# SEPTIN 4 : PURIFICATION AND PROTEIN-PROTEIN INTERACTIONS

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Septins are a family of GTP-binding proteins first identified in *Saccharomyces cerevisiae* by their involvement in cytokinesis. They are expressed in almost all eukaryotes, but seem to be absent in plants. Septin 4 is expressed in brain and some tumoral tissues have increased level of its expression. Septin 4 was found in Lewy bodies what could suggest its involvement with neurodegenerative diseases. Septin 4 was cloned in the vector pGEX5x-1 fused to GST (Garcia *et al.*, Biochemistry 2006, 45:13918). The protein was expressed in *E. coli* at 20°C for 14h. The purification was performed by affinity chromatography with Glutathione Sepharose, the GST-septin 4 was cleaved by Factor Xa and the septin 4 was submitted to size-exclusion chromatography. To perform yeast two hybrid assays, the septin 4 gene was cloned in the vector pBTM116 fused to the Lex-A DNA binding domain. The autonomous activation test for *HIS3* was performed in minimal medium plates and the ß-galactosidase filter assay to detect expression of *lacZ* reporter gene. We are seeking a better way to purify septin 4. Changes in buffer composition and chromatography proceedings have been tried. The purity was improved; nevertheless, the purity needed to crystallography assays was not yet achieved. A yeast two hybrid screen is being performed to identify septin 4 partners in cellular process. Septin 4 full length was tested for autonomous activation of the reporter gene *HIS3* and *lacZ* by capacity of growth in minimal medium and ß-galactosidase filter assays, respectively. Both results showed an autonomous activation. We are now building new constructions with different septin 4 domains to identify which regions do not cause autonomous activation.

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