Electroporation

A-2

Improving Electroporation Transformation Efficiencies using Centri•Spin™-20 separation columns.

Introduction:

The high salt concentrations present in ligation mixes are known to reduce electroporation transformation efficiency. Recommendations to reduce salt concentration prior to electroporation include float dialysis, centrifugal ultrafiltration, and dilution. Each of these are either time consuming, not completely effective (reducing the transformation efficiency), or worse, result in significant DNA loss.

A **Centri•Spin-20** *protocol for desalting ligation reactions eliminates salts and co-factors while recovering >85% of the DNA. Electroporation transformation efficiencies are increased 2-fold.*

Cells may be transformed by electroporation when mixed with DNA and placed in an electric field. The electric field is created when a charge is delivered between an anode and cathode separated a specific distance by an insulated layer. In the case of electroporation of E.coli this insulating layer consists of cells in a nonionic solution of glycerol and ddH2O. The strength of this insulating layer is indicated by the time required for the applied charge to dissipate (the time constant). Ions increase the conductivity of this layer and cause a rapid dissipation of the electric field (reducing the time constant) thereby decreasing the transformation efficiency. If salt concentrations are sufficiently high, cuvette "arcing" may occur destroying the cuvette and ruining the experiment.

Protocol:

1. Conduct ligation(s) as appropriate (generally in a volume of about 20 uL).
2. Hydrate the necessary number of **Centri•Spin-20** columns with 650 mL sterile ddH2O. Allow at least 30 min for hydration at room temp.
3. Heat the ligation mix to 65-68oC for 10 min. to inactivate the ligase and linearize any non-ligated molecules (circular due to sticky ends).
4. Place the **Centri•Spin-20** in the 2.0 mL wash tube and centrifuge at 750 x g for 2 min. to remove the interstitial fluid Blot dry any excess liquid on the end of the column (after centrifugation) with a lab wipe.
5. Apply the ligation mix (20-50 mL) to the top of the gel bed (be careful not to disturb the gel) in the **Centri•Spin** column.
6. Place the **Centri•Spin-20** column in the collection tube (1.5 mL) and centrifuge at 750 x g for 2 min. to collect the sample.
7. Discard the column and use 1-2 mL (generally not more than 0.5 mg DNA) of the cleaned ligation mix per 40-50 mL cell suspension for electroporation.
8. Carry out the electroporation experiment as recommended.

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Results:

To demonstrate the effectiveness of **Centri•Spin-20** for this application, plasmid pGEM3Zf(+) (Promega) was cut with Eco R1 (New England Biolabs), cleaned using a Centri-Spin 20 and religated using T4 DNA Ligase (New England Biolabs). The ligation was heated as described above and 20 mL desalted using a **Centri•Spin-20**. The salt free ligation mix was compared with an untreated ligation mix and a 1:4 dilution of the untreated ligation mix into sterile water for efficiency of transformation by electroporation.

40 mL of DH10B electrocompetent cells (Life Technologies) were mixed with 2 mL of ligation mix and electroporated using an EM 600 Electroporator (BTX Technologies). The data is listed in Table I.

Table I. Effect of Post-ligation treatment on Time Constant and number of CFU obtained.

Post-ligation treatment	Time Constant (msec)	CFU
Centri·Spin-20	4.70	827
No treatment	4.56	460
1:4 dilution of untreated mix	4.70	277
Cells alone (no DNA)	4.70	0

Conclusion:

Table I clearly shows the advantage of cleaning ligation mixes with **Centri·Spin-20** for maximum electroporation efficiency. The decrease in the electroporation time constant for the untreated ligation reaction mix resulted in a 45% decrease in transformed colonies. Although diluting the reaction mix 1 to 4 results in a normal time constant, the transformation efficiency is only 25% of that seen for the **Centri•Spin-20** reaction, presumably due to the reduced amount of DNA in the electroporation cuvette

Centri•Spin-20 *spin columns are a fast, effective method to obtain the maximum amount of salt free DNA for electroporation. Twofold increases in transformation efficiencies can be obtained.*

Ordering Information:

Centri•Spin-10 columns are recommended for purifying DNA primers greater than 10 bases in length and desalting peptides with molecular weights above 5 kd.

Centri•Spin-20 columns are recommended for desalting DNA fragments greater than 25 bases in length and proteins with molecular weights above 25 kd.

Centri•Spin-40 columns are recommended for removing primers from PCR reactions and desalting proteins with molecular weights above 100 kd.

PRINCETON SEPARATIONS

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