MODIFYING HUMAN PARKIN shRNAmir TO MOUSE shRNAmir and PRODUCING HUMAN PD shRNAmir CELL LINES

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and

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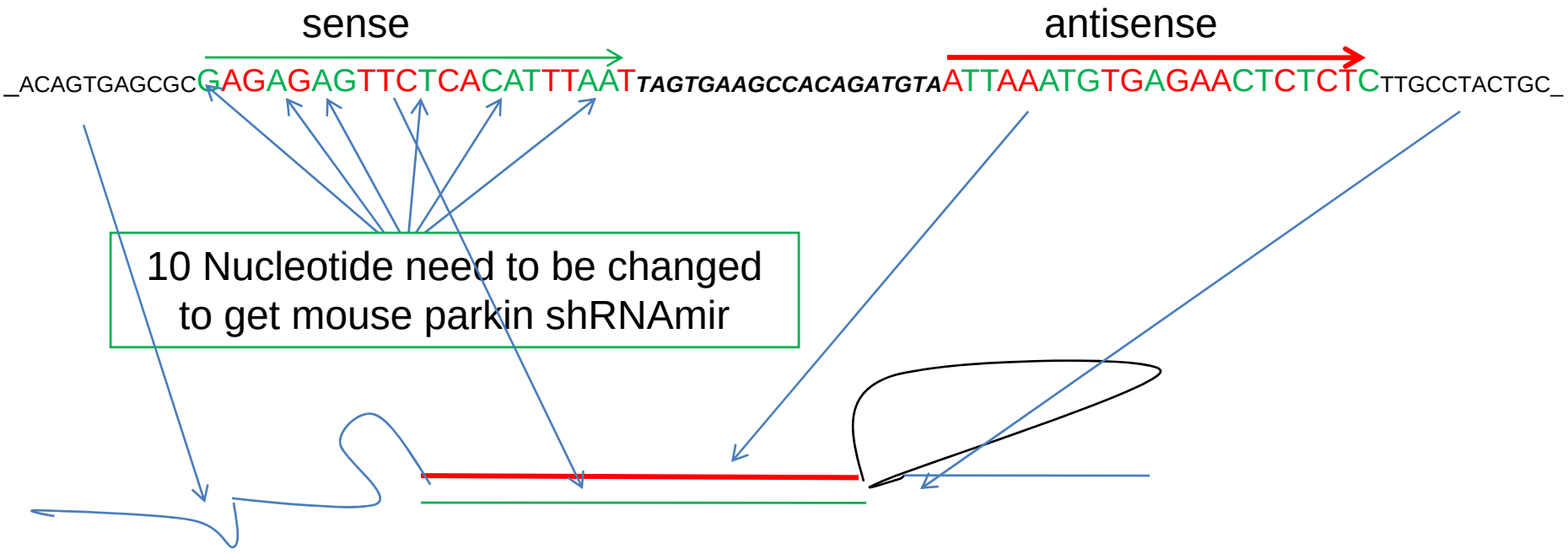
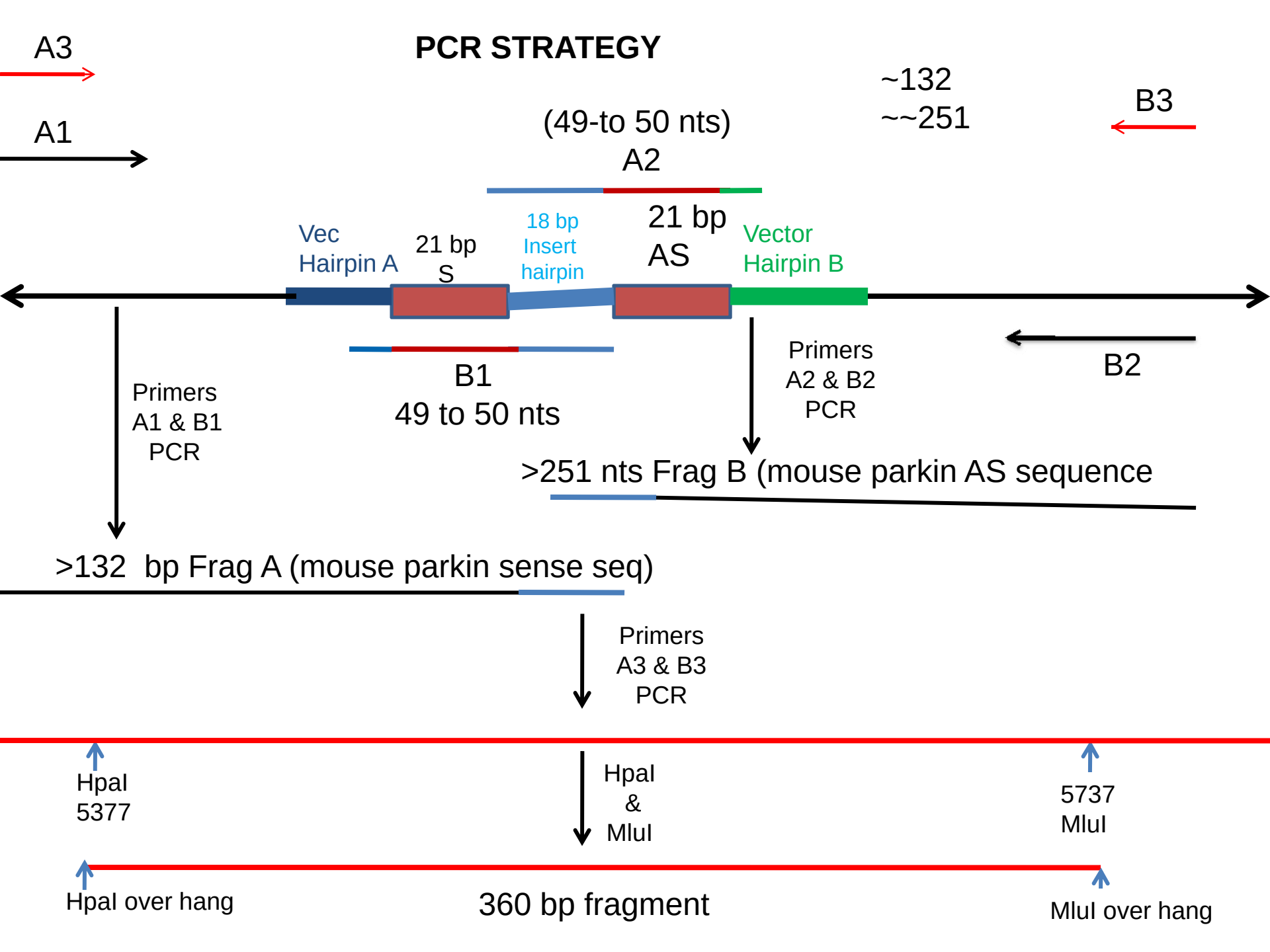
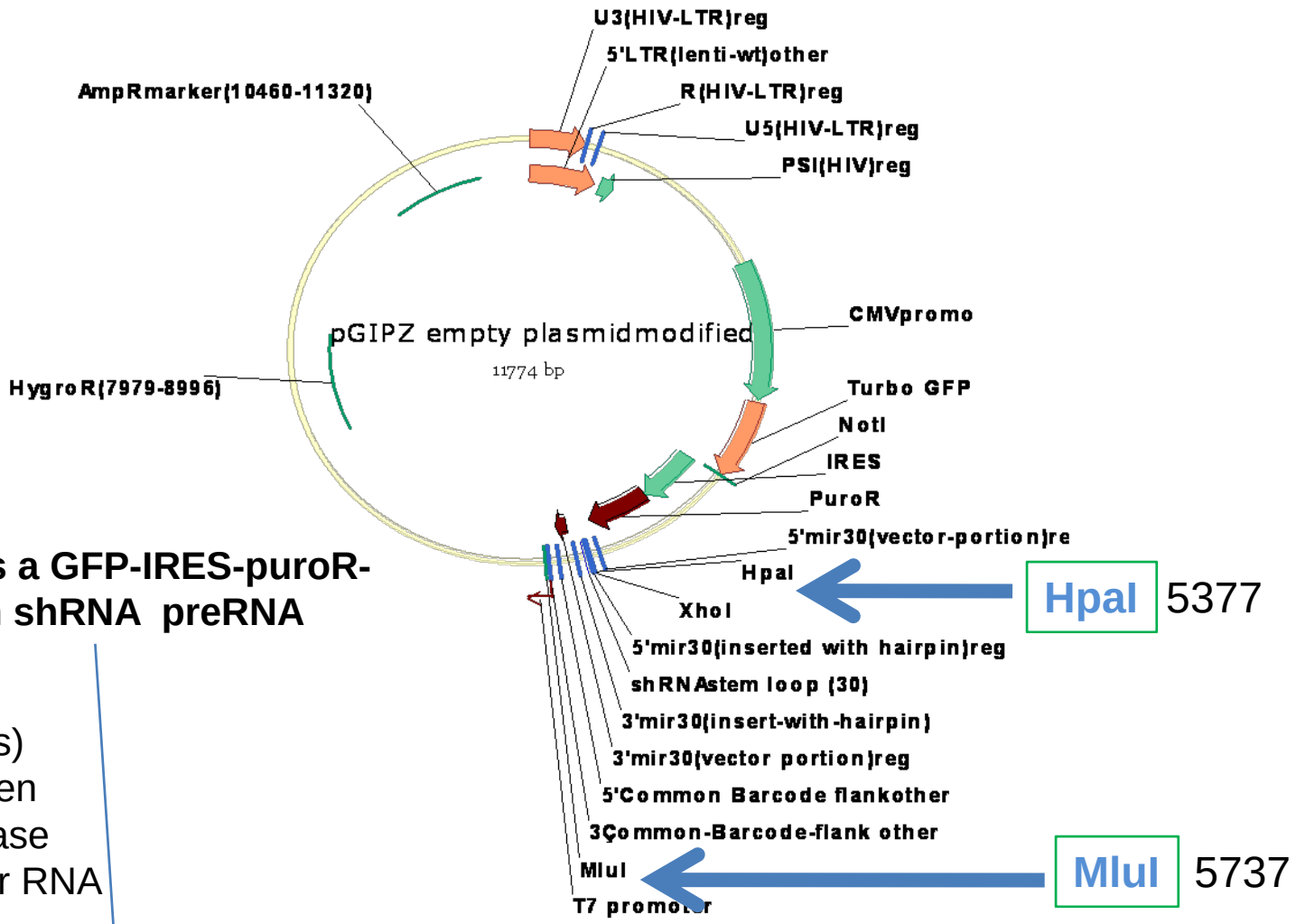
PROBLEM: Converting human to mouse shRNAmir

Human PARK2_517 shRNAmir (PARK2_518 and _520 are not shown).




A3
PCR STRATEGY
~132
~~251
B3
A1
(49-to 50 nts)
A2
21 bp
AS
18 bp
Insert
hairpin
Vec
Hairpin A
21 bp
S
Vector
Hairpin B
B1
49 to 50 nts
Primers
A1 & B1
PCR
Primers
A2 & B2
PCR
B2
>251 nts Frag B (mouse parkin AS sequence
>132 bp Frag A (mouse parkin sense seq)
Primers
A3 & B3
PCR
HpaI
5377
HpaI
&
MluI
5737
MluI
HpaI over hang
360 bp fragment
MluI over hang

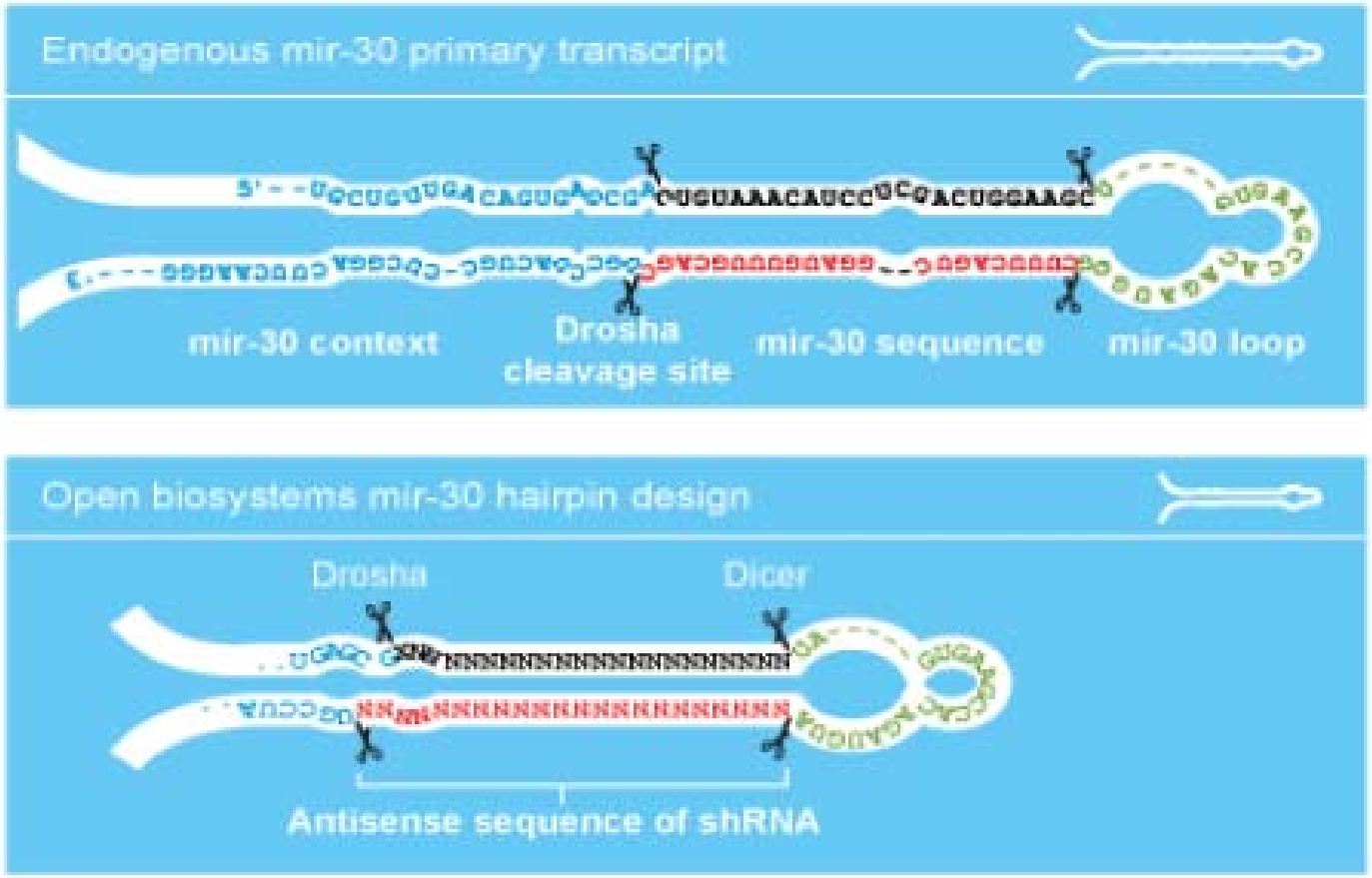


Generates a GFP-IRES-puroR-Parkin shRNA preRNA

Drosha (class 2 RNase III enzymes) to pri-mir RNA, then Dicer (endonuclease of RNase III) to mir RNA

GFP-IRES-puroR mRNA + parkin shRNAmir

How the shRNAmir is generated by Drosha and Dicer



GENERATION OF PARKIN, DJ-1, AND PINK1 EXPRESSING pGIPz shRNAmir PLASMIDS IN HUMAN SH-SY5Y CELLS

PROMEGA NORADIOACTIVE CELL PROLIFERATION ASSAYS

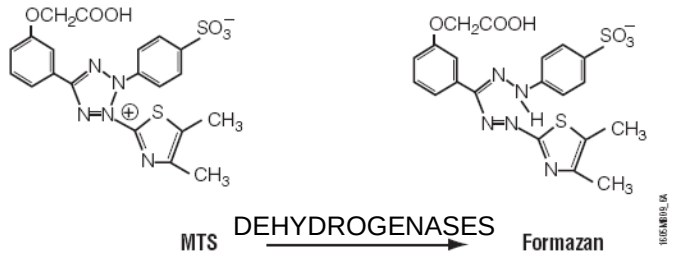


Figure 1. Structures of MTS tetrazolium salt and its formazan product.

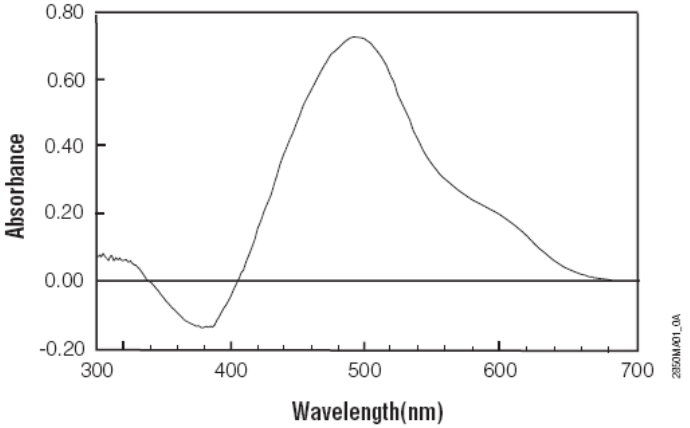


Figure 4. Absorbance spectrum of MTS/formazan after bioreduction by K562 cells. The K562 cells were cultured in RPMI 1640 supplemented with 10% FBS. The blank used to generate this absorbance spectrum was culture medium containing MTS that was not bioreduced by cells. The negative absorbance values (382nm) correspond to the disappearance of MTS as it is converted into formazan.

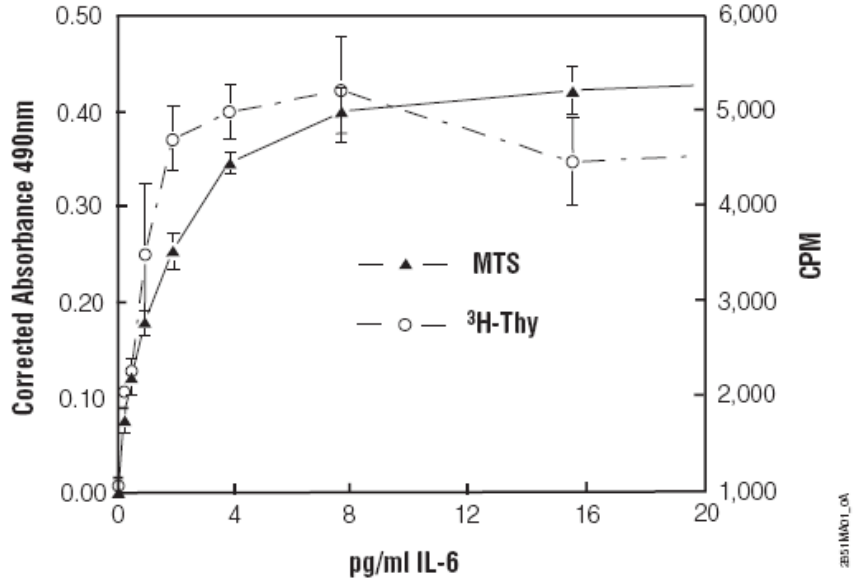
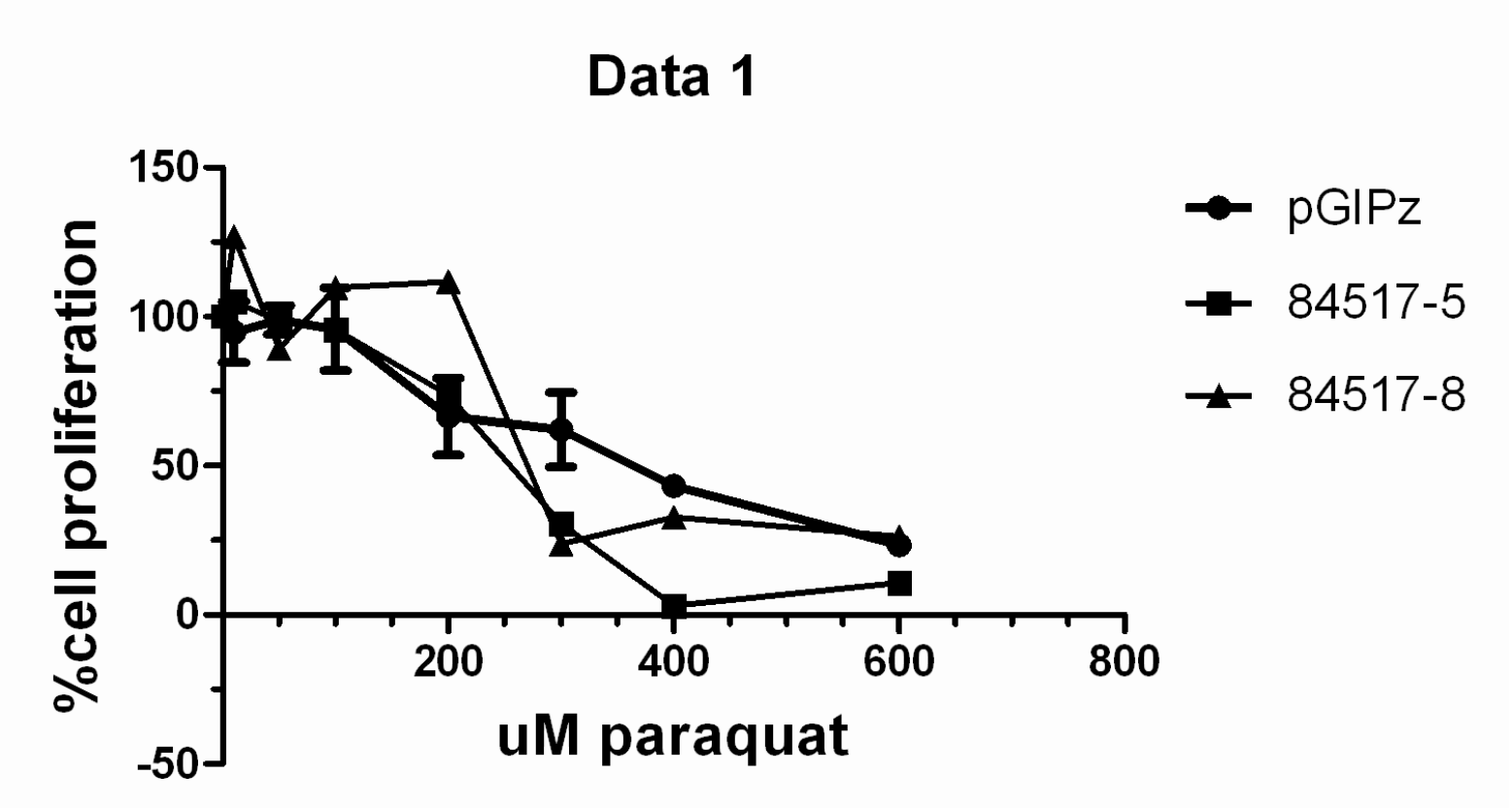
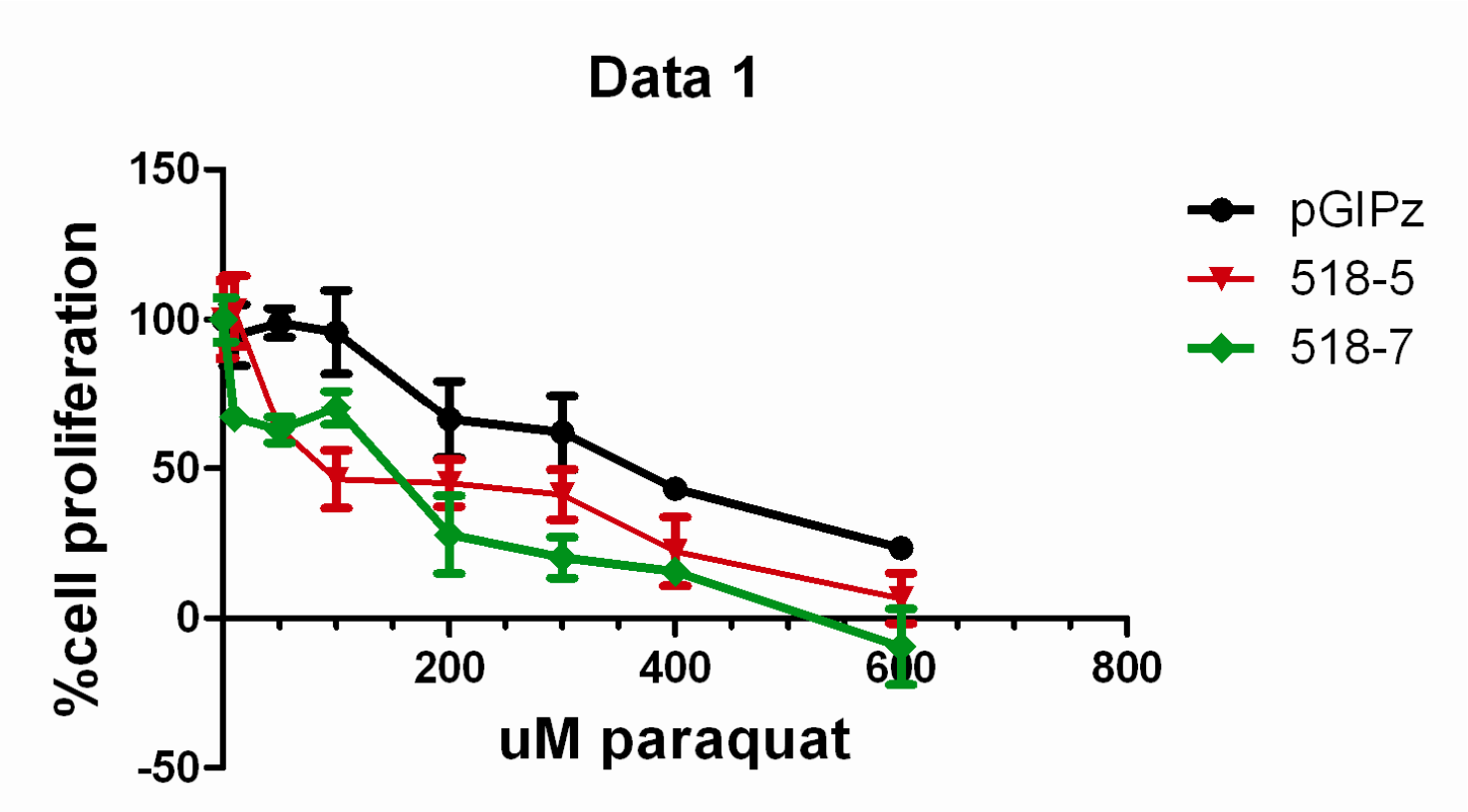


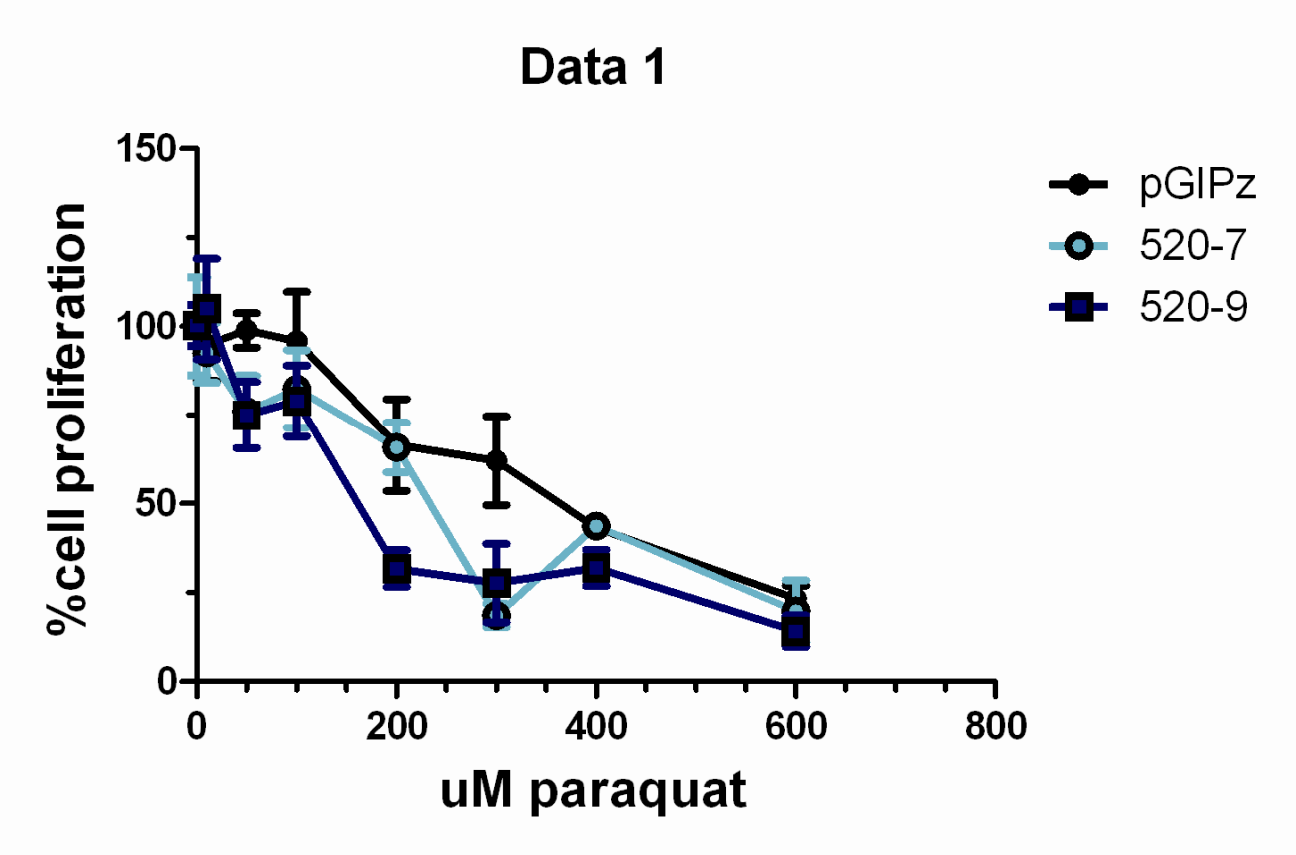
Figure 3. Proliferation of B9 cells in response to various concentrations of IL-6 measured using the CellTiter 96® AQ_{ueous} Assay and [^{3}H]-thymidine incorporation assays.

SOLUBLE, ABORBANCE AT 450-490 nm.

Abs is DIRECTLY PROPORTIONAL TO THE NUMBER OF LIVING CELLS.


Data 1
%cell proliferation
150
100
50
0
-50
200
400
600
800
uM paraquat
pGIPz
84517-5
84517-8


Data 1
%cell proliferation
150
100
50
0
-50
200
400
600
800
uM paraquat
pGIPz
518-5
518-7


Data 1
%cell proliferation
150
100
50
0
0
200
400
600
800
uM paraquat
pGIPz
520-7
520-9

CELL LINE

- PARKIN shRNAmir: started out 27 got 15 different cell lines for 3 different shRNAmirs (pGIPz)
- DJ-1 shRNAmir: started out 9 got 8 cell lines for one pGIPz shRNAmir, started 36 cell colonies, got 38 for 5 different pLKO shRNAs.
- PINK1 shRNAmir: started 18, got 15 different cell lines for pGIPZ shRNAmir
- Alpha synuclein: started at 9, got 9 for one pGIPZ
- ATP13A2: started 9, got 9 for one pGIPz
- sytI: started out 18, got 17 for 2 pGIPz
- sytII: started out 18, got 14 for 2 pGIPz
- **TOTAL TIMES: 4 MONTHS**