Mutagenesis for Studying Gene Function

Spring, 2007
Guanyi Wang, Ph.D.
POST103B
guanyi@hawaii.edu

http://www.soest.hawaii.edu/marinefungi/OCN403webpage.htm
Overview of Last Lecture

• DNA microarray hybridization
• DNA Fabrication
  – In situ synthesis using masks
    • Procedure
    • Advantage and drawback
  – Spotted microarray
    • Contact printing
      – Advantage and drawback
    • Noncontact ink jet printing
      – Advantage and drawback.
• Application and analysis
  – Fish pathogens
  – Whole-genome wide microarray of marine archarea
Why mutagenesis is necessary for functional genomics?

- Understanding the function of a particular gene within the context of a complex biological system is a multisteps process;
- Not all predicted genes using bioinformatics tools are correct;
- Some gene can not be assigned using a putative based on homology;
- Functional assignment by similarity comparison ban be misleading or incorrect;
- Gene function information from DNA microarray and proteomics is inferred. Mutagenesis can give direct information;
General Features of Transposable Elements

1. Exist in all organisms.

2. Normal and ubiquitous components of prokaryote and eukaryote genomes.

3. Transposable elements cause genetics changes and make important contributions to the evolution of genomes:
   - Insert into genes
   - Insert into regulatory sequences; modify gene expression
   - Produce chromosomal mutations.

Classification

1. Variation in size, structure, insertion specificity, and transposition mechanisms.

2. Two major groups in bacteria:
   - insertion sequence (IS)
   - Transposons (Tn)

3. Three types of transposition:
   1) Conservative transposition: the element is not replicated;
   2) Non Conservative transposition: the element is replicated;
   3) Retrotransposition: Mediated by RNA.
Insertion sequence

Insertion sequence (IS) elements:

1. Simplest type of transposable element found in bacterial chromosomes and plasmids.

2. Encode only genes for mobilization and insertion.

3. Range in size from 768 bp to 5 kb.

4. IS1 first identified in E. coli’s lactose operon is 768 bp long and is present with 4-19 copies in the E. coli chromosome.

5. Ends of all known IS elements show inverted terminal repeats
5. Most bacteria contain several IS elements and often more than one copy on the chromosome.

6. During insertion a small portion of the target sequence is duplicated.

Slide courtesy of Dr. N. Keyhani
Table 20-1. Prokaryotic Insertion Elements

<table>
<thead>
<tr>
<th>Insertion sequence</th>
<th>Normal occurrence In <em>E. coli</em></th>
<th>(bp)</th>
<th>Inverted repeat* (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS1</td>
<td>5–8 copies on chromosome</td>
<td>768</td>
<td>18/23</td>
</tr>
<tr>
<td>IS2</td>
<td>5 copies on chromosome; 1 on F</td>
<td>1327</td>
<td>32/41</td>
</tr>
<tr>
<td>IS3</td>
<td>5 copies on chromosome; 2 on F</td>
<td>1400</td>
<td>32/38</td>
</tr>
<tr>
<td>IS4</td>
<td>1 or 2 copies on chromosome</td>
<td>1400</td>
<td>16/18</td>
</tr>
<tr>
<td>IS5</td>
<td>Unknown</td>
<td>1250</td>
<td>Short</td>
</tr>
<tr>
<td>γ-δ</td>
<td>1 or more copies on chromosome; 1 on F</td>
<td>5700</td>
<td>35</td>
</tr>
<tr>
<td>(TN1000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSC101 segment</td>
<td>On plasmid</td>
<td>200</td>
<td>30/36</td>
</tr>
</tbody>
</table>

* Fraction of base pairs; for example, 18 of 23 bp, and so forth.

Transposons (Tn)

1. Composite transposable elements
2. Consists of two IS flanking a gene
3. Can mobilize the DNA between the IS
4. The IR sequences together with their contained genes have been collectively called a transposon (Tn). (Transposons are longer than IS elements)

Two different transposons having different IR regions and carrying different drug-resistance genes. (a) Tn9 has a short IR region, because the two IS1 elements are in the same orientation and each element has a short inverted repeat. (b) Tn10 has a large IR region because the two IS10 components have opposite orientations, and the entire IS10 sequence constitutes the inverted repeat.
1. Transposons are DNA elements that “hop” or transpose to different places on the DNA.

2. Transposition is the movement of the transposon.

3. This process requires special protein factors particularly to cut and ligate the DNA \(\rightarrow\) transposase

4. NO homology is required between the transposon and the target sequence.
The insertion of a transposon (Tn) into a plasmid. RTF (resistance-transfer functions) represents the resistance-transfer functional genes of the plasmid. Tn includes both the IS elements and the drug-resistance genes.
Table 13-3. Some Prokaryotic Transposons

<table>
<thead>
<tr>
<th>Transposon</th>
<th>Marker</th>
<th>Length (bp)</th>
<th>Inverted repeat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn1</td>
<td>Ampicillin</td>
<td>4,957</td>
<td>38</td>
</tr>
<tr>
<td>Tn2</td>
<td>Ampicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tn3</td>
<td>Ampicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tn4</td>
<td>Ampicillin, streptomycin, sulfanilamide</td>
<td>20,500</td>
<td>Short</td>
</tr>
<tr>
<td>Tn5</td>
<td>Kanamycin</td>
<td>5,400</td>
<td>1500</td>
</tr>
<tr>
<td>Tn6</td>
<td>Kanamycin</td>
<td>4,200</td>
<td>Not detectable with electron microscopy</td>
</tr>
<tr>
<td>Tn7</td>
<td>Trimethoprim, streptomycin</td>
<td>14,000</td>
<td>Not detectable with electron microscopy</td>
</tr>
<tr>
<td>Tn9</td>
<td>Chloramphenicol</td>
<td>2,638</td>
<td>18/23</td>
</tr>
<tr>
<td>Tn10</td>
<td>Tetracycline</td>
<td>9,300</td>
<td>1400</td>
</tr>
</tbody>
</table>

Genes encoding resistance to the antibiotics chloramphenicol (cmR), kanamycin (kanR), streptomycin (smR), sulfonamide (suR), and ampicillin (ampR) and to mercury (HgR) are clustered on the resistance-determinant segment, which consists of multiple transposable elements; inverted repeat termini are designated by arrows pointing outward from the element. A transposon encoding resistance to tetracycline (tetR) is on the resistance-transfer segment. Transposon Tn3 is within Tn4. Each transposon can be transferred independently.

Movement of Transposons

A transposon can jump from a plasmid to a bacterial chromosome or from one plasmid to another plasmid.
Specificity and Effect of Transposon

- Insertion does not rely on the similarity between donor and recipient DNA sites.
- Tn can insert into multiple sites within chromosome.
- Most elements display different degrees of selectivity, stringency, and patterns of target site selection. Some of them preferentially insert into certain sites, others avoid specific regions on the chromosome.
- Insertion elements can affect the transcription of genes located in the vicinity of the insertion.
Transposons as Tools for Mutagenesis

In vivo Mutagenesis

Suicide vector (conditional replicon)-conditional for its replication

Plasmid backbone requirements used for insertional mutagenesis:

1. Conditional for replication to allow selection for integration into the chromosome. This can be achieved by using a plasmid that is able to replicate autonomously only in permissive hosts or by using conditional replicons (e.g. a plasmid that is temperature sensitive for replication).

2. The plasmid must carry a selectable marker (e.g. antibiotic resistance).

3. Ideally, the plasmid should be transferable to a variety of other bacteria. Plasmids that can be transferred by conjugation are preferable for situations in which other means of transfer such as transformation or electroporation are not efficient.

4. It is convenient if the plasmid has an array of unique cloning sites.

it needs pi protein for replication
In vivo Mutagenesis- allelic exchange

FIG. 1. (A) Double-selection strategy. Positive selection of allelic exchange mutants in a two-step selection strategy, using a counterselectable marker. (B) Single-selection strategy. CSM, counterselectable marker; SM, selectable marker; WT, wild-type allele; mut, mutated allele.

Infection and immunity, 1998, p.4011-4017
In vivo Global Transposon Mutagenesis

Conceptually, there are two ways to identify essential genes or regions of the bacterial chromosome: (1) the ‘negative’ approach, which identifies many regions that are not essential and presumes that everything else is essential; and (2) the ‘positive’ approach, which identifies genes that are essential by generating a conditional mutation and showing that it has a lethal phenotype.

Global transposon mutagenesis. The whole bacterial chromosome is the target for transposon mutagenesis. A large number of viable insertions are analyzed by sequencing.
The positive approach to identify essential genes: identify directly genes that are essential. Transposition with a transposon containing an outward-facing inducible promoter at one edge in the presence of the inducer results in many possible transposon insertions. The horizontal arrows signify possible insertion locations on the bacterial chromosome. Screening identifies insertions that disrupt the promoter region of an essential gene (red arrow). The strain generated by such an insertion is dependent on the inducer for viability. The insertional junction is sequenced, allowing the identification of the downstream essential gene.
Abundance of Tn3, Tn21, and Tn501 Transposase (tnpA) Sequences in Bacterial Community DNA from Marine Environments

CECILIA DAHLBERG and MALTE HERMANSSON*
Department of General and Marine Microbiology, Göteborg University,
S-413 90 Göteborg, Sweden

Received 9 February 1995/Accepted 12 May 1995

The occurrence of the tnpA genes of the transposons Tn3, Tn21, and Tn501 was assessed in total bacterial community DNA isolated from different marine environments. The PCR technique was employed, together with most probable number statistics, to determine the abundance of the target tnpA genes. All three genes could be detected, and the Tn21 tnpA sequences predominated in all samples. The smallest amount of total community DNA in which the Tn21 tnpA sequence could be detected was 0.037 ng, and on the basis of our results, we estimated that this sequence was present in 1 of 1,000 to 10,000 bacteria. Hybridization of the PCR products with the respective tnpA probes verified the Tn21 and Tn501 tnpA sequences but only some of the Tn3 tnpA amplification products. The distribution and dissemination of transposons in natural bacterial communities are discussed.

![Diagram](image)

**FIG. 1.** Locations of primers and probes within the tnpA genes of Tn3, Tn21, and Tn501. The lengths of the PCR products and probes are indicated above the lines. Restriction enzymes that were used to cut out the probes are shown. The two successive rounds (I and II) of amplification in nested PCR are indicated.

![Gels](image)

**FIG. 2.** Typical products obtained in nested PCR on total community DNA with primers designed for Tn3 (A), Tn21 (B), and Tn501 tnpA (C). Each gel contained 2% agarose. Lane 1, community DNA; lane 2, positive control; lane 3, 1-kb ladder.

**TABLE 4.** PCR detection limit of tnpA genes in total community DNA verified by hybridization

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tn3 tnpA (ng)</th>
<th>Tn21 tnpA (ng)</th>
<th>Tn501 tnpA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>URB1-bulk</td>
<td>0.037 (19/29)</td>
<td>0.037 (21/29)</td>
<td>3.0 (1/4)</td>
</tr>
<tr>
<td>URB1-A/W</td>
<td>0.11 (4/11)</td>
<td>0.037 (21/29)</td>
<td>0.1 (5/12)</td>
</tr>
<tr>
<td>RUR1-bulk</td>
<td>0.11 (4/11)</td>
<td>0.037 (21/29)</td>
<td>3.0 (1/3)</td>
</tr>
<tr>
<td>RUR1-A/W</td>
<td>-</td>
<td>0.037 (14/22)</td>
<td>3.0 (1/3)</td>
</tr>
</tbody>
</table>

* Smallest amount of total community DNA for which PCR products were verified by hybridization. The number of hybridization-positive PCR products of the total number in threefold community DNA dilutions in the range between 3 ng and the detection limit shown in this table is given in parentheses.

* None of the PCR products of the expected size could be verified by hybridization in the range of 3 to 0.0014 ng of community DNA.
Overview

• Transposable elements
  – Classification and types of transposition
  – Insertion sequences
    • General features
  – Transposons
    • General features
    • Movement of transposon
    • Specificity and effect of transposon insertion

• Transposon as tools for mutagenesis
  – In vivo mutagenesis
    • Allelic exchange.
    • Global transposon mutagenesis.
  – Transposons in marine microbes.