Online risk of false results. · northern

Onlinedatabas for northern blotting is BlotBase. Mouse and human northern blots arepublished here about 700 in number.

there blots are run on 25 types of differenttissues and on 650 genes. This blot can be searched by tissues, blotID, geneidentifier and paper reference. Science community get benefits from thisdatabase and share information. Results on BlotBase give results by providinggene , its expression level, its blot ID, tissues and species from which it takenand blot view(14). BlotBase: Hereis another procedure which is variant of Northern Blotting known as ReverseNorthern Blotting. This procedure we use a fix membrane that contain substrateconsist of small fragments of DNA and radiolabeled probe is RNA, this iscollected from tissues.

Reverse Northern Blotting: · In case of northern blotting ofacetylcholinestrase, non-radioactive technique is used. It is considerd moresensitive as compared to radioactive. It is advantageous as it takes less time.

13· It helps to detect the RNA size, determine thequality and quantity of RNA on gel before blotting and reprobing of membraneafter blotting. 8· It has high specificity. It reduces the riskof false results.· Northern blot is preffered over microarrays asit helps to determine very small changes while gene expressing phenomena.

12· There are several methods instead of northernblotting which are quite useful. These include microarrays, RT PCR, RNAaseprotection assays as well as SAGE. 11Advantages: · It has low sensitivity. 8· Microarrays has advantage over northernblotting as, by using microarrays, a lot of gene

expressions could be readquickly while northern blot takes time. 9, 10· The reagents mostly used in this procedure arevery harmful to the researcher in some circumstances. Chemicals are likeformaldehyde, ethidium bromide, DEPCO and UV light.

· The disadvantage of northern blotting is thatit degrades the sample. This sample degradation could be avoided by the use ofRNAase inhibitors i. e DEPCO and glassware sterilization. Disadvantages: · It helps in recombinant screening with thehelp of mRNA Obtained by transgene.· Expression patterns help to identify thefunction of a particular gene.

· It is also used to observe oncogenes as wellas tumor suppressor gene in cancerous cells in contrast with normal cells. 8· It is used to observe gene expressions. In differentfields, helps to observe pattern of gene expression between tissues, organs anddevelopmental stages. 5, 6, 7· It is used to detect different diseases aswell as their treatments.· It is used to detect specific mRNA through anysample. Applications: To increase the sensitivity as well as detection of highly nonisotropicblots having low background and high signal/noise ratio make conjugates ofNorthernMax and BRIGHGTSTAR. These conjugates of Northern blots give best resultsalso with radiolabeled probes.

(4)Different detection kits are available with all material and reagents for detection of DNA and RNA probes attachedwith biotin (biotinylation) likeBRIGHTSTAR and Nonisotropic Detection kits are used mostly. Immediately expose to X-ray film (blotis enclosed is plastic wrap if it use

radiolabel probe to avoid its drying)forauto radiography. Use nonisotopic reagent for detection of blot which usenonisotopic probe before its exposure to auto radiography.(3)Detection: NorthernMax kits have low stringent and high stringent washing bufferswhich are certified as RNase free, these washing buffers are availableseparately as well.(2)· High stringent washes: Use high strictness instandards of washing for removal of partially hybridized probes like 0.

1X SSCand SSPE.· Low stringent washes: Use less strictness in standards of washingfor removal of unhybridized probes like 2X SSC (sodium citrate buffer)and SSPE. Next step is washing after hybridization , during this step use differentconcentrations of buffer to remove unwound or unhybridized probes. Two types ofstringent washes are available: Washing: All probes are placed in very little quantity of ULTRAhyb buffer before use , then finally addthem to the blot. This step increasesensitivity by hundred times verses all other hybridization solutions.(1) Wecan detect 10, 000 molecules minimum by this process because this process isvery fast and can be done in just 2 hours using many messages and as for ashighly sensitive.

Hybridization takes place between the DNA and RNA which is he general principle of Northern Blotting.· RNA probes· ss-DNA probe· ds-DNA before use that isdenaturedTo avoid problems after probe hybridization we have to do goodprehybridization, this is also known as blocking , coating of the probe onmembrane can be blocked by this step. Coat nonspecific molecules on membranewhere place is not occupied by mRNA. Two most

popular kits are used in labs forboth pre-hybridization and hybridization e. g., NorthernMax.

Gly and NorthernMax. Both kits are efficient because they use ultra-sensitive hybridization buffer(ULTRAhyb). Three types of probes are used in blotting: Prehybridization and Hybridization with Probe: For the detection of target RNAprepare the probes which are identical to the sequence of our target RNA andmostly present in form of Complementary DNA for Northern Blotting and about minimally25 base pairs to several thousand nucleotides . Here the most important pointof consideration is that avoid the use of very long probes this can causepartial binding of probes with non-specific sequences. We can visit data base searches this willhelp and ensure to make the probes which have unique sequences identical to ourtarget genes. To make the max.

hybridization provide large amount of probe sothat it anneals with max. amount of target sequence. If probe is in lessquantity then less hybridization occur and less signal will be detected on the digitaldisplay. Probe: