Rapid test for *in vivo* stability and DNA binding of mutated octamer binding proteins with 'mini-extracts' prepared from transfected cells

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The nuclear "mini-extract" procedure (accompanying report) can also be used to analyze the binding of octamer proteins produced from cells transfected with cloned Oct-2A cDNA (1). Transfection of increasing amounts of Oct-2A expression plasmid into HeLa cells results in a proportional amount of octamer binding activity (lanes 2-5) which is also accompanied by a similar increase in transactivation of target reporter plasmids (not shown). The sensitivity of the assay is very high when transfection is done in Cos-7 cells which express constitutively the T-Antigen protein of SV40 (2) and thus allow high replication of the expression vectors (3) bearing an SV40 origin of replication. As little as 10 ng of RNaseA treated and phenol purified DNA from boiling minipreps (4) give readily detectable signals (lane 11). With this assay the binding/stability potential of truncated Oct-2A proteins (5) can be tested: Progressive N-terminal deletions result in faster migration of the truncated protein : DNA complex (lanes 7-9). When part of the DNA-binding region is deleted, binding is abolished (lane 10). Although DNA-binding can also be monitored with in vitro translated proteins, bandshifts obtained from in vivo expressed proteins provide additional information: The protein does not only (i) bind to DNA but is also (ii) localized in the nucleus, is (iii) stable under in vivo conditions and (iv) may have undergone posttranslational modifications important for function.

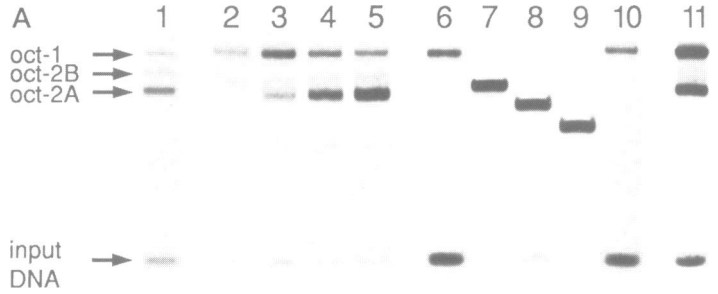

Figure legend: Autoradiography of a bandshift experiment with a radiolabelled octamer probe. Lane 1: BJAB nuclear extract. Lanes 2-5: Nuclear "mini-extracts" of HeLa cells transfected with no, 0.2 μg, 2 μg or 5 μg of Oct-2A cDNA in an expression vector. Lanes 6-10: 1 μg of nuclear "mini-extract" of Cos-7 cells transfected with no (lane 6), full length (lane 7) or truncated (lanes 8-10) Oct-2A cDNA. Lane 11: Cos-7 cell nuclear "mini-extract' transfected with 10 ng of full length Oct-2A cDNA.

Method: Transfections (lanes 6-10): 2 μg of expression vector were transfected per 10 cm dish of 80% confluent Cos-7 cells grown in DMEM supplemented with 10% fetal calf serum using the DEAE-dextran method as described in (6). After DMSO shock (3 min with 25% DMSO at 20°C) the cells were allowed to grow 48 h before harvesting and "mini-extract" preparation. 1 μg of protein was used per bandshift reaction.

References: (1) Müller, M.M. et al. (1988) Nature 336, 544-551. (2) Gluzman, Y. (1981) Cell 23, 175-182. (3) Severne, Y. et al. (1987) EMBO J. 7, 2503-2508. (4) Holmes, D.S. and Quigley, M. (1981) Anal. Biochem. 114, 193. (5) P.M. et al., in preparation. (6) Gerster, T. et al. (1987) EMBO J. 6, 1323-1330.