siRNA/DNA transfection
v.1

1. Plate the previous day. HeLa cells 0.40 x 10^6 per 35mm (six well) dish. 80-90% on day of transfection. Protocol for six well plates.
2. Dilute siRNA in RNAse-free water

siRNA; sc-36869
10µM solution.

Final Needed	Final Needed pmol	Final Needed µmol	Stock 10µM	Liters needed to added (umol/10 µM)	µl add to 35mm	
5 pmol	5	.000005	10	.0000005	0.5	
25 pmol	25	.000025	10	.0000025	2.5	
50 pmol	50	.00005	10	.000005	5	

3. Warm Opti-Mem
4. Use 2ug DNA. 4ul pcDNA1.1 Atx1[2] (500ng/ul).
5. Add siRNA and DNA to 250ul opti-MEM. Mix gently.
6. Add 4 µl (2.5-6 µl) Lipofectamine 2000 in 250ul opti-MEM. Mix gently. Incubate 5 minutes RT.
7. Mix siRNA/DNA and Lipofectamine 2000. Mix gently by slow pipetting up and down. Incubate 20 minutes RT. Solution may become cloudy.
8. Plate the cells in 2ml opti-MEM.
9. Add transfection solution to cells. Rock back and forth to mix.
10. Media may be added back in 4-6 hours.

Note: Keep hood light off when using fluorescein (fitc) labeled siRNA and cover with aluminum foil during incubations.

Do not forget proper controls.