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Title:	Expression and purification of protocadherin- 15 protein of varying domain numbers and labeling of inter- domain linkers of tip- link proteins with fluorescent dyes
Authors:	Garg, Keshav (/jspui/browse?type=author&value=Garg%2C+Keshav)
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Abstract:	<p>Hearing one of the most well developed sensory process involves conversion of sound wave into electrical signal inside inner ear. Tip-link, a protenacious bridge made up of heteromeric interaction of two calcium binding proteins Cadherin 23 and Protocadherin 15 conveys the mechanical force produced by sound wave into electrical signal. Cadherin 23 and Protocadherin 15 are having 27 and 11 extracellular domains respectively whereas only two outermost domains interact with each other. So, to find out the role of increasing domain number various fragments of protocadherin 15 containing varying number of domains have been cloned. Protein expression was performed using mammalian suspension cells. Between each domain linker is present which binds to calcium. Calcium is required to maintain the elasticity of the linker and the overall protein. Using nanosecond time resolved anisotropy measurements flexibility changes can be measured which requires attachment of a fluorophore. Cysteine mutated variants of Cdh 23 and Pcdh15 EC12 were prepared to modify fluorophore with the protein. Proteins were expressed using BL21 RIPL cells in E- Coli bacteria.</p>
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