TISSUE SELECTION AND OPTIMIZATION OF DNA EXTRACTION FOR THE CONSTRUCTION OF GENOMIC LIBRARY OF WHITE DRAGON FRUIT (*Hylocereus undatus*)

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ABSTRACT

Genomic library of *Hylocereus undatus* was successfully constructed using Lambda Bacteriophage FIX II vector kit (Stratagene) which resulted in titer of 4 x 10^4 pfu/ml. One week old leaflets from seed germination were used for DNA extraction. This method ensures sustainable supply of material for DNA extraction and the yield of the DNA was 975 µg/g of leaf material. The white colour pellet indicates that it was free from contaminants which were supported by a spectrophotometer reading (A_{260} nm/A_{280} nm ratio of 1.92) and agarose gel electrophoresis showing intact genomic DNA.

The genomic DNA was digested with *Bam*HI to produce fragments within 9-23kb due to the limitation of the vector cloning capacity. *BamHI* cleaves at the recognition sequence GGATCC which generates an overhang 5'–GATC-3' molecule. This overhang molecule has to be treated further by partial fill-in reaction according to the Klenow Fill-In Kit (Stratagene). This kit uses Klenow polymerase with the fill-in buffer, dATP and dGTP, because the Lambda FIX II vector (Stratagene) had been digested with *XhoI* enzyme and partially filled in with dCTP and dTTP leaving 3'-CT-5' overhangs. This will generate compatible end and prevent it from self ligate. In the partial digestion, 0.30U was identified as the best concentration for the digestion of 1µg of genomic DNA.

The digested DNA insert was ligated to the vector at 4°C and subsequently packaged to form virus particles using Gigapack III XL Packaging Extract, an in vitro packaging extract which is specially designed for use in constructing genomic library. It preferentially size

selects for extra large insert and thus eliminates the step to size select the genomic insert and avoid the losses of genomic samples through purification.

The system operates based on spi (sensitive to P2 inhibition) selection. Lambda phages containing active *red* and *gam* genes on the stuffer fragment are unable to grow on host strains that contain P2 phage lysogens such as XL1-Blue MRA (P2). When the stuffer fragment is replaced by an insert, the recombinant Lambda FIX II vector becomes Red–/Gam–, and the phage is able to grow on the P2 lysogenic strain. Hence, in the Lambda FIX II system, only recombinant phages are allowed to grow.

Similar strategy to construct the *Oryza sativa L. Var. Pokkali* and identify their gene of interest with the use of probes. However, the genomic information reported here was obtained using PCR method. The insert DNA was flanked by T7 and T3 promoter (5'-AAT ACG ACT CAC TAT AG-3' and 5'- ATT AAC CCT CAC TAA AG-3'). Therefore, polymerase chain reaction (PCR) can be used to amplify the insert DNA and send to sequencing centre to process.

ABSTRAK

Perpustakaan genom bagi *Hylocereus undatus* telah berjaya dibina dengan menggunakan bakteriofag Lambda FIX II kit vektor (Stratagene) yang menghasilkan titer 4 x 10^4 pfu/ml. Daun muda berumur satu minggu dari percambahan biji telah digunakan untuk ekstrasi DNA. Kaedah ini menjamin bekalan daun berterusan untuk ekstrasi DNA. Pelet yang berwarna putih menunjukkan bahawa ia adalah bebas dari kontaminasi. Fakta ini disokong oleh bacaaan spektrophotometer yang memberi nilai nisbah A_{260} nm/A_{280} nm 1.92 dan elektroforesis gel agarosa menunjukkan DNA genomic utuh.

DNA genomik dicerna dengan BamHI untuk menghasilkan serpihan dalam 9-23kb disebabkan oleh kapasiti kloning dari vektor. BamHI akan membelah di urutan pengakuan GGATCC yang menghasilkan molekul overhang 5'-GATC-3' yang harus diperlakukan secara separa lebih banyak mengisi reaksi mengikut kaedah pengisian Klenow (Stratagene) yang menggunakan polimerase Klenow dengan mengisi dalam buffer, dATP dan dGTP kerana Lambda FIX II vektor (Stratagene) telah dicerna dengan enzim XhoI dan sebahagian diisi dengan dCTP dna dTTP yang meninggalkan 3’-CT-5’ overhangs. Ini adalah untuk menghasilkan produk akhir yang serasi dan mencegah ligate sesama sendiri. Dalam pencernaan separa, 0.30U dikenalpasti sebagai tumpuan terbaik untuk pencernaan 1µg DNA genomik.

DNA sisipan yang telah dicerna dimasukkan ke dalam vektor di bawah suhu 4°C. Hasil pergabungan tersebut kemudiannya dibungkus dengan Extract Gigapack III-XL untuk menghasilkan zarah virus. Extract Gigapck III-XL ialah extract prabungkusan khas yang

digunakan dalam pembinaan perpustakaan genomik. Pemilihan DNA sisipan yang lebih besar adalah lebih cenderung menyebabkan pengabaian langkah untuk pemilihan DNA sisipan besar serta pengelakkan kerugian sampel genomik melalui purifikasi.

Sistem ini beroperasi berdasarkan spi (sensitif terhadap inhibisi P2) pemilihan. Fag lambda yang mengandungi gen aktif merah dan gam pada fragmen stuffer tidak dapat tumbuh pada jenis host yang mengandungi lysogens fag P2 seperti XL1-Blue MRA (P2). Ketika serpihan stuffer digantikan dengan penyelitan, Lambda vektor rekombinan FIX II menjadi Red-/Gam-, dan fag mampu tumbuh pada strain lisogenik P2. Oleh kerana itu, dalam sistem FIX Lambda II, hanya fag rekombinan yang dibenarkan untuk tumbuh.

Strategi yang sama untuk membina Oryza sativa L. Var. Pokkali dan menggunakan probe untuk mengenalpasti gen minat mereka. Namun demikian, maklumat genomik yang dilaporkan di sini diperolehi dengan menggunakan kaedah PCR. DNA insert diapit oleh promotor T7 dan T3 (5'-AAT ACG ACT CAC TAT AG-3 'dan 5'-ATT AAC CCT TAA CAC AG-3'). Justeru, reaksi berantai polimerase (PCR) boleh digunakan untuk menguatkan dan menyelitkan DNA sequencing dihantar ke pusat untuk diproses.

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λ	Lambda
µg	Microgram
µl	Microliters
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BSA	Bovine serum albumin
CHEF	Contour-clamped homogeneous electric field
CTAB	Cetylmethylammonium bromide
cDNA	Complementary deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanine triphosphate
dTTP	Deoxythymine triphosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
HGP	Human genome project
HCl	Hydrochloric acid
Kb	Kilobase
mRNA	Messenger ribonucleic acid
Mb	Megabase
ml	Mililiters
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate

NaCl	Sodium chloride
Pfu	Plaques forming units
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidones
TE	Tris- ethylenediaminetetraacetic acid
UV	Ultraviolet
YAC	Yeast artificial chromosome

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