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Structural Classification of Pax7 Using Homology Modeling: A Functional Approach

KEYWORDS: PAX7: Paired box7, NCBI: National Centre for Biotechnology Information

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ABSTRACT *PAX7 gene belongs to the class of transcription factor and has a significant role in neural crest development. 1PDN.pdb was used as a template for 3D structure prediction of PAX7 domain with identity 80% and 91% positivity using Discovery Studio version 3.1. The predicted model was then subjected to check internal consistency and reliability. Qualitative and quantitative analysis suggests that the predicted model was reliable and stable with best quality and resolution along with volume. Overall fractional accessible surface area, residues volume and packing quality index was found to be good using VADAR server. Resolution of the predicted model is 2.803A°. Structural classification suggests that this super family represents winged helix DNA binding domains CATH ID: 1.10.10.10.*

Summary: PAX7 gene beongs to the class of transcription factor. In this proposed study homology modeling of PAX7 protein has been done. The predicted model was then subjected to check internal consistency and reliability. Structural classification suggests that this super family represents winged helix DNA binding domains CATH ID: 1.10.10.10.

Introduction:
PAX7 is transcription factor involved in embryonic development. Nine members of PAX family have been characterized into four subgroups with paralogous gene PAX3 and PAX7 under same third subgroup. It is characterized by the presence of paired domain (~128 amino acids long), two DNA binding motif (C. Walther, 1991; Noll, 1993), paired type homeodomain and octapeptide chain (A. Mansouri, 1996; Erickson, 1993; Gros, 1997; H.M. Berman, 2000; Underhill, 2012; Walther, 1996). PAX7 gene has been mapped on chromosome 1p36.2(E Vorobyov, 1997). PAX family members are specific to multicellular vertebrates having role in differentiation of myogenic lineages. C terminal end of PAX7 is evolutionary conserved and are expressed in myogenic and neural tumor cell lines (FG Barr, 1999). PAX7 play significant role in regulation of myogenic potential and satellite cells (P Seale, 2000). Translocation of PAX7 with fork head gene domain (FKHR) showed expression in alveolar rhabdomyosarcoma (RJ Davis, 1994). In this proposed study we have demonstrated the homology modeling of PAX7 gene and then the quantitative and qualitative analysis of predicted model was done using different insilico techniques.

Materials and Methods:
PDB advanced BLAST (H.M. Berman, 2000) (http://www.rcsb.org/pdb/home/home.do) was used to select template sequence. Homology modeling of target protein was done using Discovery studio version 3.1 (Rashmi, 2013; S. K. Shahi, 2013) (http://www. accelrys.com, Discovery Studio 3.1, 2011, Accelrys Inc, San Diego, CA, USA) and submitted in PMDB database. The secondary elements were determined using UCSF chimera (E. F. Pettersen, 2004) and PDB Sum server (Laskowski RA, 2005). The predicted 3D model was tested for internal consistency and reliability. Quality check of model was done using Verify3D (Roland Luthy, 1992) and Errat server (C. Colovos, 1993) (www.nihserver.mbi.ucla.edu/ERRATv2) and refinement using RAMPAGE (S.C. Lovell, 2003) (http://mordred.bioc.cam.ac.uk /~rapper / rampage.php). Quantitative analysis was determined using Volume Area Dihedral Angle Reporter (VADAR) (Leigh Willard, 2003)(http://vadar.wishartlab.com/). Resolution quality was determined using ResProx (Resolution-by-proxy) (Mark Berjanskii, 2012). CATH (Orengo, 1997)(www.cathdb.info/) and SCOP database (Murzin, 1995)(scp.mrc-lmb.cam.ac.uk/scop) determined structural classification of predicted model.

Result
1PDN was used as template for modeling of PAX7 protein having 80% identity and 91% positivity. Ramachandran study showed no residue in outlier region, 95.9% residues in favored region and 4.1% residues in allowed region with good stereo-chemical quality. Model having more than 90% residue in favored region are determined to be best model. Successfully refined, validated model of PAX7 protein from Homo sapiens was deposited to PMDB database (PM0078620). The predicted structure has 7 helices (53-65, 69-76, 80-93, 108-110, 113-123, 130-140, 156-159) 6 helix-helix interacs, 2 β-strand (36-38, 44-46), 1 β-sheet, 1 β-hairpin, 1 β-bulge, 1 β-turn and 10 coils (Figure 1 A, B and C) as secondary elements.

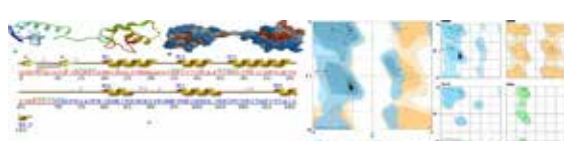

Figure 1: Structure of PAX7 protein from Homo sapiens. (A) 3D structure, (B) surface structure (C) Secondary structure. Ramachandran plot statistics of PAX7.

Modeled structure for PAX7 with best scores had PDF Total energy 498.2125, DOPE score -10840.472656 and PDF physical energy 282.12083 (Table 1).

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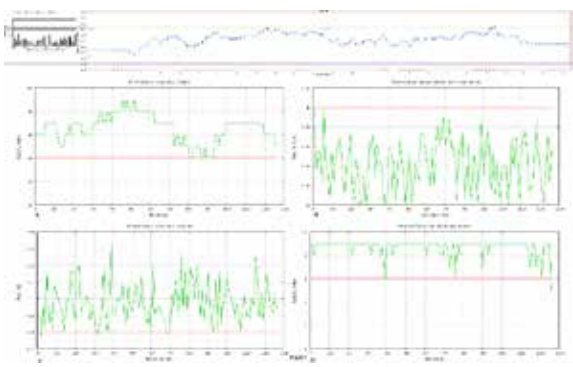
"Table 1 about here"

Model Scores			
Name	PDF Total energy	PDF Physical Energy	DOPE Score
Untitled1. M0003	498.2125	282.12083	-10840.472656
Untitled1. M0002	499.4792	275.004056	-10821.746094
Untitled1. M0005	539.8435	291.98816	-10713.703125
Untitled1. M0004	627.2080	314.1519694	-10807.924805
Untitled1. M0001	682.7383	295.3083511	-10738.113281

Table 1: DOPE score, PDF physical energy of predicted model of protein using Discovery studio3.1 for PAX7 Quality factor was 96.364% suggesting that model had a good quality. Verify 3D had a range between 0.40-0.12.



"Figure 2 about here"

Figure2: The 3D profiles of Homo sapiens PAX7 protein verified using ERRAT server, expressed as the percentage of the protein to which the calculated error value falls below the 95% rejection limit. Verification of PAX7 protein structure using Verify 3D. Fractional accessibility surface area (A) Profile quality, (B) Surface area (C) Residual volume (D) and Stereo/ Packaging Quality index of PAX7 protein using VADAR server.

VADAR statistics demonstrated modeled structure having 50% Helices, 5% Beta, 44% Coils and 28% turns. Overall fractional accessible area, residues volume and packing quality index were found to be approved for PAX7.Resprox results inferred that the resolution of the predicted model was 2.803A° for PAX7.

Discussion:

PAX7 is associated to class of transcription factor and has a significant paired domain. Insilco techniques were used for structural classification of PAX7 gene using homology modeling. RAMPAGE study showed that the model having more than 90% was best model, based on this it was found that model was good (Arora N, 2012; Shahi SK, 2013). ERRAT server suggested that PAX7 model had a good quality as a score higher than 50 was acceptable(Alisaraie L, 2012; Yu H, 2013). Qualitative and quantitative analysis of the predicted model represented the best quality model which is reliable and stable. In the predicted model, hydrogen bonds (mean H bond distance, mean H bond energy and residues with H bonds) are arranged with accessible confirmations. Overall fractional accessible area, residues volume and packing quality index was found to be good using VADAR server for PAX7. Resolutions of the predicted models are 2.803A°. Structural classification resulted that this super family represents winged helix DNA binding domains CATH ID: 1.10.10.10.

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