pGEM®-T Easy Vector System is an Easy Tool for Preparing Gel Shift Probes

ABSTRACT

The interactions between transcription factors and specific DNA sequences present in the regulatory region of genes play important roles in gene expression. The Promega pGEM®-T Easy Vector (Cat.# A1360), with two EcoRI sites in the MCS and flanking the cloning site, makes it easy to clone DNA fragments and excise the inserts using EcoRI.

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The interactions between transcription factors and specific DNA sequences present in the regulatory region of genes play important roles in gene expression. Electrophoretic mobility shift assay (EMSA or gel shift assay) is one of the most convenient methods to test DNA-protein interactions. For example, EMSA can be used to test whether a known DNA binds protein. Also, it is a powerful tool to reveal what kind of DNA motif that a transcription factor preferentially binds. Cloning and labeling the DNA sequences is necessary for the EMSA experiments. DNA can be end labeled with [γ-^{32}P]ATP or by incorporating [^{32}P]dNTPs by PCR. A convenient method to label DNA fragments uses Klenow DNA polymerase or *Taq* DNA polymerase to fill-in EcoRI cut cleavage sites with [α-^{32}P]dATP. The pGEM®-T Easy Vector (Cat.# A1360) has two EcoRI sites in the MCS that flank the A/T cloning site. This makes it easy to clone the DNA fragment and excise the inserts using EcoRI. The 5´ overhang left by EcoRI digest can be filled in to generate radiolabeled probes.

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AGL15, a MADS-domain protein, accumulates to the highest level during embryogenesis. Identifying the genes that are regulated by AGL15 is important for understanding the roles this DNA-binding protein plays in development. In our research, we have found a putative target of AGL15 that has a CArG motif in the regulatory region. The CArG motif is the DNA sequence preferentially bound by MADS-domain proteins. We cloned the DNA fragment into pGEM®-T Easy Vector, which was then excised by EcoRI digestion. Next, we labeled the fragment with [^{32}P]dATP by fill-in with Klenow DNA polymerase, and used this probe in gel shift experiments with AGL15 (Figure 1). We were able to show that AGL15 interacts with this DNA fragment in a specific manner.

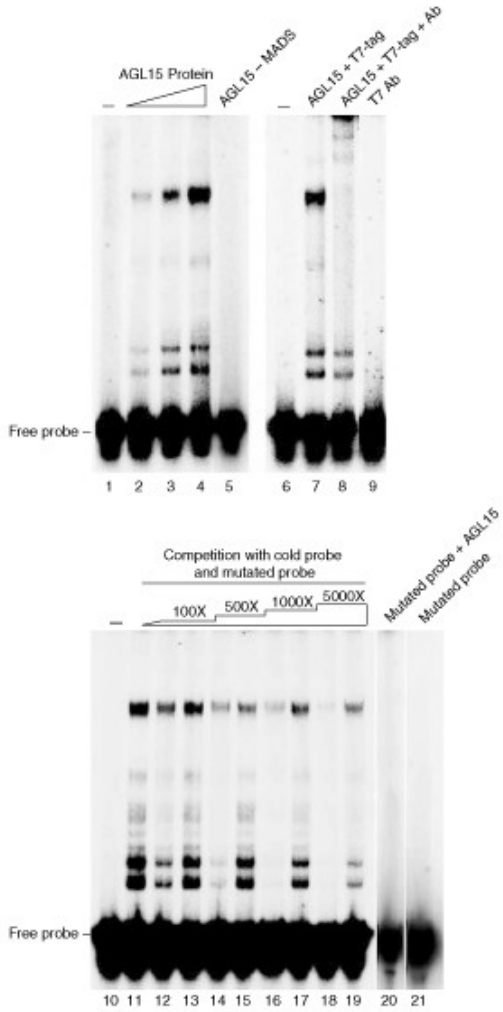

Figure 1. A DNA sequence in the regulatory region of a putative target gene serves as a specific recognition site for AGL15 binding. The radiolabeled DNA fragment was tested in binding reaction with AGL15 protein, and resulting complexes were resolved by 5% nondenaturing PAGE (containing 2.5% glycerol) with 0.5X TBE. Lanes 1, 6 and 10 are ^{32}P-labeled probe only (negative control). Lanes 2–4 are binding reactions with increasing amounts (~20ng, 60ng and 180ng) of AGL15. Lane 5 is a binding reaction with ~180ng AGL15 minus the DNA-binding MADS-domain. Lanes 7–9 show a supershift of the complexes. Lanes 10–21 show the competition binding reaction with cold, nonmutated DNA (12, 14, 16 and 18) and mutated DNA (13, 15, 17 and 19). Lane 11 is noncompetition reaction between nonmutated probe with AGL15. Lane 20 is mutated probe with AGL15. Lane 21 is mutated probe only. The mutation was made by changing two conserved bases in the CArG motif to create a binding site that AGL15 should not specifically bind.

We have used an in vitro binding method to enrich the DNA motif that AGL15 preferentially binds from a random population of oligonucleotides. We have cloned the enriched PCR amplified DNAs (approximately 70bp in length) into pGEM®-T Easy Vector. We found Promega's pGEM®-T Easy vector possesses very high efficiency and is fast for cloning PCR products, especially using their Rapid Ligation System. It is very convenient to sequence the inserts using T7 or SP6 Primer.

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