

# Expression and Purification of Histidine-Tagged VPS10 Domain of sorCS1 in a Baculovirus System

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## ABSTRACT

SorCS1 is a recently discovered member of a family of proteins in mammals known as the VPS10 receptor family. SorCS1 is a trans-membrane protein found near the cell surface as well as in the trans-Golgi network. It has been localized mainly to the CNS, but has been found as well in kidney, liver and heart tissue. The VPS10 domain of sorCS1 is soluble and is found in the lumen of the Golgi, and is believed to have a potential for binding proteins inside the secretory vesicles for transport to the cell surface. Through a collaboration project with Dr. Alan Attie at UW-Madison, we expressed the VPS10 domain (C-terminally His-tagged) of sorCS1 in a baculovirus system and purified it using a nickel column. The purified VPS10 domain will be used to determine binding partners within mammalian cells, as well as for antibody production.

## INTRODUCTION

SorCS1 belongs to a family of receptor proteins known as the VPS10 receptor family. SorCS1 is a trans-membrane protein, consisting of a leucine-rich domain, a single trans-membrane domain, and a short cytoplasmic domain, and an N-terminal VPS10 domain. Localized largely to the cell surface but also to the trans-Golgi network, it has been hypothesized that sorCS1 is involved in binding proteins within the cell and transporting them to the cell surface. SorCS1 is a secreted protein, and has a pro-peptide form that is converted to mature sorCS1 in the Golgi by furin, a serine protease.

The VPS10 domain of this protein is approximately 130 kD; the DNA sequence for this domain is approximately 3.3 kbp in length. The VPS10 domain is believed to be the function protein binding domain of sorCS1; in yeast it is involved in the binding and sorting of carboxypeptidase Y.

To elucidate the function of VPS10 in sorCS1, it will be necessary to purify it in large quantities to use in binding assays. Due to the fact that this protein requires secondary modifications, and is secreted, the use of insect cells will be necessary to obtain functional protein. Therefore, a baculoviral system of expression will be used to obtain functional VPS10 protein.

## METHODS

### *Cloning of VPS10 domain*

The VPS10 domain sequence (3.3 kb) was received in a pCR-Blunt II-TOPO cloning plasmid. Prior to receiving the plasmid, the VPS10 sequence was tagged on its 3' end with a sequence encoding for a histidine tag (Fig. 1a).

### *Digestion of plasmids (Fig. 1b)*

The plasmid with the VPS10 insert was digested with *SpeI* and *NotI*. In addition, pVL1393 plasmid (~9kbp) was digested with *XbaI* and *NotI*. The VPS10 insert was ligated into the pVL1393 vector. Presence of insert was checked by digesting the VPS10/pVL1393 vector with *EcoRI* and *BglII* (Fig. 2).

### *Homologous Recombination*

The VPS10 insert was inserted into a baculovirus via homologous recombination, and the baculovirus was used to infect SF-9 insect cells. The media was collected and used to infect 200 mL of 3EX insect cells. The infected media from the 3EX cells was harvested for further analyses.

### *VPS10 purification*

The 200 mL of infected media was run on a nickel affinity chelation column. The column was washed with 10 mM imidazole, 50 mM imidazole, and 250 mM imidazole washes to elute protein. The 250 mM wash was expected to elute the protein. Prior to running the column, 0.5 mL of PMSF and 15 mg of benzamidine and trypsin inhibitor were added to inhibit protease activity. After running the column, 0.1 M EDTA was added directly to the 50 and 250 mM fractions to prevent aggregation of His tag ends.

### *Check for presence of VPS10 protein*

Immunoblot assays were performed using rabbit anti-sorCS primary antibodies, and goat anti-rabbit-alkaline phosphatase secondary antibodies (Fig. 3). Purity of the protein in the 50 and 250 mM fractions was determined using a Coomassie stain (Fig. 4).

### *Concentration of purified VPS10 protein*

The absorbencies at 280 nm of the 50- and 250-mM imidazole washes were determined, and used to calculate the concentration (mg) of protein (Table 1). The extinction coefficient for VPS10 was calculated using a bioinformatics program (Gill and von Hippel). Concentration of the protein was determined as follows:

$$\text{Concentration (mg/mL)} = (\text{Abs}_{280}) / (\text{Extinction coefficient (M}^{-1} \text{ cm}^{-1}\text{)})$$

## **RESULTS AND DISCUSSION**

The subcloning procedure (Fig 1b) was successful—a band corresponding to the 3.3 kbp VPS10 sequence was seen in three of the four restriction digests (Fig. 2).

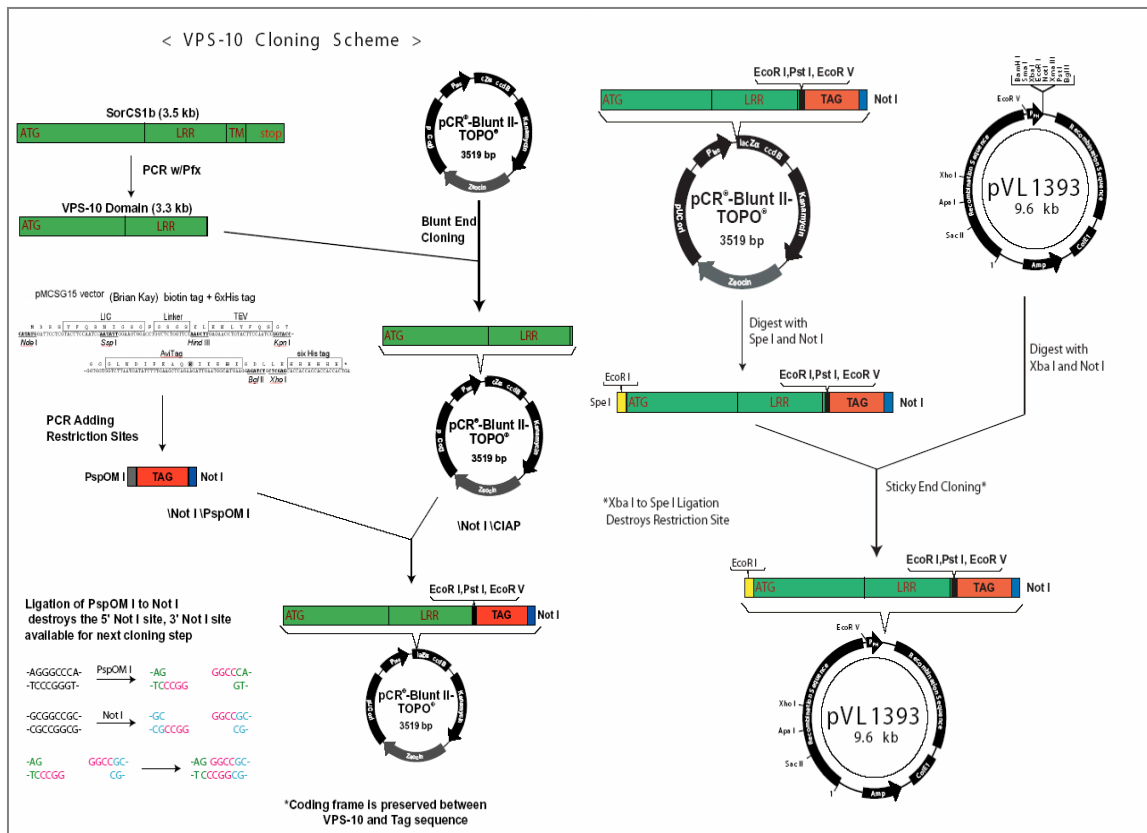
During the purification of the 200-mL baculovirus transfection, some of the protein was lost due to precipitation (Fig. 3); this may have been due to aggregation of the his-tagged ends. In addition, the SorCS1 protein has been shown to have some affinity for itself (Hermey et al.), resulting in the precipitation of aggregated protein.

Some of the protein was eluted in the 50mM imidazole wash of the nickel column (Fig 3). This also may be due to his-tag aggregation of the VPS10 protein, leading to decreased affinity for the nickel beads and earlier elution.

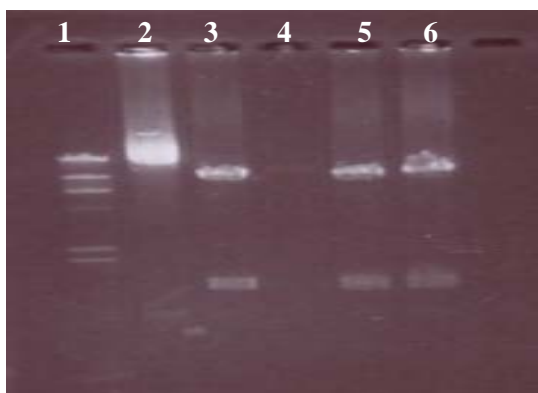
It appears from Fig. 4 that there may be some issue with the purity of the VPS10 domain protein---it was proposed that the 75 and 25 kD bands were being seen due to furin cleavage in the insect cells prior to secretion, despite the addition of serine protease inhibitors (Fig. 4).

From the 200-mL baculovirus preparation, approximately 0.15 mg of VPS10 protein was harvested (Table 1). The extinction coefficient for VPS10 was calculated using a bioinformatics program (Gill and von Hippel). In addition, a 2-L was also prepared, but only resulted in a 2-fold increase in protein production compared to the 200-mL preparation (data not shown).

The purified protein from the 200-mL preparation was taken to Denmark, and through surface plasmon resonance analyses it was determined to be active. A 10-Liter preparation using the baculovirus transfection system is being planned for future endeavors.



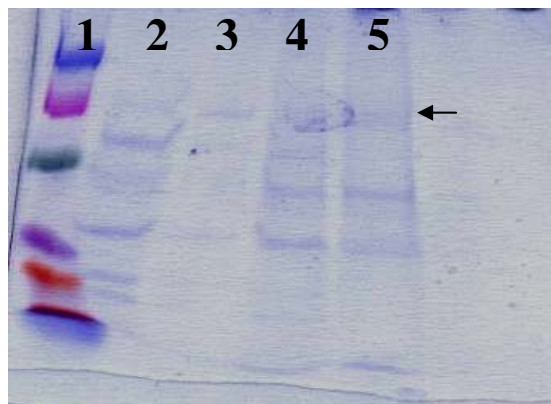
**Figure 1:** Subcloning protocol. 1a; attachment of his-tag to 3' end of VPS-10, insertion of vector into pCR-Blunt II-TOPO plasmid. 1b; subcloning of VPS10 vector into pVL1393 plasmid.



**Figure 2:** Check for presence of VPS10 insert in pVL1393 on a 0.8% agarose gel. Lane 1: Molecular Weight Marker; lane 2: uncut plasmid; lanes 3-6: *EcoRI* and *BglIII* digested Vector+insert



**Figure 3:** Immunoblot assay. Fractions collected from the nickel column were run on a 10% SDS-PAGE gel, then transferred to nitrocellulose. Lane 1: MWM; lane 2: flow through nickel column; lane 3: 10mM imidazole wash; lane 4: precipitated protein from the 50mM imidazole wash; lane 5: precipitated protein from the 250 mM imidazole wash; lane 6: 50 mM imidazole wash; lane 7: 250 mM imidazole wash



**Figure 4.** Check for VPS10 purification. A 10% SDS-PAGE gel was run and stained with Coomassie Blue to determine if the 50- and 250-mM imidazole fractions were free of contaminants. Lane 1: MWM; lane 2: 50mM imidazole wash; lane 3: 250 mM imidazole wash; lane 4: precipitated protein from the 50 mM wash; lane 5: precipitated protein from the 250 mM wash.

**Table 1.** Concentration of VPS10 collected from 200 mL baculovirus infection

Fraction	Abs280	Extinction Coefficient ( $M^{-1} \text{ cm}^{-1}$ )	Volume Collected (mL)	Concentration (mg)
50 mM imidazole	1.019	1.305	1.2	1.54
250 mM imidazole	0.41	1.305	0.5	1.59

## REFERENCES

- Gill and von Hippel. (1989). *Anal. Biochem.* 182, 319-326.  
 Hervey, G. et al. (2002). *Journal of Biological Chemistry.* 278, 7390-7396.

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