

## Cloning of Lis1 and CKAP5

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**Abstract:** Lis1 and CKAP5 are proteins that are both linked to type 1 Lissencephaly. Lis1 is a causative gene for Lissencephaly which causes defects in neural positioning. The regulation of Lis1 is still unknown however. CKAP5 is a microtubule associated protein that is essential for spindle orientation. In order to eventually perform mass spectroscopy experiments to identify any post-translational modifications to the proteins, we had to clone Lis1 and CKAP5. This involved doing a polymerase chain reaction (PCR) amplification, ligation of the PCR fragment into the cloning vector and transformation of the vector into E. coli and plating of the transformed bacteria onto plates for selection of transformed colonies. Transformants were inoculated into liquid media for plasmid preparation, restriction digest of the purified plasmid, and running a gel to screen for inserts. Our results DNA sequencing results verified that we have successfully cloned Lis1. We have also obtained an insert for CKAP5. After we have verified the plasmid carries the insert for CKAP5, we can move on to doing a transfection of cell cultures with Lis1 and CKAP5, performing a cell lysate, running SDS-PAGE, western blotting, and mass spectroscopy experiments to study post-translational modifications of the proteins.

**Keywords:** Lis1, CKAP5, Lissencephaly, Cloning, Plasmid

### Introduction

Lis1 has been identified as a gene product of type 1 Lissencephaly and is required for cell proliferation, neuronal positioning, and cell survival (Yang et al. 2009). However, the regulation of Lis1 is still largely unknown (Zhu et al. 2010). Lissencephaly is a developmental brain abnormality characterized by defects in neural positioning which can be fatal (Yang et al. 2009). Children who suffer with the brain malformation often experience severe retardation, epilepsy, and death due to a mutation in the Lis1 gene (Emes and Ponting 2001). When Lis1 is overexpressed, spindle misorientation occurs (Faulkner et al. 2000). CKAP5 is the mammalian homolog to the yeast protein Stu2, which is a microtubule-associated protein that is essential for spindle orientation (Kosco et al 2001, Basto et al. 2007). Post-translational modifications have been shown to play a role in regulation of these proteins in yeast

(Alonso et al. 2010). In order to identify any post-translational modifications to the proteins (Lis1 and CKAP5) in mammalian systems, we will need to clone the proteins. Once the proteins have been cloned, we will be able to move on to other steps like performing transfections of the cell cultures with MCF7 using the two plasmids, determining if the cells will express functional proteins by making a cell lysate, running SDS-PAGE, western blotting, and eventually mass spectroscopy experiments where we look for the post-translational modifications.

### Methods

We began with a plasmid prep and ran a 1% agarose gel (40 milliliters and 0.40 g agarose) in Tris/acetic acid/EDTA (TAE) buffer in order to verify that the commercial plasmid carried the DNA inserts with the correct band/insert size for Lis1 and CKAP5. After running a gel for each, the

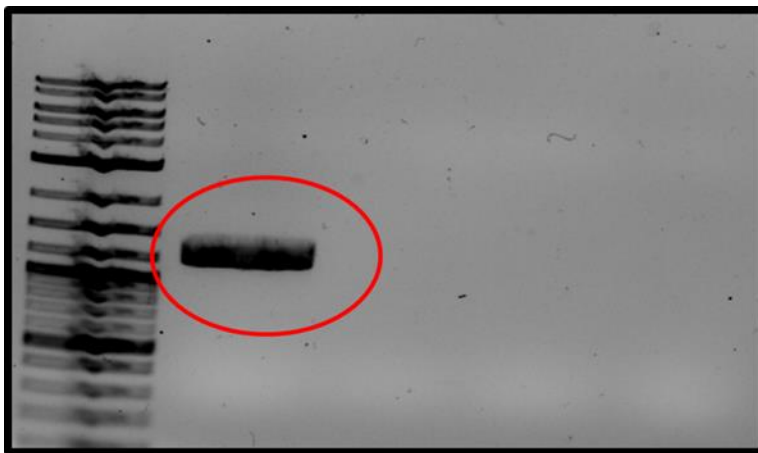
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protocol for CKAP5 was adjusted slightly to accommodate the its longer length of DNA. Next, we did a PCR amplification for Lis1 and CKAP5 with CKAP5 diluted 1 to 10 with water due to the higher concentration and the protocol slightly changed due to the size of the protein's coding sequence. We used four different tubes for each protein where the volume ratios were slightly altered depending on the additives. Tube 1 contained no extra additives. Tube 2 contained 1.5 microliters of DMSO. Tube 3 contained 1.5 microliters of MgCl<sub>2</sub>. Tube 4 contained 1.5 of each additive. After the PCR amplification, we ran a gel to determine which condition the primers worked best under. After that, we performed a ligation and transformation for each plasmid in order to grow colonies under selection for antibiotic resistance code dfor on the plasmids. Then we recovered antibiotic resistant colonies for Lis1 and CKAP5 and used them for an inoculation. Once each had been inoculated, we performed a plasmid prep and a restrictive digest check followed by agarose gel electrophoresis in order to screen for plasmids containing inserts for Lis1 and

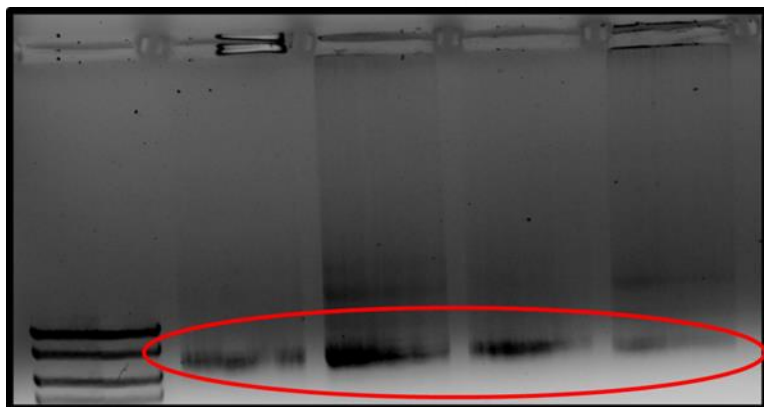
CKAP5. One colony was found with the proper insertion size for Lis1. We subsequently repeated the PCR amplification, the ligation and transformation, the inoculation, the plasmid prep and restrictive digest, and the agarose gel for CKAP5 and have obtained a plasmid with an insert of the correct size.

## Results

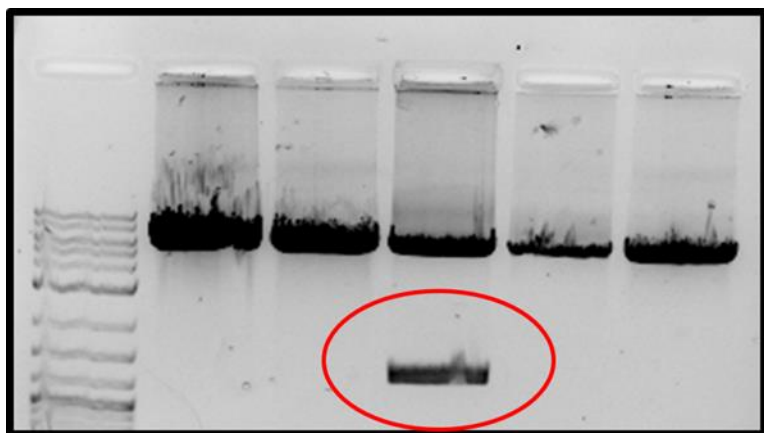
When we did a plasmid prep to determine whether or not the primers appeared on the correct band/insertion size, CKAP5 was more concentrated meaning that the PCR amplification protocol for CKAP5 had to be altered. After doing a plasmid prep for Lis1 and CKAP5 under the four different conditions (no extra additives, 1.5 microliters of MgCl<sub>2</sub>, 1.5 microliters of DMSO, and 1.5 microliters of each additive), an insert appeared for Lis1 that showed that the amplification of Lis1 worked best with no extra additives while inserts appeared under all conditions for CKAP which showed that the amplification of CKAP5 could work under any condition. Lastly, when we screened for inserts after the restrictive digest, a single colony with the proper insertion size for Lis1 appeared. DNA sequencing confirmed the plasmid carried a DNA insert coding for full length Lis1 with no mutations. Since an insert did not appear after screen for CKAP5, we repeat the process and have obtained a colony that carries a plasmid that appears to be the proper insertion size.



*Figure 1 - The lis1 insert check after the PCR amplification showed that the amplification worked best with no extra additives.*



*Figure 2 - The CKAP5 insert check after the PCR amplification showed that the amplification could work under any condition.*



*Figure 3 - The Lis1 insert check after the restrictive digest yielded one colony with the proper insertion size.*



## Discussion

The best condition for the amplification of Lis1 appeared to need no extra additives for the PCR (as shown in Figure 1). For CKAP5, inserts appeared under all conditions meaning that any condition would work for amplification (Figure 2). When the Lis1 PCR product was ligated into the pcDNA3 1D/V5-His TOPO vector and transformed, we recovered 11 different bacterial colonies. Both colonies had to be put into the pcDNA3.1D/V5-His Topo vectors because in the original plasmids (pCS6 Lis1 and pCRbluntII Topo CKAP5), there were no epitope tags for

doing future tests like western blotting and immune-precipitation pulldowns. When we grew up the colonies and screened for the insertion of Lis1, we found a single colony with the proper insertion size (Figure 3). Screening of a second set of bacterial colonies for the insertion of CKAP5 indicates we have a bacterial colony containing a plasmid with an insert size expected for CKAP5. Once we verify the insert for CKAP5 by DNA sequencing, we will be able to move on to the next procedure. First, we will do a transfection of the cell cultures using MCF7 and possibly others. Then we will make a cell lysate, run SDS-PAGE, and do western blotting in order to determine if the cells will express functional protein from the plasmids. Lastly, we will perform mass spectroscopy experiments on affinity purified proteins in order to look for changes in post-translational modifications to the proteins.

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