www.ibc-2012.org

Santosa Hotel, Senggigi Beach
Mataram, Lombok Island
Province of West Nusa Tenggara, Indonesia

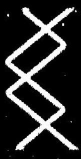
July 4th-7th 2012

The 5th Indonesia Biotechnology Conference
An International Forum

PROGRAM BOOK & ABSTRACTS

"Green Industrial Innovation through Biotechnology"

organized by:



Indonesian Biotechnology Consortium

The 5th Indonesia Biotechnology Conference

List of Participant

	Name Participation Number Institution Institution Address Email
1	Abinubli Tariswafi Mawarid Participant Universitas Indonesia Jl. Marga Indah 1 No. 2 Buah Batu, Bandung archaeathemighty@gmail.com
2	Abu Amar P 42 BIOTEEN-ITI Jalan Raya Pupspiptek Serpong 15320 Tangerang Selatan aamar3884biugm@yahoo.com
3	Abu Nasar Siddique O 22 Institute of Biotechnology and Genetic Engineering (IBGE) Khyber PukhtoonKhwa, Peshawar, Pakistan abunasarfarabi@yahoo.com
4	Ade Andriani O 15 Research Centre for Biotechnology Jalan Raya Bogor Km 46, Bogor 16911 andriani_chemie@yahoo.com
5	Ade Mulyana Participant PT. East West Seed Indonesia Desa Benteng Kecamatan Campaka Purwakarta - Jawa Barat 41181 Telp.: +62 264 201871 ; Fax : +62 264 201875 ade.mulyana@ewsi.co.id
6	Adi Santoso P 65 Indonesian Institute of science Jl. Raya Jakarta Bogor Km 46 Bogor, Cibinong adi_santoso1@yahoo.com

130	Meilinah Hidayat Participant Maranatha Christian University Jalan Terusan Prof Sutami 19 Bandung mellahidayat@yahoo.com
131	Melisa Intan Barliana O 12 Faculty of Pharmacy Padjadjaran University Jl. Raya Jatinangor KM 21, Jatinangor-Sumedang melisabarliana@yahoo.com
132	Melva Louisa O 17 Faculty of Medicine, University of Indonesia Jl Salemba Raya No 6 , Jakarta Pusat melva.louisa@gmail.com
133	Merisa Bestari Faiz O 20 Chemical Engineering Department, Faculty of Engineering, University of Indonesia, Depok 16424, Indonesia merisabestarifaiz@hotmail.com
134	Miftahudin O 8 Departemen Biologi, FMIPA IPB, Gedung Fakultas Peternakan Lt.5 Wing 1 Kampus IPB Darmaga, Bogor miftahudinm@yahoo.com
135	Miranti Nurindah Sari Participant Research Center for Biotechnology- Indonesian Institute of Sciences; Jln. Hm. Ashary Rt01/Rw08 No. 11, Cibinong 16911. Bogor- Jawa Barat; mia1chby@yahoo.com
136	Misri Gozan O 55 Program Studi Teknologi Bioproses - Departemen Teknik Kimia, Fakultas Teknik, Universitas Indonesia, Kampus UI Depok 16424 mrgozan@gmail.com

137	Mohamad Amin P 27 Biology Department, State University of Malang Jalan Semarang 5 Malang rizalamin@yahoo.com
138	Mohamad Taufik Fauzi O 33 The University of Mataram Jl. Majapahit, Mataram, Lombok offiek@gmail.com
139	Mohammad Sadikin O 13 Departemen Biokimia dan Biologi Molekuler Fakultas Kedokteran UI Jl. Salemba Raya No 6, JAKARTA PUSAT, 10430 biokimiafkui@gmail.com
140	Morina Riauwaty S WD 6 Fishery and Marine Science Faculty, Riau University. Pekanbaru; Kampus Bina Widya km 12,5 Simpang Panam, Pekanbaru, 28293 morinariauwaty@yahoo.co.id
141	Muhamad Sahlan O 56 Department of Chemical Engineering, University of Indonesia sahlan@che.ui.ac.id
142	Muhammad Ali P 74 Mataram University; Jl. Majapahit 62 Mataram maliqh@yahoo.com
143	Muhammad I Anwaruddin Participant Universitas Indonesia Jl. Manukan Rejo Iii 1c/10 Surabaya ian.uud@gmail.com

P 74

High-throughput Construction of Human Monoclonal Antibody Libraries

Muhammad Ali

Mataram University
Jl. Majapahit 62 Mataram, Indonesia
maliqh@yahoo.com

Monoclonal antibodies are among the first approved therapeutic molecules and most successful reagents in molecular targeting therapy dedicated by modern biotechnology. However, the use of rodent antibodies has been hampered by the inherent immunogenicity of these biopharmaceutical molecules, which can lead to rapid clearance, reduced efficacy, and allergic reaction risk in human patients. Since the immune system in human is capable of creating wide variety of antibodies, the use of patient's blood as repertoires of antibody library will be helpful to generate antibody with useful specificities. Here, we present a method for rapid construction of human antibody libraries from large numbers of single plasma cells of patient's. Since the method entirely bypasses most of the biological processes and allow robotic automation, the burden of a novel antibody discovery will dramatically reduced. Therefore, we considered this method is convenient and applicable for high-throughput generation and the screening of monoclonal antibodies with high-affinity and specificity.

Keywords: *biopharmaceutical molecules,monoclonal, antibodies*

P 75

Production of Xyloologosaccharides Using Free and Immobilized Xylanolytic Enzymes

Budiasih Wahyuntari

Badan Pengkajian dan Penerapan Teknologi
LAPTIAB, PUSPIPTEK Serpong
build 610, Tangerang Selatan, Indonesia
budiasih.wahyuntari@bppt.go.id

Xylooligosaccharides (XOS) a linier polymer of 1,4-beta linked xylose, has been reported having beneficial effects such as reducing blood cholesterol and suppressing blood glucose level. XOS can be produced by enzymatic degradation of xylan. The aim of this experiment was to produce XOS enzymatically using free and immobilized enzymes. Xylan extracted from sugarcane baggase was used as a raw material. The enzymes used were Arabinofuranosidase (ABFA) and endoxylanase produced by *Bacillus subtilis* AQ1. In this experiment, ABFA and endoxylanase were immobilized in nitrocellulose membrane disc. Immobilization efficiency of ABFA was only 8.56% (membrane #1A) and 35.19% (membrane #2A) whilst the immobilization efficiency of endoxylanase was 63.58% (membrane #1E) and 69.29% (membrane #2E). The highest reducing sugar of XOS produced using free enzyme was 5 mg/ml only after incubating xylan with ABFA for 1 hour followed by endoxylanase for 5 hours. Xylan hydrolysis using immobilized ABFA (membrane 1A) for 1 hour followed by immobilized endoxylanase for 3 hours (membrane #1E) and immobilized ABFA (membrane 2A) for 1 hour followed by immobilized endoxylanase for 4 hours (membrane #2E) resulted in 6.69 mg/ml and 12.22mg/ml respectively. The immobilized enzyme on nitrocellulose membrane disc could only be applied once since the performance of the used membrane was decreased drastically as compare to the fresh immobilized membrane.

Keywords: *Bacillus subtilis* AQ1, xylooligosaccharides, *immobilized*

This is to certify

Muhamad Ali

as Presenter

The 5th Indonesia Biotechnology Conference

An International Forum

"Green Industrial Innovation through Biotechnology"

July 4th - 7th 2012

Mataram, Lombok Island, Indonesia

Indonesian Biotechnology Consortium

Prof. Dr. Ir. Bambang Prasetya
President

Organizing Committee

Dr. Ing. Misri Gozan
Chairman

KONSORSIUM BIOTEKNOLOGI INDONESIA

Indonesian Biotechnology Consortium

This is to certify

Muhamad Ali

as Presenter

The 5th Indonesia Biotechnology Conference

An International Forum

"Green Industrial Innovation through Biotechnology"

July 4th - 7th 2012

Mataram, Lombok Island, Indonesia

Indonesian Biotechnology Consortium

Prof. Dr. Ir. Bambang Prasetya
President

Organizing Committee

Dr. Ing. Misri Gozan
Chairman

High-throughput Construction of Monoclonal Antibody Libraries using Ingesc-Sicrex Technology

Muhamad Ali[1,2] and Yunita Sabrina[3]

[1]Laboratory of MicroBiotechnology, Faculty of Animal Sciences, Mataram University, Indonesia

[2]Laboatory of Molecular and Cellular Biology, Graduate School of Science and Engineering, Toyama University, Toyama, Japan

[3]Faculty of Medical, Mataram University, Indonesia

ABSTRACT

Monoclonal antibodies are among the most successful diagnostic and therapeutic molecules in molecular targeting therapy dedicated by cutting-edge biotechnology. However, the use of conventional rodent antibodies has been hampered by the inherent immunogenicity of these biopharmaceutical molecules, which lead to rapid clearance, reduced efficacy, and allergic reaction risk in human patients. To generate human monoclonal antibodies, single-cell RT-PCR and cell-free expression (Sicrex) technology has been established (Ali *et al.*, 2006) and successfully used for anti-hepatitis B monoclonal antibodies generation (Sabrina *et al.*, 2010). Here, we have developed Immunoglobulin Gene-Selective Cassette and single-cell RT-PCR linked cell-free expression (Ingesc-Sicrex) technology to improve the sensitivity of our previous Sicrex technology. In this method, immunoglobulin cassette of Lc and Hc were designed to have all necessary elements for rapid cloning of 3'-random nucleotide-tailed immunoglobulin variable gene fragment; which allows the insertion of PCR-amplified immunoglobulin variable gene selectively, even in the presence of nonspecifically amplified PCR products. The resulting constructs can be used directly as template for monoclonal antibodies generation using cell-free expression system. Since this method entirely bypasses most of the biological processes and allow robotic automation, the burden of a novel antibody discovery will be dramatically reduced. This method undoubtedly is unsurpassed high-throughput method for generation and screening of monoclonal antibodies libraries with high-affinity and specificity.

1. Introduction

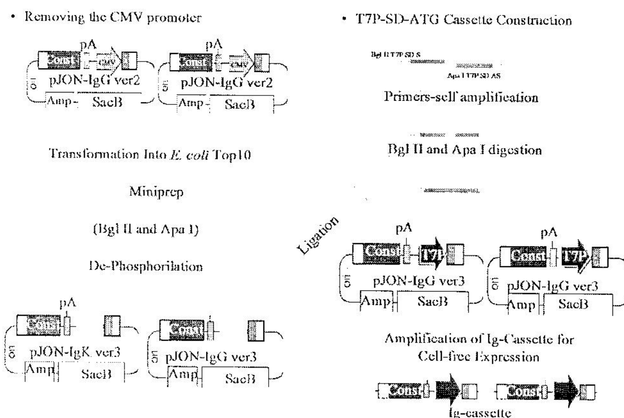
Monoclonal antibodies are magic bullet for molecular therapy. However, hybridoma technology is only established method to produce the molecule. Since the hybridoma technology has several drawback, some effort to find out other technology are conducted. single-cell RT-PCR and cell-free expression (Sicrex) technology has been established (Ali *et al.*, 2006) and successfully used for anti-hepatitis B monoclonal antibodies generation (Sabrina *et al.*, 2010). However, nonspecific PCR-amplified V gene were obtained in the method. Moreover, the PCR-amplified V gene fragments must be purified to remove nonspecifically amplified DNA fragments. In addition, both V gene fragments and nonspecifically amplified PCR products are joint to the cassette. This tedious process sometimes results in a low yield of Ig-expression. Therefore, more effective technologies are necessary for screening and generation of recombinant monoclonal antibodies. In this research, we designed Immunoglobulin Gene-Selective Cassette to overcome these problems. Then, cell-free expression was conducted to confirmed the obtained gene.

2. Ingesc-Sicrex Technology

Immunoglobulin Gene-Selective Cassette linked Single-Cell RT-PCR and cell-free Expression system (Ingesc-Sicrex) Technology consist of three steps i.e.: construction of immunoglobulin gene-selective cassette, single cell RT-PCR, and cell-free expression system. The immunoglobulin gene-selected cassette was designed to has long overlapping regions of immunoglobulin gene-specific homology on its ends and contains all essential element for expression of the antibody-encoding gene, including promoter, Ribosome binding site, start codon, tag, and stop codon. Variable gene fragment was tailed with random nucleotides on its 3 ' end using terminal deoxynucleotidyl transferase to avoid ... ... Then, joint PCR was conducted for joining of the tailed V gene fragment to the Ig-cassette. The homology sequences facilitate assembled PCR-amplified V gene fragment selectively into a linear Ig-expression construct, even in the presence of nonspecifically amplified DNA fragments.

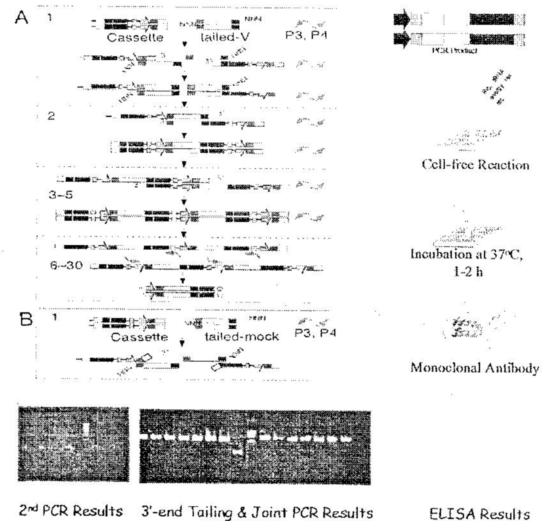
3. Immunoglobulin Gene Selective Cassette Construction

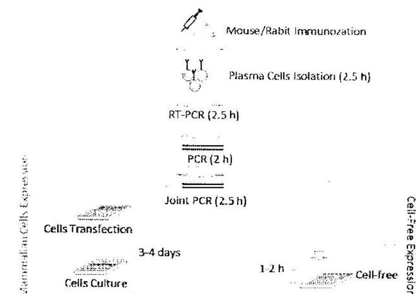
The DNA cassette for the mouse VL (IgK-cassette) and VH (IgH-cassette) were generated from pJON-mIgK ver.2 and pJON-mIgG ver.2 plasmid, respectively. CMV promoter in these plasmids were replaced by T7 promoter fragment containing T7 promoter, Shine Dhalgarno sequence, start, and stop codon for expression in E. coli cell-free expression system. Construction of these cassette were shown in the below illustration.



4. 3'-end Tailing, Joint PCR Ingesc Sicrex Technology Application for anti-GFP Monoclonal Antibody Production

PCR product was mixed with TdT solution (1 x PrimeStar GC buffer containing 0,2 mM of each dNTPs and 2 units of TdT) and incubated at 37°C for 30 min for 3'-end random nucleotide tailing. TdT was heat inactivated at 94°C for 5 min. The random nucleotide-tailed PCR product were mixed with PCR mixtures containing IgK- or IgG-cassette, 0.2 mM of each dNTPs, 1.25 U of PrimeStar DNA Polymerase and 400 nM of mini CMVf1 (F & R) primers in 1x PrimeStar GC buffer. Joint PCR was performed for 5 cycles at 95°C for 30 s, 70°C for 4 min followed by 30 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min. The 3'-end Tailing and Joint PCR were illustrated below. The DNA fragments results were then used as template for expression in cell-free expression system.





2nd PCR Results 3'-end Tailing & Joint PCR Results ELISA Results

5. Next Work

Mouse/Rabit Immunozation

Plasma Cells Isolation (2.5 h)

RT-PCR (2.5 h)

PCR (2 h)

Joint PCR (2.5 h)

Cells Transfection

3-4 days

Cells Culture

1-2 h

Cell-free

Mammalian Cells Expression

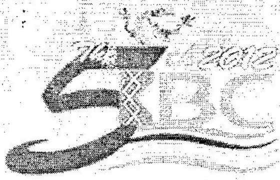
Cell-Free Expression

6. Merits of Ingesc-Sicrex Technology

- Reaction is finished within several hours.
- Synthesis of toxic proteins is possible.
- Incorporation of unnatural amino acids is possible.
- Control of the reaction conditions is easy.
- Use of PCR amplified DNA directly as the template.
- Miniaturization of reaction scale is easy.

This poster was presented at International Biotechnology Conference, Mataram, July 4-7, 2012

Lamp I : 6



The 5th Indonesia Biotechnology Conference
An International Forum
Lombok, July 4 – 7th 2012

Green Industrial Innovation through Biotechnology

Depok,May 3rd, 2012

ACCEPTANCE LETTER

Dear
Muhammad Ali
Mataram University

We are delighted to inform you that your abstract :

Title : High-throughput Construction of Human Monoclonal Antibody Libraries

Reg. number :ISB58B

Field :Health & Medical Biotechnology

is accepted as poster presentation.To ensure your paper to be included in the proceeding, please kindly submit your full paper to our website www.ibc-2012.org (menu : Full Paper Registration) before May 18th, 2012.

We expect you to pay your registration fee before June 30, 2012 to the following account :

c/q : Konsorium Bioteknologi Indonesia
133-00-0521555-3, SWIFT CODE BMRIIDJA
Bank Mandiri Kantor Kas Cibinong, Bogor, Indonesia

For your information, the earlybird will due on May 11, 2012. Please send your receipt by email toibcv11.secr@gmail.com and cn275@yahoo.comafterward.

Please consult our website on regular basis for updated information.

Sincerely,

Dr.-Ing. Ir. Misri Gozan, M.Tech
Chairman of IBC V 2012