Fig.1.

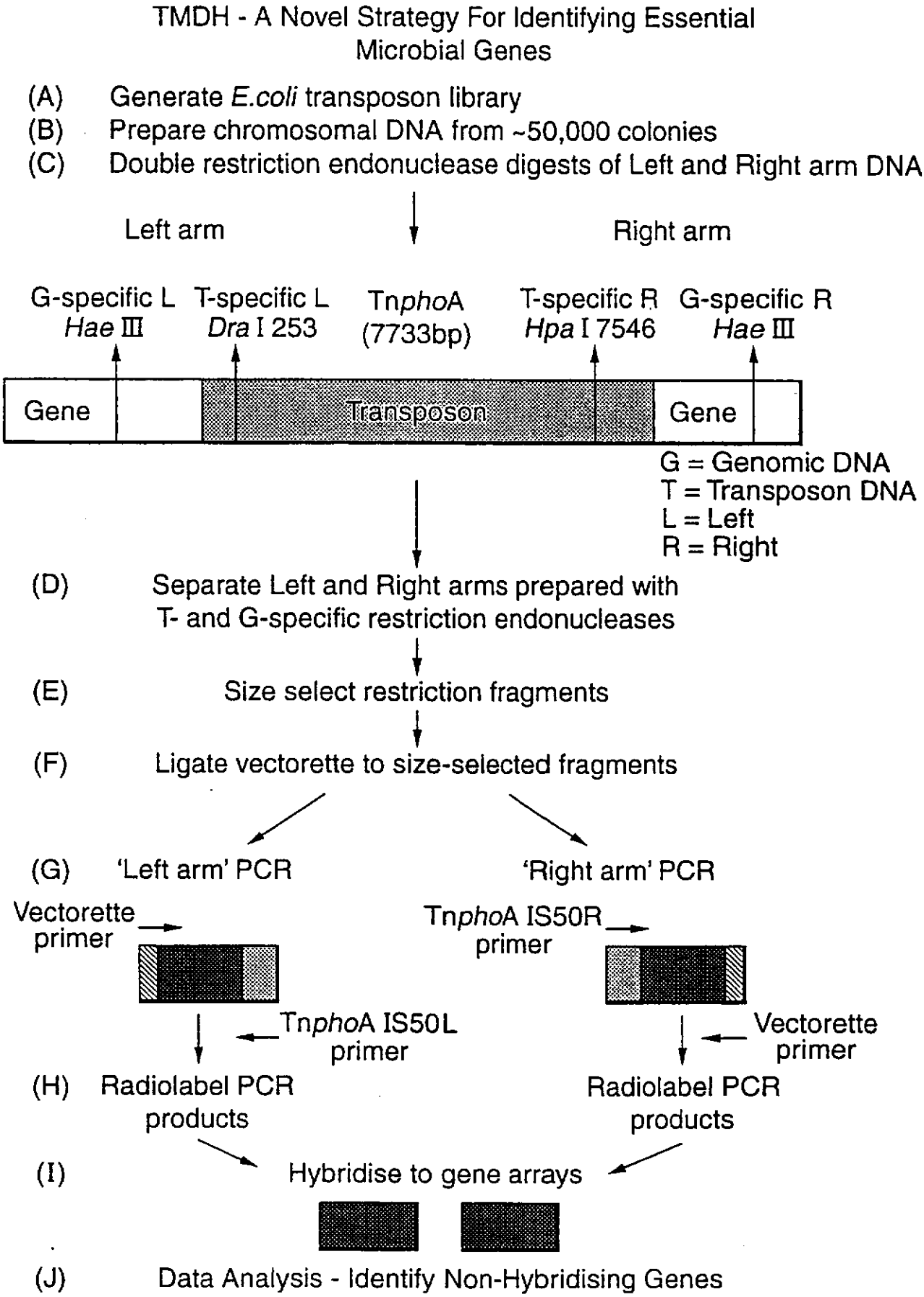

TMDH - A Novel Strategy For Identifying Essential Microbial Genes
(A) Generate E.coli transposon library
(B) Prepare chromosomal DNA from ~50,000 colonies
(C) Double restriction endonuclease digests of Left and Right arm DNA
Left arm
Right arm
G-specific L Hae III
T-specific L Dra I 253
TnphoA (7733bp)
T-specific R Hpa I 7546
G-specific R Hae III
Gene
Transposon
Gene
G = Genomic DNA
T = Transposon DNA
L = Left
R = Right
(D) Separate Left and Right arms prepared with T- and G-specific restriction endonucleases
(E) Size select restriction fragments
(F) Ligate vectorette to size-selected fragments
(G) 'Left arm' PCR
'Right arm' PCR
Vectorette primer
TnphoA IS50R primer
TnphoA IS50L primer
Vectorette primer
(H) Radiolabel PCR products
Radiolabel PCR products
(I) Hybridise to gene arrays
(J) Data Analysis - Identify Non-Hybridising Genes

Fig.2. Generation of Left and Right arm hybridisation probes: Interpretation of signal intensity

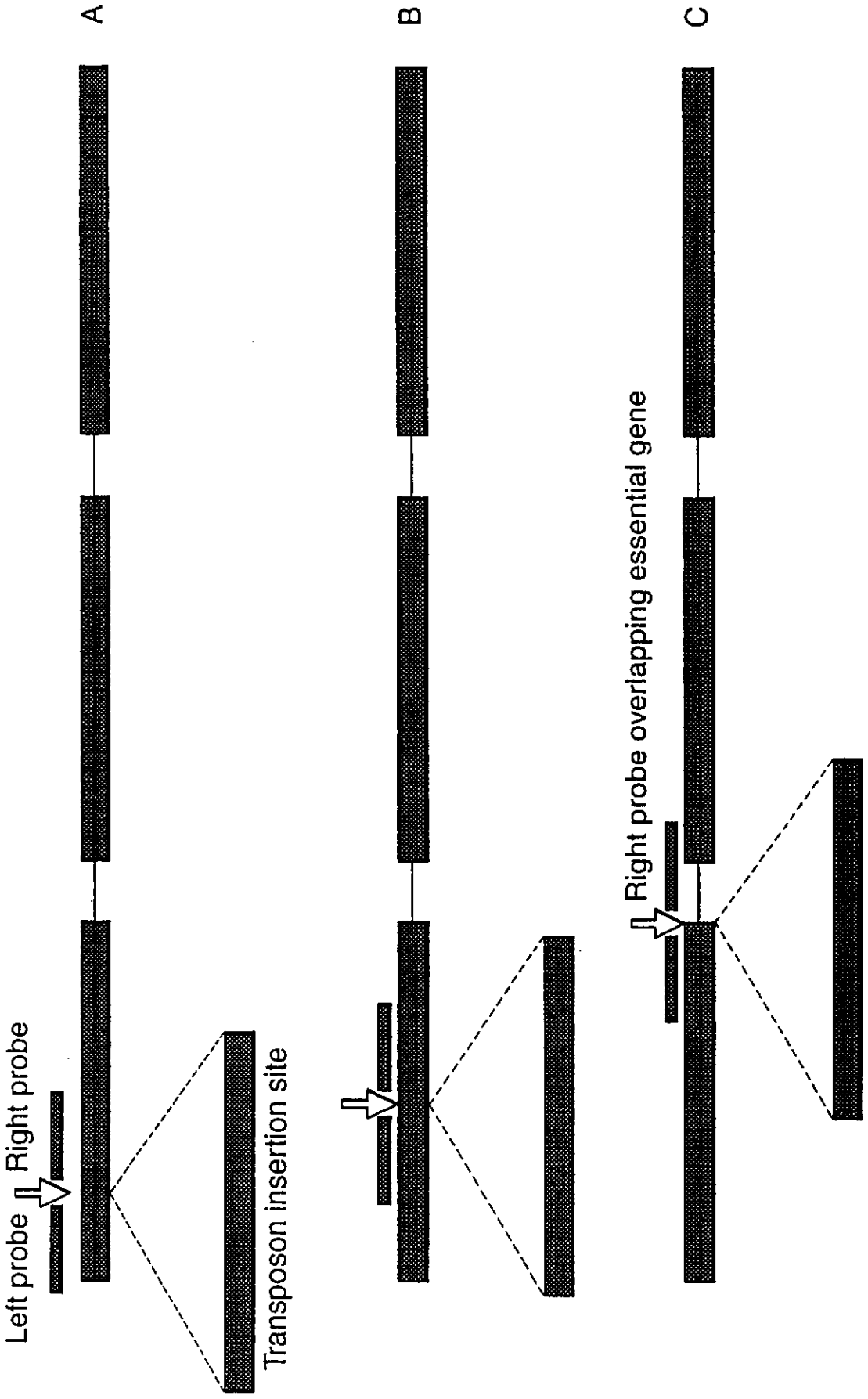


Fig.3.

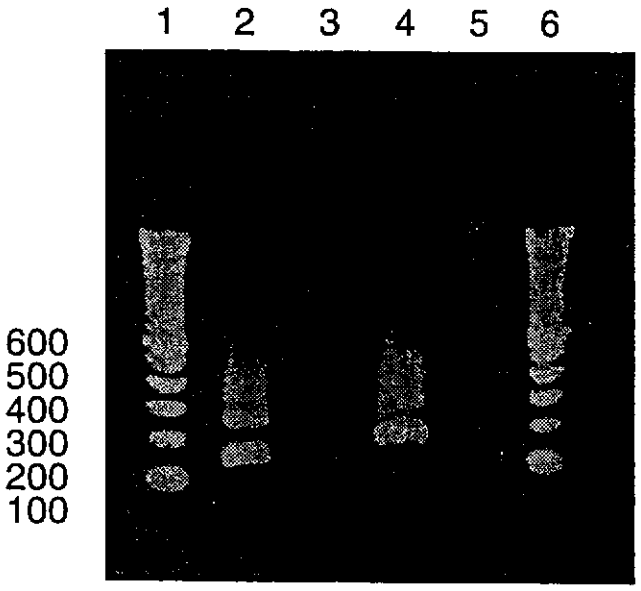


Fig.4a.

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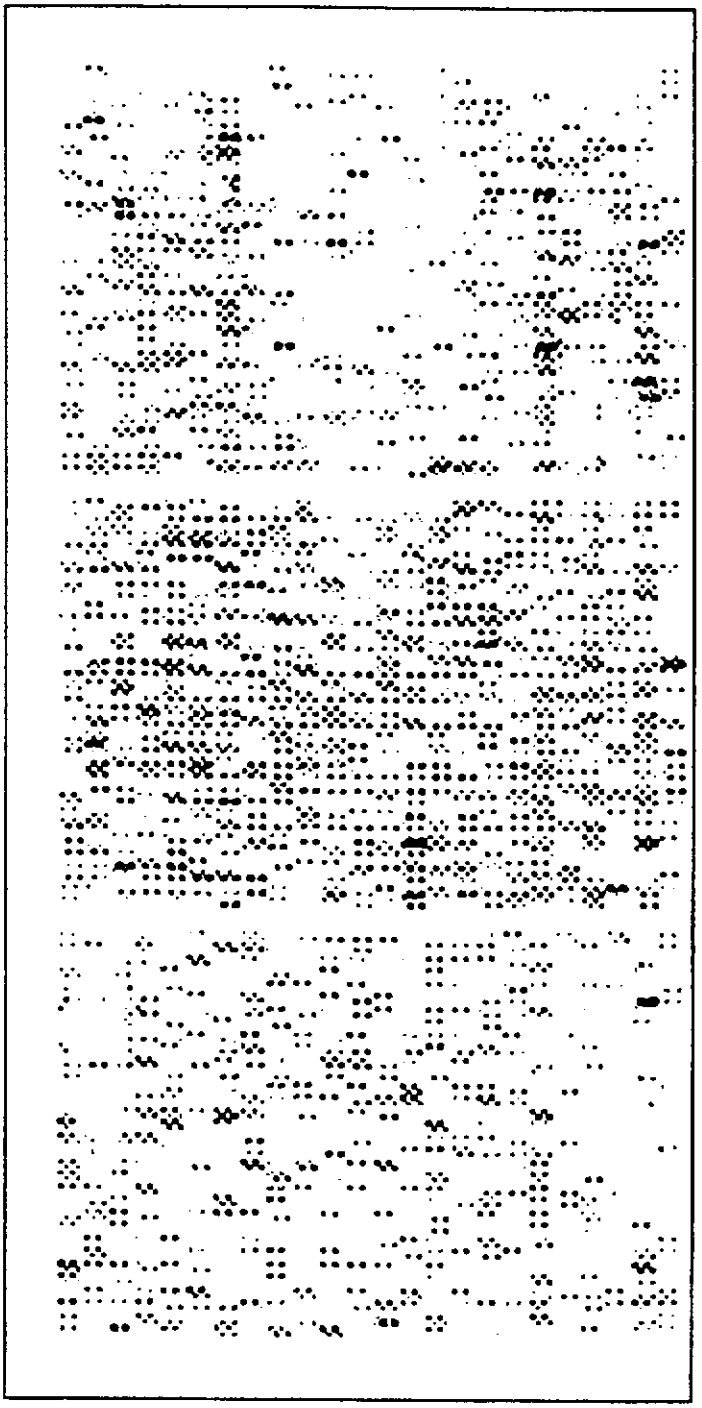


Fig.4b.

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