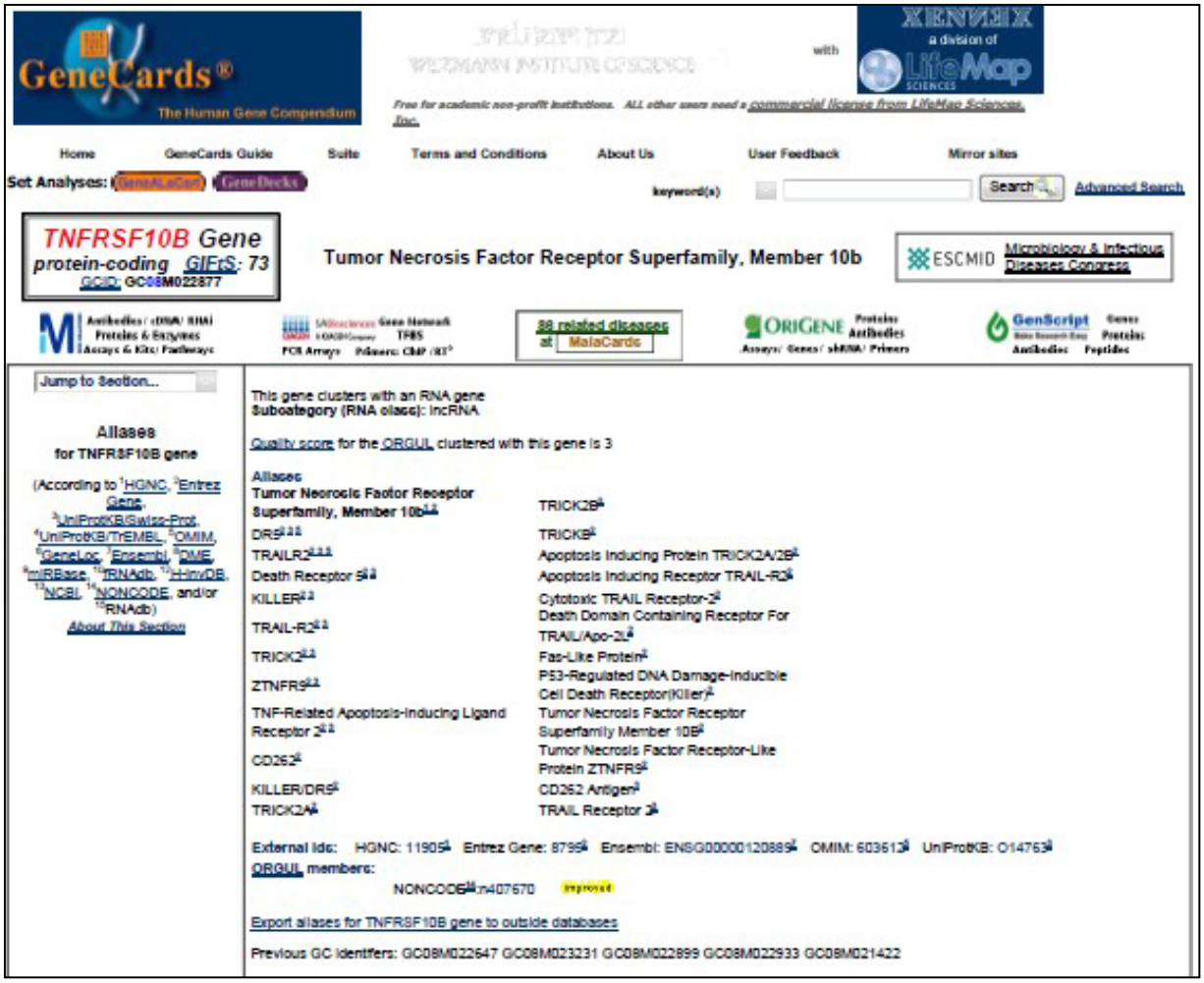
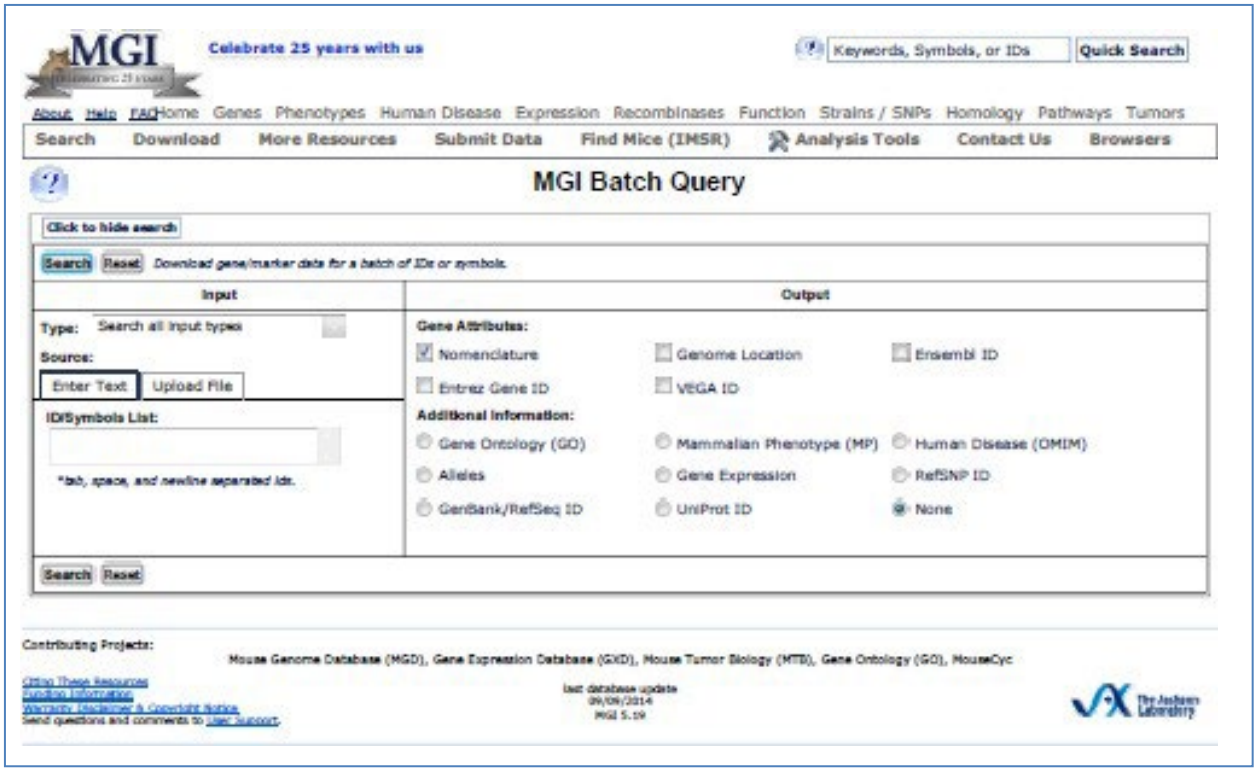
# How to Complete a Risk Assessment for si/shRNA Experiments in Animals and Cell Culture

1. **Agent:** siRNA specific for [insert species]. Discuss how the siRNA will be dosed: viral vector, nanoparticle, plasmid, liposome, etc.
2. **Synonym:** Gene silencing
3. **Containment Requirements:** Usually BSL-1 and chemical hygiene practices, containment equipment and facilities for all activities involving non-virus dosing. For virus-vectored siRNA, BSL-2 practices including biological safety cabinets are recommended. Centrifuge safety precautions, secondary containers for transport between incubator and BSC. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.
4. **siRNA dosing precautions:** Systemic *in vivo* siRNA delivery in mammals was first demonstrated using hydrodynamic tail vein injections in mice. In this procedure, unmodified siRNAs are rapidly injected into the tail vein in a large volume of aqueous solution, resulting in localization within hepatocytes. Although not clinically relevant, this procedure does permit gene function and drug target validation studies within the rodent liver, until more effective delivery technologies are developed. In support of this technique, Song and colleagues found that hydrodynamic injection of a Fas siRNA resulted in silencing of Fas in mouse hepatocytes for a period of 10 days. Similarly, hydrodynamic tail vein injection of a caspase 8 siRNA protected mice against acute liver failure induced by Fas antibody or expression of Fas ligand.
   More recently, low volume, normal pressure intravenous delivery of a modified siRNA targeting apolipoprotein B in mice resulted in gene silencing in the liver and jejunum. The siRNA was conjugated with cholesterol to provide targeted delivery, and included backbone and sugar modifications to enhance serum stability. Although route of administration is an important variable, systemically administered naked nucleic acids generally accumulate in the organs of the reticuloendothelial system (RES) such as the liver, lung, spleen, and kidneys (43), and recent reports suggest that siRNAs also behave similarly (44–46). Braasch et al. (44) first showed that siRNAs in mice accumulate in the liver and kidneys following systemic administration. Additional studies (45, 46) also showed that naked siRNAs accumulate in the kidneys of mice and are detectable in the urine as early as 5 minutes after i.v. injection. Taken together, these studies suggest that siRNAs behave as typical macromolecules of less than 50 kDa and 6 nm, in that they are susceptible to glomerular filtration in the kidney and excretion in the urine. (Akhtar et al, Journal of Clinical Investigation V117 N12 December 2007) The use of sharps should be minimized. Safe-sharp technology is highly recommended during animal dosing.
5. **Spills:** If non-virus vectored, cleanup per the chemical hygiene plan.
6. **Biohazardous Waste:** Collect in double red bags and transport in a rigid container.
7. **Approved Disinfectants:**
   i. Non-virus vectored siRNA: soap and water
   ii. Virus-vectored; disinfectants appropriate for the virus.

8. **Disposal:** Non-virus vectored, as a chemical. Virus-vectored: Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.
9. **Storage:** Store as per the chemical hygiene plan.
10. **Pathogenicity:** Mucous membranes, ingestion, broken skin and injection. Reasons can be sharps contact, failure to wash hands, skin contamination from dirty gloves or work surfaces.
11. **Modes of Transmission:** Liposomes and plasmids may cross the cell membrane of individual cells. If the gene target is present, it could result in silencing. Liposomes and plasmids are not infectious; once integrated into cells, they do not reproduce.
12. **Length of gene expression:** Non-viral vectored RNA silencing is transient, lasting between 5-7 days in rapidly dividing cells; silencing can last up to 3 weeks in non-dividing cells. (Bartlett et al, Nucleic Acids Research V 34, N 1 Pp. 322-333)
13. **Communicability:** Not communicable.
14. **Medical surveillance and clinical treatment procedure**: No medical surveillance is required. GOH Clinical Operating Procedure "Lentivirus Vectors" must be listed on risk assessment if used to vector siRNA.
15. **Stability in Environment:** Samples shipped as dried pellets are stable at room temperature for 2-4 weeks. Neither repeated freeze/thaw cycles, extended incubations (over 1 year at 21°C), nor shorter incubations at high temperatures (up to 95°C) have any effect on siRNA integrity as measured by non-denaturing polyacrylamide gel electrophoresis and functional activity assays. Degradation was also not observed following exposure to hair or skin at 37°C. However, incubation in fetal bovine or human sera at 37°C led to degradation and loss of activity. Therefore, siRNA in the bloodstream is likely inactivated, thereby limiting systemic exposure (Oligonucleotides. Dec 2008; 18(4): 345–354).
16. **siRNA concentration, dosage per experiment:** State your stock concentration, and the amount used per experiment or kg animal weight.
17. **siRNA shedding from animals:** Animals will not shed siRNA if dosed by intravenous route. For viral vector dosing route, refer to specific viral vector risk assessment.
18. **Transgene Information:** Discuss effects of transgene on animal or cell line. A good source for understanding the transgene being silenced or over-expressed is GENE CARDS (http://www.genecards.org/). A snapshot of a sample gene card is shown below:




To better understand potential human outcomes from accidental silencing, you can see if information exists in the JAX Mouse Genome Informatics: (http://www.informatics.jax.org/batch). Enter the gene designation, and then look to see if a mouse knockout phenotype exists. If so, add that information to the risk assessment.




You must discuss the potential effects due to accidental worker exposure. If unknown, state that. Is the gene sequence or siRNA specific to animals, humans or could it affect both?