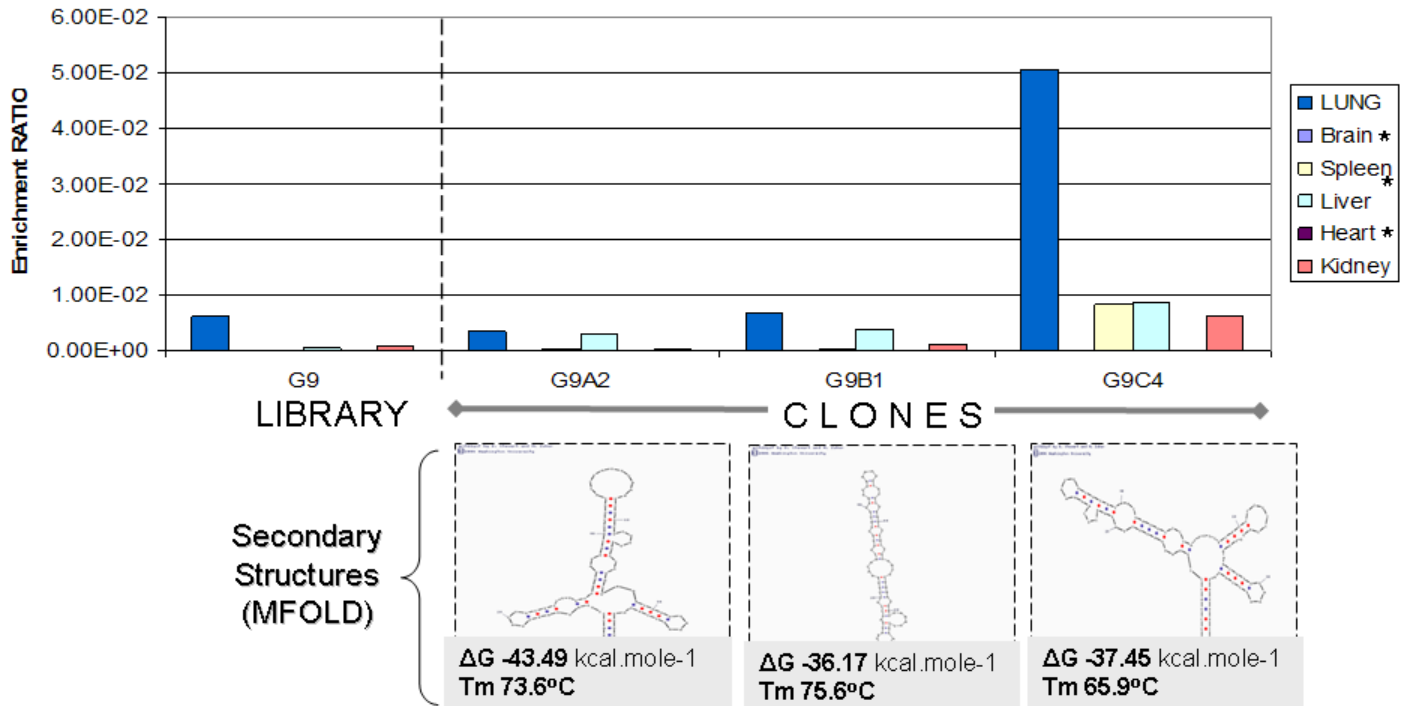
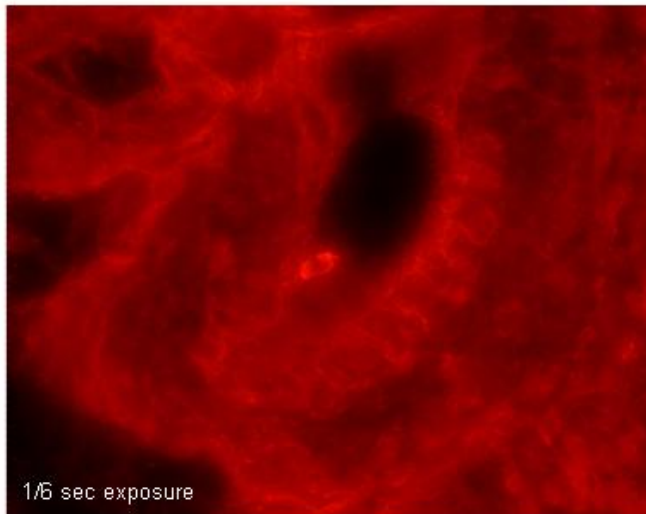


Figure 1. Presence of Aptamer Targeting Across a Panel of Tissues Followed by Enrichment of Lung Specific Aptamers. A 10^{13} random library of 2'-F-RNA was tail-vein injected into a balb/c mouse, and after forty minutes the mouse was perfused with PBS (pH 7.4), and various tissues (brain, lung, heart, liver, spleen, and kidney) were harvested for RT-PCR analysis. **(Top)** Gel electrophoresis of aliquots from the RT-PCR samples. + and (-) represent amplicon from RNA isolated from tissue lysate which has and has not been exposed to IV injection of 2'-F-RNA library, respectively. Controls consist of sham and no template as negative controls, and stock library as a positive control (not shown). Molecular weight marker (left) is 100bp ladder; MW ladder (right) is Hyperladder V (Bioline). Notice the control tissues which have not been exposed to the aptamer library (-) do not show any bands as expected, whereas the (+) exposed tissues do show a corresponding band. Each band represents an amplifiable pool of aptamers that bind in vivo to the indicated target organ. **(Bottom)** Charting the enrichment of 2'-F-RNA aptamers specific to murine lung tissue. For each round of selection (x-axis) a 10^{13} library of 2'-F-RNA molecules were administered to a mouse (tail injection); at varying time lengths from 5 to 40 minutes, the mouse was perfused with PBS (pH7.4), followed by necropsy to harvest lung tissue and to purify 2'-F-RNA. Preparative RT-PCR generates an enriched 2'-F-RNA library for another round of selection. Through analysis by real-time qRT-PCR, the molecular library becomes increasingly enriched (y-axis) with 2'-F-RNA species that target lung tissue; the enrichment from G1 to G9 was three orders of magnitude (i.e. $\sim 7,400$ fold). The enrichment ratio, as defined previously, for all tissues was determined for each round of selection. Background tissues were analyzed beginning at round 6. At rounds seven through nine the enrichment of library has reached a plateau and contains 2'-F-RNA aptamers that target lung tissue approximately ten-fold more than other background organs and tissues. (*) Note: G8 (spleen) and G9 (brain & spleen) tissue samples could not be analyzed by qRT-PCR because of insufficient data points to meet the standards for quantitative measurement. Rejected data points include samples with a significant primer-dimer formation as indicated by melting curve analysis or unacceptable real-time PCR reaction efficiencies (i.e. not within 90-110%) for linear regression analysis and interpretation of the enrichment ratio.

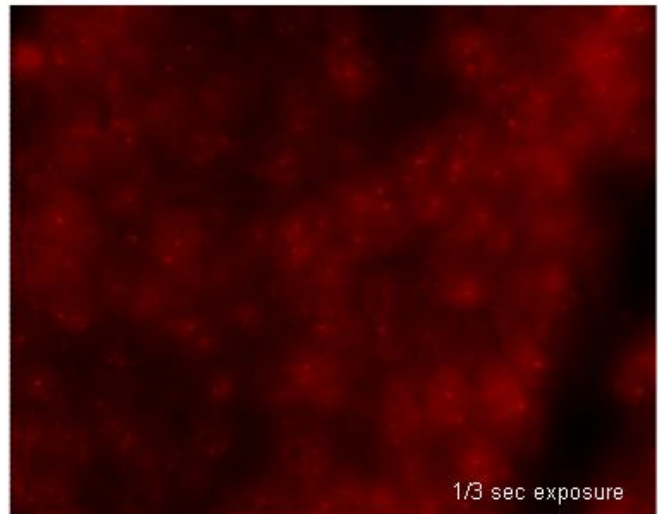


Family	# of Clones	5'- gggcgaccugaugag [Consensus Sequence] cgaaacggugaaagccguagguugccc -3'
Group A	12	[UGACUGCUCCGUUCGGUUAUGACAGCUGCACCCAGUUAAGC:GGUUCUGGGUCCGGA] G9A2
Group B	7	[CCUUUUUGAACAAACUGUGCGAUUUUGAUUG:AAAUUUCUCUCUGAUCCCACCGUGACG] G9B1
Group C	2	[UCUAGAGCGCAGAAACUUCUCUCAACGAUUCGCCACGUCCUCGCCCGCCCGGU] G9C4

Figure 2. Sequence Analysis of Lung G9 Clones. (Top) A bar graph comparing the relative Enrichment Ratio, as defined previously, of the G9 library to the individual clones within G9. All tissue samples were normalized at 0.5 mg/ul for enrichment ratio analysis. This preliminary trial indicates that clone G9C4 is the best binder for lung tissue. (*) Note, the following are tissue samples that could not be analyzed by qRT-PCR because of insufficient data points to meet the standards for quantitative measurement: G9 library (brain & spleen), G9A2 (brain), G9C4 (brain & heart). Rejected data points include samples with a significant primer-dimer formation as indicated by melting curve analysis or a correlation coefficient of less than 0.98 for linear regression analysis and subsequent interpretation of the enrichment ratio as defined. Also, note, the bar graph for G9C4 represents a second trial analysis. The first trial analysis exhibited an elevated liver background for enrichment ratio. Subsequent trials to reproduce the results shown above have not yet been attempted. **(Middle)** Predicted (mfold) secondary structures of the individual G9 clones. **(Bottom)** Consensus sequence of G9 clones. Underlined bases indicate guanines involved a predicted G-quartet structure.



LUNG



LIVER

Figure 5. Fluorescence Microscopy of Lung-Aptamer. 5'-end labeled G9C4 RNA aptamer with ADO™550/570 (Adegenix, Monrovia, CA); washed with PBS; fixed tissues with acetone, followed by *in situ* binding of (~4 mg) aptamer for 40 minutes at room temperature; washed. (not shown) Brain, spleen, heart, kidney were negative. The labeled-aptamer fluorescence indicates binding throughout the lung tissue, and is significantly brighter than liver as indicated by the microscope camera exposure setting. However, the low level of aptamer binding *in situ* to liver possibly indicates localization within the nuclear compartment. Liver and lung tissues were examined repeatedly from different trials, and results remain consistent.