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## **Collagen IV Assembly: Production of a Recombinant Construct for Mechanistic Studies**

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### **ABSTRACT**

Basement membranes, specialized extracellular matrix, provide mechanical support for epithelial cells and shape cell behavior by interacting with cell receptors. Collagen IV, the predominant component of basement membrane, comprises six genetically distinct alpha chains ( $\alpha 1$  to  $\alpha 6$ ) that assemble to form protomers, which associate to form hexamers, in the scaffold. Non-assembly due to mutations within alpha chains give rise to kidney diseases. Yet, the mechanism of assembly is poorly understood.

Preliminary data in our lab indicated that amino acid 78 of collagen IV  $\alpha 2$  NC1 (non-collagenous) domains may play a role in a chloride-dependent switch that supports hexamer formation. We hypothesized that this particular amino acid is critical for hexamer assembly. To this end, we sought to generate a mutated construct of collagen IV  $\alpha 2$  truncated protomer, TP(D78A). We anticipate this construct will be a critical tool in understanding the mechanism of collagen IV  $\alpha 2$  domain assembly.

Using molecular biology techniques, we introduced a point mutation in TP(D78A) and identified optimal conditions for protein expression. We generated PCR products of expected molecular weight and sent them for sequence verification. We then investigated cell lines for optimum protein expression to later look for hexamer formation. We found that COS-7 cells as compared to HEK-293 cells were better for expression of our wild-type construct WT(TPD78). In conclusion, we produced candidate TP(D78A) constructs for sequencing and determined COS-7 cells were better for expression of WT(TPD78). These results are significant in producing a tool to investigate the mechanism of collagen IV  $\alpha 2$  NC1 domain assembly.

**Keywords:** Basement membrane, Collagen IV, and  $\alpha 2$

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