Application of genetically engineered microorganisms (GEM) in soil to improve plant growth and bioremediation and accidental release of GEM into soil have caused a concern about the potential possibility of transferring and spreading introduced novel genes in soil.

Understanding the nature of DNA interactions with soil components is a basis for studying microbial ecology by analysis of DNA composition in soil.

DNA fate in soil has an implication in evolution of microbial cells.

DNA transfer between cells living in soil.

Interaction of extracellular DNA with organic and inorganic substances in soil.
DNA Transfer in Soil

- Conjugation
  DNA in one cell enters another cell through cell contact

- Transduction
  DNA enters a cell with bacteriophage

- Transformation
  DNA enters a cell directly
PROCEDURE FOR DETERMINATION OF DNA COMPOSITION IN SOIL

1. Extraction
2. Purification
3. Amplification
4. Quantification and Characterization
DNA EXTRACTION FROM SOIL

- Direct extraction: *in situ* lysis of cells and then separation of DNA from soil and cell debris.

- Indirect extraction: separation of cells from soil, lysis of cells and then separation of DNA from cell debris

- Advantages and disadvantages: compared to indirect extraction, direct extraction is less time consuming, recovers higher amount of total DNA and thus is more representative for microbial community, but can shear DNA and contaminate DNA with more humic materials.

- Methods for lysis of cells: detergents (SDS, sarkosyl), lysozyme, heating, shaking, grinding, and sonication.
### Efficiency of Recovery of the Bacterial Fraction from Soil

<table>
<thead>
<tr>
<th>Sample or Parameter</th>
<th>Number of Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct Counts</td>
</tr>
<tr>
<td>SN1</td>
<td>$1.7 \times 10^6$</td>
</tr>
<tr>
<td>SN2</td>
<td>$5.0 \times 10^8$</td>
</tr>
<tr>
<td>SN3</td>
<td>$1.1 \times 10^9$</td>
</tr>
<tr>
<td>Total recovered (SN1 + SN2 + SN3)</td>
<td>$3.3 \times 10^9$</td>
</tr>
<tr>
<td>Number remaining (P3)</td>
<td>$6.3 \times 10^9$</td>
</tr>
<tr>
<td>% Recovered $\left( \frac{SN1 + SN2 + SN3}{SN1 + SN2 + SN3 + P3} \right)$</td>
<td>34.9</td>
</tr>
</tbody>
</table>
REMOVAL OF HUMIC MATERIALS

- Binding humic materials
  Polyvinylpolypyrrolidone (PVPP), polyvinylpyrrolidone (PVP), hexadecylmethyl-ammonium bromide (CTAB), hexametaphosphate, hydroxylapatite, and ion exchange resin

- Precipitation of DNA
  Iso-amylalcohol and polyethyleneglycol (PEG)

- Separation of DNA from humic materials
  Agarose gel electrophoresis and CsCl equilibrium density gradient centrifugation.
Nanometers

Absorbance

Soil

Pure Culture

Soil before PVPP

0.00

0.35

220.0 320.0

III

I

II

I

I

Soil

Pure Culture

Soil before PVPP

Absorbance

Nanometers

220.0

320.0
QUANTIFICATION AND CHARACTERIZATION

Quantification

Spectrophotometer to measure absorbance at 260 nm and densitometer to measure fluorescence and radioactivity.

Characterization

Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), agarose gel electrophoresis, denaturing and temperature gradient gel electrophoresis (DGGE and TGGE), probing, and sequencing
## DNA ADSORPTION ON SAND

### Kinetics of Plasmid DNA Adsorption

<table>
<thead>
<tr>
<th>Incubation Time (minutes)</th>
<th>DNA Adsorbed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCC</td>
</tr>
<tr>
<td>1</td>
<td>92.9</td>
</tr>
<tr>
<td>2.5</td>
<td>97.5</td>
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<tr>
<td>10</td>
<td>98.7</td>
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<tr>
<td>60</td>
<td>99.1</td>
</tr>
</tbody>
</table>
pH EFFECT ON DNA ADSORPTION

DNA Adsorbed (%) vs pH

- 5 mM Mg
- 0.5 mM Mg

DNA Adsorption decreases with increasing pH for both 0.5 mM and 5 mM Mg concentrations.
EFFECT OF ADSORPTION ON DNA DEGRADATION

Acid Precipitabable Fraction (%)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10000</th>
<th>100000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>80</td>
<td>50</td>
<td>40</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

DNA in solution

Sand absorbed DNA

DNA in solution

Dnase I (ng ml⁻¹)

Acid Precipitable Fraction (%) vs. Dnase I (ng ml⁻¹)
EFFECT OF ADSORPTION ON DNA TRANSFORMATION

The graph shows the effect of Dnase on the number and frequency of transformants. The x-axis represents the Dnase concentration (μg), while the y-axis shows the Log10 number of transformants and the Log10 transformation frequency. Two lines are plotted: one for the number of transformants (black dots) and one for the frequency (green squares). The graph indicates a decrease in transformants and an increase in frequency as the Dnase concentration increases.
DNA PROBES

1. Use DNA probes to identify a specific component of the microbial community.

2. To follow the fate of a specific piece of DNA such as an introduced gene into plants or microorganisms
CRITERIA OF METHODOLOGY TO PROBE SOIL DNA

1. Rapid enough to permit the processing of a large number or samples
2. Isolated DNA must be of sufficient purity and size for precise characterization
3. Probe must be sensitive and specific (PCR reaction)