# Arpit Mishra, Ph.D.

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- An experienced hybrid biomedical scientist with a demonstrated history of working in a professional and academic setting for over 8 years.
- Currently, working on autoimmune disease associated non-coding variants for their possible target genes and underlying disease mechanism.
- Prioritized regulatory variants by combining GWAS hits and epigenomic features such as enhancer marks, ATAC-Seq peaks.
- Identified possible target genes/genomic regions for 5600 autoimmune disease related enhancer variants using capture Hi-C in Th1, Th2 and Th17 cells.
- Allelic activity for regulatory variants in disease relevant primary cell types such as Th1, Th2 and Th17 using allelic MPRA
- Validated MPRA hits and Capture Hi-C regulatory variant-target gene pairs using arrayed and pooled genome editing approaches in primary T cells and Jurkat T cells.
- Previously successfully completed projects in the areas ranging from protein engineering, Structure-functional studies, biophysics of small molecule-DNA interactions, protein purification and activity optimization for novel high fidelity CAS9 enzymes for genome editing, optimizing RNP approaches for genome editing, Off target CRISPR integration and on target analysis for Adenovirus mediated CRISPR edits.

## Key Skills:

Benchwork: Functional genomics using ChIP seq, RNA-Seq, Sc RNA-Seq (droplet and combinatorial indexing based approach), Hi-C, Capture HiC, MPRA, STARR-Seq, CRISPR screens, On target off target library preparation, library sequencing using illumina platform (Next-seq 550)

## Computational work:

NGS data analysis for RNA-Seq, Sc RNA-Seq, HI-C, Capture Hi-C, CRISPR off target on target analysis, and data visualization using Shell scripting, R and Python packages, version control with GIT, (https://github.com/bioarpit1)

# Professional Experience (Recent)

#### **University of Washington Seattle**

Senior Fellow, 2017 to Present

- Delineating target genes for autoimmune disease-associated regulatory variants using 3D genomics, MPRA and genome editingOptimization and implementation of CRISPR-CAS9-mediated gene editing in primary T helper cells.
- Generated NGS libraries for different omics assays such as ChIP-Seq, RNA-Seq, ScRNA-Seq (Split-pool), Hi-C, capture Hi-C, MPRA libraries, CRISPR off target NGS libraries.
- Library QC using bioanalyzer and NGS run using Next-Seq 550 platform.
- Demultiplexing data and NGS data analysis using appropriate pipeline such as HiCUP, Chicago (Hi-C, capture Hi-C), DE-Seq 2 (RNA-Seq), MACS and Deeptools (ChIP-Seq), Scanpy, ScPrep, Seurat and monocle3 (ScRNA-Seq), MPRAnalyze (MPRA, STARR-seq), CRISPRESSO2 (CRISPR NGS libraries).

Postdoctoral Associate, 2015-2017

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#### Genome editing:

- Heterologus Purification and characterization of naturally accuring high fedelity CAS9 from francisella novicida (FnCAS9)
- Optimized in vitro cleavage assay (IVC) for comparing SpCAS9 and FnCAS9
- Comparison of enzyme kinetics for cleavage using IVC and bio-physical methods.
- In vivo genome editing optimization for FnCAS9 in mouse embryonic stem cells.
- Off target comparison between FnCAS9 and Sp CAS9 using ChIP-seq.
- supplied FPLC Purified and activity tested SpCAS9 and FnCAS9 to many labs across the institute.

#### Synthetic biology:

• investigated the effects of various ionic liquids on DNA-small molecule interection using bio physical method such as CD spectroscopy,UV melting and isothermal titration calorimetry(ITC)

# **Educational Background**

**PhD in Bio-Technology** Institute of Genomics and integrative biology, New Delhi and Savitribai Phule Pune University, Pune India

Masters (MSc) in Microbiology-The Maharaja Sayajirao University of baroda, Vadodara, India

Bachelors (BSc) in Zoology - The Maharaja Sayajirao University of baroda, Vadodara, India

# **Research Publications**

- ND Jayavelu, A Jajodia, A Mishra, RD Hawkins Nature communications, 2020, Candidate silencer elements for the human and mouse genomes
- Acharya S, Mishra A, Paul D, Ansari AH, Azhar M, Kumar M, Rauthan R, Sharma N,Aich M, Sinha D, Sharma S, Jain S, Ray A, Jain S, Ramalingam S, Maiti S,Chakraborty D. Francisella novicida Cas9 interrogates genomic DNA with very highspecificity and can be used for mammalian genome editing. Proc Natl Acad Sci U SA. 2019 Oct 15;116(42):20959-20968. doi: 10.1073/pnas.1818461116. Epub 2019 Sep30. PubMed PMID: 31570623; PubMed Central PMCID: PMC6800334.(co-first author)
- Li C, **Mishra AS**, Gil S, Wang M, Georgakopoulou A, Papayannopoulou T, HawkinsRD, Lieber A. Targeted Integration and High-Level Transgene Expression in AAVS1Transgenic Mice after In Vivo HSC Transduction with HDAd5/35++ Vectors. Mol Ther.2019 Dec 4;27(12):2195-2212. doi: 10.1016/j.ymthe.2019.08.006. Epub 2019 Aug 19.PubMed PMID: 31494053; PubMed Central PMCID: PMC6904827.
- Mishra A, Hawkins RD. Three-dimensional genome architecture and emergingtechnologies: looping in disease. Genome Med. 2017 Sep 30;9(1):87. doi:10.1186/s13073-017-0477-2. Review. PubMed PMID: 28964259; PubMed Central PMCID:PMC5623062.
- Mishra A, Ekka MK, Maiti S. Influence of Ionic Liquids on Thermodynamics of Small Molecule-DNA Interaction: The Binding of Ethidium Bromide to Calf ThymusDNA. J Phys Chem B. 2016 Mar 17;120(10):2691-700. doi: 10.1021/acs.jpcb.5b11823.Epub 2016 Mar 4. PubMed PMID: 26907668.
- Kumar A, Kumar S, Kumar D, Mishra A, Dewangan RP, Shrivastava P, RamachandranS, Taneja B. The structure of Rv3717 reveals a novel amidase from Mycobacteriumtuberculosis. Acta Crystallogr D Biol Crystallogr. 2013 Dec;69(Pt 12):2543-54.doi: 10.1107/S0907444913026371. Epub 2013 Nov 19. PubMed PMID: 24311595; PubMedCentral PMCID: PMC3852659.
- Mishra A, Vij M, Kumar D, Taneja V, Mondal AK, Bothra A, Rao V, Ganguli M, Taneja B. Integration host factor of Mycobacterium tuberculosis, mIHF, compactsDNA by a bending mechanism. PLoS One. 2013 Jul 26;8(7):e69985.

# Awards & Fellowships

- Senior Research Fellowship Council for Scientific and Industrial Research, India
- Junior Research Fellowship Council for Scientific and Industrial Research, India
- Best poster/oral presentation at the EMBO global exchange lecture course on "structural and bio-physical methods for biological macromolecules in solution" held at CSIR-CCMB, hydrabad india 2012
- Selected in prestigious STEP(science teaching experience for post-doctoral).
- Designed and taught senior year 2 credit course on genome editing in disease treatment as a STEP fellow.

#### **Technical Proficiencies**

Molecular Biology	
Tools & Techniques	

PCR | Primer/probe design | quantitative real time PCR (qRT-PCR) | CRISPR-Cas9 | Gene editing analysis | DNA, RNA and protein isolation, plasmid library preparation | Gibson assembly of highthroughput MPRA and CROP-Seq libraries | In vitro transcription | Gene cloning | Site-directed mutagenesis | vector design using Benchling tool | ChIP-Seq libraries | Hi-C and Capture Hi-C libraries | ATAC-Seq libraries | RNA-Seq libraries | Single cell RNA-Seq libraries (combinatorial indexing based) | lentiviral packaging

#### **Protein Analysis**

Recombinant protein expression and purification in *E. coli*, animal cells | western blots | Immunoprecipitation | EMSA | FPLC | CD spectroscopy | ITC titrations

# Bioinformatics and NGS analysis

ChIP-Seq | ATAC-Seq |RNA-Seq | Sc RNA-Seq | Hi-C, Capture Hi-C |MPRA data analysis using R and Python packages from Bioconductor and Bioconda repositories | Input data preparation for variety of R/Python packages using Awk | Pandas and Numpy | reproductive pipeline environment stored using Jupyter notebooks and pushed them to git repository

## Mammalian Cell Culture Assays

Mammalian cell culture (representing adherent cell lines such as HEK 293-T, mESCs, Hela, and suspension cell lines such as K562, Jurkat, primary T cell culture ) | PBMC to CD4 isolation | T cell activation/proliferation | Genome-editing in naïve CD4 T Cells | electroporation using NEON system | transduction using lentiviral and adenoviral particles | Stable cell line generation using lentivirus | Flow cytometry | Cryopreservation