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

[*Science*, 257:967-971]

Presented by Liang

Abbreviation

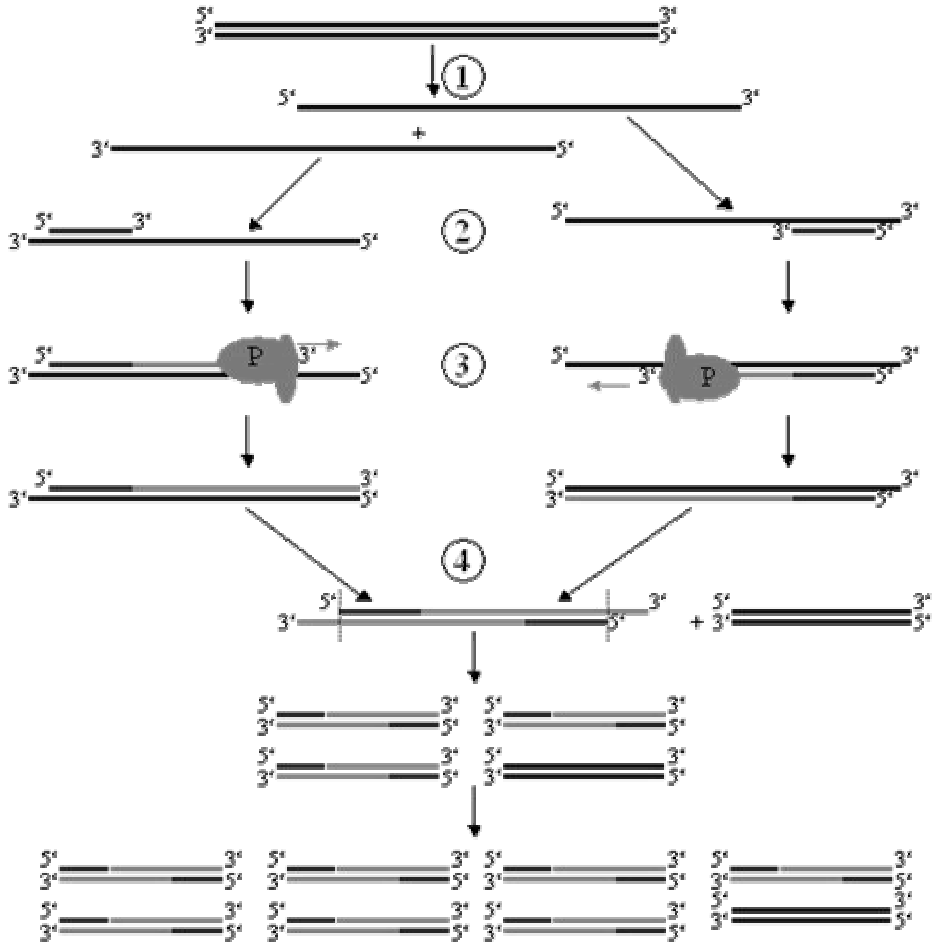
- **DD:** differential display
- **mRNA:** messenger RNA
- **SS/DS:** single strand / double strands
- **RT:** reverse transcription
- **PCR:** polymerase chain reaction
- **SH:** subtractive hybridization
- **RDA:** reductive differential analysis
- **SSH:** suppression subtractive hybridization

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

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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.
- ……

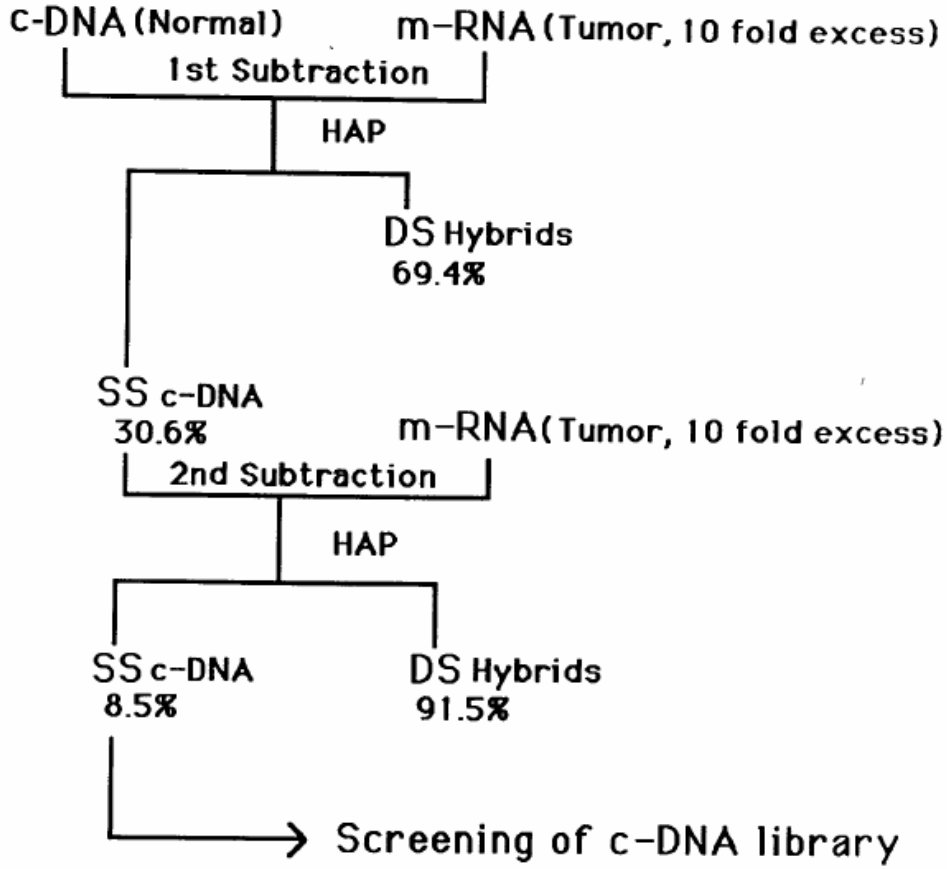


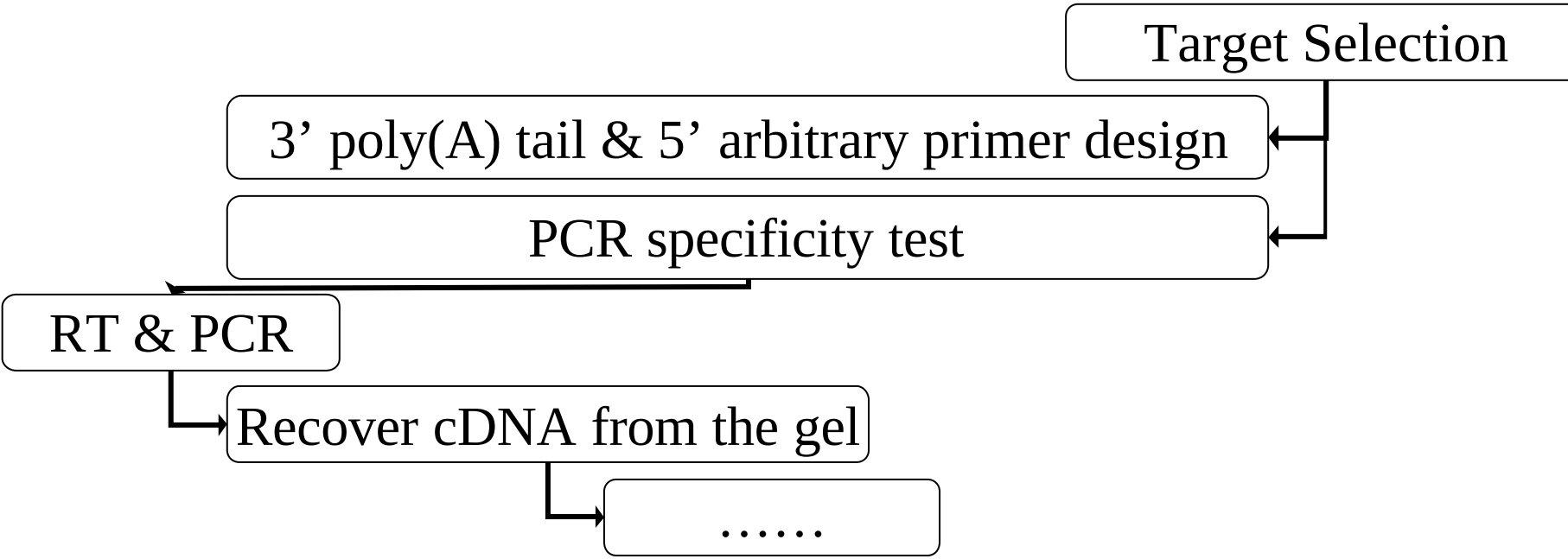
FIG. 1. Flow diagram of subtractive hybridization and yields of the recovered single-stranded cDNA. The proportion of single-stranded (SS) and double-stranded hybrid (DS) after each round of subtraction is indicated. HAP, hydroxylapatite.

[*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



3’ poly(A) tail primer design

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

5’ arbitrary primer design

Table 1. Theoretical calculation and experimental data of the number of mRNA species that can be amplified by arbitrary primers with different lengths in combination with an anchored oligo(dT) primer that binds to one-twelfth of the mRNA 3′ termini. The theoretical calculation is based on the estimation that a mammalian cell expresses about 15,000 different mRNA species (*8*) and that only amplified cDNA fragments with sizes smaller than 500 bp are visualized by a DNA sequencing gel.

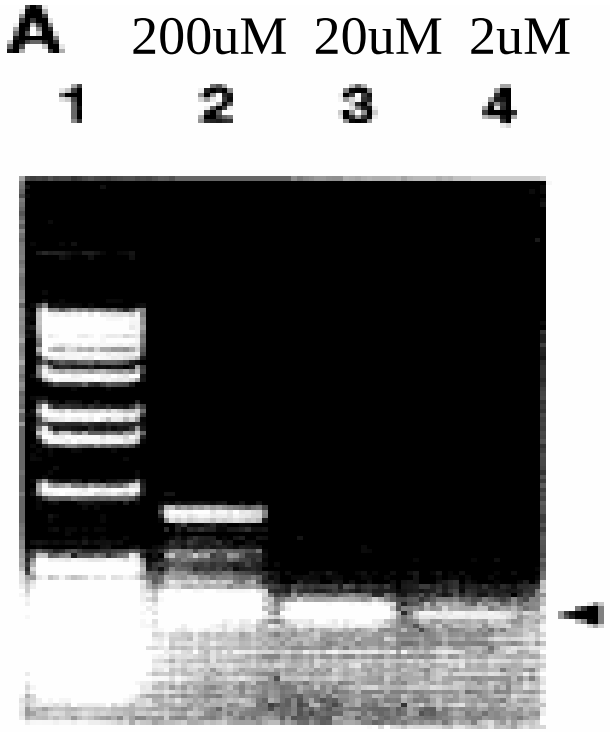
Length of arbitrary primer (bases)	Kilobases per binding site	mRNA displayed (no.)	
		Theory	Experimental
6	4	150	0
7	16	38	0
8	65	10	0
9	262	2	20–30
10	1049	<1	50–100

- Standard PCR uses primers of 20 or more.
- Experiment here showed 10-mer primer could give specific amplification.

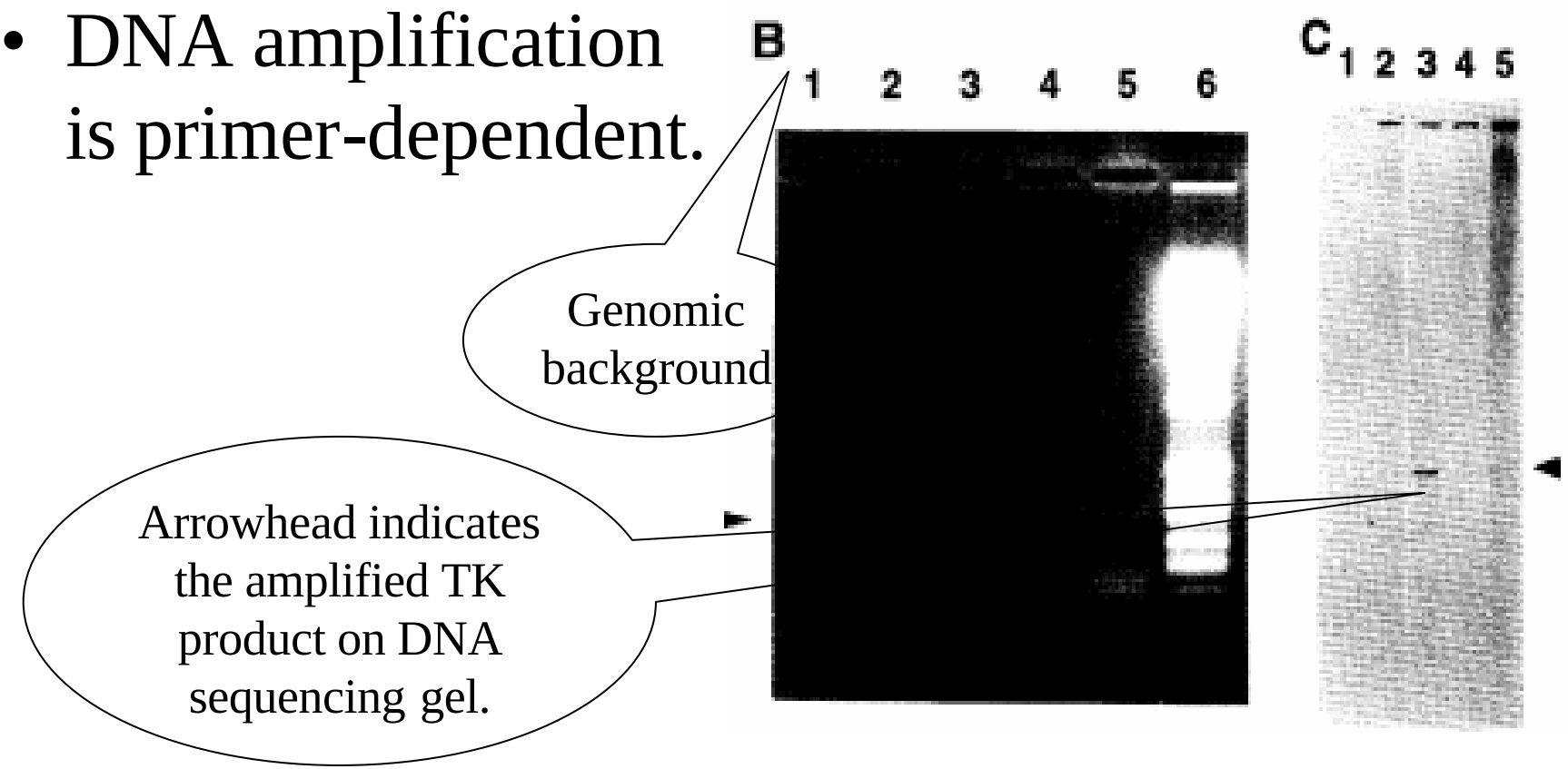
$4^n/1000$

Specificity of PCR

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

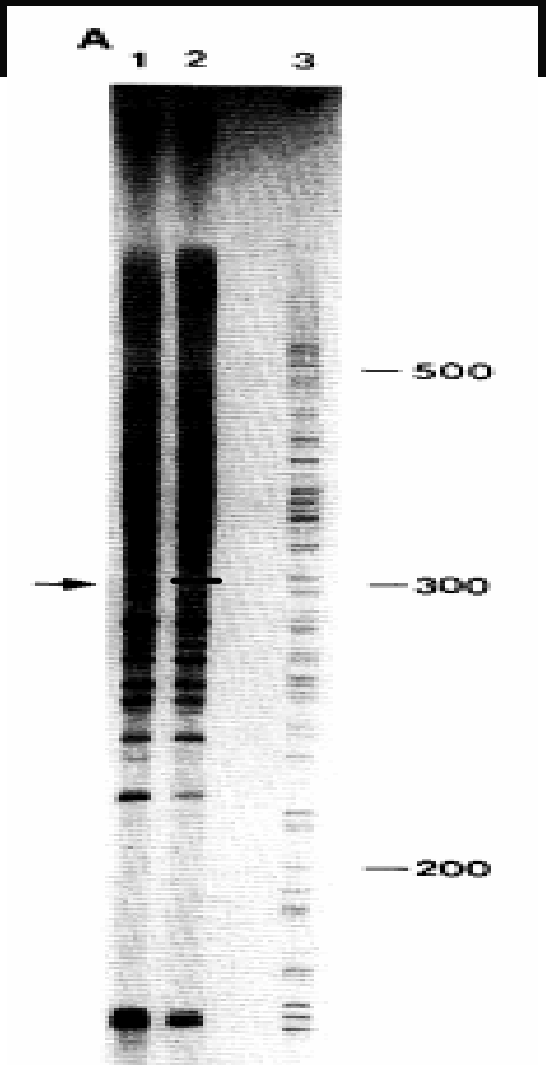


- DNA amplification is primer-dependent.

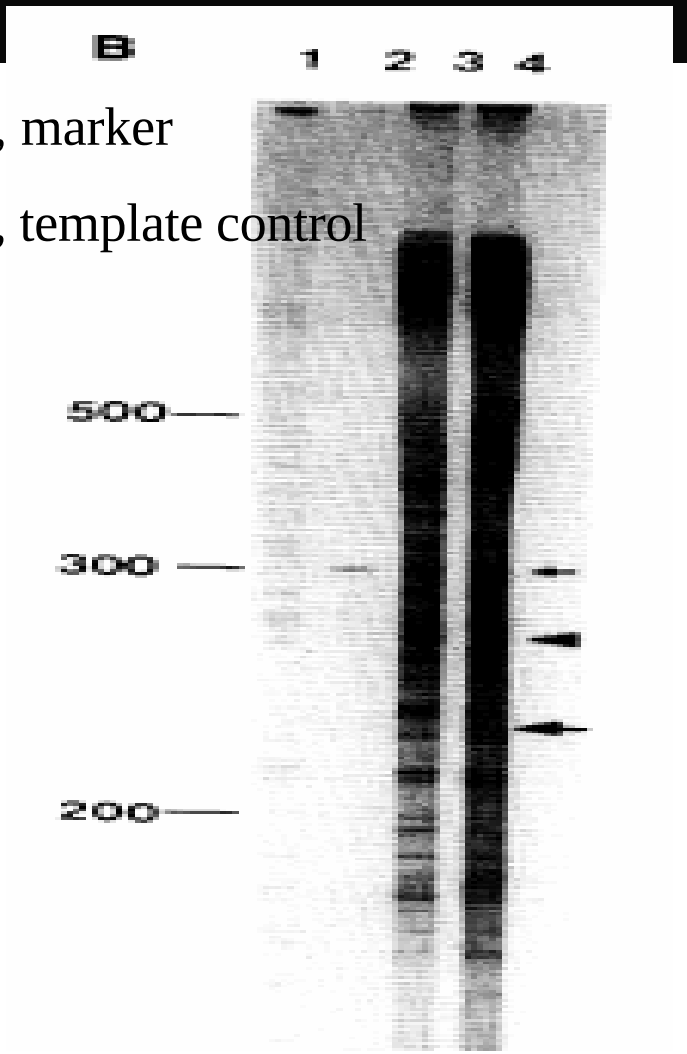


DD: cycling vs. quiescent

- TK mRNA only present in the cycling cells (*lane 1*, arrowhead).



DD: normal vs. tumorigenic



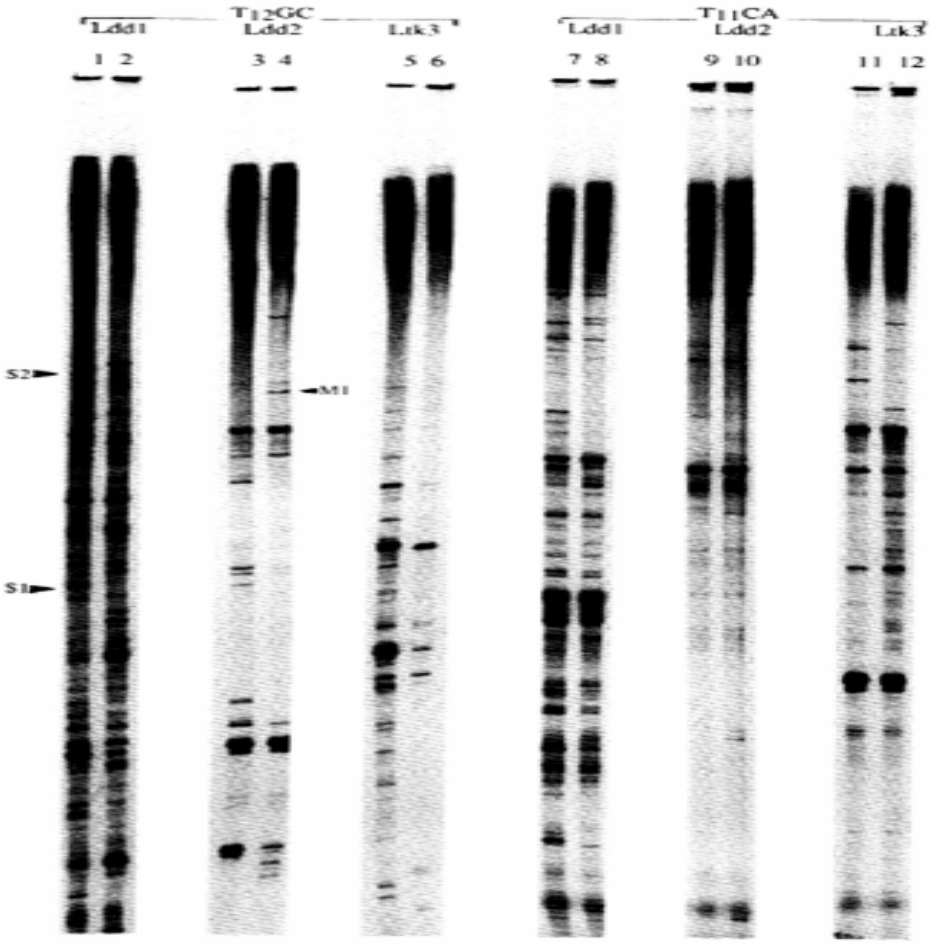
1, marker

2, template control

- 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).

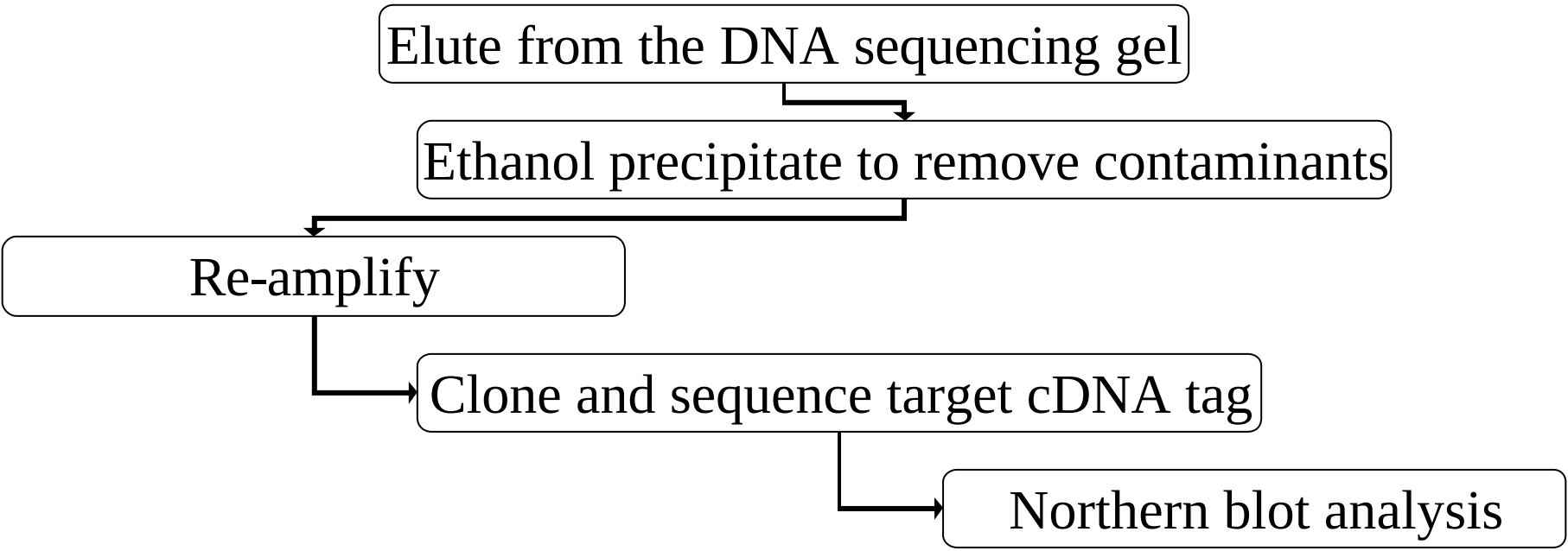
DD with different primers

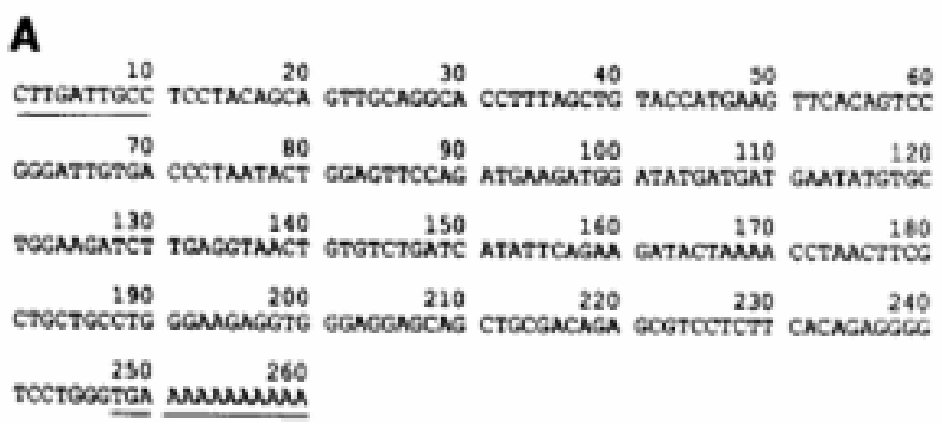
- Amplified with different primer sets exhibited totally different patterns.
- Arrowheads show some candidate cDNA tags with differentially expression.

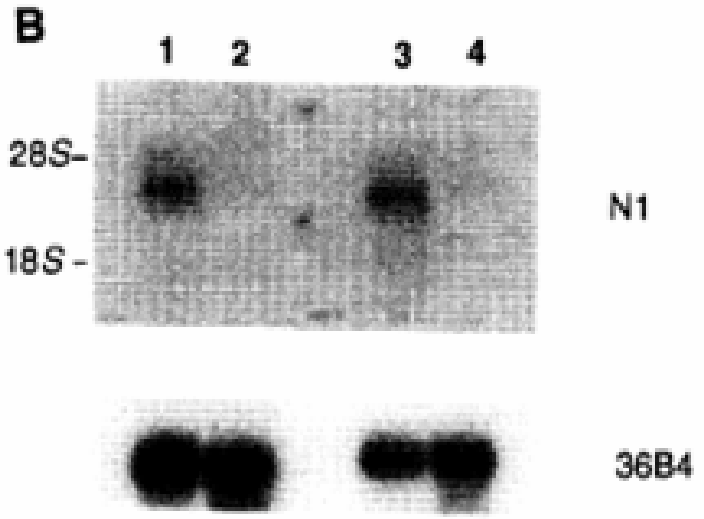


- Calculation showed that 20 arbitrary 10-mers (priming as 6- to 7-mers) should statistically cover all mRNA upstream of 12 possible anchored oligo(dT) primers.

Recover of cDNA




A
10 20 30 40 50 60
CTTGATTGCC TCCTACAGCA GTTGCAGGCA CCTTTAGCTG TACCATGAAG TTCACAGTCC
70 80 90 100 110 120
GGGATTGTGA CCCTAATACT GGAGTTCCAG ATGAAGATGG ATATGATGAT GAATATGTGC
130 140 150 160 170 180
TGGAAGATCT TGAGGTAACT GTGTCTGATC ATATTCAGAA GATACTAAAA CCTAACTTCG
190 200 210 220 230 240
CTGCTGCCTG GGAAGAGGTG GGAGGAGCAG CTGCGACAGA GCGTCCTCTT CACAGAGGGG
250 260
TCCTGGGTGA AAAAAAAAAA


B
1 2 3 4
28S-
18S-
N1
36B4

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3. Discussion

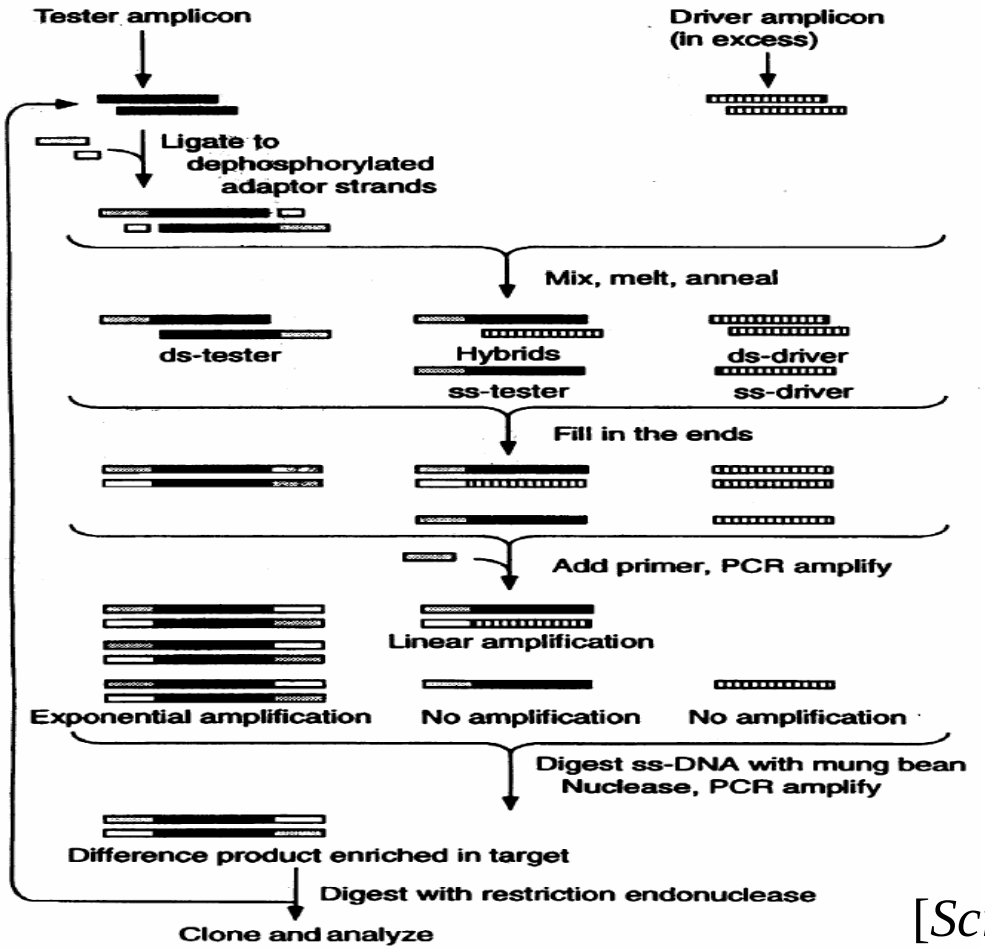
- **Advantages of DD**
  - Simplicity
  - Sensitivity
  - Speed
  - Reproducibility
  - Versatility

- **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.

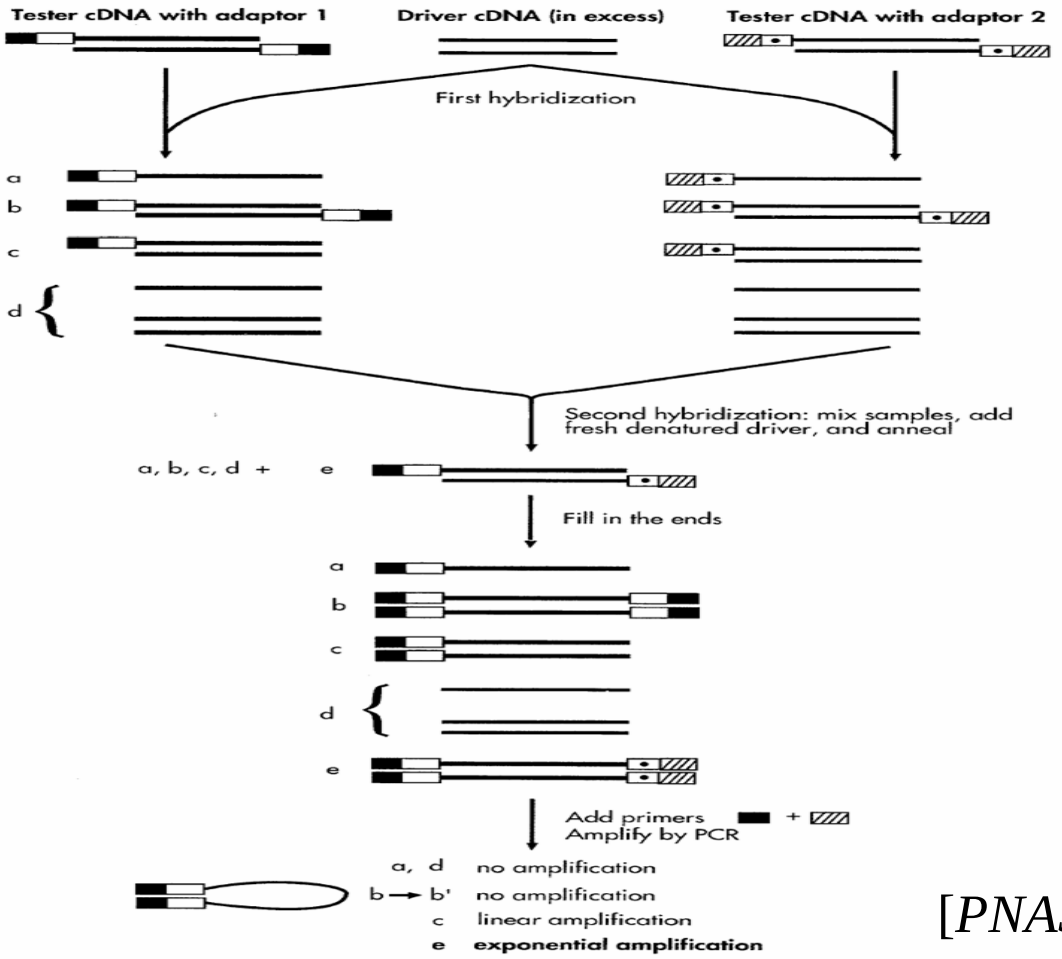
- Quickly sequence a tag for each mRNA, which has a different expression pattern, and compare in data banks.
- Clone individual band and use as probes for northern/southern blotting or isolating genes from libraries.

RDA



[*Science*, 259:946-51]

SSH



[*PNAS*, 93:6025-30]

Q & A

Thanks !