DNA Technology and Genomics

Ch. 20

Genetic engineering

- **Genetic engineering**: the direct manipulation of genes for "practical" purposes
- When genes from two different sources are combined *in vitro* into the same molecule it is called: **Recombinant DNA**
  - Such as the introduction of a desired gene into the DNA of a host that will produce more of the gene of a desired protein
  - This is done by cloning

- Basic cloning technique begins with inserting a foreign gene into a bacterial plasmid.

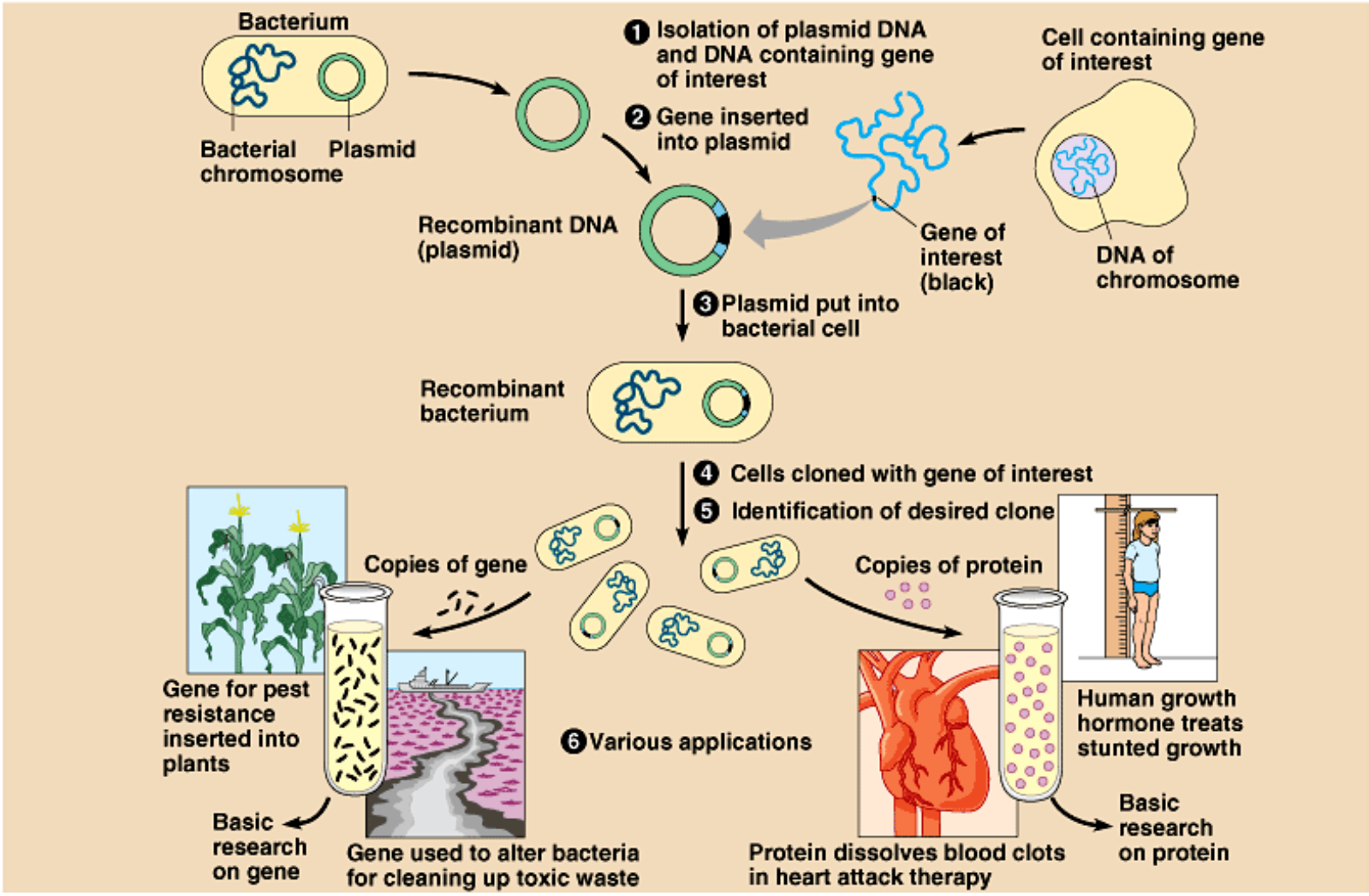


Fig. 20.1

Restriction Enzymes

- All this made possible by the discovery of **restriction enzymes**
- R**estrictions enzymes** recognize and cut specific short DNA nucleotide sequences
- In nature, bacteria use **restriction enzymes** to cut foreign DNA, such as from phages
  - Bacteria protect their own DNA by methylation.

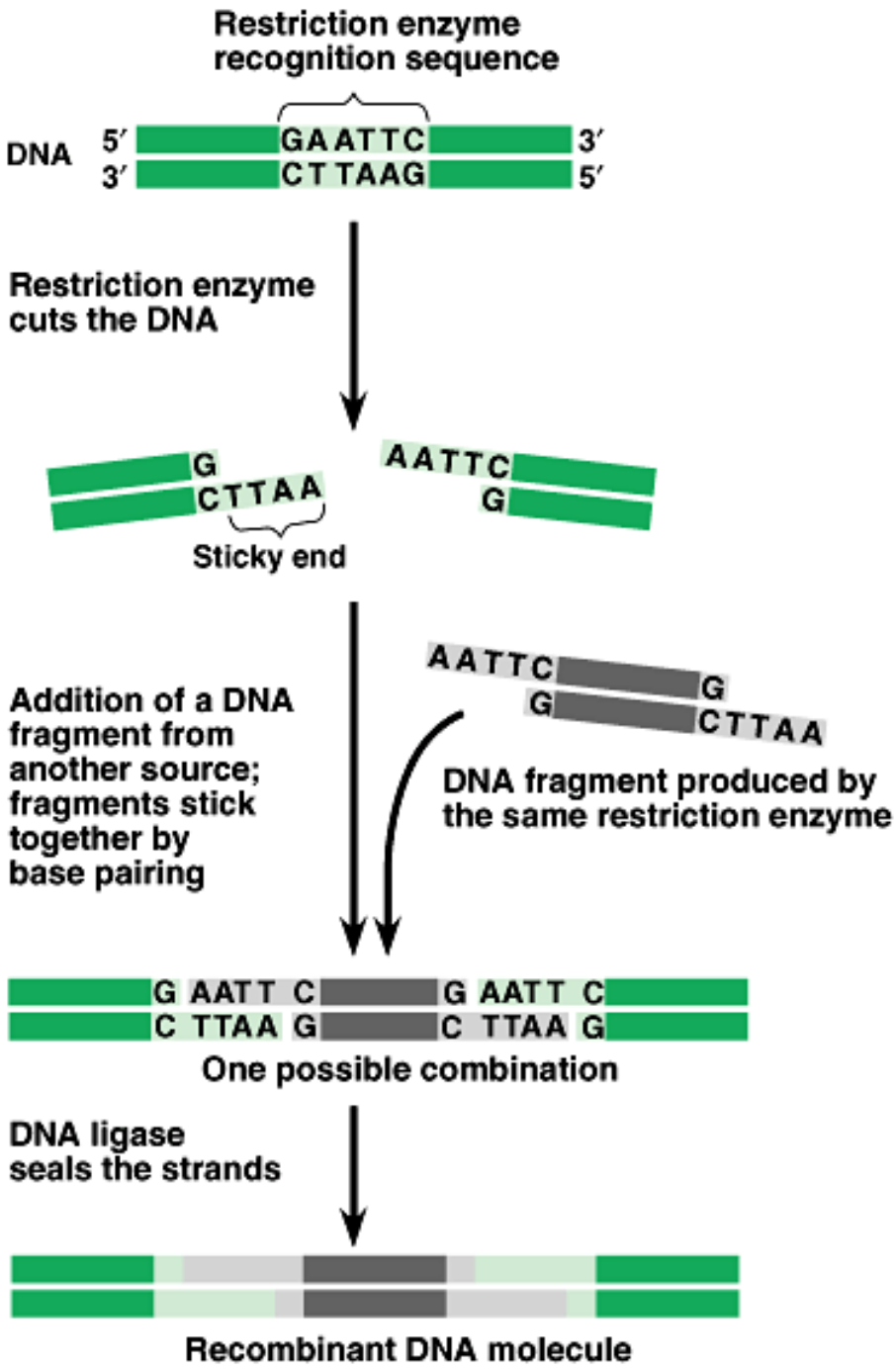

Restriction enzyme recognition sequence
DNA
5′
3′
GAATTC
CTTAAG
3′
5′
Restriction enzyme cuts the DNA
G
CTTAA
AATTC
G
Sticky end
Addition of a DNA fragment from another source; fragments stick together by base pairing
AATTC
G
G
CTTAA
DNA fragment produced by the same restriction enzyme
G AATT C
C TTAA G
G AATT C
C TTAA G
One possible combination
DNA ligase seals the strands
Recombinant DNA molecule

Fig. 20.2

- How we find the transformed bacteria

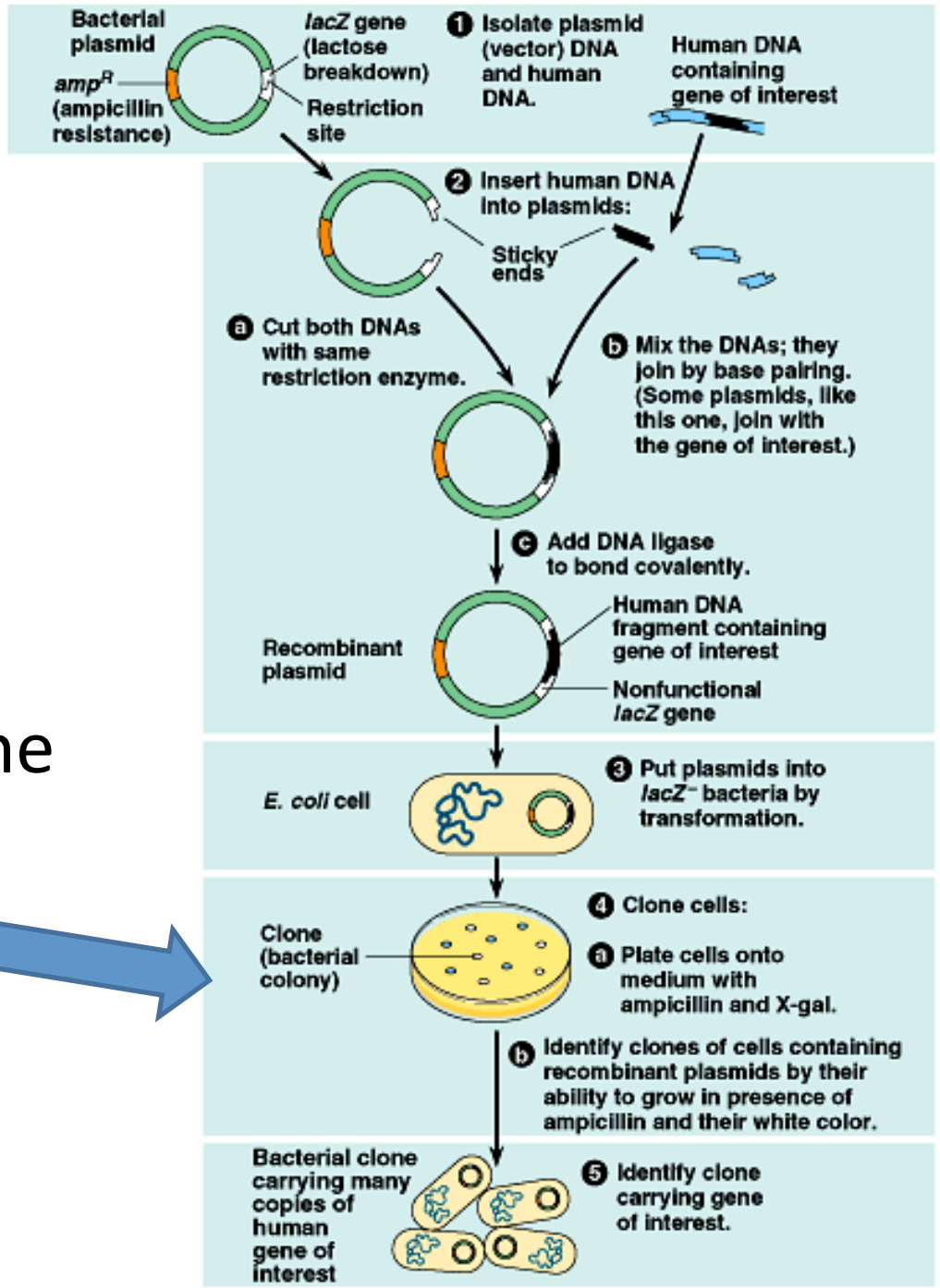


Fig. 20.3

Will the bacteria make the protein?

- Getting a cloned eukaryotic gene to work in a prokaryotic host is difficult
- So? Use an **expression vector**: contains a prokaryotic promotor upstream of the restriction site
- The bacterial host recognizes the promotor and expresses the foreign gene that has been linked to it, making the eukaryotic proteins

What about using eukaryotic cells as hosts?

- Why are yeasts plasmids better vectors?
  - Scientists have constructed **yeast artificial chromosomes** (**YACs**) that can hold more foreign DNA than bacterial plasmids
  - Yeast will do protein modifications
- How does DNA get in?
  - DNA enters with aid of electric pulse to open membrane: **electroporation**
  - Or, injected with a microscopic needle

What if you just want lots of the DNA fragment?

- No need for organism vector- just amplify it!
- Polymerase Chain Reaction (PCR)
- The DNA is incubated in a test tube with:
  - special DNA polymerase
  - a supply of nucleotides
  - short pieces of single-stranded DNA as a **primer**
- Makes BILLIONS of copies of the DNA in a few hours- faster than even bacteria!

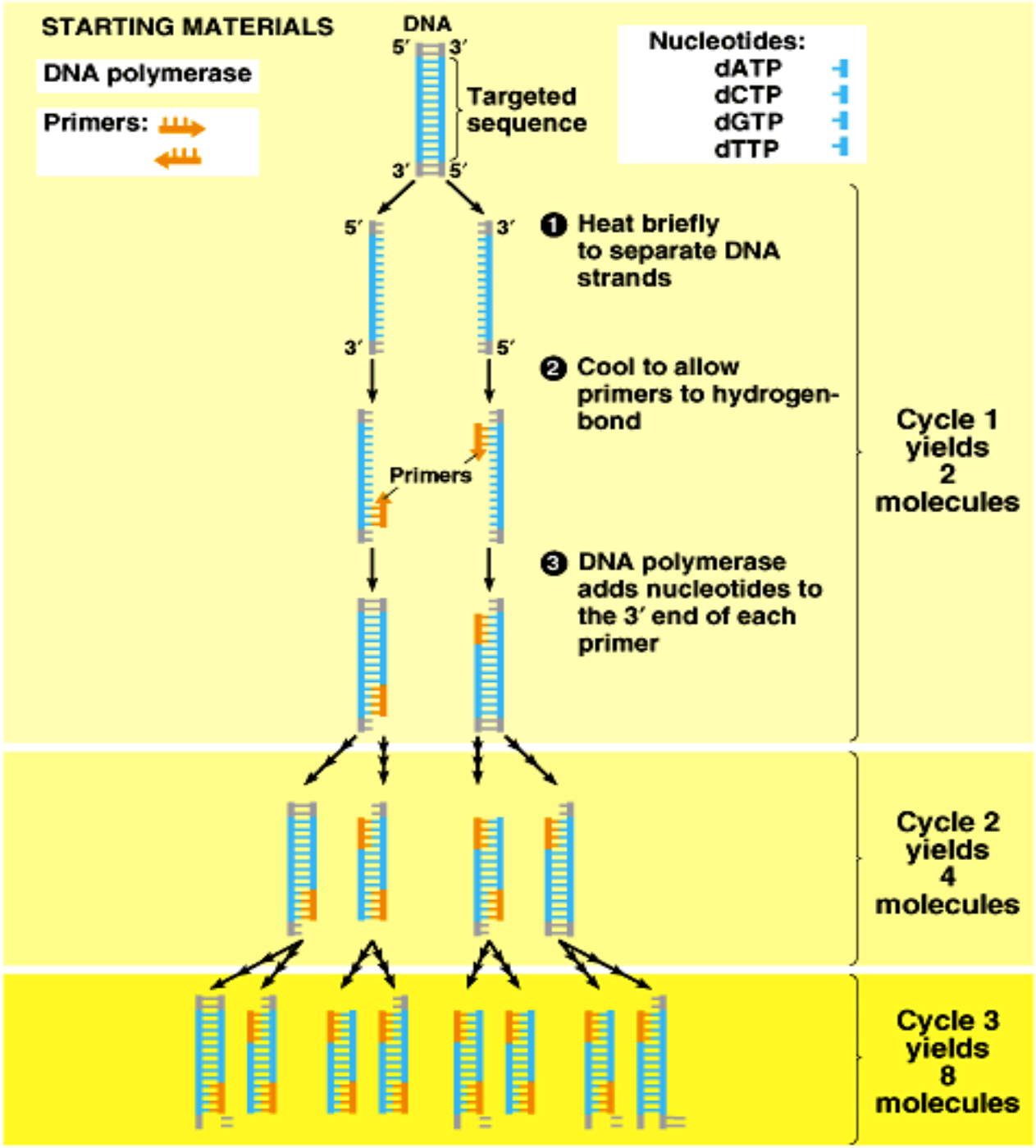

STARTING MATERIALS
DNA polymerase
Primers:
DNA
5′
3′
Targeted sequence
3′
5′
Nucleotides:
dATP
dCTP
dGTP
dTTP
1 Heat briefly to separate DNA strands
2 Cool to allow primers to hydrogen-bond
Primers
3 DNA polymerase adds nucleotides to the 3′ end of each primer
Cycle 1 yields 2 molecules
Cycle 2 yields 4 molecules
Cycle 3 yields 8 molecules

Fig. 20.7

How do we visualize amplified DNA?

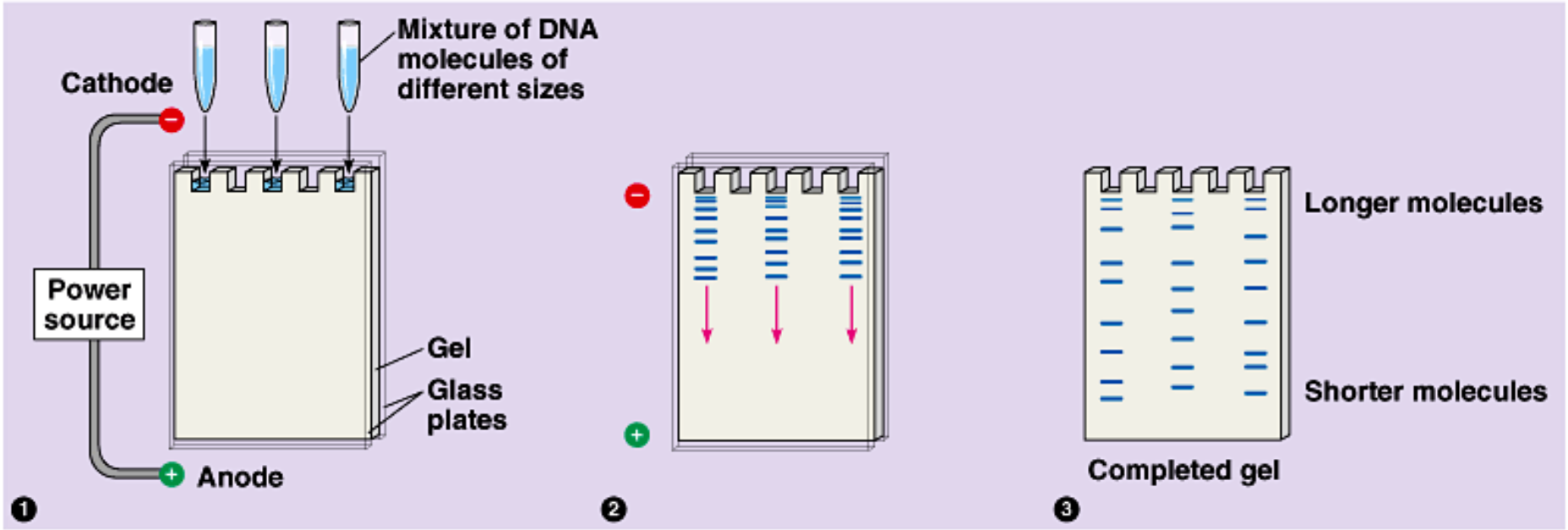


Fig. 20.8

Comparing DNA from 3 people

- Start by adding the restriction enzyme to the three DNA to produce restriction fragments
- Separate the fragments by gel electrophoresis.
- **Southern blotting** transfers the DNA fragments from the gel to a sheet of nitrocellulose paper
- Attach a radioactive probe to the DNA sequence of interest to visualize bands

- For our three individuals, the results of these steps show that individual III has a different restriction pattern than individuals I or II.

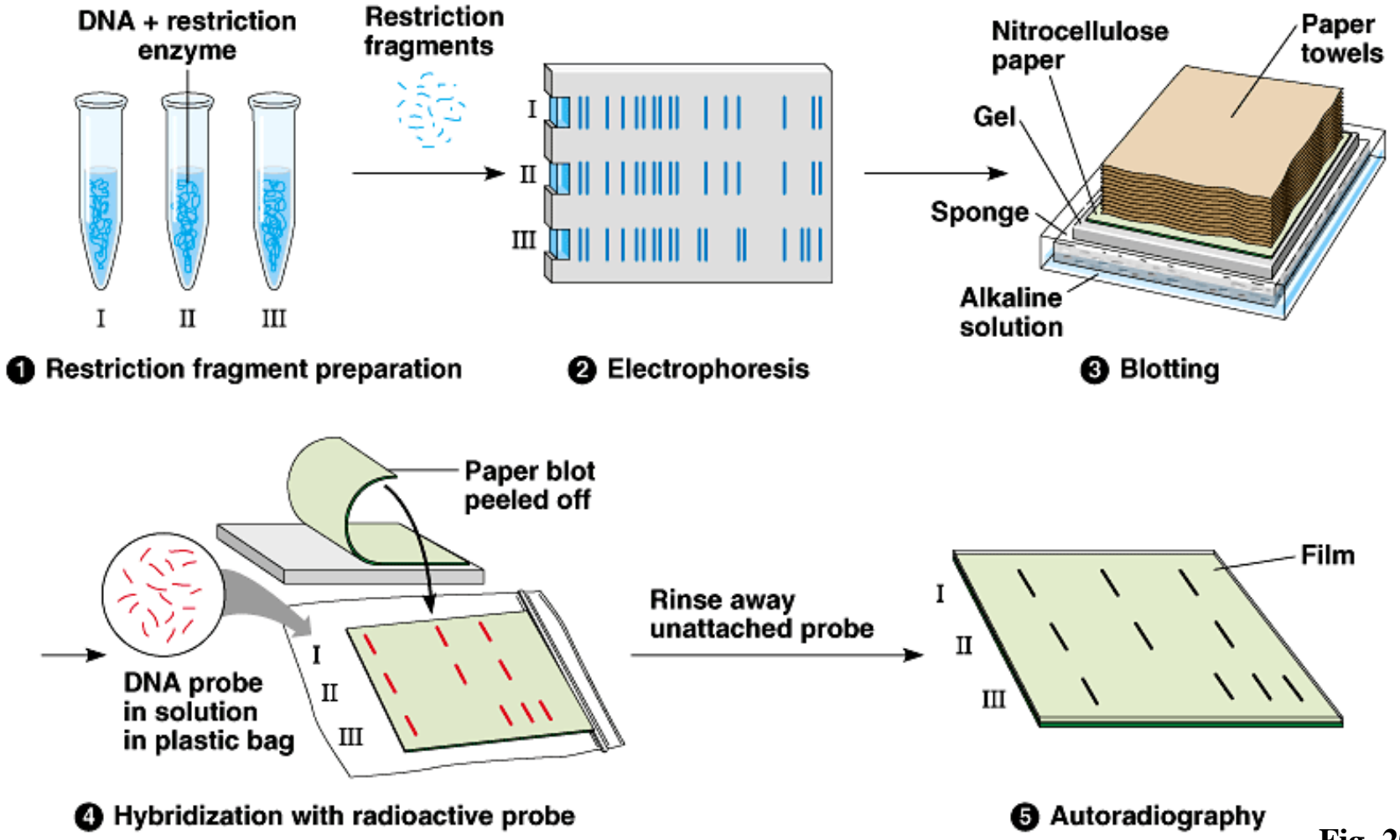


Fig. 20.10

Mapping the Genome

- A physical map is made by cutting the DNA of each chromosome into restriction fragments and then determining the original order of the fragments
- The key is to make fragments that overlap and then use probes of the ends to find the overlaps
- By mid-2001, the genomes of about 50 species had been sequenced
- There are still many gaps in the human sequence
  - Areas with repetitive DNA and certain parts of the chromosomes are hard to map with the methods

Surprising Results?

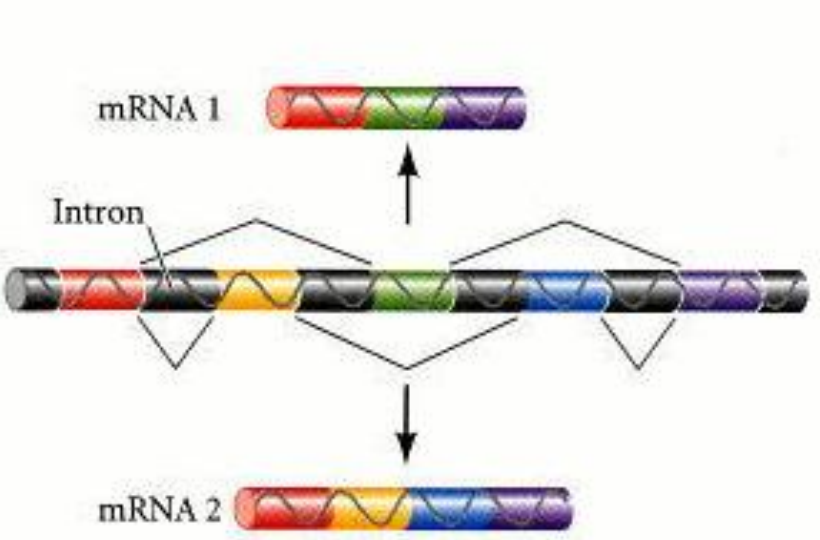
Table 20.1 Genome Sizes and Numbers of Genes

Organism	Genome Size	Estimated Number of Genes	Genes per Mb*
H. influenzae (bacterium)	1.8 Mb*	1,700	950
S. cerevisiae (yeast)	12 Mb	6,000	500
C. elegans (nematode)	97 Mb	19,000	200
A. thaliana (plant)	100 Mb	25,000	200
D. melanogaster (fruit fly)	180 Mb	13,000	100
H. sapiens (human)	3,200 Mb	30,000–40,000	10

*Mb = million base pairs

But there is more to a number

- The typical human gene probably specifies two or three different proteins by using different combinations of exons (coding region)
- Also more protein diversity via post-translational processing
- More protein diversity than invertebrates



Comparing Genomes have:

- Confirmed strong evolutionary connections between even distantly related organisms
  - For example, yeast has lots of genes close enough to our versions that they can substitute them in our cells
- Revealed how genes act together to produce a functioning organism through a complex network of interactions among genes and their products

How to find the function of a gene

- ***In vitro* mutagenesis** disables the gene and watch the consequences
  - Returned the mutated gene to a cell and it may be possible to determine the function of the normal gene by examining the phenotype of the mutant
- The next step after mapping and sequencing genomes is **proteomics**, the study of full protein sets (*proteomes*) encoded by genes
  - Why is this difficult?

Practical Applications of all this!

- The government needs your expert help to come up with ethical guidelines for use of DNA technology, they need:
- Positive uses of DNA technology
- Potential negative uses, if any
- Your recommendations for ethic guidelines
- You are asked to review main issues in the topics and become experts, giving pros and cons
- Review scenarios and ask yourself if it is ethical or not.