REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1.	Name and designation of ICMR- IF	Nishith Gupta, Professor and HoD, Department of Biological Science
2.	Address	Department of Biological Sciences, Birla Institute of Technology & Science - Pilani, Hyderabad, Telangana, India, 500078
3.	Frontline area of research in which training/research was carried out	CRISPR-mediated deletion of SIRT6 (Histone Deacetylase) in HEK293T Cells and Advanced Imaging
4.	Name & address of Professor and host institute	Dr. Sergio Grinstein, Department of Cell Biology, Hospital for the Sick Children (SickKids), Toronto, Canada
5.	Duration of fellowship with exact date	: 01-04-2023 to 30-06-2023, 90 days
6.	Highlights of work conducted	: CRISPR knockout and Imaging
	i) Technique/expertise acquired	:

(a) Establishing the mammalian cell culture (HEK293T)
(b) Construction and transfection of gene-specific Cas9/sgRNA plasmids
(c) Sorting and seeding of clonal transgenic cells by fluorescence-based cytometry
(d) Genomic screening of mutant clones and sequencing
(e) Advanced biosensor transfection and imaging

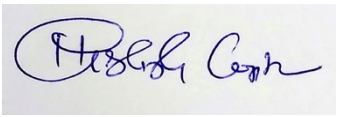
ii) Research results, including any papers, prepared/submitted for publication :

The online program Benchling predicted 6 different guide RNA, which were cloned in pX458 vector. These clones (sg1, sg2, sg3, sg4, sg5 and sg6) were divided into four combinations (sg1+sg3, sg2+sg3; sg4+sg6; sg5+sg6) and transfected into HEK293T cells. PCR screening of HEK293T gDNA revealed the efficacy of these combinations of sgRNA. The most efficient combination sg2+sg3 and sg4+sg6 were again transfected into HEK293T cells and positive clones

were selected using Fluorescence assisted cell sorting (FACS) for further screening and isolation of the hSIRT6 mutant lines. In addition, as series of biosensors were transfected in HEK293T cells to visualize different organelles.

iii) Proposed utilization of the experience in India :

Our home lab at Birla Institute of Technology and Science (Hyderabad Campus) focuses on investigating the host cell reprogramming and subversion by intracellular parasites of the protozoan phylum Apicomplexa. We have long been using *Toxoplasma gondii* as a model to decipher the network design principles of intracellular parasitism. It is considered as one of the most successful pathogens due to its exceptional ability to infect and reproduce in a wide range of host cells, offering an excellent opportunity to explore pathogen-host interactions in unprecedented details. In the current project, we have learned an advanced CRISPR method to disrupt host-cell genes, such as SIRT6, which can now be implemented to identify the host determinants of intracellular infection. For example, the SIRT6 knockout cell line generated during this work can be deployed to understand a role of SIRT6 protein on *T. gondii* infection and to test whether SIRT6 enzyme can be a potential host-directed therapeutic target. Likewise, biosensor-transfected cells can be deployed to determine the parasite-induced subversion of host-cell metabolism and signaling networks.



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