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Chromatin Assembly Protocol (S150 extract)

Components

10X McNAP (use 10 µl per 100 µl assembly reaction)

water	1	**Order of addition IS important!**
300 mM ATP	1	
0.1 M DTT	1	
0.1 M MgCl2	3	
1 M creatine phosphate (CP)	3	
ExB-5/50	0.9	
1 µg/µl creatine phosphokinase	0.1	
	for 10 µl	

Assembly reaction:

Order of addition IS important, mix gently after addition of each component

Extract (S-150)	70 µl (determine empirically for each extract)
ExB-5/50	16.4 µl (adjust depending on volume of DNA and extract)
McNAP	10 µl

Mix
Add DNA to side of tube

pHspXX3.2 (0.1 µg/µl)	1 µl (6.6 kb fragment; -1.5 to +1.8)
ØX DNA (0.25 µg/µl)	2.6 µl (8 kb double strand)

Spin briefly to mix
Mix gently after spin

Incubate for 6 hours at 26° C (fly room)

Add 0.05% Sarkosyl to inactive endogenous chromatin remodeling complexes

Purify chromatin over Sepharose CL-4B spin column
Collect purified fraction into SILICONIZED tube and add BSA to 0.5 mg/ml

Stock solutions

300 mM ATP, pH 7.0	10 µl aliquots at -80° C
1 M creatine phosphate (CP)	30 µl aliquots at -80° C, (327.2 g/mol)
1 µg/µl creatine phosphokinase (CPK)	prepare in ExB-5/50, 0.1% BSA; 10 µl aliquots at -80° C do not freeze and thaw CPK, discard after use
0.1 M DTT	store at -20° C
0.1 M MgCl2	store at room temp

Buffer ExB-5/50	10 mM Hepes, pH 7.6 0.5 mM EGTA, pH 7.9 5 mM MgCl2 50 mM KCl 10% glycerol