Transcript Isolation Techniques

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Points to Consider

Advances in the end game during the last decade: The incremental and the quantum

How is/will the Genome project changing the process.

If you have a region of interest now, what is the best way to find "your" gene

The problem with complex traits vs. Mendelian

What size interval is feasible to tackle
- genetic vs. physical maps
- gene density considerations

Technical and person year requirements

Lecture Outline

Phases of a project that has gone "Post Linkage"

I. Contig building
Reagents
Methods

II. Gene Hunting

Physical methods
- functional cloning
-direct screening
- direct selection
- exon trapping
- sequencing

Virtual Methods
- database
- virtual evolution

Completing the gene file
- cDNA based
- genomic based

III. Candidate gene triage
-strategies
- informatics considerations

IV. Searching for mutations

- methods of choice circa 1997
- confirmation that this is the correct gene
- material and computational considerations

Transcript Isolation Techniques

FUNCTIONAL CLONING (complementation cloning)

- variation on old theme
- can use large insert DNA clones
  - YAC, PAC, BACS, P1
- requires robust assay
- not practical for many phenotypes
- false positives

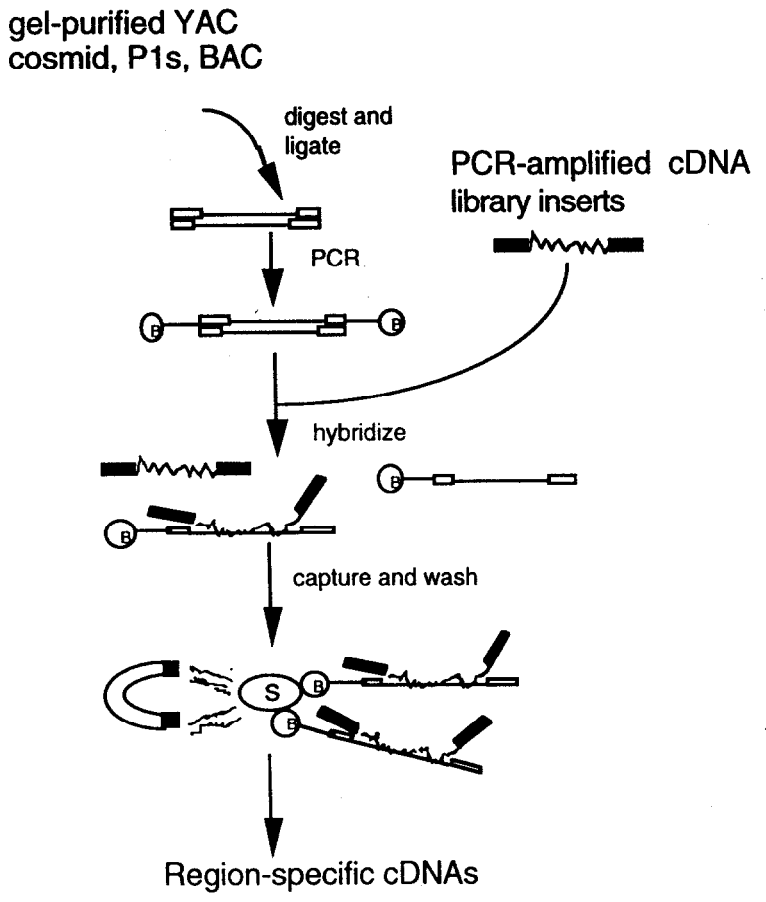
DIRECT SCREENING

Screen libraries directly with large insert clones
- cheap
- fast
- clone size a function of the library

- low yield
- requires repeat suppression

Direct Selection

- a.k.a. magnetic capture, cDNA selection
  - variation: Gene Trapper (LTI)
- utilizes cDNA libraries or primary RT-PCR product
- acts to normalize up to 10^5
- rare and absent messages missed
- significant background ~50%
- short fragments
- YACs, BACs, P1s, cosmids



Magnetic Bead Trapping of Region-Specific cDNAs.

Exon Trapping

- a.k.a. Exon Amplification
  - internal and 3' kits (BRL)
- utilizes genomic DNA
- independent of expression level
- ultimate form of normalization
- significant false positives
- short fragments, average exon 200-400 bp
- YACs, BACs, P1s, cosmids
  - intact cosmid vector avail.(van Ommen)

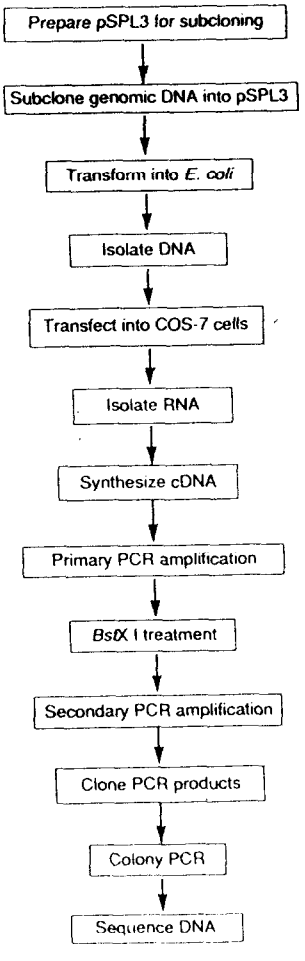


Figure 1. Schematic of the exon trapping procedure

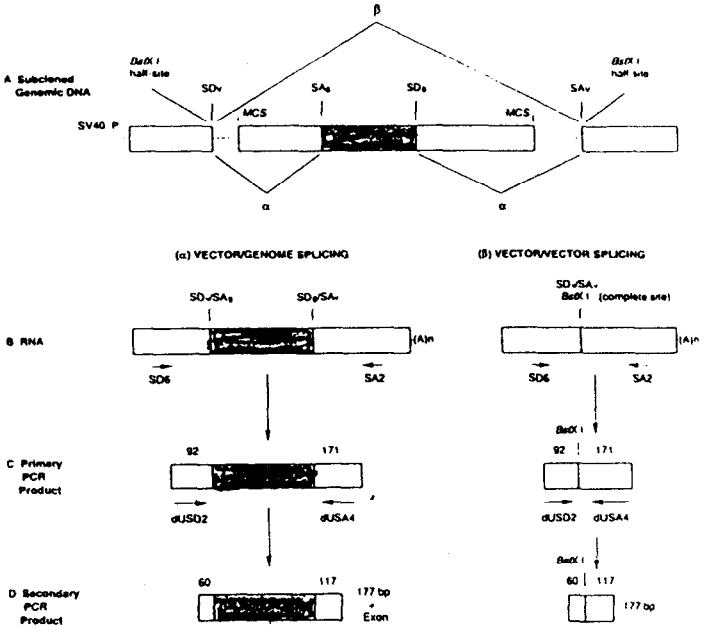


Figure 3. Potential splicing events in the exon trapping procedure. *Panel A*, Subcloned genomic DNA containing an exon. *Panel B*, RNA from two types of splicing events (α and β) are shown, and the products of these splicing events are depicted in the subsequent panels. *Panel C*, RNA from transfected COS-7 cells is isolated, converted to cDNA, and amplified in the primary PCR reaction. *Panel D*, The amplified DNA is reamplified by PCR. SD_v=vector splice donor, SD_g=genomic DNA splice donor, SA_v=vector splice acceptor, SA_g=genomic splice acceptor, MCS=multiple cloning site.

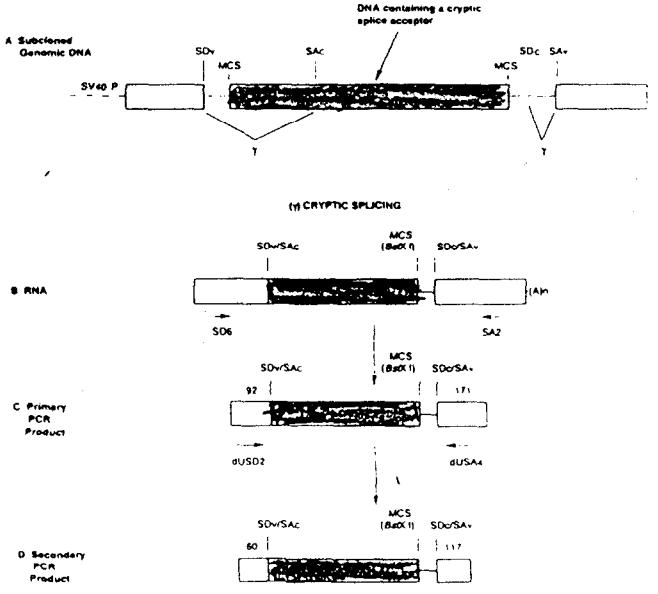


Figure 5. Cryptic splicing. A genomic DNA fragment containing a cryptic splice acceptor (SA_c) is subcloned into pSPL3. With cryptic splicing, part of the MCS containing a *BstX* I recognition site is retained, allowing digestion by that enzyme (not shown), which prevents PCR amplification. See figures 2 and 4 for a description of symbols.

SEQUENCING

"Complete" sequence not needed

"Single pass" sequence methods

- shotgun clone insert
- sequence a set number of clones

- cost considerations
  - 100 kb @ ~ $ 25/ run
    400 runs > $ 10,000

Locating genes in raw genomic sequence

- gene identification software
- dbest approaches

- data management and informatics

Transcript Isolation - References

* recomended reading

GENERAL REVIEW

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INTERNET RESOURCES

GRAIL
http://avalon.epm.ornl.gov/Grail-bin/

GENEFINDER
http://dot.imgen.bcm.tmc.edu:9331/gene-finder/gf.html

GENETICS LINK OF LINKS
HARVARD http://golgi.harvard.edu/genome.html
BAYLOR http://gc.bcm.tmc.edu:8088/home.html

DIRECT SELECTION

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