Differential Display of Eukaryotic Messenger RNA by Means of the Polymerase Chain Reaction

[Science, 257:967-971]
Presented by Liang
Abbreviation

- **DD**: differential display
- **mRNA**: messenger RNA
- **SS/DS**: single strand / double strands
- **RT**: reverse transcription
- **PCR**: polymerase chain reaction
- **SH**: subtractive hybridization
- **RDA**: reductive differential analysis
- **SSH**: suppression subtractive hybridization
Content

• **Rationale**

• **Methodology**
  – Challenges before
  – The research strategy in DD

• **Discussion**
  – Advantages, drawbacks and usages
  – Future improvement
1. Rationale

- **Basis: PCR.**
  [www.wikipedia.org/wiki/Polymerase_chain_reaction]
• The **aim** of DD is:
  – Identification
  – Isolation
• **Rationale**

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2.1 what challenges faced

• SH is used to distinguish mRNAs in comparative studies, such as positive selection of candidate tumor suppressor genes.

• A fingerprinting for mRNA by 2-D electrophoresis is used in detecting cellular protein species.

• ……
Fig. 1. Flow diagram of subtractive hybridization and yields of the recovered single-stranded cDNA. The proportion of single-stranded (SS) and double-stranded hybrid (DS) after each round of subtraction is indicated. HAP, hydroxylapatite.

[PNAS, 88:2825-9]
• **Drawbacks of SH:**
  – mRNA extraction: rigorous
  – Time: consuming
  – Comparison & repetition: lacking
  – Amount of sample: too large
  – Validity of subtraction: unstable
• **Drawbacks of fingerprinting:**
  – reproducibility
  – inability to obtain enough protein for characterization
2.2 research strategy of DD

- **Target Selection**
  - 3' poly(A) tail & 5' arbitrary primer design
  - PCR specificity test

- **RT & PCR**
  - Recover cDNA from the gel

- **......**
• Most eukaryotic mRNAs have poly(A) tails.
• 3’ primer is designed as:
  \textbf{5’-poly(T)CA matches 3’-poly(A)GT}
• There are 12 different 3’ primers, omitting
  \textbf{5’-poly(T)TN}. 
5’ arbitrary primer design

- Standard PCR uses primers of 20 or more.

- Experiment here showed 10-mer primer could give specific amplification.

<table>
<thead>
<tr>
<th>Length of arbitrary primer (bases)</th>
<th>Kilo-bases per binding site</th>
<th>mRNA displayed (no.)</th>
<th>Theory</th>
<th>Experimental</th>
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<tr>
<td>6</td>
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<tr>
<td>10</td>
<td>1049</td>
<td>&lt;1</td>
<td>50–100</td>
<td>0</td>
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</tbody>
</table>

4^n/1000
Specificity of PCR

- The DNA amplification dramatically increased with decreasing [dNTP].
- DNA amplification is primer-dependent.

Arrowhead indicates the amplified TK product on DNA sequencing gel.
DD: cycling vs. quiescent

- TK mRNA only present in the cycling cells (lane 1, arrowhead).
• TK mRNA was amplified as a control (small arrow).

• Arrowhead indicates an amplified mRNA only in normal A31 cell (lane 3).

• Large arrow indicates an mRNA only in tumorigenic BPA31 cell (lane 4).
DD with different primers

- Amplified with different primer sets exhibited totally different patterns.
- Arrowheads show some candidate cDNA tags with differentially expression.
• Calculation showed that 20 arbitrary 10-mers (priming as 6- to 7-mers) should statistically cover all mRNA upstream of 12 possible anchored oligo(dT) primers.
Recover of cDNA

1. Elute from the DNA sequencing gel
2. Ethanol precipitate to remove contaminants
3. Re-amplify
4. Clone and sequence target cDNA tag
5. Northern blot analysis
• **Rationale**

• **Methodology**
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3. Discussion

- Advantages of DD
  - Simplicity
  - Sensitivity
  - Speed
  - Reproducibility
  - Versatility
• **Drawbacks of DD**
  – Differential display depends on the resolution of the gel
  – Need re-amplification to obtain enough amount of target cDNA tag for cloning, and the length of tag is only about 500bp
  – False positives
• Usages of DD
  – Visualize mRNA compositions of cells by displaying subsets of mRNAs as short cDNA bands, such as identifying alterations in gene expression.
– Quickly sequence a tag for each mRNA, which has a different expression pattern, and compare in data banks.

– Clone individual band and use as probes for northern/southern blotting or isolating genes from libraries.
Differential Display of Eukaryotic mRNA by PCR

[Science, 259:946-51]
Differential Display of Eukaryotic mRNA by PCR

[PNAS, 93:6025-30]
Q & A
Thanks!!