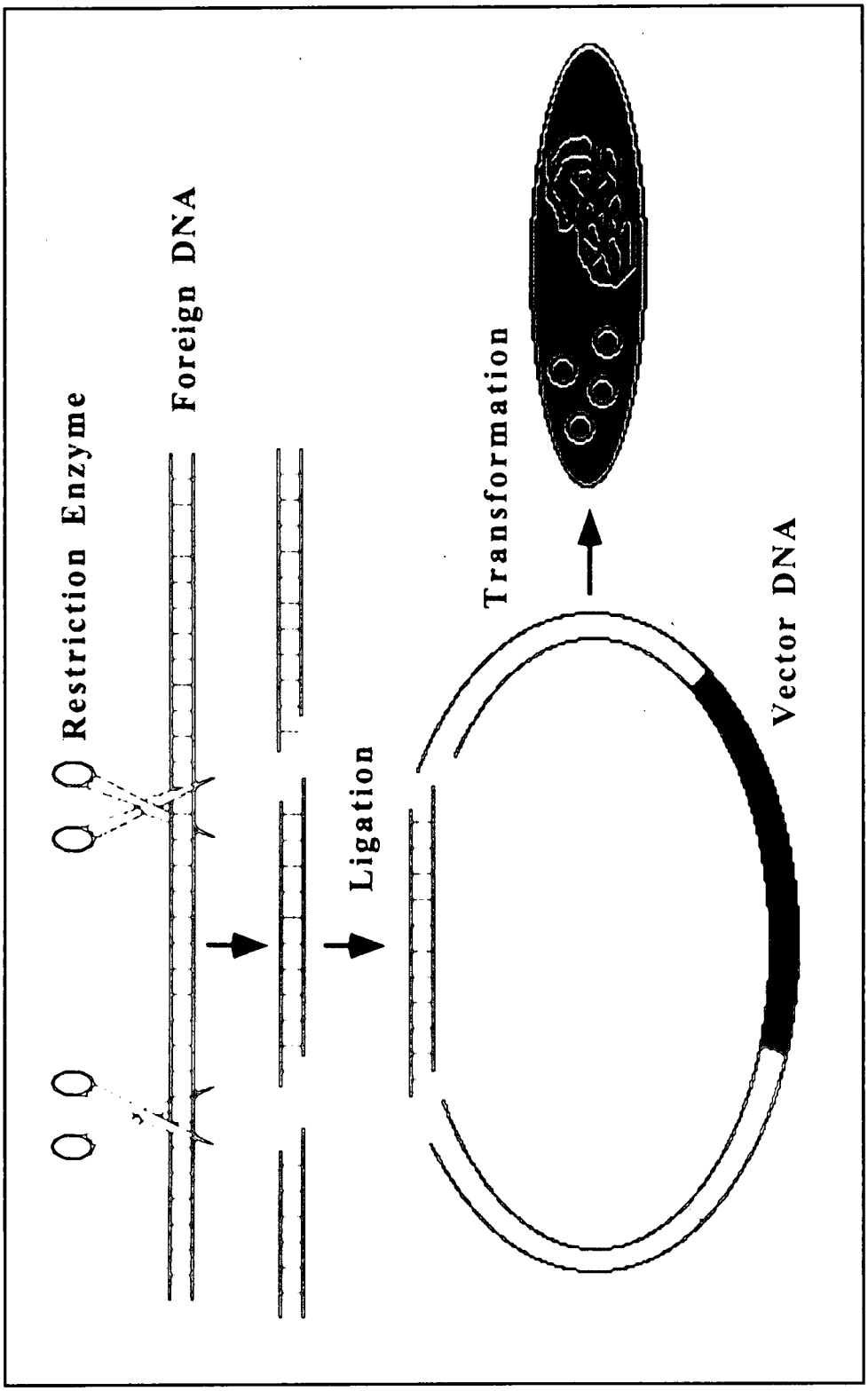
Restriction Enzyme
Foreign DNA
Ligation
Transformation
Vector DNA

Fig. 1

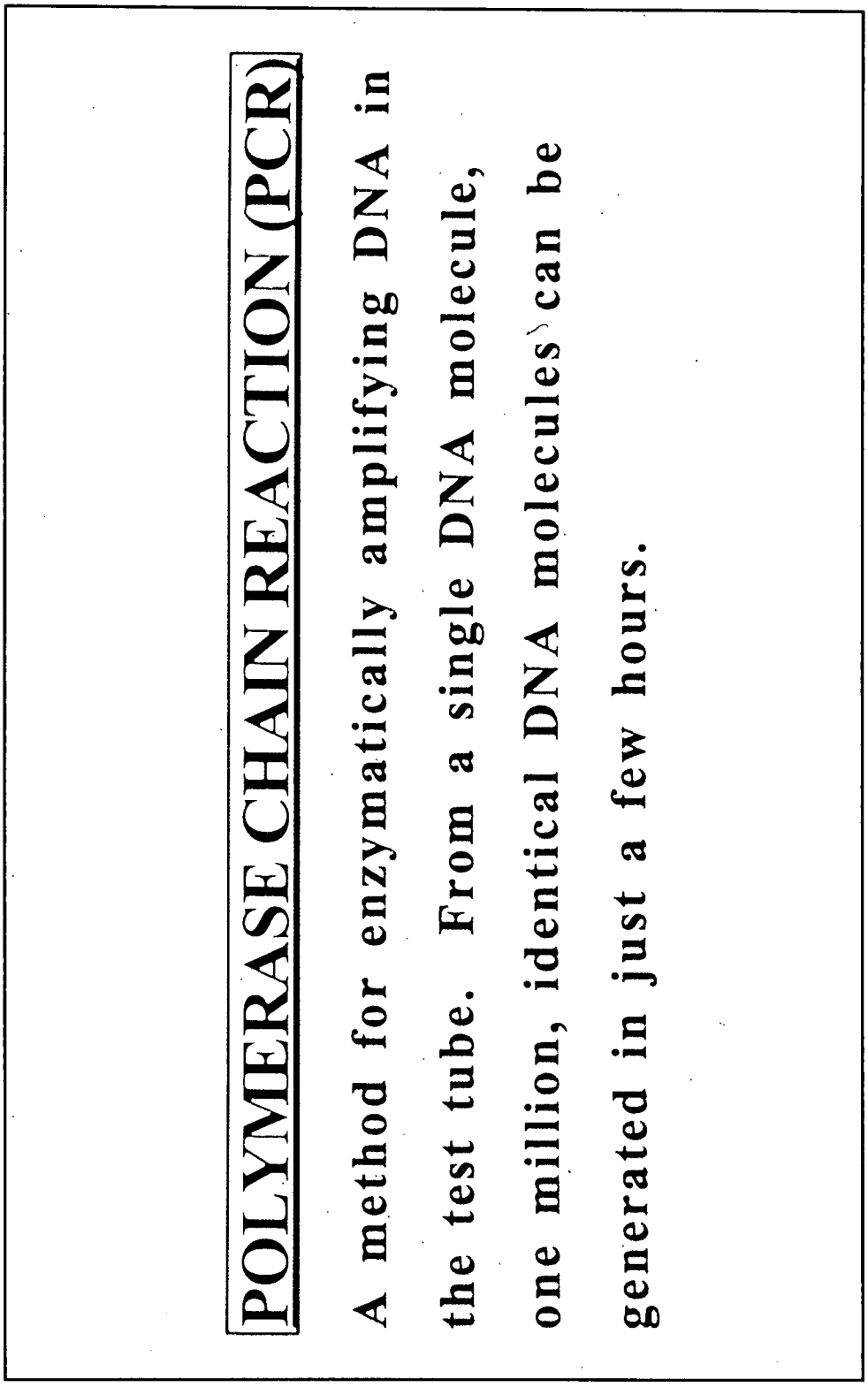
POLYMERASE CHAIN REACTION (PCR)
A method for enzymatically amplifying DNA in the test tube. From a single DNA molecule, one million, identical DNA molecules can be generated in just a few hours.

Fig. 2

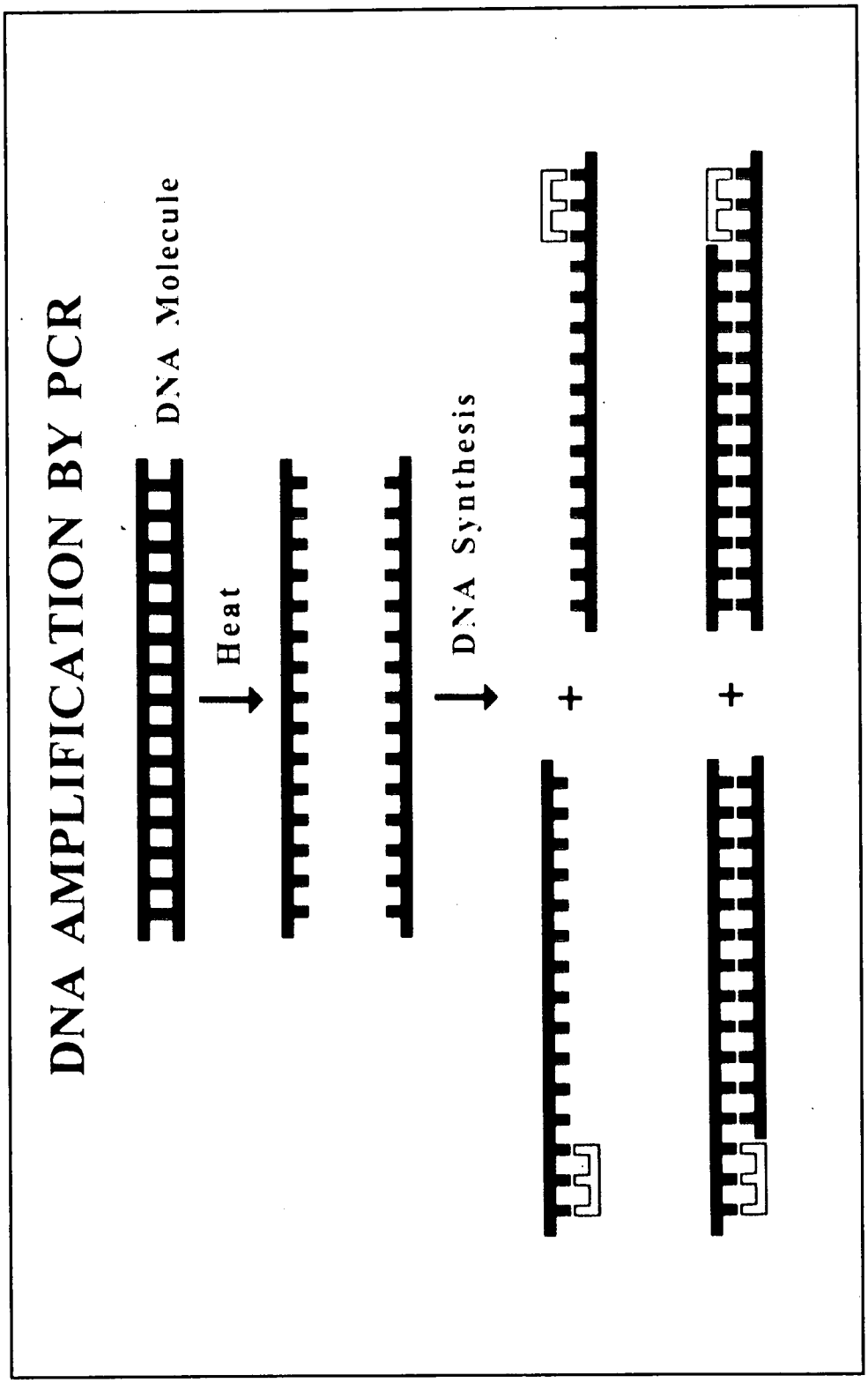

Fig. 3

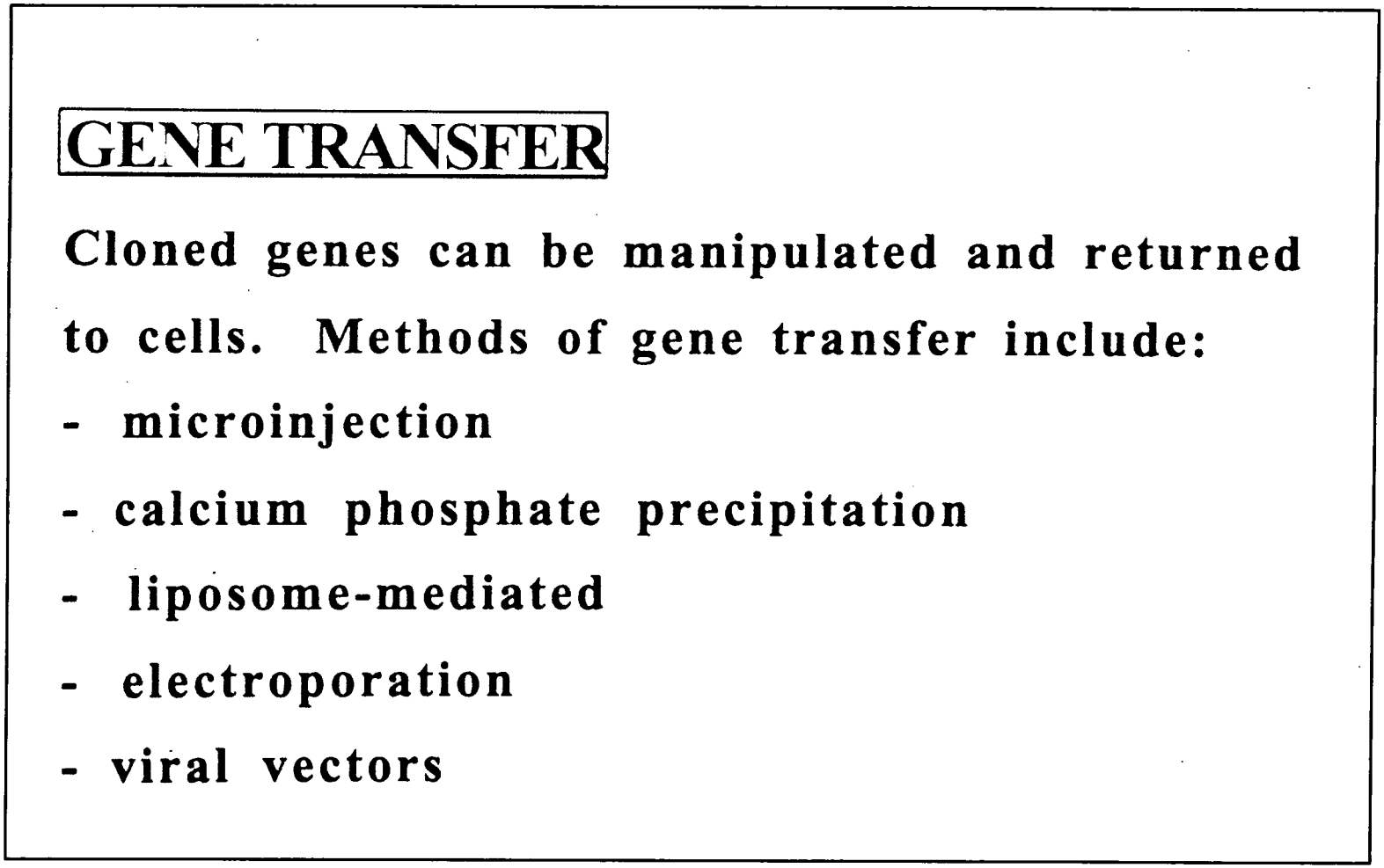
GENE TRANSFER
Cloned genes can be manipulated and returned to cells. Methods of gene transfer include:
- microinjection
- calcium phosphate precipitation
- liposome-mediated
- electroporation
- viral vectors

Fig. 4

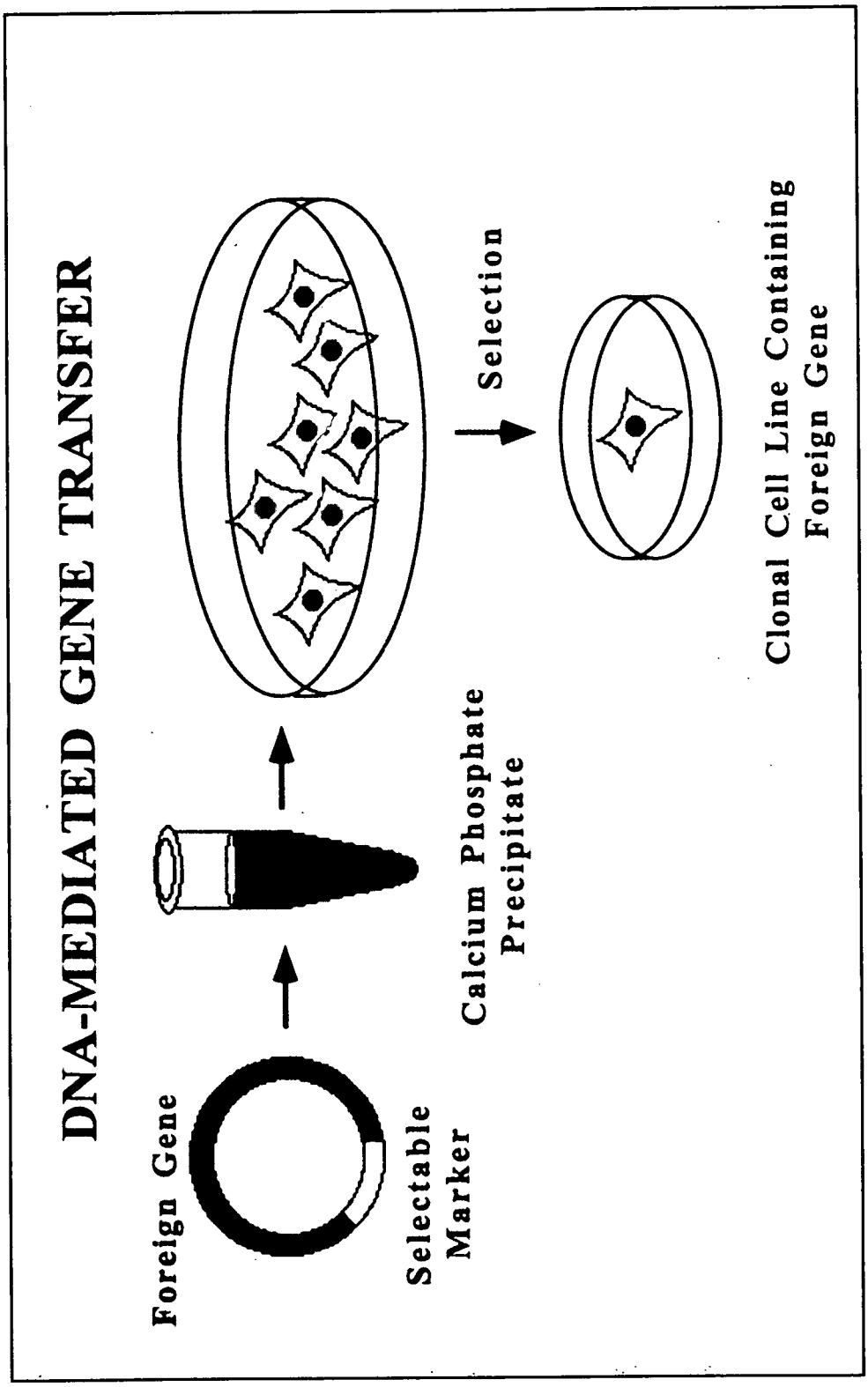
DNA-MEDIATED GENE TRANSFER
Foreign Gene
Selectable Marker
Calcium Phosphate Precipitate
Selection
Clonal Cell Line Containing Foreign Gene

Fig. 5

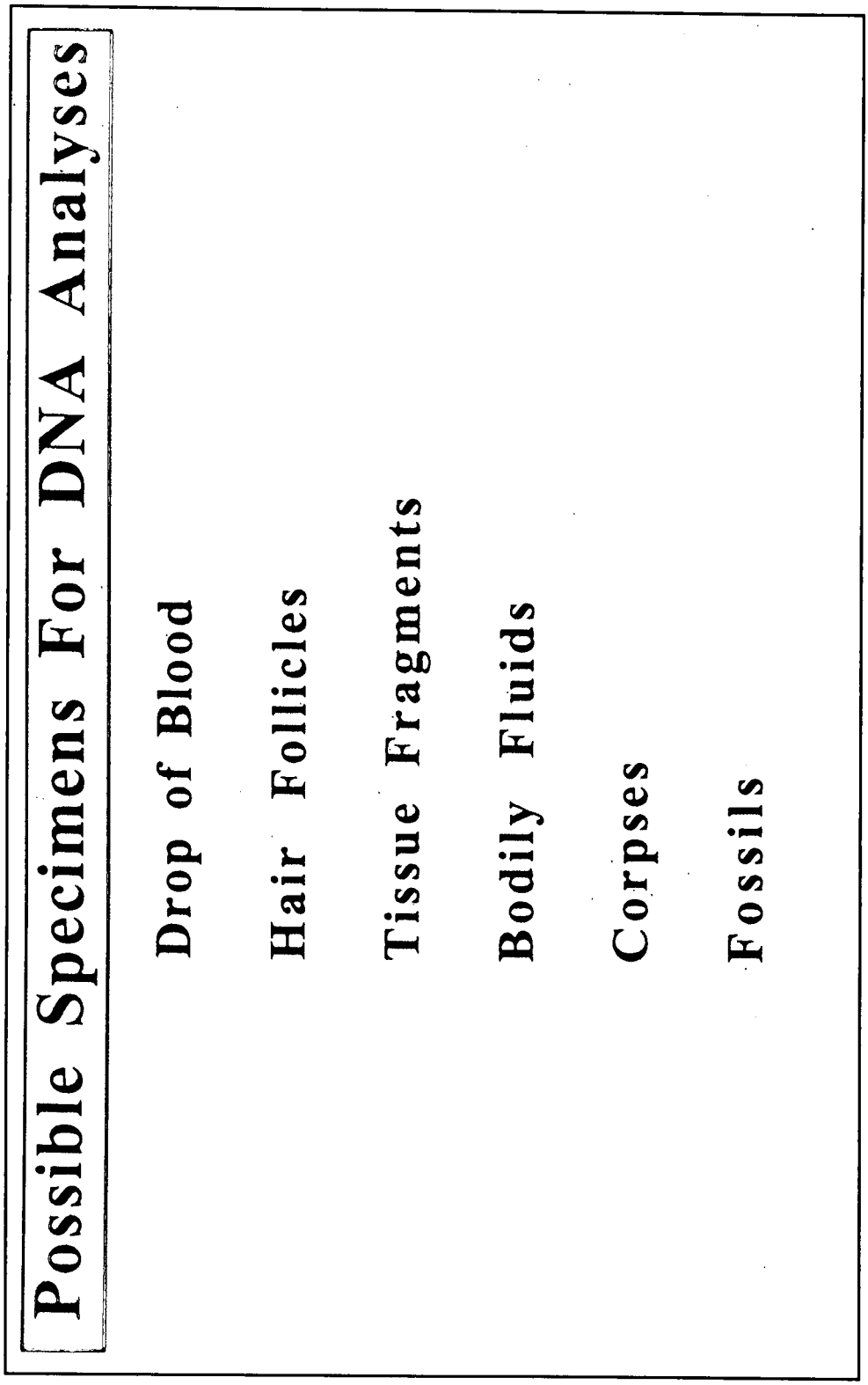
Possible Specimens For DNA Analyses
Drop of Blood
Hair Follicles
Tissue Fragments
Bodily Fluids
Corpses
Fossils

Fig. 6

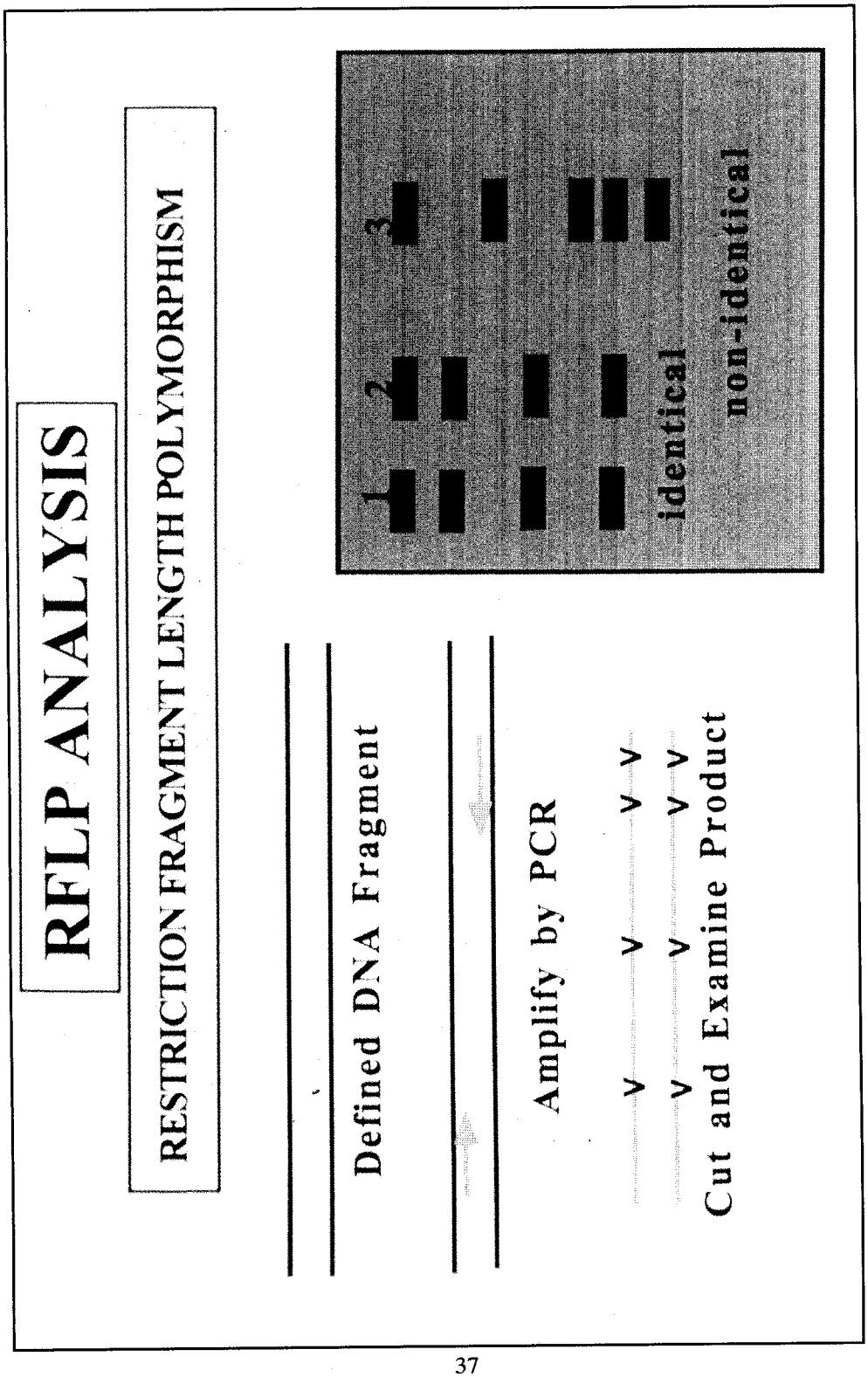
RFLP ANALYSIS
RESTRICTION FRAGMENT LENGTH POLYMORPHISM
Defined DNA Fragment
Amplify by PCR
Cut and Examine Product
1
2
3
identical
non-identical

Fig. 7

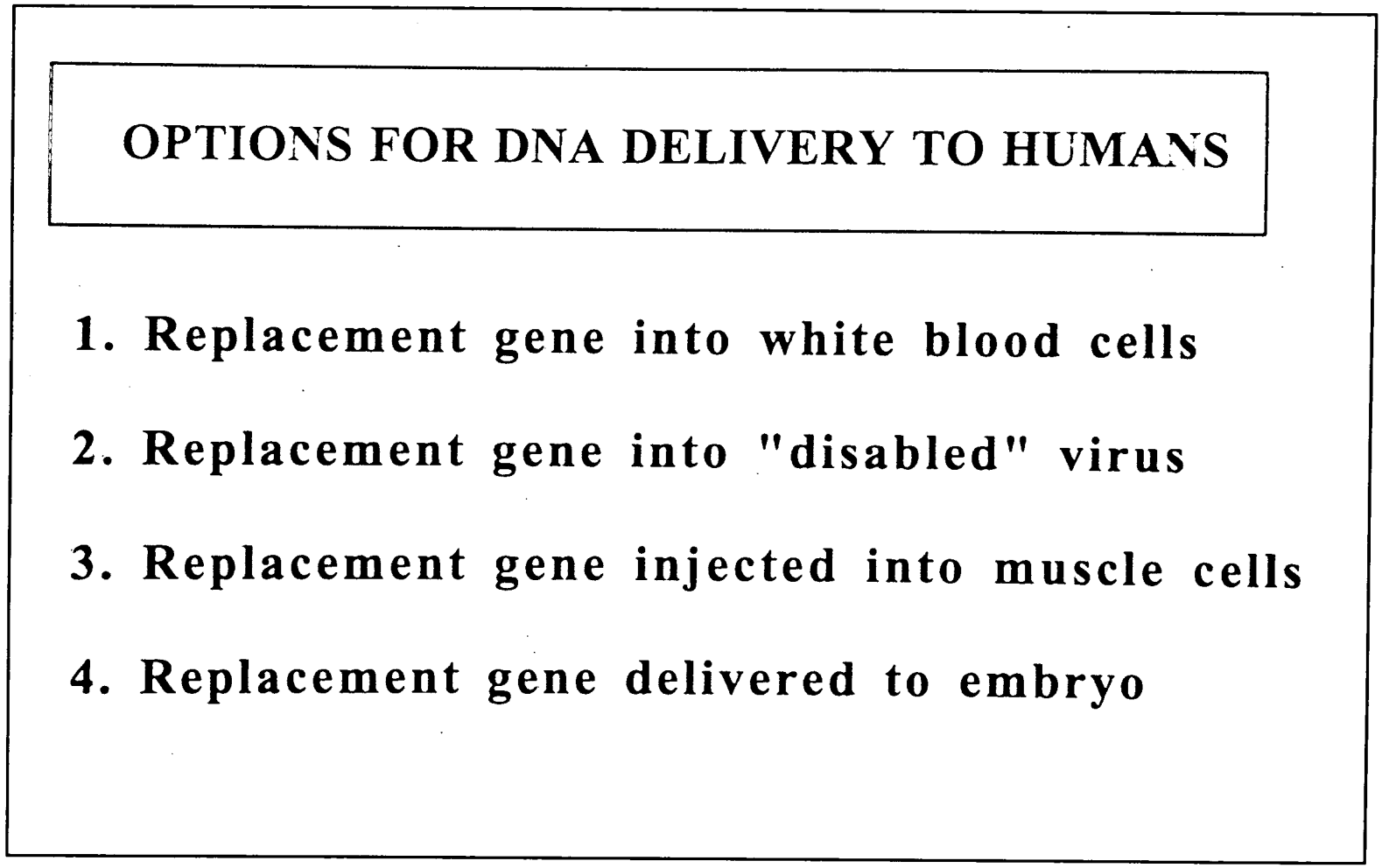
OPTIONS FOR DNA DELIVERY TO HUMANS
1. Replacement gene into white blood cells
2. Replacement gene into "disabled" virus
3. Replacement gene injected into muscle cells
4. Replacement gene delivered to embryo

Fig. 8