Phage Display Technique

Principle of Phage Display

- The coat protein of Ff phage might tolerate being fused to foreign polypeptides with out losing its function

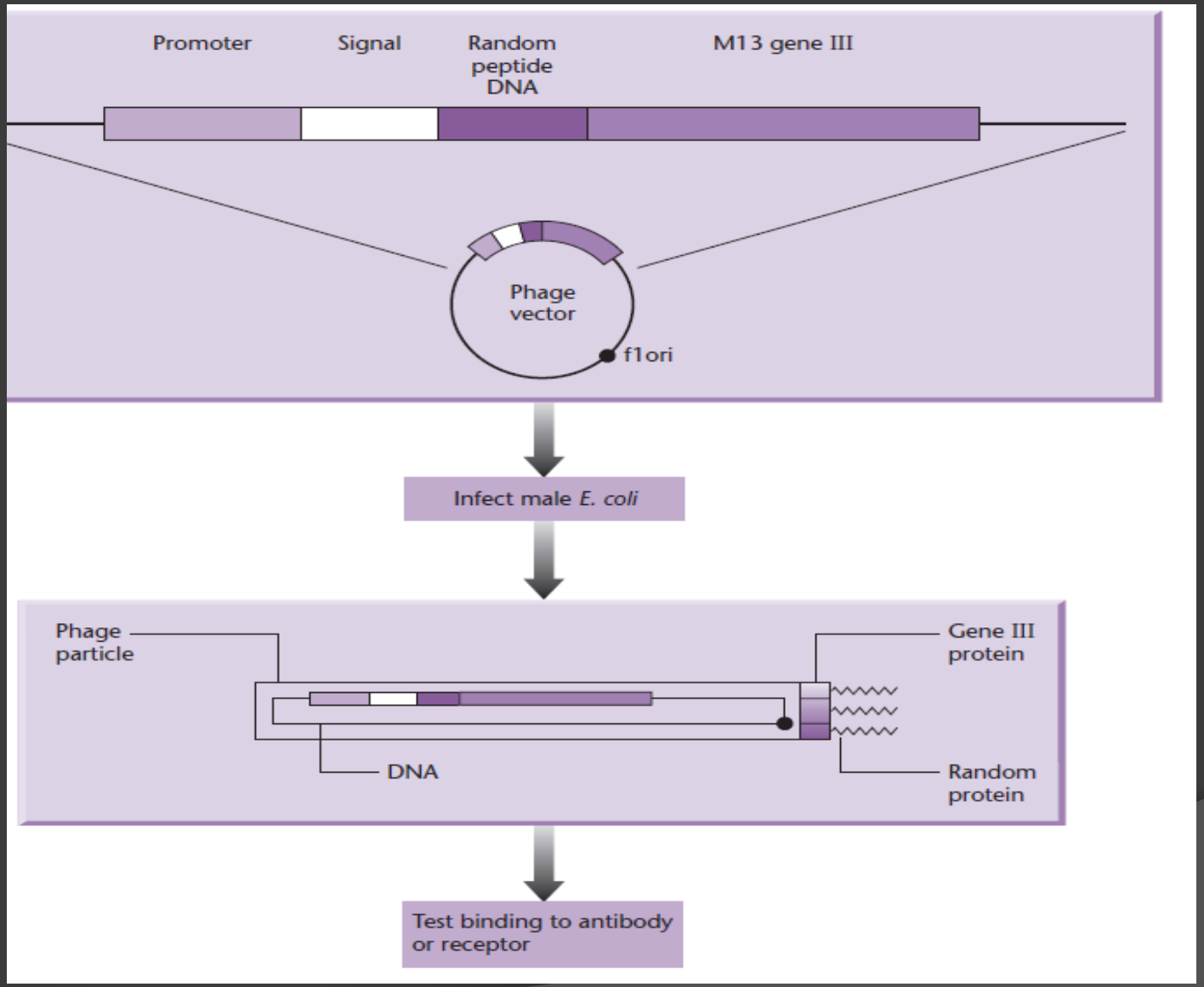
- The fused foreign polypeptide can be displayed on the surface of the filamentous phage

Phage Display Library

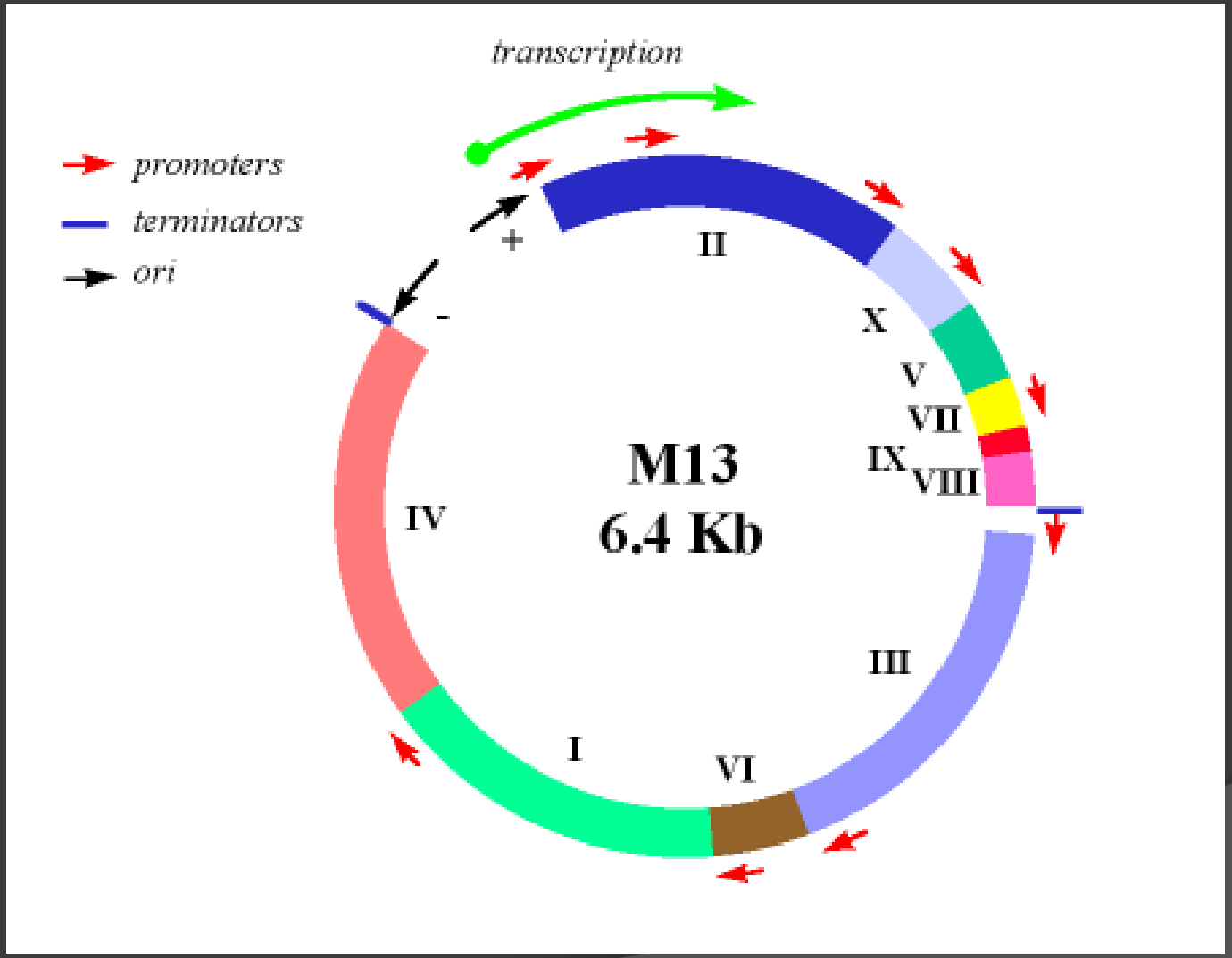
A large number of virions just occupy a small volume. So it's possible to construct foreign polypeptides libraries of the required diversity using recombinant DNA technology. In such libraries, each phage displays a unique random peptide.

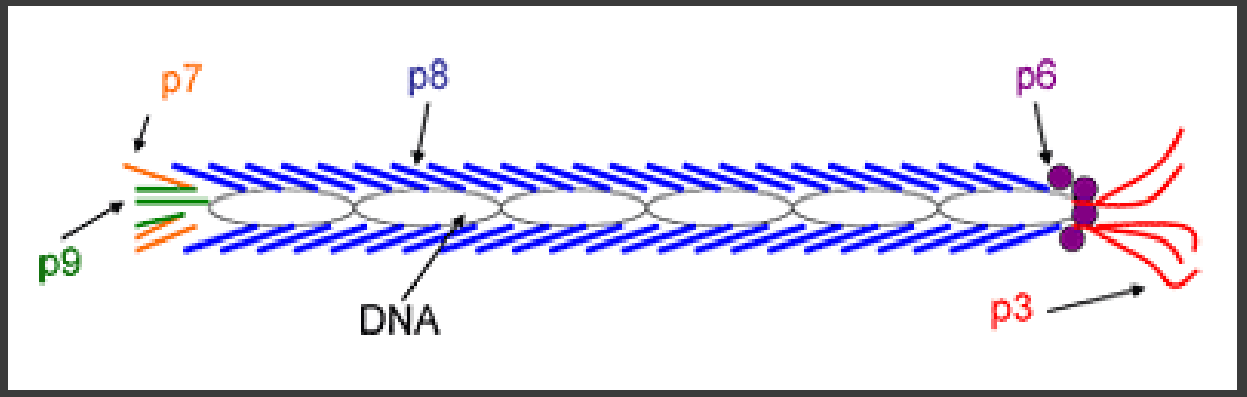
Those phage displaying polypeptides of interest can be screened using the affinity purification.

Displaying peptides or proteins on the surface of bacteriophage



Philamentous Bacteriophage





it encodes 11 genes.[DNA replication(2,5,10), Capsid(3,6,7,9] and assembly of the phage(1,4,11)

M13 phage particle: single stranded DNA molecule surrounded by a coat consisting of several thousand copies of the major coat protein,P8

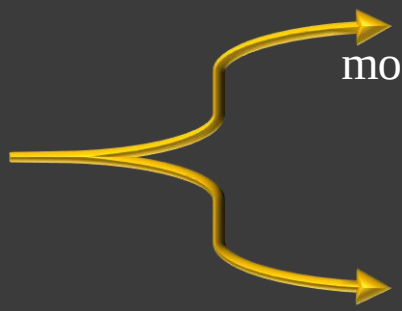
at the end of the particle are five each of the two minor coat proteins P9 and P7 and at the other end of five copies each of P3 and P6.

Principle and Application of Phage Display

I. Using recombinant DNA → Large Libraries

II. Phage Display → Genotype
Phage Display → Phonotype

Phage Display of Natural Peptide



Mapping epitopes of monoclonal and polyclonal antibodies

Generating Immunogens

Phage Display can be used to localized the antigenic epitope relatively quickly.

Fragment of different proteins have been displayed on M13 for the purpose of eliciting antibodies against the certain parasites or viruses.

Phage Display of Random Sites

- Synthetic oligonucleotides, fixed in length but unspecified codons, can be cloned as fusions to gene III or VIII of M13 where they are expressed as a plurality of peptides:capsid fusion proteins.

- Biopanning→ Incubation of library with a target molecule, amplification and selection for binding to target again and enriching for those phage that bind the target molecule.

- Random Peptide Library→ bind to the combining site of antibody, cell surface receptors, cytosolic receptor, extracellular and intracellular proteins and DNA and many other target.

Phage Display of Random Sites

A. Mapping epitopes of monoclonal and polyclonal antibodies
   - 1) Peptide competitors of antigen-antibody interaction
   - 2) Functional sites of numerous antigens
   - 3) Immunodominant peptide sequences of antigen
B. Identifying peptide ligands(drug)
C. Defining post-translational substrate sequences

Peptide Display of Protein and Protein Domains

1. Directed Evolution of Protein
2. Isolation of high affinity antibodies
3. Defining protein-protein interaction

conclusion

Phage Display

I. Immunology
II. Protein biochemistry
III. Protein engineering
IV. Cell biology
V. Library of random peptide ,protein and protein domain
VI. Mapping epitopes
VII. Identifying of antagonist and agonists of various target molecules
VIII. Engineering of human antibodies
IX. Optimizing antibody specificities
X. Novel binding activities