RMRCT UPDATE

Vol. 8, No.1, April 2011

BIANNUAL NEWSLETTER OF
REGIONAL MEDICAL RESEARCH CENTRE FOR TRIBALS
JABALPUR

Inside

Application of DNA Sequencing in Infectious disease

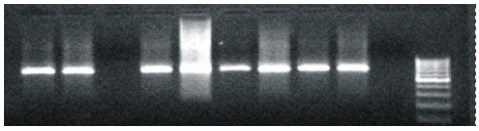
Praveen Kumar Bharti

Introduction

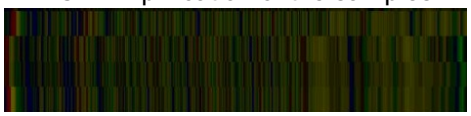
DNA sequencing is the process of determining the exact order of the nucleotide bases A, T, C and G in a segment of DNA. In fundamental nature, the DNA is used as a template to generate a set of fragments that differ in length from each other by a single base. The fragments are then separated by size, and the bases at the end are identified, recreating the original sequence of the DNA. The most commonly used method of DNA sequencing is the dideoxy or chain termination method developed by Frederick Sanger in 1977 (for which he won the Nobel Prize). The key to the method is the use of modified bases called dideoxy bases; when a piece of DNA is being replicated and a dideoxy base is incorporated into the new chain, it stops the replication reaction.

Sanger sequencing:

The DNA to be sequenced is provided in single-stranded form acts as a template. DNA synthesis requires a supply of the four nucleotides (the building blocks of DNA), the enzyme DNA polymerase and a primer (a short sequence annealed to the template which initiates the new DNA strand). The nucleotides added to the growing DNA strand are complementary to those in the template strand. Sequencing is achieved by including in each reaction a nucleotide analogue that Cannot be extended and thus acts as a chain terminator. The Sanger method is a mixed-mode process involving synthesis of a complementary DNA template using natural 2-deoxynucleotides (dNTPs) and termination of synthesis using 2,3-dideoxynucleotides (ddNTPs). Balanced appropriately, competition between synthesis and termination processes results in the generation of a set of nested fragments, which differ in nucleoside monophosphate units. The ratio of dNTP/ddNTP in the sequencing reaction determines the frequency of chain termination, and hence the distribution of lengths of terminated chains. The nucleotides included in the reactions contain different fluorescent labels allowing DNA strands terminating at each of the four bases to be identified. The reaction products are then separated by gel electrophoresis, according to size. As the DNA strands pass a specific point, the fluorescent signal is detected and the base identified. The whole process can be extensively automated.

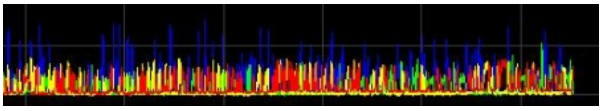


PCR Amplification of the samples

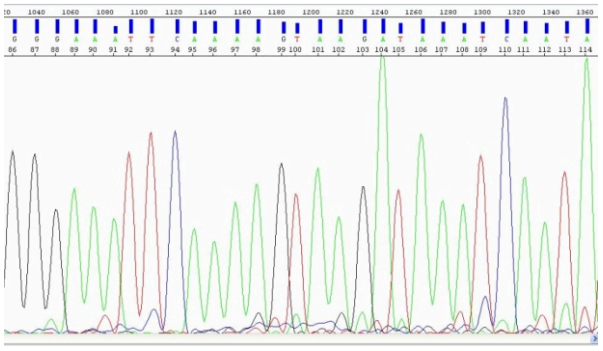


Electrophoresis data of DNA sequence

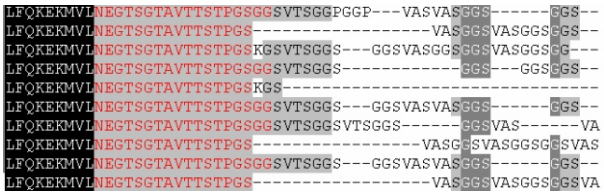
Regional Medical Research Centre for Tribals
(Indian Council of Medical Research)
Nagpur Road, Garha
Jabalpur - 482 003
Madhya Pradesh, India
Ph: 0761-2370800/818, Fax: 0761-2672835
Email: rmrctjabalpur@rediffmail.com
www.rmrct.org



DNA sequencing raw data



Electropherogramme of DNA Sequence



DNA Sequence alignments

Steps involved in the DNA Sequencing

DNA Sequencing applications

Knowledge of DNA sequence can help identify the genetic makeup of an organism and by using DNA sequence researchers have been able to associate vulnerability to certain diseases with specific genetic makeup. The DNA sequence of individuals can help to determine their vulnerabilities, as well as the type of treatment and gene therapy, that replaces defective genes. Today DNA sequencing Has been in greater need, with applications spanning diverse research sectors including comparative genomics and evolution, forensics, epidemiology, and applied medicine for diagnostics and therapeutics.

Identification of novel human pathogens;

The first novel pathogen to be identified by sequence-based methods was *Rochalimaea henselae*, the organism responsible for bacillary angiomatosis (BA). 16S rRNA gene sequence from tissue samples obtained from bacillary angiomatosis patients was partially amplified (Fredericks and Relman et al. 1990) and analysed. The result of this 16S sequence suggested a novel species most closely related to *Rochalimaea spp*. further confirmed the novelty of the isolated BA agent, *Rochalimaea henselae* (later moved into the genus Bartonella) (Regnery et al. 1992). The same strategy was soon applied to other potentially infectious diseases and led to the identification of *Ehrlichia chaffeensis*, a new species associated with tick bites that causes a febrile illness. Ehrlichiosis is clinically similar to Rocky Mountain spotted fever, another tick-borne disease caused by the intracellular parasite, *Rickettsia rickettsii*.

Novel viral pathogens

Larger numbers of viral pathogens have remained to be identified because of their growth conditions, they are far harder to determine and nucleic acid techniques based on sequence conservation was not available. Nonetheless there are several examples of successful application of culture-independent methods for the identification of novel viral pathogens. These include the use of DNA subtraction techniques to identify the Kaposi's sarcoma virus (Chang et al. 1994), expression screening of a cDNA library with patient serum to identify the hepatitis-C agent (Choo et al. 1989), and more recently hybridization-based screening to identify the SARS virus as a coronavirus (Ksiazek et al. 2003).

Role of Genomics in Modern Vaccine and Drug Design

DNA sequencing approaches vary depending on the nature of the pathogen and are based on several accepted principles. The key requirements of vaccines and therapeutics, including the need for targets to be (i) expressed and accessible to the host immune system, or to a therapeutic agent, during human disease; (ii) genetically conserved; (iii) important for survival or pathogenesis; and (iv) free of measurable homology or similarity to host factors. Although many of these approaches are described for focus on vaccine development, which involves screening of candidates for immunogenicity, they are largely applicable to drug development by altering the selection criteria used and screening candidates against compound libraries (Kaushik et al 2008). New sequencing technologies will also open up opportunities for monitoring pathogen vaccine escape by screening for evidence of immune selection in the genomes of pathogen populations before and after vaccine selection.

Malaria

The availability of Plasmodium genome sequence has the potential to reveal a high number of new drug targets and genes important to parasite biology and pathogenesis. However, only a third of the predicted genes code for proteins and the function of which can be identified. Due to the complexity of parasite life cycle conventional methods cannot be routinely applied for functional studies. Recent advances in genome-wide transcription profiling

through microarray technology proteome analysis of different developmental stages (Gardner et al 2002) and subcellular compartments as well as computational biology, will allow exploitation of genome sequence data in a systematic way leading to a more comprehensive survey of the Plasmodium life cycle.

The Regional Medical Research Centre for Tribals is equipped with 16 capillary DNA sequencer (ABI 3130xl model) instrument for the study of *Plasmodium falciparum and P. vivax vaccine and drug resistance markers*. We have carried out theSeveral studies on polymorphism in *P.candidate antigens falciparum* Merozoites surface proteins and *P. vivax* circumsporozoite proteins as these are potent vaccine candidate antigens.

Merozoite Surface Protein-1 (MSP-1):

MSP-1 is the most abundant protein present on the surface of merozoite and is involved in erythrocyte invasion. The gene is subdivided into 17 blocks according to the genetic diversity. The block 2 is highly polymorphic and has three types of allelic families K1, MAD 20 and RO33 types. Alleles in K1 and MAD 20 contain a specific three amino acid repeats that varies in numbers and arrangement, where as Ro33 lacks any repeats.

Block 2 region of msp1 gene was amplified and sequenced from 167 *P. falciparum* isolates from central India region. The allelic family specific distribution of isolates for K1, MAD20 and RO33 were 46%, 24% and 30% respectively. It was observed that there are a total of 36 different types of K1 alleles , 24 different MAD20 type of alleles and 9 different RO33 alleles in the parasite population.

Merozoite Surface Protein-2 (MSP-2):

The MSP-2 is one of the abundant merozoite surface protein that is a target for naturally acquired immunity. Antibodies against MSP-2 have been reported to inhibit in vitro merozoite invasion (Genton et al 2002). The major portion of msp-2 is polymorphic, only the N- and C-terminal domains are conserved. The central region of msp-2 belongs to either of two highly polymorphic repetitive domains identified as 3D7-type and FC27-type allelic families.

A total of 92 samples have been sequenced for the central repeat region of *P. falciparum* msp-2. Sequences for both 3D7 and FC27 type allelic families were present among the isolates Isolates with Fc27 type were more prevalent (73%) followed by 3D7 (27%) . In 3D7 type, 9 alleles were found while 15 alleles in FC27 family with having 32 amino acid repeats and 12 amino acids repeats.

Merozoite Surface Protein-3 (MSP-3):

MSP-3 is encoded by a single locus on chromosome no.10 of the parasite. It is divided in to two regions N-terminal polymorphic region and C-terminal conserved region. N-terminal region is composed of three blocks of alanine heptad repeats. Studies have suggested that the N-terminal domain of MSP-3 is more immunogenic than C-terminal domain.

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A total of 45 clinical isolates were sequenced for MSP-3 gene (polomorphic region covering all of the three heptad repeat) from the central India region. Sequence analysis showed that there were three types of MSP-3 alleles (K1, FC27, 3D7) in the parasite population. Isolates with K1 type were most prevalent followed by FC27 and 3D7.

***Plasmodium vivax* Circumsporozoite protein (CSP):**

The CSP is one of the best characterized and widely accepted leading malaria vaccine candidate and is the most abundant protein on sporozoite's surface with a molecular weight of 58-kDa. CS protein is a multifunctional molecule that plays a crucial role at various stage's of the malaria parasite. The sequences of central immunodominant repeat region of *P. vivax* CS protein are 3 variant VK247, Vk210 and *P. vivax* like, which differ in the amino acid composition of the central repetitive region of the gene. We studied the polymorphism in central immunodominant repeat region of *P. vivax* circumsporozoite protein to determine the prevalence of variant VK247, VK210 or P. vivax like. Sequences from 80 samples were analyzed and the sequences were found to be of VK210 type with the nona-peptide repeat sequence GDRA(A/D)GQPA. Indicating high prevalence of VK210 type allele among the isolates from the central India. Detailed analysis of the sequences revealed that the presence of 5 different variants of the VK 210 at the aminoacid level. Variant type I is prevalent (65 %) among the all 5 variant followed by type II (23.75%) and remaning 3 were low in number.

Drug resistance marker genes

Plasmodum falciparum chloroquine resistance transporter (Pfcrt):

The increased resistance to antimalarial drugs, especially chloroquine (CQ), shown by *Plasmodium falciparum* is one of the principal factors contributing to the worldwide increase in morbidity and mortality due to malaria. Certain point mutations in the *Plasmodium falciparum* chloroquine resistance transporter gene (PFCRT), present in the food vacuole membrane, have been implicated in rendering this parasite less susceptible to this drug (Fidock et al 2000). The pfcrt gene was partially sequenced from 112 clinical isolates to study the mutation at codons 72-76. These codons of *pfcrt*

have been shown to be associated with chloroquine resistance worldwide. Only 2.5% (3 isolates) were found to contain the wild-type genotype CVMNK whereas 95.5% (107 isolates) possessed double mutant SVMNT genotype and 2% (2 isolates) with triple mutant CVIET genotype at these codons of *pfcrt*.

Conclusion

Genomics will greatly aid the control of emerging infectious diseases. Genome-based approaches increased the efficiency for identification of vaccine and therapeutic targets.Genome-based approach may have increased the understanding of microbial pathogenesis and development of effective vaccines and therapeutics .

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ACKNOWLEDGEMENT

The author acknowledge Dr. Rajasubramaniam, Scientist-D at the centre for his suggestions and editing the manuscript.

Praveen Kumar Bharti- Scientist 'C'

Publications

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Conference/Workshop/ Meeting attended

Dr. Neeru Singh

1. Attended a meeting on molecular biology of malaria parasites at AIIMS Delhi on 24th & 25th November 2010.
2. Attended meeting with state health officers regarding malaria situation and other operational project discussion at Bhopal on 1st December 2010.
3. Attended and delivered a lecturer in a international meeting at ICGEB, Delhi on 2nd & 3rd December 2010.
4. Attended a meeting on Bivalent Kits at NVBDCP, Delhi on 17th & 18th February 2011.
5. Delivered a lecture on 'Transmission dynamics-recent trends in malaria' at Patna on 24th & 25th March 2011.
6. Attended expert committee meeting on estimation of malaria deaths at NIMR, Delhi on 30th March 2011.

Dr. R. S. Balgir

1. Attended and delivered Dr. Malti Sathe Oration at 51st annual conference of the Indian Society of Hematology and Transfusion Medicine (ISHTM) held during 18th - 21st November 2010, at Kolkata.
2. Attended and presented a research paper in 4th international congress on sickle cell disease: management and prevention of sickle cell disease in developing societies held during 22nd-27th November 2010, at Raipur.

Dr. V. G. Rao

1. Attended and presented a paper at 65th National Conference on Tuberculosis and Chest Diseases (NATCON 2010) held at Bengaluru during 10th - 12th January, 2011.

Dr. T. Chakma

1. Attended ICMR expert committee meetings on fluorosis at ICMR New Delhi on 31st July & 1st August 2010, 6th & 7th December 2010 and 13th December 2010 at ICMR New Delhi.
2. Attended ICMR expert committee meeting on Hyertension at ICMR New Delhi on 8th December 2010.
3. Attended and co-chaired a session during the dissemination workshop on IDSP non communicable disease risk factor survey, held at New Delhi on 15th December 2010 organized by NCD Division of ICMR.
4. Attended Institutional ethics committee meeting as a member on 17th February 2011of Institute of Biotechnology, JNKVV Jabalpur.

Dr. R. B. Gupta

1. Attended as Guest Speaker at International Congress on Sickle Cell Disease, held at Raipur, Chhattisgarh during 22nd - 27th November 2010.
2. Attended and presented a paper at International conference of Human Genetics held at Manipal during 14th - 16th February 2011.
3. Attended and presented a paper at National Conference on Strategies for Tribal Development of Central India, organized by Department of Anthropology, H.S. Gaur University Sagar.

Dr. Suneel Qamra

1. Attended and presented a paper at 9th National Conference on Growth, Development and Behaviourial Pediatrics (IAP Chapter) at CMC, Ludhiana in October 2010.
2. Attended and delivered an invited talk at Seminar on The Globalization, Urbanization and Migration: Anthropological Dimensions of Trends and Impacts in January 2011.
3. Attended and delivered an invited talk at National Seminar on Tribal Health and Nutrition: Status, Challenges and Possibilities at Bhopal in March 2011.

Dr. K. B. Saha

1. Attended and presented a paper at the 32nd annual conference of Indian Association for the Studies in Population (IASP) held at Utkal University, Bhubaneswar, Odisha during 28th - 30th November 2010.

Dr. Jyothi Bhat

1. Attended and presented a paper at 65th National Conference on TB & Chest Diseases held at NIMANS, Bangalore during 10th - 12th January 2011.

Dr. R. K. Sharma

1. Attended and presented a paper at the 32nd annual conference of Indian Association for the Studies in Population (IASP) held at Utkal University,Bhubaneswar, Odisha during 28th - 30th November 2010.

2. Attended a technical workshop for Technical/Nodal Officers on National Knowledge Network (NKN) at Delhi University, Delhi on 25th March 2011.

Dr. P. K. Bharti

1. Attended and delivered a lecture at International meeting at ICGEB, New Delhi on 2nd - 3rd December 2010.

Dr. Rajiv Yadav

1. Attended and presented a paper at 65th National Conference on TB & Chest Diseases held at NIMANS, Bangalore during 10th - 12th January 2011

Mr. S. B. Singh, Mr. Vijay Gadge, Mr. Lalit Sahare

1. Attended a workshop on awareness of HIV/AIDS among the employees working in ICMR & its Institutes, held at RMRIMS, Patna on 28th & 29th January 2011

Workshops/Training/Meetings conducted

A Biosafety training workshop for the students of the centre was also conducted on 22nd January, 2011. Eighteen students were trained on various aspects of biosafety

A training workshop on vector borne diseases for Medical Officers of various districts of Madhya Pradesh was organized during 25th - 27th March 2011 jointly with NIMR FS Jabalpur and Directorate of Health Services.

Training for laboratory technicians of ICTC and Primary health centers was conducted in four batches during 11th - 15th October, 22nd - 26th November, 29th November - 3rd December 2010 and 7th - 11th March 2011 in association with M.P. State AIDS Control Society at RMRCT. Fifty three technicians participated in the training which involved theory as well as hands on training for HIV testing.

Team of experts from Central and State TB division evaluated and accredited the Centre's TB Laboratory as Intermediate Reference laboratory on 25th October, 2010.

A workshop on Integrated Fluorosis Mitigation was organized on 13th November, 2010. The Workshop was organized jointly by RMRCT Jabalpur and sponsored by UNICEF Bhopal.

Project review committee (PRC) meeting was held on 20th & 21st December, 2010. The progress of ongoing projects and recently completed research projects were discussed in the meeting.

Awards

- Dr. R. S. Balgir received Dr. Malti Sathe Oration Award 2010 from the Indian Society of Haematology and Transfusion Medicine (ISHTM) during 18th -21st November 2010, Kolkata.
- Dr. K. B. Saha received Prof. S. N. Singh Memorial Award for the paper on qualitative aspects of reproductive health dimensions of Sahariya Tribal youth of Gwalior district of Madhya Pradesh at IASP conference held at Utkal University, Bhubaneswar, Odissa during 28th - 30th November 2010.

Joining/Promotions/Transfer

- Dr. S. Raja Subramanian joined as Scientist 'D' on 01.11.2010.
- Smt. Flomina Lakra was promoted to Assistant on 05.10.2010.
- Mr. S. K. Yadav was promoted to Personal Secretary on 30.12.2010.
- Mr. S. C. Muduli was promoted to Personal Assistant on 31.12.2010.
- Mr. S. K. Vinodia transferred to Virus Research Unit, Kolkatta on 21.03.2011.

Events

National vigilance week was observed during 25th October-1st November 2010 to engender awareness among the employees for sincere and dedicated service free from corruption. Oath was taken by all employees of the centre not to indulge in corrupt practices.

23rd Scientific advisory committee (SAC) meeting held on 5thJanuary, 2011. The ongoing projects and new research proposals were discussed in the meeting. The list of new equipments was also discussed in the meeting.

The centre celebrated 62nd Republic Day on 26th January, 2011 with great enthusiasm. Dr. Neeru Singh, Director of the centre hoisted the National Flag at the main building of the centre.

The centre celebrated its Foundation Day on 1st March, 2011. Dr. Ved Prakesh Mishra, Vice-Chancellor, Datta Meghe Institute of Medical Sciences (Deemed University), Wardha delivered the foundation day lecture.

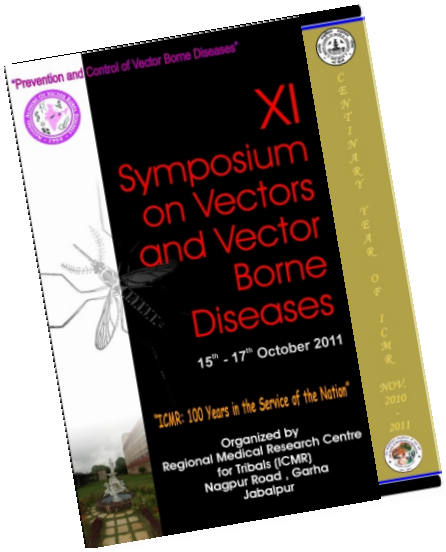
ICMR Centenary Year Celebration

Indian Council of Medical Research (ICMR) is celebrating its 100th year in existence. During the year various activities are being carried out as a part of its centenary year celebration. The centre also organized various activities including health camps under ICMR centenary year celebration.

Public awareness camps were organized by Department of Information and Broadcasting, Government of India on 29th and 30th January, 2011 at Sihora town of Jabalpur district and at Dindori on 27th and 28th February, 2011 under the banner of Bharat Nirman Jan Suchna Abhiyan. Scientists and staff of the Centre actively participated in the camps. Exhibition stall was established at the camps. Rural folk were communicated about the preventive measures against malaria, tuberculosis, scabies, malnutrition and genetically transmitted diseases (Sickle cell anemia, Thallessemia and G-6 PD deficiency).

Visits

Dr.Y. D. Sharma, Prof. and Head, Dept. Of Biotechnology, AIIMS, New Delhi visited the centre on 5th January, 2011.



Centre will organize XI Symposium on Vectors & Vector Borne Diseases on 15-17 October 2011, Details are available in the web site: www.rmrct.org