

Webcast Q&A: High-Throughput, High-Content RNAi Assays

DATE: 05/05/04

Transcript of Questions and Answers:

Asked: how to quantify si RNA in plasma using non-isotopic methods?

Answered: Radiolabeled oligos for RNA protection assays are currently the best method for quantitation. Multiple companies are preparing detection kits. I suggest looking at the miRNA and RNAi resource pages at www.ambion.com. It has significant links to papers, etc....

Asked: are the siRNA specific for proteins involved in signal transduction pathways are patented?

Answered: To my knowledge, none of the major siRNA suppliers have patented their individual siRNA sequences. The IP surrounding any single gene could require some type of license for research use. Ambion transfers the rights to use its products to its customers.

Asked: why not use dsRNA for mammalian studies as well?

Answered: dsRNA caused very rapid apoptosis (programmed cell death) via the interferon pathway with very, very low amounts of dsRNA (low pM to sub pM concentrations).

Asked: Did they screen all of the genome in this fashion or just genes of interest?

Answered: Every gene. 19,400+. In at least duplicate. It was quite a setup during that process.

Asked: Could you do your screen first with a pool than narrow down to single gene?

Answered: The dsRNAs were single dsRNAs with lengths of 300-800 bps.

Asked: is there evidence for developing resistance to siRNA molecules?

Answered: Not that I have heard about.

Asked: why did u choose such long siRNAs? Most people use very short 19-23 bp siRNAs?

Answered: C. elegans and Drosophila do not have an interferon response to long dsRNAs. siRNAs are required in mammalian cells.

Asked: have you blocked interferon inducible enzyme and then tried longer siRNAs?

Answered: Great question. We have not tried that yet. The IFN response is pretty broad, so I do not know if you can knock all of them down.

Asked: free siRNA difficult to deliver in cell then how it is possible to see effect by soaking the worm in siRNA SOLUTION?

Answered: Worms can be soaked in a dsRNA solution to get entry into the organism.

Asked: Is RISC complex formed in fly and worms as well or just mammalian cells

Answered: The RISC complex is conserved from fission yeast through worms through mammals.

Asked: HOW EFFECTIVE ARE SINGLE -STRANDED siRNA IN SILENCING SPECIFIC TRANSCRIPTS (OR GENES)?

Answered: Very poor in our hands. (David D.)

Asked: What proliferation assay did you use for the HeLa cells?

Answered: This experiment was a "snapshot" experiment where cells were counted using software from a microscopic image. The next slides will make it clear why this was done...

Asked: how many % silencing is good enough to qualify for particular siRNA?

Answered: The cutoff was set at 70%, which is an arbitrary cutoff.

Asked: How is mitotic index measured?

Answered: Histone H3 phosphorylation state using a phospho-specific Ab.

Asked: What is the concentration of siRNA used in these experiments?

Answered: 100 nM to reduce the false negative rate.

Asked: What do you use for negative controls? Scramble? Mock? Could you comment on the pro/cons of each?

Answered: Scrambled siRNAs from Ambion. The use of lipids without nucleic acids is very toxic to cells and probably does not represent a true control. I is likely just a different experiment.

Asked: how many siRNA you design and test for one transcript?

Answered: 3 siRNAs/transcript

Asked: isn't 100 nM high? I thought that many people were recommending 10nM or less?

Answered: It all depends on the false negative to false positive rate. Every screen is different. If the screen has a 20% hit rate, then the concentrations need to reduced. This should be addressed in pilot experiments before screens thousands of genes.

Asked: siRNA knocksdown the mRNA level. Protein level knockdown is dependent on protein turnover. In your experience, do protein and mRNA always correlate to each other?

Answered: If you wait long enough, yes. The mRNA and protein turnover rates for any one gene can be very different.

Asked: how did you account for the mitotic cells coming off the plate

Answered: They do not detach to my knowledge. They will ball up a little bit, depending on the cell type.

Asked: how many different genes can be hit at once?

Answered: At least 5 (performed at Ambion). David D.

Asked: Is there a correlation between siRNA knock down efficacy and mRNA turnover in the cell?

Answered: I have not seen much data on this.... David D.

Asked: Several reports used invivo siRNA generation from DNA emplate with hair pin structure. Given the low cost of DNA production compare to RNA, why should one chose SiRNA than plasmid with hair pin structure for RNAi assay?

Answered: The transfection efficiency is much higher for chemically synthesized siRNAs compared to plasmids. Also, many promoters (H1, U6, CMV, etc...) behave differently in different cell types. Finally, siRNA is not that expensive anymore (competition between multiple reagent companies)....

Asked: Is there a correlation between message abundance and knock down efficiency?

Answered: No.

Asked: Can you differentiate a normal apoptosis vs. neoplastic from the cell morphology or dividing pattern?

Answered Privately: Yes. Please see www.cellomics.com and their competitors to get an idea of what algorithms are out there.

Asked: have u tried RNAi arrays onto which one can grow cells and look at effect of many siRNA at once

Answered: I have seen this type of data and it appears to work quite well. My understanding from the researchers doing those experiments is that the matrix is important to get the experiments to work.

Lisa Licameli Asked: using a common siRNA for the homologous genes (members of the same gene family), would the knockdown be the same for all the genes?

Answered: I do not know. David D.

Asked: So the SiRNA efficacy is based on the phenotype of affected cells? Have nothing to do with actually how much transcript is knock down?

Answered: The siRNA efficacy is a function of transfection efficiency and siRNA efficacy. The protein knockdown will differ by gene.

Asked: What lipid/transfection conditions do you use for the kinase study?

Answered: oligofectamine. Our competitor!

Asked: what transfection reagent do you use?

Answered: It depends on the cell line. For the HeLa experiments shown here, OligofectAMINE was used - not an Ambion product :(.

Asked: Are H1 and U6 interchangeable in terms of function in various cell types?

Answered: No.

Asked: Did you count the transcript number to determine the SiRNA efficacy? I thought you were looking at mitotic index to decide the 70%.

Answered: relative mRNA levels

Asked: What is the duration of action of these chemically syn-siRNA libraries and how does it relate to phenotype effects you measures such as proliferation, necrosis/mitotic index

Answered: The activity lasts for days and is dependent on how many cell divisions are allowed (dilution effect).

Asked: have u ever observed negative silencing (activation of a gene) in some cases knocking down a gene could result in the cell trying to compensate for expression or enhanced activity of a specific protein?

Answered: I do not know.

Asked: Is there any microarray data available to see how one-gene knockdown would affect overall gene expression?

Answered: Yes. A great reference list can be found at the RNAi resource page at www.ambion.com/RNAi . See Jackson et al., Semizarov et al., a paper from Pat O Brown's lab and Michael Green's lab.

Asked: your opinion on using cationic lipids for delivering siRNA in cells or in vivo?

Answered: I hope it works! David D I'll ask Chris if time permits.

Asked: how much it costs and how long it takes to knock down one gene?

Answered: see www.ambion.com/sirna