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KGB: a single buffer for all restriction endonucleases

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Most recommended restriction buffers contain Na^+ and Cl^-. However, in bacteria the most abundant intracellular cation and anion are usually potassium and glutamate, respectively (1). Furthermore, restriction endonucleases cleave DNA in potassium glutamate (KGlu) over a much broader concentration range than they do in NaCl (2). These facts encouraged us to investigate the possibility that we could use KGlu in a chloride-free buffer and achieve normal levels of activity for all restriction endonucleases. We have tested fifty-five restriction endonucleases for their ability to cleave DNA in a series of KGlu buffers (KGB, see Table 1) and compared the level of activity with that found under conditions recommended by the vendors (New England Biolabs, Boehringer Mannheim Biochem. and International Biotech. Inc.). Assays were performed as partial digests (0.2 units per ug of DNA in 30 ul for 30 min.) and as overnight digestions with excess enzyme to ensure that no loss of specificity (star activity) occurred. Most restriction endonucleases, polymerases and ligase showed broad KGlu concentration optima and all enzymes functioned in 100 mM KGlu (1X KGB). Reducing agent was not normally required. Some enzymes worked well in concentrations of KGlu over 400 mM (data not presented). KGB can be used to simplify laboratory procedures including double digests, DNA cleavage followed by end-labeling, or the digestion of DNA embedded in agarose prior to pulsed field gel electrophoresis DNA in KGB can be phenol extracted and ethanol precipitated using standard protocols.

Enzyme	0.5X	1.0X	1.5X	2.0X	Enzyme	0.5X	1.0X	1.5X	2.0X	Enzyme	0.5X	1.0X	1.5X	2.0X	KGB
Aat II	3	3	3	2	Hae III	3	3	2	2	Sac II	3	2	2	1	
Aha II	0	2	1	1	Hgi AI	0	1	2	3	Sal I	0	1	3	3	
Alu I	2	3	2	1	Hha I	2	3	2	1	Sau 3A	2	3	3	4	
Apa LI	3	1	0	0	Hin dIII	3	3	3	3	Sau 96I	3	3	2	1	
Avr II	3	3	3	2	Hin fI	2	3	2	1	Sca I	1	3	3	2	
Bam HI	3	3	2	2	Hpa II	3	1	1	1	Sfa NI	0	1	2	3	
Ban I	3	3	3	2	Kpn I	3	2	0	0	Sfi I	3	3	2	1	
Bcl I	2	3	1	0	Mbo I	2	3	3	4	Sma I	3	3	3	2	
Bgl II	2	3	2	1	Mlu I	3	3	4	4	Ssp I	2	2	3	2	
Bss HII	3	3	2	1	Msp I	2	1	1	0	Sty I	2	2	1	1	
Bst NI	2	2	2	1	Mst II	3	3	2	1	Xba I	3	3	3	1	
Cla I	2	3	2	2	Nar I	2	1	0	0	Xho I	2	3	3	1	
Dde I	2	3	3	3	Nde I	2	3	2	1	Xmn I	3	3	0	0	
Dpn I	2	2	2	2	Nhe I	3	3	3	2	T4 DNA Polymerase					
Dra I	3	3	1	1	Not I	2	2	2	2		2	2	3	3	
Eag I	0	1	2	3	Nru I	2	3	3	3	*E. coli* DNA Pol. I					
EcoO109	2	3	1	1	Pst I	3	3	3	3		2	2	3	3	
Eco RI	3	3	3	3	Pvu I	2	3	3	3	Pol. I Klenow					
Eco RV	2	3	3	3	Pvu II	3	3	2	1		2	3	3	3	
Fok I	3	3	1	0	Rsr II	4	1	0	0	T4 DNA Ligase					
Fsp I	3	3	3	1	Sac I	3	3	1	0	(1 mM ATP)	3	2	1	1	

Table 1: 2X KGB denotes a buffer (K^+ Glutamate$^-$ Buffer) which contains 200 mM potassium glutamate, 50 mM Tris-acetate (pH 7.6), 20 mM magnesium acetate, 100 ug/ml bovine serum albumin, 1 mM 2-mercaptoethanol (filter sterilized and stored at 4°C). 1.5X, 1.0X and 0.5X KGB are dilutions of 2X KGB with distilled water. Symbols represent activity relative to recommended buffer: 4 = More active in KGB. 3 = 80-100% activity in KGB. 2 = 50-80% activity in KGB. 1 = 20-50% activity in KGB. 0 = less than 20% activity in KGB.

References (1) Richey B., Cayley D.S., Mossing M.C., Kolka C., Anderson C.F., Farrar T.C. and Record M.T. (1987) J. Biol. Chem 262:7157-7164. (2) Leirmo S., Harrison C., Cayley D.S., Burgess R.R. and Record M.T. (1987) Biochemistry 26:2095-2101.

