Step 3

The affinity purified scDNA was bound to Smac Q40 under certain conditions, and the endotoxin was removed by the flow-through mode, and finally high purity scDNA was obtained, with endotoxin residue ⩽2 EU/mg.

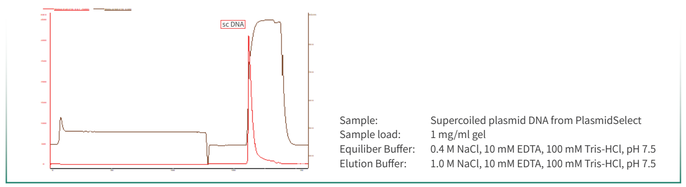

Figure 5. SmacQ 40 chromatogram.

Conclusion

After three-step purification, the purity of scDNA increased to over 98% (CGE), with a total recovery of approximately 70% from the chromatography process.The data is shown in Table 3.

Table 3. Purity and recovery of scDNA after purification by three-step method.

Sample	%scDNA (CGE)	Recovery (%)
Start Material Alkaline Lysate	74	N/A
Smartarose 6FF	92	95.6
Smac PlasmidSelect	98	76.73
Smac Q 40	98	93.59

Case 2：Smac Core 700+Plasmid affinity chromatography in a two-step process

Due to its large molecule, pDNA is excluded from the Core 700 packing microspheres and is separated in a flow through mode, while RNA, HCD, HCP and endotoxin enter the microsphere and bind to the octylamine group to achieve the purpose of separation. The purified sample was processed by Smac PlasmidSelect affinity chromatography results as shown in Figure 6. After two-step purification, the purity of scDNA exceeded 96%.

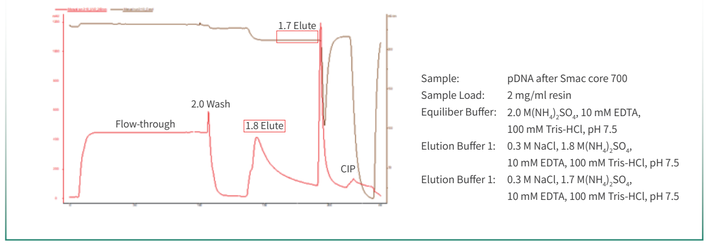

Figure 6. Smac PlasmidSelect affinity chromatogram.

Product Recommendation

Type	Product	Article Number (Optional)
Gel-filtration	Smartarose 6FF	SEC011 (25ml/100ml/500ml/1L/10L)
Affinity	Smac PlasmidSelect	SA104 (5ml/25ml/100ml/500ml/1L)
Ion Exchange	Smac Q 40	SI035 (25ml/100ml/500ml/1L/10L)
	Plasmid Purification MaxiPrep Kit	SI014K (5/10 Assays)
	Plasmid Purification Beads	SI014 (30ml/100ml/300ml/500ml/1L)
	DEAE 70M Phmac Beads	SI050 (5ml/25ml/100ml/500ml/1L/10L)
Hydrophobic	Smac Butyl 40	SH014 (25ml/100ml/500ml/1L/10L)
	Smac Phenyl 40	SH013 (25ml/100ml/500ml/1L/10L)
Mix Mode	Smac Core 700	SEC028 (25ml/100ml/500ml/1L)
Hydroxyapatite	Hydroxyapatite 60 (CHT II 60)	SA111 (5g/20g/100g/500g/1kg)
	Hydroxyapatite 40 (CHT II 40)	SA107 (5g/20g/100g/500g/1kg)
	Hydroxyapatite 20 (CHT II 20)	SA114 (5g/20g/100g/500g/1kg)

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2024.11

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Guide to Plasmid Purification

Professional Manufacturer of Chromatography Resins

Product Introduction

Plasmids are circular double-stranded DNA molecules that reside in cells and are independent of chromosomes and have the ability of self-replication and stable inheritance. It can be used as a nucleic acid vaccine or therapeutic drug alone, as a packaging raw material for the production of viral vectors, and as a template for in vitro transcription in the production of mRNA vaccines. In these applications, high-quality and high-purity superhelix plasmid (scDNA) needs to be used as a raw material. With the vigorous development of cell and gene therapies, plasmid applications are rapidly entering commercial drug production.

scDNA Production Process

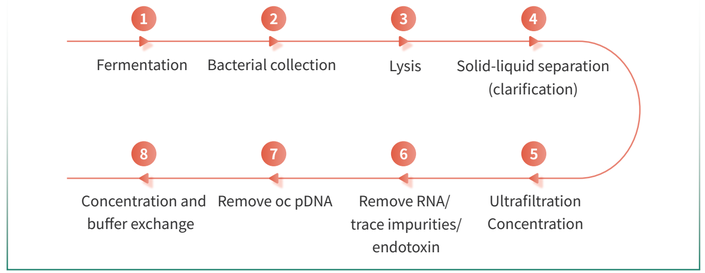

Figure 1. Preparation process of scDNA.

Fermentation and Collection of Bacterial

The method for collecting bacteria can be selected based on the volume of the fermentation broth, with options including centrifugation or hollow fiber tangential flow filtration. For bacterial collection and washing, hollow fibers with a molecular weight cutoff of 750 kDa or a pore size of 0.1–0.2 μm are recommended.

Sample Preparation

There are many methods for bacterial lysis, with alkaline lysis being the most commonly used. The collected bacterial solution is treated with 0.2 M NaOH and 1% SDS . Under alkaline conditions, the RNA、HCP、HCD and plasmids are released into the solution and denatured. During this process, slow stirring is required when adding alkali to avoid excessive local pH, and to monitor the lysis time to ensure sufficient lysis of the bacterials and prevent irreversible damage to the plasmid, which may affect the yield of pDNA. After alkaline lysis, the solution is neutralized with 3.0 M potassium acetate at pH 5.5. Controlling the mixing speed during the neutralization and precipitation process is equally important for ensuring the quality of pDNA.

In the presence of 0.2% -1% SDS, 1.0-1.5% CaCl2 can be added to promote RNA precipitation, or (NH4) 2SO4 can be added to neutralize buffer. A comparison of the different processes for RNA removal is shown in Table 1 below:

Table 1. Different methods for RNA removing.

Method	Advantage	Disadvantage
Gel-filtration	Effective for separating DNA and RNA with large molecular weight differences	Not suitable for removing RNA of similar size to DNA, especially with small sample loads
$CaCl_2$ or$(NH_4)_2SO_4$ precipitation	Selective precipitation of RNA by adjusting the ion strength and pH in the solution for separation from DNA	Conditions need to be explored. If RNA removal is not thorough, it will affect the pDNA capacity in affinity or hydrophobic chromatography
	Reduce process steps (improve recovery) and increase productivity	Complicated operation, relatively open, and low automation

Clarification and Ultrafiltration

During the alkaline lysis process, the addition of a large volume of buffer significantly increases the sample volume. Therefore, concentration and washing before chromatographic purification not only greatly reduces the volume of the sample, but also effectively removes impurities such as RNA, pigment and part of host cell protein (HCP) and host cell DNA (HCD), reducing the load at the chromatography stage. For ultrafiltration concentration of plasmids, hollow fibers are generally selected according to the following criteria (see Table 2 below). The low shear force of hollow fibers can effectively protect the supercoiled conformation of plasmids.

Table 2. Recommended Selection of Hollow Fibers.

Plasmid Molecular Weight (kbp)	Hollow Fiber Cutoff Molecular Weight (kDa)
3	100
6	300
>10	500

Chromatography of scDNA

In the preparation process of supercoiled plasmid DNA, in addition to removing impurities such as proteins, endotoxins, and RNA, separating open- circular plasmid DNA (oc pDNA) and linear plasmid DNA (linear pDNA) with similar plasmid structures is a difficult point in the purification of supercoiled plasmid DNA.

According to the plasmids application , stable chromatography processes are essential to obtain scDNA that meets different product requirements. The downstream production process is shown in Figure 2.

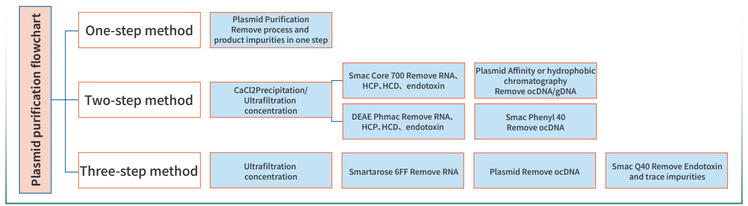

Figure 2. Different process routes for purifying scDNA.

Case 1：The classic three-step method gel filtration (Smartarose 6FF) +Plasmid affinity (Smac PlasmidSelect) + Ion exchange (Smac Q40)

Step 1

In the process of gel filtration chromatography, the plasmids initially elute through the volume of external water. The high concentration of ammonium sulfate in the buffer allows the RNA and other impurities to achieve better separation effect from the plasmids. After separation by Smartarose 6FF, there is no RNA impurity in the pDNA sample (see electrophoretic map).

Sample: Clarified alkaline lysate
Sample Load: 0.3 CV
Buffer: 2.1 $M(NH_4)_2SO_4$, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

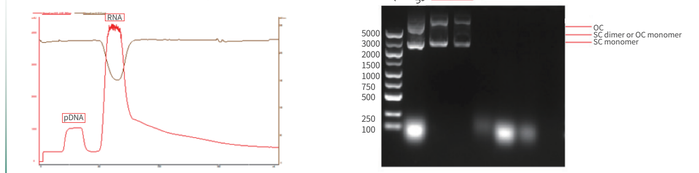

Figure 3. Smartarose 6FF chromatogram and electrophoretogram.

Step 2

Purification is performed using Smac PlasmidSelect affinity chromatography media, where scDNA binds to the packing, while ocDNA is found in the effluents and washes, and thus the separation of scDNA and ocDNA can be achieved.

Sample: Plasmid DNA after group separation
Sample load: 2 mg/ml gel
Equiliber Buffer: 2.1 M(NH4)2SO4, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5
Elution Buffer: 0.3 M NaCl, 1.7 M(NH4)2SO4, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5

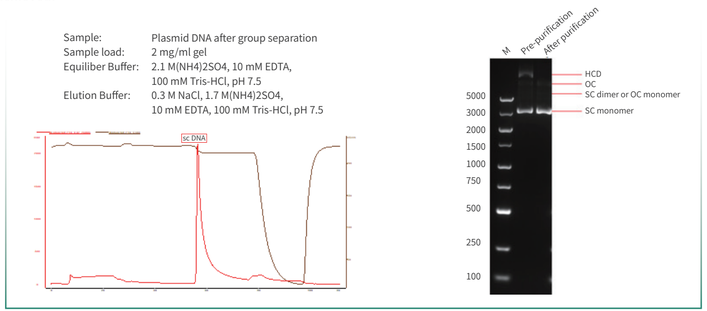

Figure 4. Smac PlasmidSelect chromatogram and electrophoretogram.