INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
PhD-17 SHORT ABSTRACT OF THESIS

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Thesis Title:
Elucidating the role of human ZSCAN4 in the maintenance of induced pluripotent stem cells

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SHORT ABSTRACT

Induced pluripotent stem cells (iPSCs) hold transformative promise for regenerative medicine, disease modelling, and drug discovery. However, the translational utility of iPSCs remains constrained by genomic instability and accumulating genetic abnormalities encountered during reprogramming and prolonged culture. While the zinc finger transcription factor ZSCAN4 has been established as a critical regulator of telomere elongation and chromosomal integrity in murine models, its functional role in human cellular reprogramming and maintenance has remained uncharacterized. This thesis addresses this critical gap through a dual-pronged approach combining protein engineering, non-integrative iPSC derivation, and CRISPR-Cas9-mediated gene knockout.

First, to enable direct intracellular delivery without genomic insertion, a cell-permeant recombinant human ZSCAN4 fusion protein (comprising nuclear localization signal, TAT cell-penetrating peptide, and octahistidine tags) alongside domain-deleted non-functional controls (ΔDB and ΔSD) was expressed in *Escherichia coli* and purified under native conditions. Circular dichroism (CD) spectroscopy demonstrated that high salt concentration (450 mM NaCl) and storage supplementation with 20% glycerol were vital to maintain the native secondary structure, prevent self-aggregation, and achieve efficient nuclear translocation in human cells.

Second, an integration-free human iPSC line (IITGi001-B) was derived from adult dermal fibroblasts using episomal Y4 vectors. The established iPSC line displayed hallmark pluripotent morphology, robust expression of core nuclear (OCT4, SOX2, NANOG, REX1, ZSCAN4) and

surface (TRA-1-60, TRA-1-81, SSEA4) markers, an integration-free status, a normal 46,XX karyotype, and functional *in vitro* trilineage differentiation potential.

Finally, a dual-gRNA-based CRISPR-Cas9 strategy was optimized to generate target-specific *ZSCAN4* gene knockouts. Systematic evaluation revealed that chromatin accessibility significantly governs Cas9 editing efficiency, with global DNA demethylation via 5-Aza-2'-deoxycytidine and open chromatin configurations (iPSCs) markedly enhancing cleavage rates compared to primary fibroblasts. Clonal expansion yielded homozygous *ZSCAN4* knockout iPSC lines. Functional characterization of ZSCAN4-deficient iPSCs demonstrated divergent phenotypic outcomes: while certain clones retained short-term pluripotency, others underwent spontaneous multi-lineage collapse skewed heavily toward mesodermal fates, accompanied by a universal loss of NANOG expression. Telomere length measurement indicated alternative compensatory maintenance mechanisms in human iPSCs.

Taken together, this work establishes an optimized molecular toolbox for overexpression of ZSCAN4 via protein transduction and CRISPR-Cas9-based knockout of human *ZSCAN4*. This study provides fundamental insights into its necessity for human iPSC maintenance, pluripotency network stability, and cellular homeostasis holding immense potential for unravelling mechanistic role of human ZSCAN4 in reprogramming and iPSCs in future.