

A white line-art illustration of a large, multi-story building with many windows and a central entrance, set against a dark grey background.

MOL.911
DNA Therapeutics

DNA as Therapeutic Agent

→ DNA Vaccines

Application form: Plasmid DNA → CCC-form

Main Challenge: ultra high purity; no contamination with non-desired genetic material

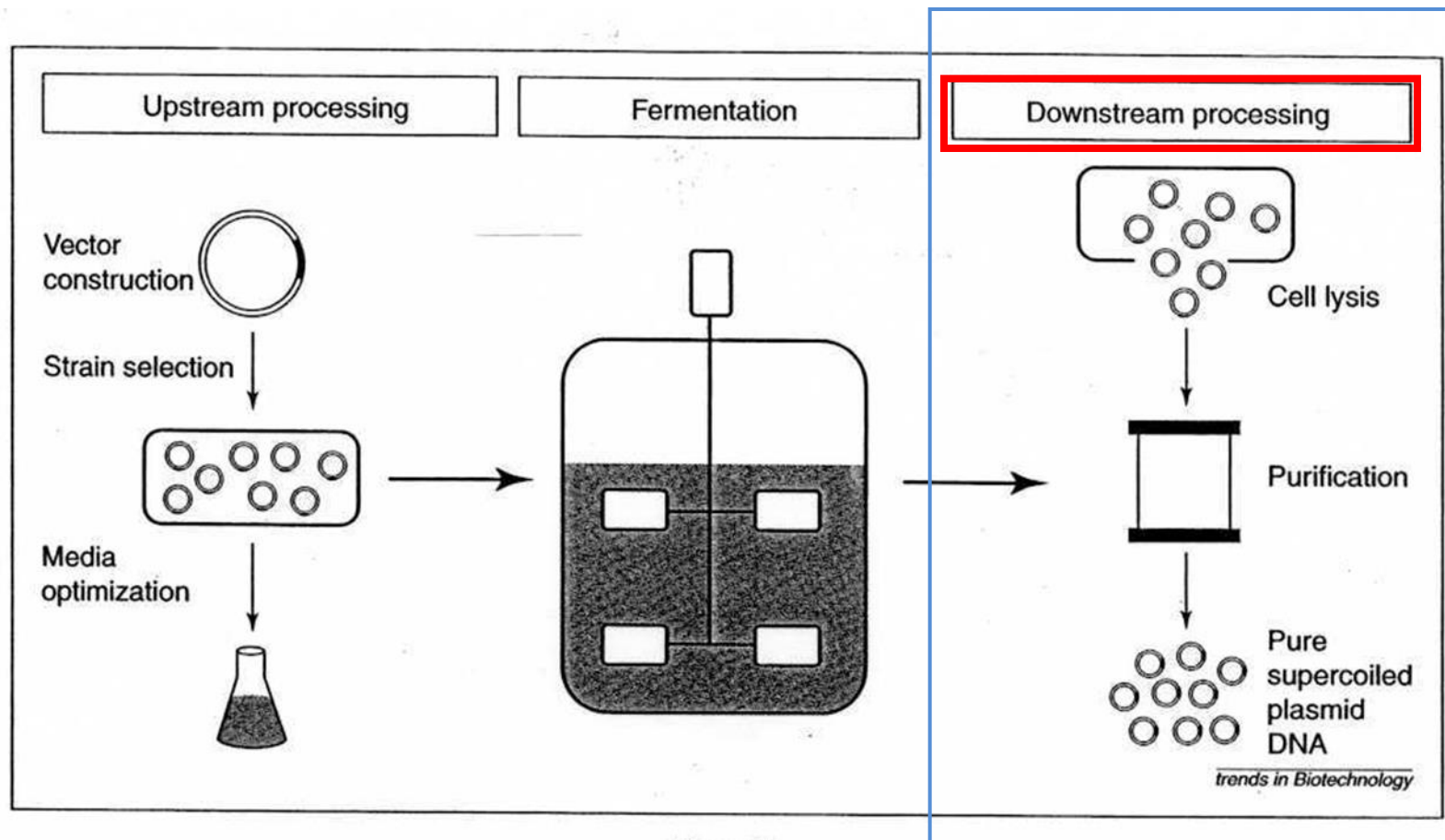


Figure 1

The three stages of plasmid-DNA process development.

3 Production of DNA

Downstream Process Steps

Table 1. Potential unit operations and process options for the downstream recovery and purification of plasmid DNA

| Unit operation | Process options |
|------------------------------|---|
| Lysis | Chemical Thermal Mechanical |
| Solids removal | Centrifugation Filtration Membrane separation Flotation |
| Intermediate purification | Fractional precipitation Membrane fractionation Adsorption |
| High-resolution purification | Chromatography: Ion-exchange Reversed phase Gel filtration Affinity |
| Finishing | Drying Formulation Vialing |

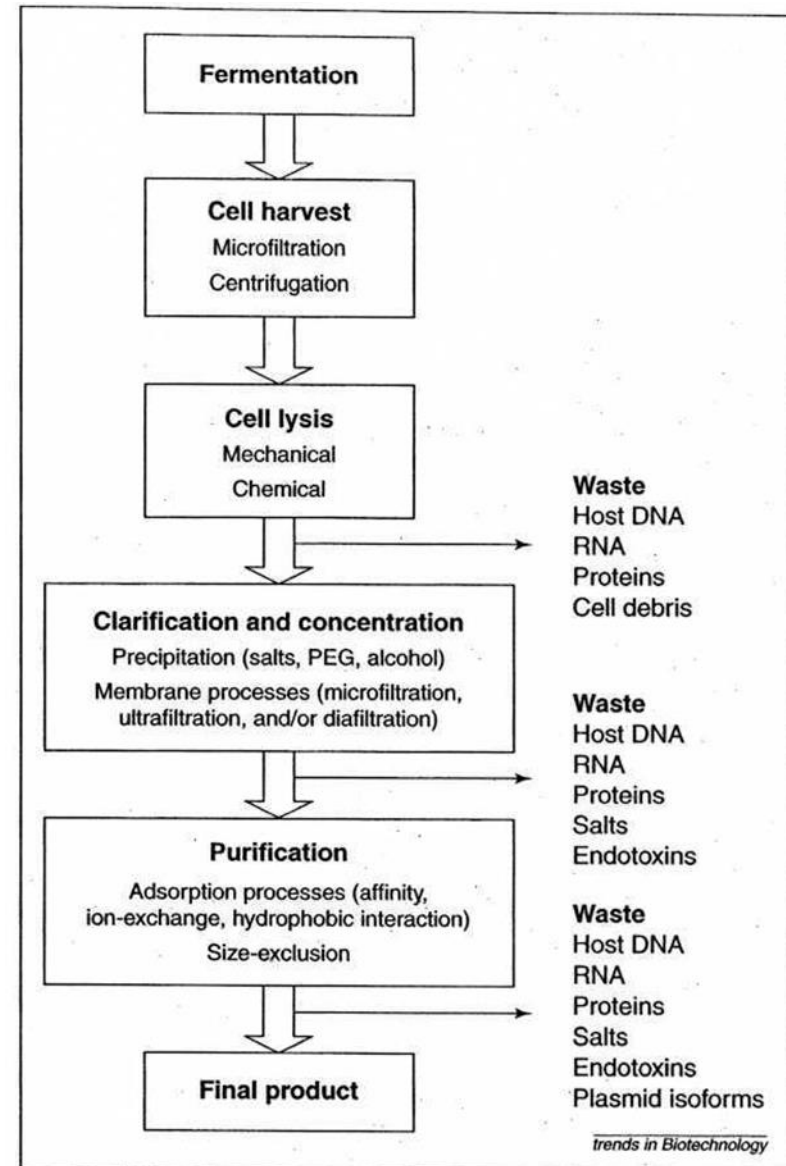


Figure 2

Process flow sheet for the large-scale purification of supercoiled plasmid DNA. Unit operations to be considered during process development are indicated together with the eliminated impurities.

Production of DNA

Purity requirements

Table 1. The principal approval specifications and recommended assays for assessing the purity, safety and potency of DNA preparations for gene therapy and DNA vaccines^a

| Impurity | Recommended assay | Approval specification |
|---|-----------------------------|--|
| Proteins | BCA protein assay | Undetectable |
| RNA | Agarose-gel electrophoresis | Undetectable |
| gDNA | Agarose-gel electrophoresis | Undetectable |
| | Southern blot | <0.01 μg (μg plasmid) ⁻¹ |
| Endotoxins | LAL assay | <0.1 EU (μg plasmid) ⁻¹ |
| Plasmid isoforms (linear, relaxed, denatured) | Agarose-gel electrophoresis | <5% |
| Biological activity and identity | Restriction endonucleases | Coherent fragments with the plasmid restriction map |
| | Agarose-gel electrophoresis | Expected migration from size and supercoiling |
| | Transformation efficiency | Comparable with plasmid standards |

^aAbbreviations: BCA, bichoninic acid; LAL, *Lymulus ameobocyte* lysate; EU, endotoxin units.

Table 2. A comparison of laboratory methods and large-scale pharmaceutical processes for purifying supercoiled plasmid DNA^a

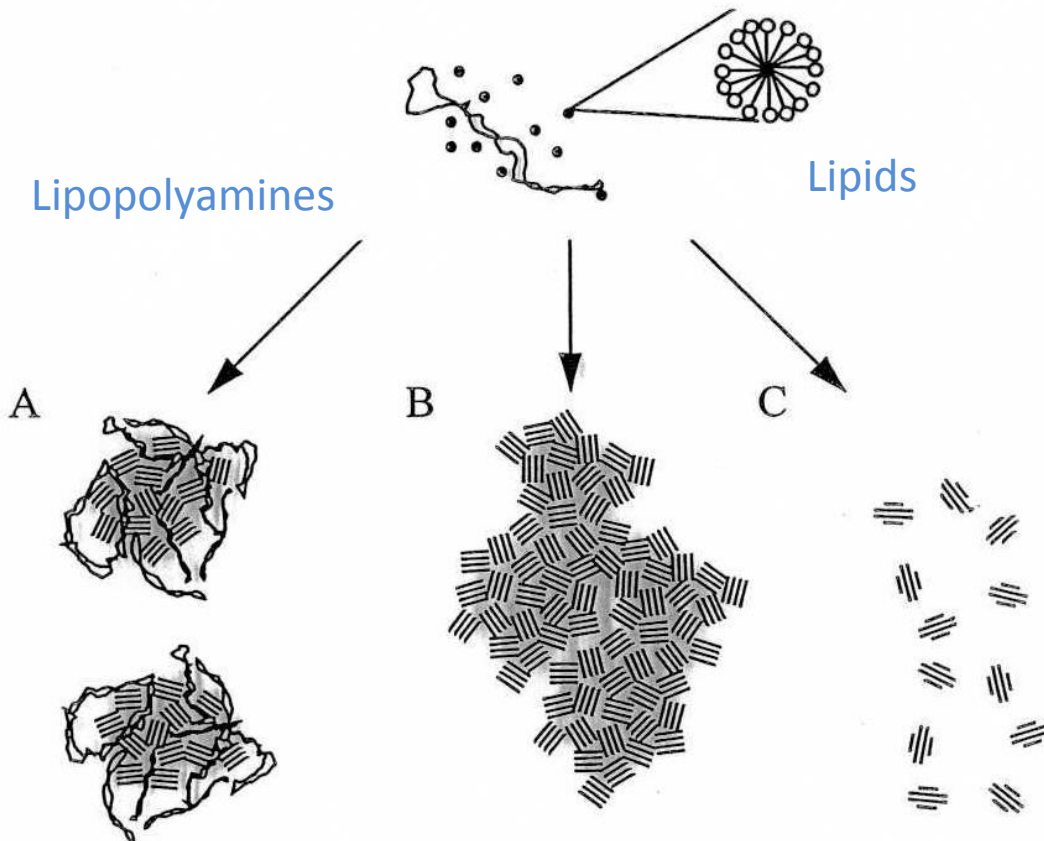
| Process step | Laboratory method | Large-scale process |
|---|--|---|
| Cell lysis | RNase, lysozyme | No enzymes Only GRAS reagents |
| Removal of cell debris | Centrifugation | Filtration, centrifugation or expanded bed chromatography |
| Removal of host impurities (RNA, gDNA, proteins and endotoxins) | RNase, proteinase K, organic solvents (phenol and chloroform) | Salting out PEG precipitation |
| Concentration | Alcohol precipitation | Alcohol precipitation, PEG precipitation |
| Plasmid purification | Ultracentrifugation (mutagenic reagents and ethidium bromide) IEC (gravity flow columns provided in commercial kits) RPC (organic, toxic solvents) | IEC and/or SEC (use only GRAS reagents) |

^aAbbreviations: GRAS, generally regarded as safe; IEC, ion-exchange chromatography; PEG, polyethylene glycol; RPC, reverse-phase chromatography; SEC, size-exclusion chromatography.

Improvement of DNA Transfer and Expression

Formulation of DNA

DNA in complexes with



Charged polymeric microparticles

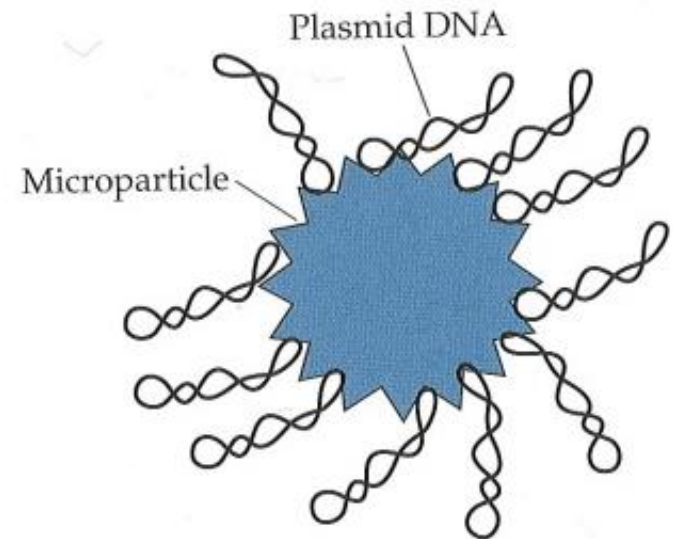


FIGURE 12.15 Schematic representation of the binding of plasmid DNA to the cationic surface of a polymeric microparticle.

DNA Vaccines

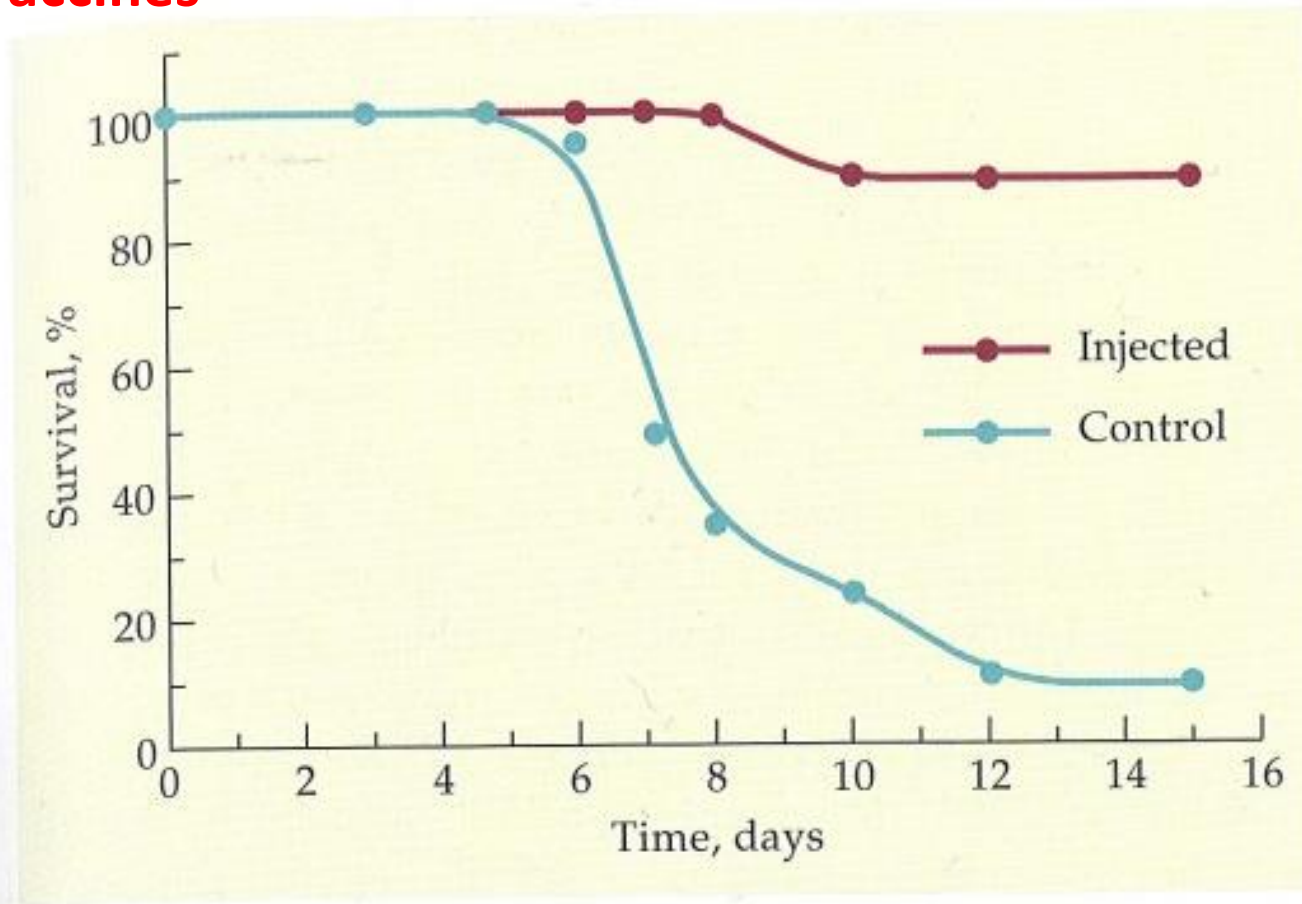


FIGURE 12.14 Survival of DNA-immunized mice. Injected mice were immunized with DNA that contained the influenza A virus nucleoprotein gene under the control of the Rous sarcoma virus promoter on an *E. coli* plasmid. The control mice were injected with plasmid DNA only. The x axis represents the number of days after the animals were challenged with the live influenza virus.

TABLE 12.3 Advantages of genetic immunization over conventional vaccines

Cultivation of dangerous agents is not required.

Since genetic immunization does not utilize any viral or bacterial strains, there is no chance that an attenuated strain will revert to virulence.

Since no organisms are used, attenuated organisms that many cause disease in young or immunocompromised animals are not a problem.

Approach is independent of whether the microorganism is difficult to grow or attenuate.

Production is inexpensive because protein does not need to be produced or purified.

Storage is inexpensive because of the stability of DNA.

One plasmid could encode several antigens/vaccines, or several plasmids could be mixed together and administered at the same time.