Teknik Analisis Biologi Molekuler dan Aplikasinya

Kuliah Pengantar TABM

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Biotechnology and Recombinant DNA

There's a long train of DNA rounding the bend...
Oh-oh! There's a stray bit of genetic material on the tracks!
Look out!... There's going to be a collision!...
The late Dolly, the most famous sheep in the world, produced by cloning techniques.
Biotechnology

- The use of microorganisms, cells, or cell components to make a product
  - Foods
  - Vaccines
  - Antibiotics
  - Vitamins
  - Biodegradation
- Selective breeding
Useful Properties of DNA

• DNA sequences specify gene locations and protein amino acid sequence
• Restriction endonucleases cut at specific nucleotides; size of pieces gives us information about DNA sequence
• Nucleotides hydrogen bond with complementary nucleotides
• DNA hybridization allows recognition of specific genes
In-Situ Hybridization

• Target nucleic acid found in intact cells.
• Provides information about presence of specific DNA targets and distribution in tissues.
• Probes must be small enough to reach nucleic acid.
• Radioactive or fluorescent tags used.
• Requires experience.
Polymerase Chain Reaction (PCR) Amplifies DNA

- Primers specify what DNA is copied
Useful Properties of DNA

- The complementary strands of DNA can be separated and re-associated by heating and cooling
- One strand of DNA specifies the sequence of the other strand
  - mRNA specifies the sequence of the gene (DNA)
PCR Amplifies DNA

• Diagnosis
• Epidemiology
• Genetic engineering
DNA Sequencing

- Primer / 3’ Template of unknown sequence
- DNA polymerase, four dNTPs, four ddNTPs
- Denature

Dye-labeled segments of DNA, copied from template with unknown sequence

DNA migration

Dye-labeled segments are applied to a capillary gel and subjected to electrophoresis.

Detector

Laser beam

Computer-generated result after bands migrate past detector

Fatchiyah, Biologi Dept, UB, 2/20/2012
DNA Sequencing

Primer

5' OH
3' CTAAGCTCGACT

Template

+ dCTP, dGTP, dATP, dTTP

+ ddATP

- GATTTCGAGCTGddA
- GATTTCGddA
- GddA

+ ddCTP

- GATTTCGAGddC
- GATTddC

+ ddGTP

- GATTTCGAGCtdddG
- GATTcdG
- ddG

+ ddTTP

- GATTTCGAGCddT
- GATCddT
- GAAddT

Autoradiogram of electrophoresis gel

Sequence of complementary strand

(c)
Manipulating an organism’s genome to:
  - alter microbes, plants, and animals for our benefit
  - correct genetic defects in humans
Recombinant DNA

- Combining DNA from two different organisms
Useful Properties of DNA

- Restriction endonucleases can cut DNA at specific sites, leaving sticky ends for insertion of new DNA.
Selection of Altered Cells

• Antibiotic resistance gene used to identify recombinant cells
Genetically Modified Organisms

- Herbicide-resistant plants
- Bt cotton/corn (toxin gene from *Bacillus thuringiensis* that kills insects)
- Flavr-Savr tomatoes
- Golden rice (beta-carotene)
- Plant-based vaccines
A transgenic tomato plant
Transgenic Animals

- “Knock-out” and transgenic mice: used to study immune system and genetic diseases
- Pigs: blood clotting Factor VIII, organs for transplantation
- Others: Human IL-2 (cancer), albumin (blood volume), growth hormone, tPA (dissolves clots)
• Two adult female *Anopheles gambiae* mosquitoes (ventral view).

• The one on the left is a mutant.

• Scientists are attempting to produce strains of these *mutant* mosquitoes, which are *unable to transmit malaria to humans*, in hopes that they will replace the malaria carriers.
Genetically Engineering Humans

- Bone marrow supplies stem cells
- Successful replacement of gene for enzyme needed for lymphocyte development
Difficulties in Genetically Engineering Humans

- Inserting gene in correct cells
- Inserting gene so it is expressed correctly
  - Orientation
  - Regulation
- Controlling virus vector
- Ethical issues
Fluorescent In-Situ Hybridization FISH
Metaphase FISH

DNA Probe:

Green = Internal control

Red = DiGeorge region

Dual-color detection of DiGeorge/Velo-Cardio-Facial/CATCH 22/Shprintzen Syndrome which is caused by a microdeletion on chromosome 22. The green signal is an internal control. The red signal is located at the DiGeorge region at 22q11.2.
FISH of Telomeres

Metaphase Spread Stained by Q-FISH

Cell 2001, 107, 67-77
Aneuploid Screen Test Using Interphase FISH

Normal male

Female fetus with trisomy-21

Probe:

Green 1 = Ch 13
Red 1 = Ch 21
Aqua = Ch 18
Green 2 = Ch X
Red 2 = Ch Y
Restriction Fragment Length Polymorphism (RFLP)

Chromosomal arrangement

Allele 1

\[ \begin{align*}
& a_1 \quad b_1 \quad b_2 \quad a_2 \quad a_3 \\
\end{align*} \]

Allele 2

\[ \begin{align*}
& a_1 \quad b_1 \quad b_2 \\
\end{align*} \]

Mutation at site \( a_2 \) prevents cleavage

Hybridization banding pattern from individual with both allele 1 and allele 2

Enzyme A

Enzyme B

Restriction endonuclease A

Restriction endonuclease B

Probed single-copy region
DNA Fingerprinting: Forensics

(a) Cells from different samples are processed to isolate their DNA. The DNA samples are exposed to endonucleases which snip them at specific sites into a series of different fragments.

(b) Electrophoresing the fragments of DNA sorts them by size (larger fragments near the wells, smaller ones farther from the wells). The relative positions of these fragments are made visible by labeled DNA probes designed to attach to specific DNA markers. The developed gel appears with a series of visible bands that correspond to the sample's pattern.

(c) An actual DNA fingerprint used in a rape trial. Control lanes with known markers are in lanes 1, 5, 8, and 9. The second lane contains a sample of DNA from the victim's blood. Evidence samples 1 and 2 (lanes 3 and 4) contain semen samples taken from the victim. Suspects 1 and 2 (lanes 6 and 7) were tested. Can you tell by comparing evidence and suspect lanes which individual committed the rape?
DNA Fingerprinting: Epidemiology

- Comparison of DNA from
  - patients
  - food
Southern Hybridization

Chromosomal DNA (e.g., Suspect 1)

Cleave with restriction endonucleases.

DNA fragments

Separate fragments by agarose gel electrophoresis (unlabeled).

DNA markers

Suspect 1

Support 2

DNA markers

Suspect 2

Evidence

DNA markers

DNA markers

Denature DNA, and transfer to nylon membrane.

Incubate with probe, then wash.

Radiolabeled DNA probe

Expose x-ray film to membrane.
RFLP as Genetic Marker

The diagram illustrates the inheritance of alleles from grandparents to children and their corresponding fragment lengths.

- **Grandparents:** 2, 1, 2, 2
- **Parent:** 1, 2
- **Children:**
  - Alleles: 2, 2, 2, 3, 2, 2, 2, 2, 2, 3, 1, 2, 1, 2
  - Fragment lengths: 10 kb, 7.7 kb, 6.5 kb

The diagram shows how the alleles are passed down through generations and how these alleles correspond to specific fragment lengths in the gel.
RFLP and Southern Blot Analysis

<table>
<thead>
<tr>
<th>Allele</th>
<th>$Bgl\ II/\zeta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha\alpha$</td>
<td>12.6 or 5.2</td>
</tr>
<tr>
<td>$\alpha^{3.7}$</td>
<td>16</td>
</tr>
<tr>
<td>$\alpha^{4.2}$</td>
<td>10–11.3</td>
</tr>
<tr>
<td>$\alpha^{20.5}$</td>
<td>8.0</td>
</tr>
<tr>
<td>MED</td>
<td>13.9</td>
</tr>
<tr>
<td>SEA</td>
<td>10.5</td>
</tr>
<tr>
<td>SA</td>
<td>7.0</td>
</tr>
<tr>
<td>BRIT</td>
<td>7.5</td>
</tr>
<tr>
<td>THAI</td>
<td>None</td>
</tr>
<tr>
<td>FIL</td>
<td>None</td>
</tr>
</tbody>
</table>
Nutrigenomics

• The study of how different foods can interact with particular genes to increase the risk of diseases such as type 2 diabetes, obesity, heart disease and some cancers

• Goal: Use of personalized diets to prevent or delay the onset of disease and optimize and maintain human health

http://nutrigenomics.ucdavis.edu/pressarticles.htm
Nutrigenomics and nutrigenetics: two sides of a coin

- For personalized nutrition:
  - effects of diet on body-metabolism
  - influence of genotype on nutritionally related diseases

Mutch, FASEB 2005
Nutrigenomics

Screening for new functional food bioactives in vitro

Safety testing
Efficacy testing
Biomarker development
Genotyping

Animal

Human

Quality and authenticity of foods
Food processing
Production of food ingredients

Fatchiyah, Biologi Dept., UB, 2/2012
Health effects of food compounds mostly are related to specific interactions on molecular level.

<table>
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<th>DNA</th>
<th>RNA</th>
<th>protein</th>
<th>metabolite</th>
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<td>gene regulation, SNP’s transcriptional control, histone interaction</td>
<td>translational control, processing, stability, transport of mRNA</td>
<td>receptor interaction gene control, signal transduction, enzyme regulation inhibition, modification transport regulation channel or pump interaction</td>
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Health effects of food compounds mostly are related to specific interactions on molecular level.

- **DNA**: gene regulation, SNP’s transcriptional control, histone interaction
- **RNA**: translational control, processing, stability, transport of mRNA
- **Protein**: receptor interaction, gene control, signal transduction, enzyme regulation, inhibition, modification, transport regulation, channel or pump interaction
- **Metabolite**: multitude of functions

**Functional genomics**

- **Sequencing, genotyping**
- **Transcriptomics ("genomics")**
- **Proteomics**
- **Metabolomics**

Food compound
Interrelated strategies for research on nutraceuticals and functional foods
Fate and activities of nutrients in the cell

May be involved in gene regulation or cell-signaling

Kaput J, Physiol Genomics 2004
Thank you