BND-Cellulose Absorption

Numbers:

5% of total DNA are single stranded.
0.2ml of packed BND-cellulose (about 0.1g) absorb 10ug of ssDNA.(use 0.5 to 0.6ml(2ml sln) for 1 to 2x10^8 c.e.)
For 50 to 100ug DNA prep., we use 0.5ml of packed BND-cellulose (about 0.25g)

Buffers:

BND Wash: 5M NaCl, 10mM Tris (pH8.0), 1mM EDTA

BND Loading: 0.3M NaCl, 10mM Tris (pH8.0), 1mM EDTA

BND DS Elution: 0.8M NaCl, 10mM Tris (pH8.0), 1mM EDTA

BND SS Elution: 1M NaCl, 10mM Tris (pH8.0), 1mM EDTA, 1.8% Caffeine

Preparing Resins:

Weigh out 2g of BND cellulose. Place in a 15ml conical tube

Add 10ml of BND Wash Buffer, suspend and make sue all particles are wet.

Centrifuge at 2000rpm for a few min and wash with BND Wash Buffer 4 more times

Wash 5 times with BND SS Elution Buffer

Wash 5 times with BND Loading Buffer

Resuspend in a Final Volume of 10ml of BND Loading Buffer. Store at 4o.

Running the Column:

Pour 0.5ml (bed v.) columns in Bio-Rad (cat no. 731-1550). Use pateur pippet (cutoff).

Take the restriction digest. (200ul add 800ul loading buffer).

Gently load the 1ml mixture onto the resin.

Wash with 3ml of loading buffer

Wash and collect with 3ml of ds elution. Add 3ul of glycogen and 7.5 ml of ethanol. -20o o/n

Wash and collect 3ml of ss elution. Add 3ul of glycogen and 7.5 ml of ethanol. -20o o/n