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

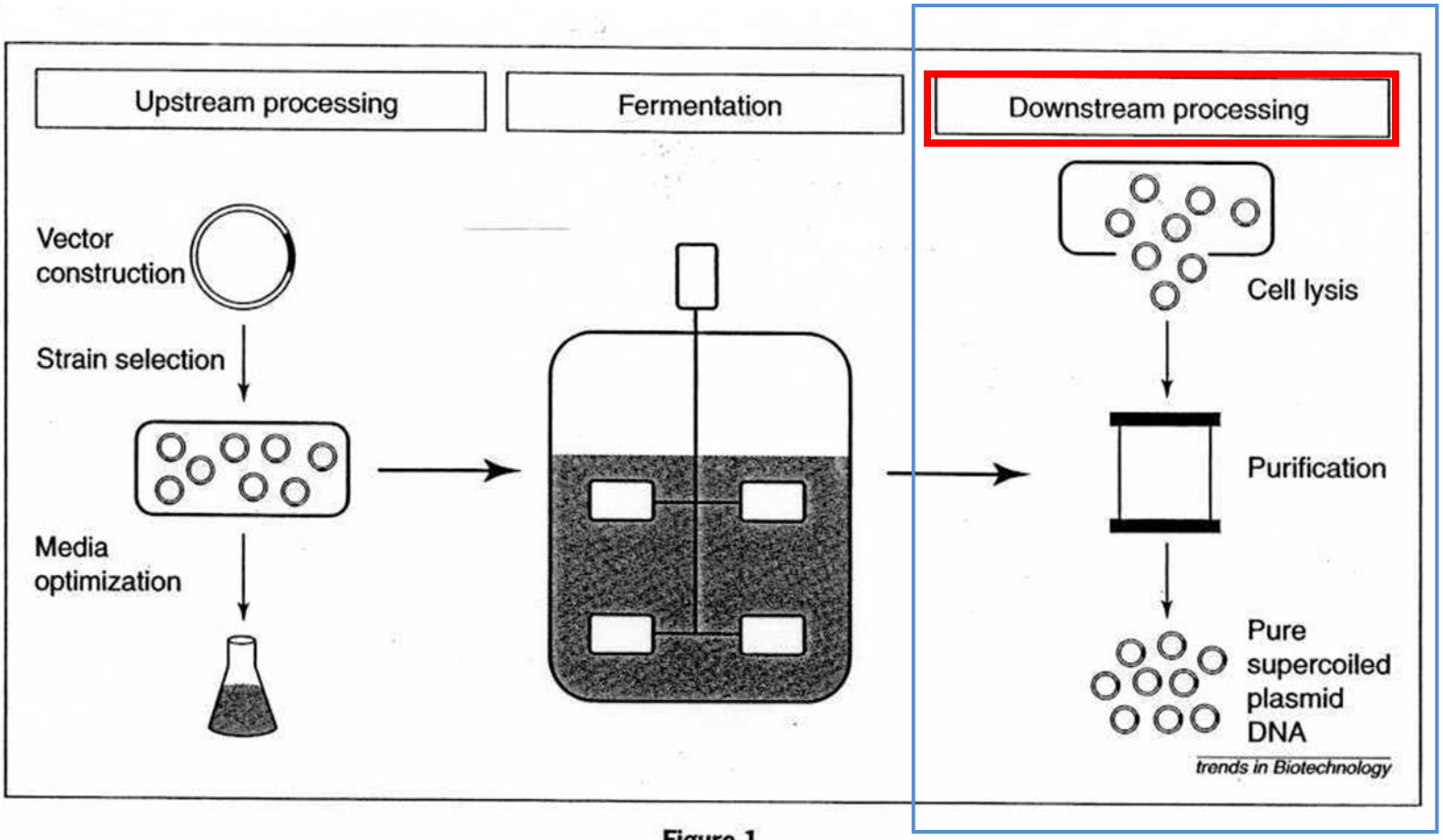


Figure 1
The three stages of plasmid-DNA process development.

Most Important Process steps

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

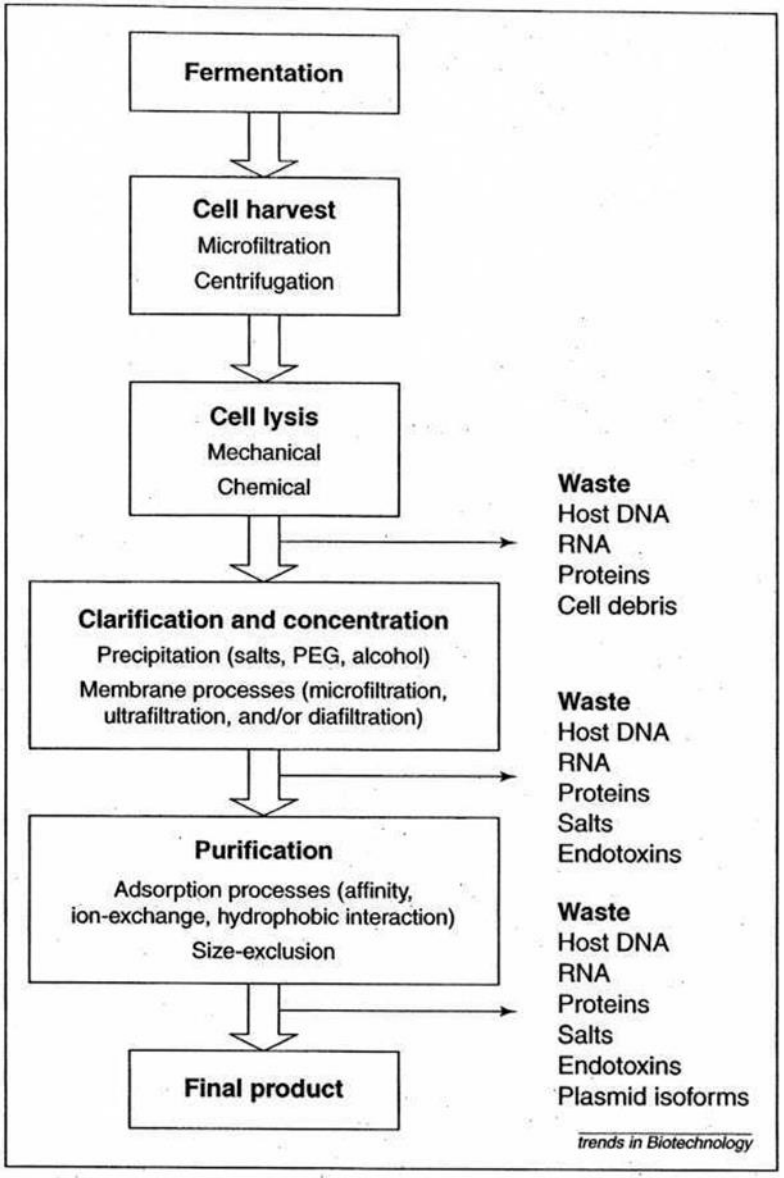


Figure 2
Process flow sheet for the large-scale purification of supercoiled plasmid DNA. Unit operations to be considered during process development are indicated together with the eliminated impurities.

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

[a]Abbreviations: BCA, bicinchoninic acid; LAL, *Lymulus amebocyte* 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)

[a]Abbreviations: GRAS, generally regarded as safe; IEC, ion-exchange chromatography; PEG, polyethylene glycol; RPC, reverse-phase chromatography; SEC, size-exclusion chromatography.

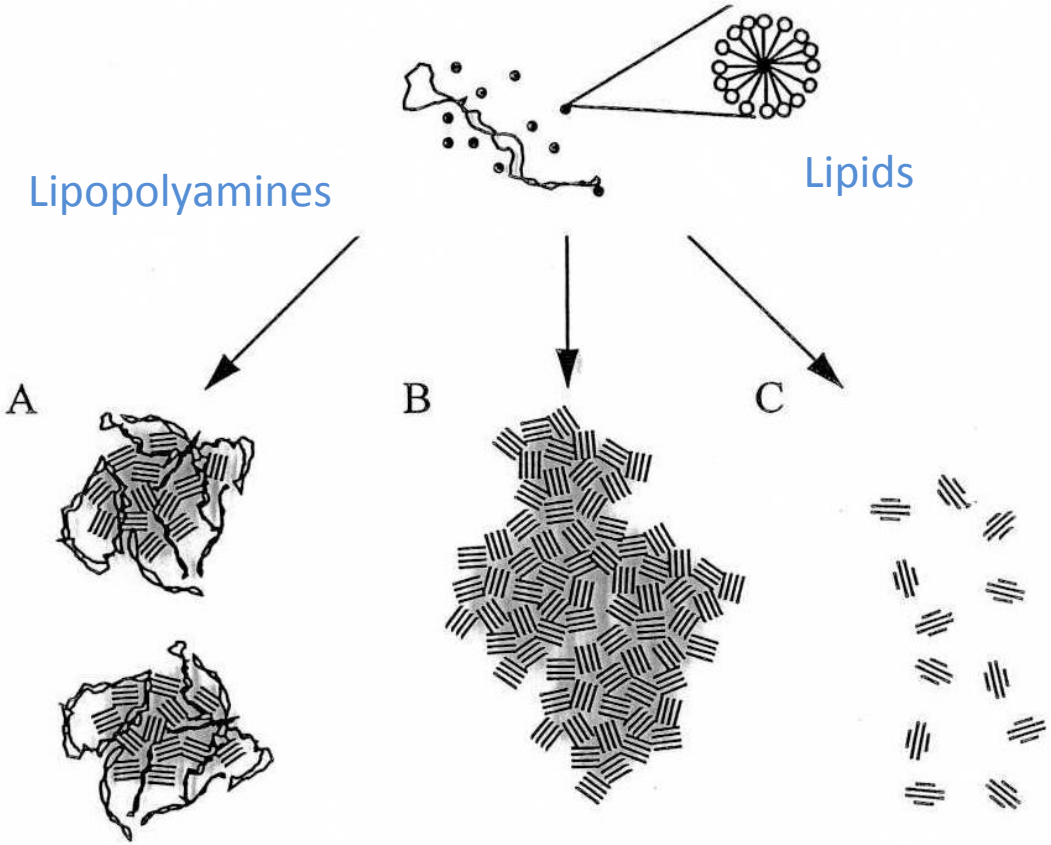
Improvement of DNA Transfer and Expression

Formulation of DNA

DNA in complexes with

Lipopolyamines

Lipids



Charged polymeric microparticles

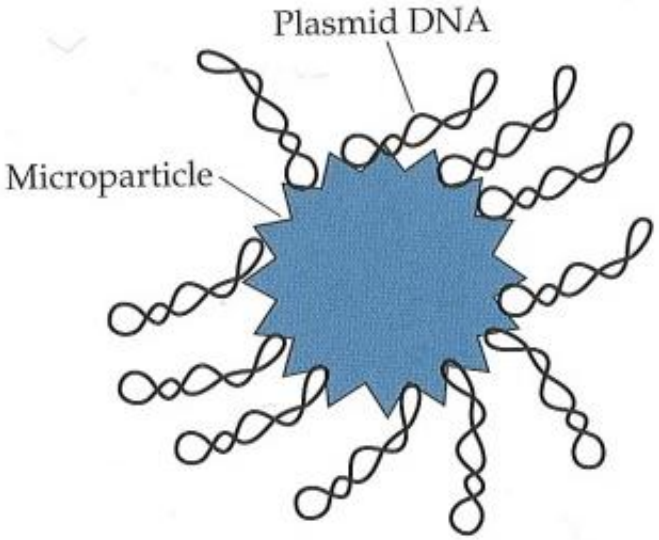


FIGURE 12.15 Schematic representation of the binding of plasmid DNA to the cationic surface of a polymeric microparticle.

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DNA Vaccines

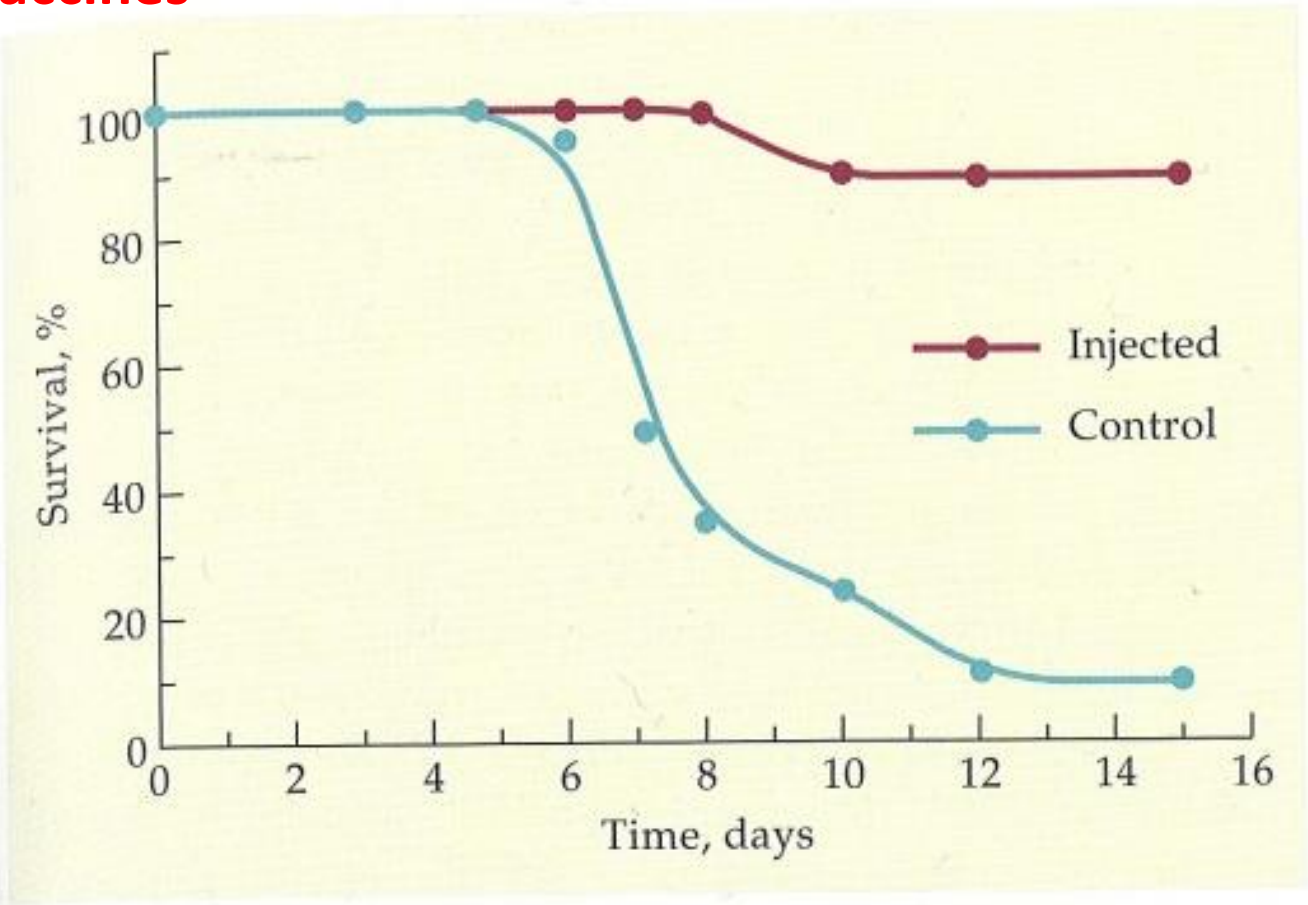

FIGURE 12.14 Survival of DNA-immunized mice. Injected mice were immunized with DNA that contained the influenza A virus nucleoprotein gene under the control of the Rous sarcoma virus promoter on an *E. coli* plasmid. The control mice were injected with plasmid DNA only. The *x* axis represents the number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

B.R. Glick, J.J. Pasternak, C.L. Patten; Molecular Biotechnology, 4th edition; ASM Press

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.

As of June 2015 one human DNA vaccine had been approved for human use, the single-dose Japanese encephalitis vaccine called IMOJEV, released in 2010 in Australia.[7] A veterinary DNA vaccine to protect horses from West Nile virus has also been approved.[

B.R. Glick, J.J. Pasternak, C.L. Patten; Molecular Biotechnology, 4th edition; ASM Press