ISSUE - 2, DECEMBER 2016

NORTH EAST BIOLINE

NEWSLETTER OF GUWAHATI BIOTECH PARK

GUWAHATI BIOTECH PARK

Discovering through partnership

Department of Science and Technology, Government of Assam

Save Earth, Save Yourself

IndianOil is committed to conduct business with a strong sense of environment conservation. And success has been achieved at every stage. Simply because environment management is not just an action plan. It is a mission firmly rooted in our mind.

इंडियनऑयल

IndianOil

Indian Oil Corporation Limited
Guwahati Refinery

Review Meeting with
Sri Sarbananda Sonowal
Hon'ble Chief Minister of Assam

Launch of the 1st issue of NORTH EAST BIOLINE by SHRI KESHAB MAHANTA Hon'ble Minister, Science & Technology Department, Government of Assam, on 1st July 2016

Hon'ble Minister, Science & Technology Department, Government of Assam, Shri. Keshab Mahanta visited Guwahati Biotech Park currently located in the IIT Guwahati campus and also visited the site in Amingoan where the permanent campus of Guwahati Biotech Park will be established on 15th June, 2016. Hon'ble Minister visited the different facilities in the

Incubation Centre like ready-to-lease laboratory and equipment facilities. Shri. Vinod Seshan, IAS, CEO, Guwahati Biotech Park and the officials of the park appraised him on the activities and various services offered by the Incubation Centre. He also interacted with the start up Entrepreneurs associated with Guwahati Biotech Park. In a short interaction thereafter Shri. Seshan also briefed the Hon'ble Minister on the status and progress of the Incubation Centre and the establishment of the Park in the permanent site. Shri. Biswaranjan Samal, IAS, Commissioner & Secretary to the GoA, Science & Technology Department was also present during this visit. Hon'ble Minister assured full support of the Government for the establishment of Guwahati Biotech Park.

सत्यमेव जयते

Shri Vinod Seshan, IAS
CEO, Guwahati Biotech Park

FROM THE CEO'S DESK

So then…it's time for GBP's second newsletter. As usual I'm excited and hoping like the last time that this edition too would have something that would make the reader satisfied. There has been a delay in releasing this on time, but then, we never had any fixed time-line or a target date. We were only resolved to release the next issue at the earliest. However, in the future, we strongly intend to make this a regular quarterly affair.

The last 6 months have been a very testing time for GBP. Some entrepreneurs at GBP have quit and some have joined freshly. The technical review committee spent a considerable time reviewing the performance of all the projects at the incubation centre and the performance of GBP itself as a whole. Some projects have registered good progress while some have progressed without a direction, probably common in research sometimes. But, their sincerity towards research progress hasn't been a point of question, which gives a lot of credibility to GBP and its incubation centre.

Revenues have been coming in and progressing steadily. Expenditure has been steady like the last fiscal. We do intend to register significant incomes in the upcoming 4th quarter to close this critical fiscal on a high. Raising revenues though is not our primary job. Our primary job is to sustain research work and enable start-ups. Hence, some important plans have been laid out and execution has commenced. Financially, the Department of Biotechnology (DBT) has extended their support to the incubation centre project of GBP by another two years and that has been a major boost to GBP in the last 6 months. IIT, Guwahati too have allowed the lease of space arrangement to GBP for another 24 months which is another positive feather. The incubation centre project is now relatively comfortable and secure and looking forward to add more new-innovative projects in the days to come.

GBP recently signed a MoA with the Sualkuchi Tat Silpa Unnayan Samiti in Kamrup in order to promote silk testing. As part of the MoU, a technological grant has also been provided to the Samiti. The Detailed Project estimates (DPR) for the permanent building of the Technology Incubation centre have been prepared

and submitted to the Science & Technology Department, Government of Assam, while the Master plan for the entire area of the biotech park is now nearing completion. Some new instruments like the Fermenter (5L & 40 L), FPLC, Chromatography Unit etc. have been added to the list of research equipment available at GBP. Many senior researchers, many investment and start-up specialists have visited GBP in the last few months, primarily to help and to see the incubation centre grow further. Universities like Cotton College State University have signed agreements to promote biotech study and research at GBP and many of their students are seen visiting the facility frequently. Many other researchers and students, irrespective of the university they belong to, have also been using the infrastructure at the incubation centre to complete their project reports and to undergo internship and dissertation work. A National Talent Search competition had been announced and over 50 research proposals have been received from various places in the country. Our scientists have participated in various technical seminars as well as research forums and presented interesting ideas while also evaluating a few others.

So, overall, though not thoroughly sparkling, very satisfying progress has been made in the last five months. As we move forward towards the close of the financial year, we intend to do a few more interesting activities, other than just raising revenues. A biotech networking conclave, with technical and business sessions, is scheduled for January while a Children Science Festival is planned for February. GBP also plans to learn from organizations like NIPER (National Institute of Pharmaceutical Education and Research), which is coming up in North Guwahati and explore mutual areas of interest. There are discussions with industry consortiums like ABLE (Association of Biotech-Led Enterprises) and FINER (Federation of North Eastern Industries) to explore how GBP may benefit from their expertise to improve the bio-tech presence and growth in the North Eastern region and India overall. GBP is also exploring opportunities to set up a sericulture biotech incubation lab to understand and promote, pre-cocoon silk work, especially for MUGA, the famed Golden Fiber of Assam. We are also hoping that the Government of Assam would sanction the construction of the permanent building for the incubation centre.

So, this general report apart, I sincerely hope that this edition is living up to your expectations. Please give us your feedback as we intend to keep improving.

Vinod Seshan

CONTENTS

ARTICLES

7 CRISPR-CAS System and its Future Applications
Dr. Probodh Borah

9 Search for novel drug molecules and energy potential of biodiversity of N E India
Prof. M C Kalita

13 Biorefineries: Towards a Sustainable Future
Professor Kannan Pakshirajan

15 The Growing Impact Of Cancer Genomics And Personalized Cancer Care In India
Souvik Ghatak and Nachimuthu Senthil Kumar

18 Kaziranga National Park and Tiger Reserve: A Refuge of Myriad Wildlife and Storehouse of Biodiversity
Suvasish Das IFS

21 Microbes in Hot Water Springs
Priyanka Dutta, Juthika Saikia, Hari P Deka Baruah, Ratul Saikia

24 Actinobacteria: a yet persuasive source of Antimicrobial metabolites
Debajit Thakur

28 Horticulture in Assam Achievements & New Initiatives
Mowsam Hazarika

32 Exploration of potential microalgae in biodiesel production in present milieu
Madan K. Mandal and Neha Chaurasia

35 Silk Moth Biodiversity: A Paradise of NE India
Dr. Mahananda Chutia

38 Insect cellulolytic systems A stirring biofuel technology for future
Dr. Dip Jyoti Haloi

42 Traditional food additive and fermented rice beer of selective tribes of Assam
Jayashree Goswami and Avik Panda

46 Awareness of Electronic Cigarettes among the youth of the North-Eastern region of India
Yashmin Choudhury, Hirakjoti Roy and Sambuddha Das

50 Pharmacogenetics An Approach for Personalized Medicine
Dr.Ranadeep Gogoi, Madduri Madhuri, G. Naresh Kumar and Arundhati Gogoi

52 Turtles: The armoured warrior fighting for existence
Jayaditya Purakayastha

57 Non-Mulberry Silk Of Assam And Its Position In Market
Dr. Mamata Sarma

62 Sustainable Disease Management Practices In Tea: Need Of The Hours
Satya Ranjan Sarmah and Sosanka Protim Sandilya*

65 Orchids of Assam: the beauty of nature
Dr. Nabanita Bhattacharyya

68 Entrepreneur's Corner

70 Entrepreneurs Working at Guwahati Biotech Park Incubation Centre

71 Nobel Prizes 2016

73 Activities at GBP

76 Training Programs

77 Internship/Dissertation programs

78 Some Of The Equipment Facilities At Gbpic

81 List of Various Types Of Instruments/ Equipment Currently Available At Gbpic

CRISPR-CAS System
and its Future Applications

Dr. Probodh Borah
Professor & Head, Dept. of Animal Biotechnology
& Coordinator, State Biotech Hub (Assam)
College of Veterinary Science, AAU, Khanapara, Guwahati-22

Biomedical researchers have been constantly searching for a precise tool for targeted manipulation of the genome of an organism. The CRISPR-CAS System has recently been shown to be a promising tool in this direction which may revolutionize the area of genome editing which may have far-reaching implications in food industry, molecular medicine, crop productivity and livestock breeding in the near future.

The **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats (CRISPR) are the segments of DNA containing short repetitions of base sequences found in Bacteria and Achaea. Each repetition is followed by short segments of spacer sequences usually derived from previous exposures to a bacteriophage or mobile genetic element like plasmids. It is indeed recognized as a prokaryotic immune system that confers resistance to foreign genetic elements as that of bacteriophages and plasmids, and thereby provides a form of adaptive immunity to the host. CRISPR associated proteins (Cas) are the nucleases that use the CRISPR spacers to recognize and cut the exogenous genetic elements in a precise targeted manner similar to RNA interference in eukaryotes. CRISPR, in combination with Cas proteins, forms the CRISPR/Cas systems. CRISPRs have been identified in nearly 40% of the bacterial genomes and 90% of the archaea sequenced so far. The size of CRISPR repeats and spacers varies between 23 to 47 base pairs (bp) and 21 to 72 bp, respectively. CRISPR repeat sequences are generally highly conserved within a given CRISPR locus, but the sequences may show distinct variations across different microbial species. Most of these sequences are partially palindromic and has a tendency to form stable, highly conserved secondary structures like hairpins.

Six core *cas* genes (designated as *cas1 to cas6*) have been identified, including the universal markers of CRISPR/Cas systems, *cas1* and *cas2*. Besides these, subtype-specific genes also exist. On the basis of this variability in their genetic regulation, CRISPR-Cas systems are currently classified into two classes, class 1 and class 2. Class 1 systems use a complex of multiple Cas proteins to degrade exogenous sequences. On the other hand, Class 2 systems use a single large Cas protein for the

same purpose. Class 1 is divided into three types (I, III, and IV), while class 2 is divided into two types (II and V). The five system types are further divided into 16 subtypes.

There are a number of Cas enzymes, but so far the best known is Cas9. It was the first CRISPR nuclease protein discovered in *Streptococcus thermophilus* and *S. pyogenes*, followed by Cpf1 in *Francisella novicida*. CRISPR/C2c2 is the third system identified in the bacterium *Leptotrichia shahii*; however, it is a system that targets RNA rather than DNA. The first experimental evidence of CRISPER/CAS system being the adaptive immune mechanism in bacteria was demonstrated in 2007. It was observed that the spacer sequences of CRISPR cluster are acquired by *Streptococcus thermophilus* from invading bacteriophages. By deliberately manipulating the spacer sequences, researchers could alter the resistance of the bacteria to bacteriophages.

The Cas9 endonuclease has been recognized as a four-component system which includes two small RNA molecules. The Cas9 endonuclease was re-engineered in 2012 into a more manageable two-component system by fusing the two RNA molecules into a "single-guide RNA" (sgRNA). When mixed with Cas9, this engineered sgRNA could find and cut the DNA target specified by it. The artificial Cas9 system could be programmed successfully to target any sequence in DNA for cleavage by manipulating the sequence of the guide RNA. Since then the CRISPR-Cas9 systems have been used for genome editing in a wide range of organisms including yeasts, nematodes, plants, animals and human embryos. Using purified components of Cas9 (protein, RNA and DNA), it could be shown that it functions as an enzyme which uses RNA molecules to specifically target double-stranded DNA sequences for site-specific cleavage.

The target DNA site of Cas9 endonulcease is flanked by a 2- to 4- base-pair long conserved sequence called protospacer-adjacent motif (PAM), which is first recognized by the RNA-guided Cas9 endonuclease. After specific binding to the PAM sequence, Cas9 searches the flanking DNA sequences for complementarity to the sequence of the guide RNA. When complementarity between the target DNA strand and the first 12 base pairs of the guide RNA is detected, the DNA undergoes unwinding locally to form an R-loop. Then the catalytic domains of the Cas9 endonulcease, RuvC and HNH, cleave each of the DNA strands precisely to generate a blunt double-strand DNA break at a position three base pairs upstream of the 3′ edge of the PAM. Based on the recent understanding of the structural details and biochemistry of Cas9 endonuclease of bacteria and archaea, many variants with altered PAM-targeting or modified DNA affinity have been engineered that could experimentally be used to accomplish targeted cleavage of specific DNA sequences in a wide variety of organisms. The main advantage of CRISPR technologies is the ease with which CRISPR single-guide RNAs (sgRNAs), which are only around 80 nucleotides long, can be synthesized to have a precise specificity of Cas9 to different target sequences. CRISPR-based screens have also enabled identification of essential genes and drug targets.

Scientists today believe that CRISPR-CAS technology will in future have wide ranging applications in the food industry, biomedical sciences, crop and livestock production and many other areas of applied biology. It has so far been used successfully in microbial genotyping, manipulation of antimicrobial and bacteriophage resistance in bacteria as well as for targeted engineering of probiotic bacteria. CRISPR-Cas9 is expected to be useful for engineering industrially important bacteria, yeast and archaea to mediate increased production of biofuels, biochemicals and other biomaterials. In the biomedical field, CRISPR-Cas9 technology is expected to potentiate targeted genome editing of animals and human cells leading to efficient engineering of tissues and organs for xenotransplantation. It will also pave the way for correction of genetic disorders like thalassemia. Targeted delivery of Cas9 and sgRNA to cells or tissues may in future be used to derive therapeutic benefit against a wide spectrum of genetically controlled diseases.

For full version, please subscribe our newsletter by sending an email to

ceogbp@guwahatibiotechpark.com

Subscription charges :

- *For educational/research institute/corporate houses : ₹160.00 INR per copy*
- *For students : ₹ 40.00 INR per copy*