Methods in gene vector production for vaccine clinical trials

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Abstract
Methods of gene vector purification vary widely based on application. The three main types of gene vectors discussed here are: DNA, RNA, and Virus. DNA gene vectors are primarily composed of supercoiled plasmid DNA, due to their efficient transfection rate. RNA gene vectors can vary from mRNA, siRNA, or miRNA, but all have similar purification methods. Purification of viral vectors is the most unique of the three, due to the protein encapsulation of viruses. Large volumes (up to 2000 liters) of gene vector are necessary for emerging vaccine and non-viral vector technologies. As such, efficient industrial-scale purification techniques are valuable for pharmaceutical industry and genetic research. This review outlines the different methods of nucleic acid preparation based on preferred finished product.

Common Methods

Lysis:
- Bench: Organic, Alkaline (pDNA only)
- Industrial: pH/heat combination

Purification:
- Bench: Centrifugation or magnetic bead-binding
- Industrial: Filtration (most common) or chromatography. Limited by filter clogging.

Washing:
- Industrial-scale DNA/RNA purification may include a phase-separation centrifugation technique

Elution:
- Bench: TE buffer Centrifugation
- Industrial: May vary depending on type of filtration or chromatography

Treatment:
- Industrial-scale diafiltration is used to further concentrate DNA after purification
- RNA may be treated with DNase to remove confounding nucleic acids

Conclusion
Vector production is currently a major bottleneck in gene-therapy and vaccine research, so efficient methods of vector production or needed. For effective scale-up up vector purification, each step should be analyzed to determine the most effective method for obtaining the desired product. This review outlines the effective techniques for scale-up of each vector type.

Citations

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