Case Study Using Transcreener EPIGEN Methyltransferase Assay for DNA Methyltransferase DNMT1

Customer's goal: To identify DMNT1 inhibitors

Disease implications: Cancer

Hypermethylated tumor suppressor genes are a direct result of aberrant DNMT targeting

Epigenome may play a role in tumor cell resistance to oxidative stress and highlights a potential role for DNMT1 as a potential molecular target in cancer therapy.

DNMT1 and DNMT3b cooperate to silence genes in human cancer cells

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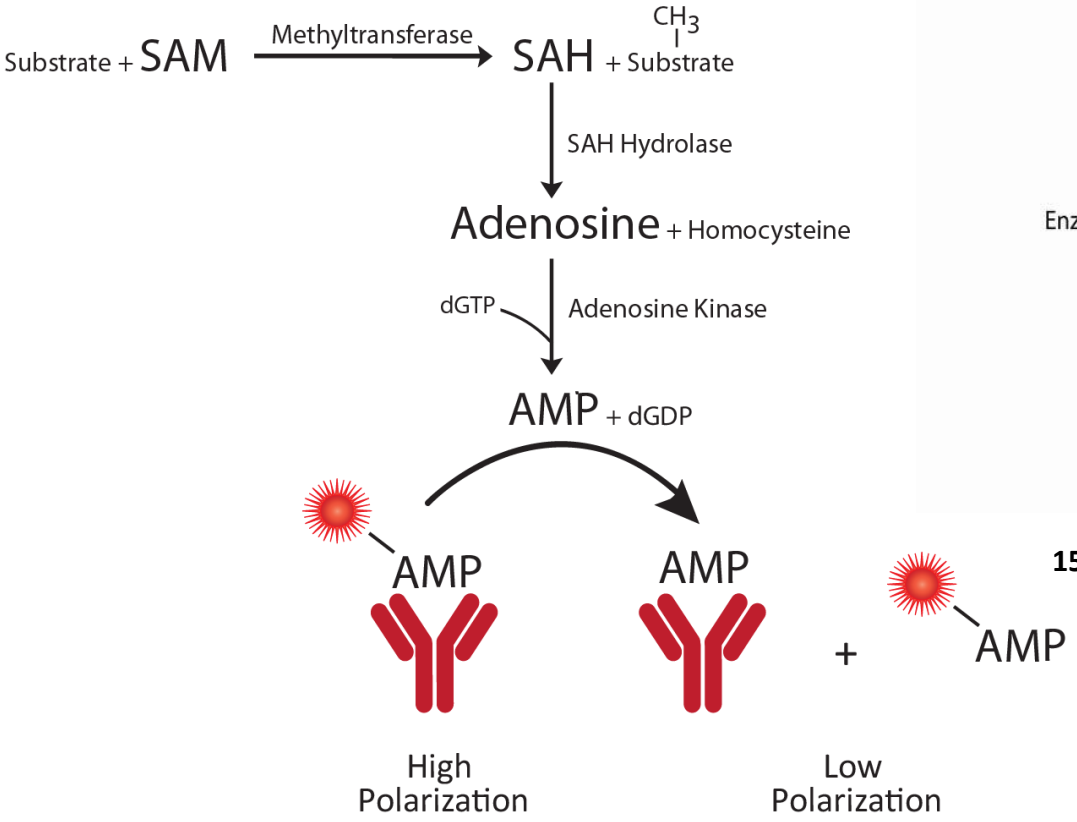
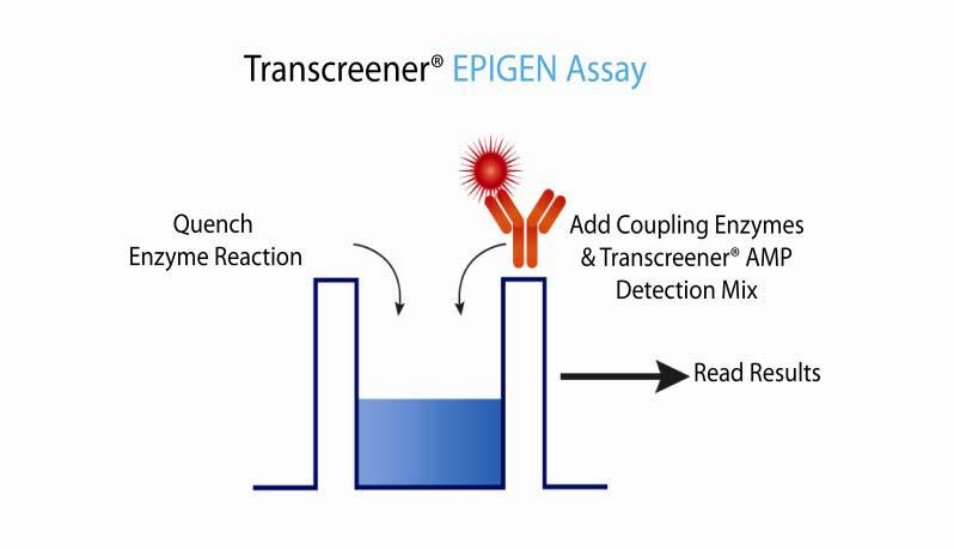
Inactivation of tumour suppressor genes is central to the development of all common forms of human cancer[1]. This inactivation often results from epigenetic silencing associated with hypermethylation rather than intragenic mutations[2–7]. In human cells, the mechanisms underlying locus-specific or global methylation patterns remain unclear[8,9]. The prototypic DNA methyltransferase, Dnmt1, accounts for most methylation in mouse cells[10,11], but human cancer cells lacking DNMT1 retain significant genomic methylation and associated gene silencing[12]. We disrupted the human *DNMT3b* gene in a colorectal cancer cell line. This deletion reduced global DNA methylation by less than 3%. Surprisingly, however, genetic disruption of both *DNMT1* and *DNMT3b* nearly eliminated methyltransferase activity, and reduced genomic DNA methylation by greater than 95%. These marked changes resulted in demethylation of repeated sequences, loss of insulin-like growth factor II (*IGF2*) imprinting, abrogation of silencing of the tumour suppressor gene $p16^{INK4a}$, and growth suppression. Here we demonstrate that two enzymes cooperatively maintain DNA methylation and gene silencing in human cancer cells, and provide compelling evidence that such methylation is essential for optimal neoplastic proliferation.

Transcreener® EPIGEN Methyltransferase Assay





15 µL enzyme + 2.5 µL stop + 2.5 µL AMP Detection Mix

Basic Steps for Optimizing the MT Assay

Exp 1 Determine optimum antibody concentrations and perform standard curve with EPIGEN Methyltransferase reagents.

Exp 2 Perform DNMT1 titrations in 3 buffers and determine optimum enzyme concentration for HTS.

Exp 3 Calculate Z' values.

Exp 4 Assay improvement for catalytic domain.

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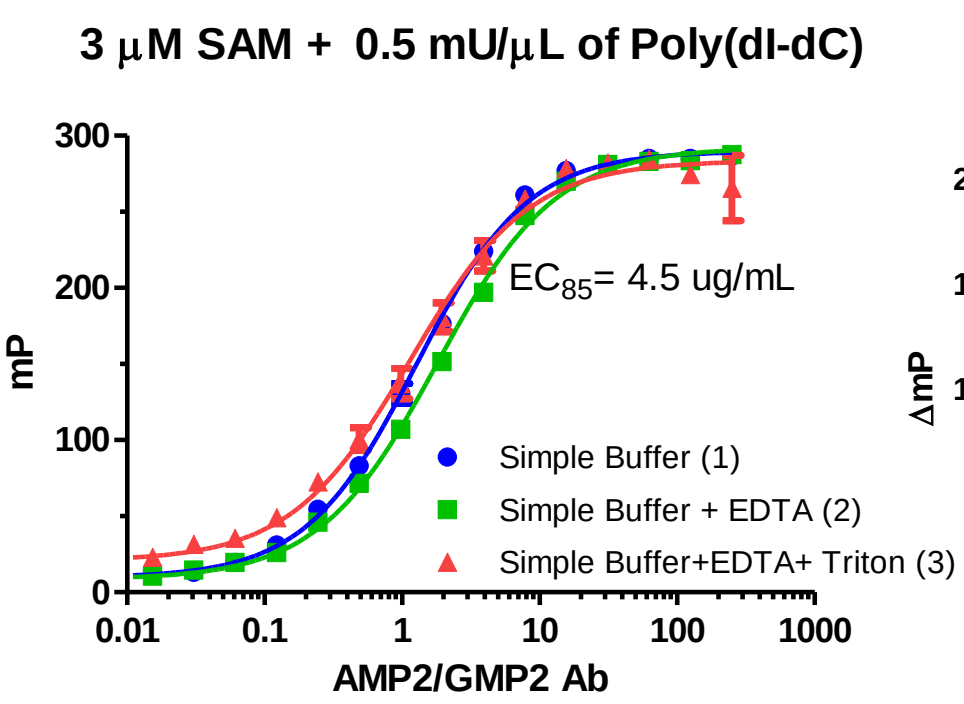
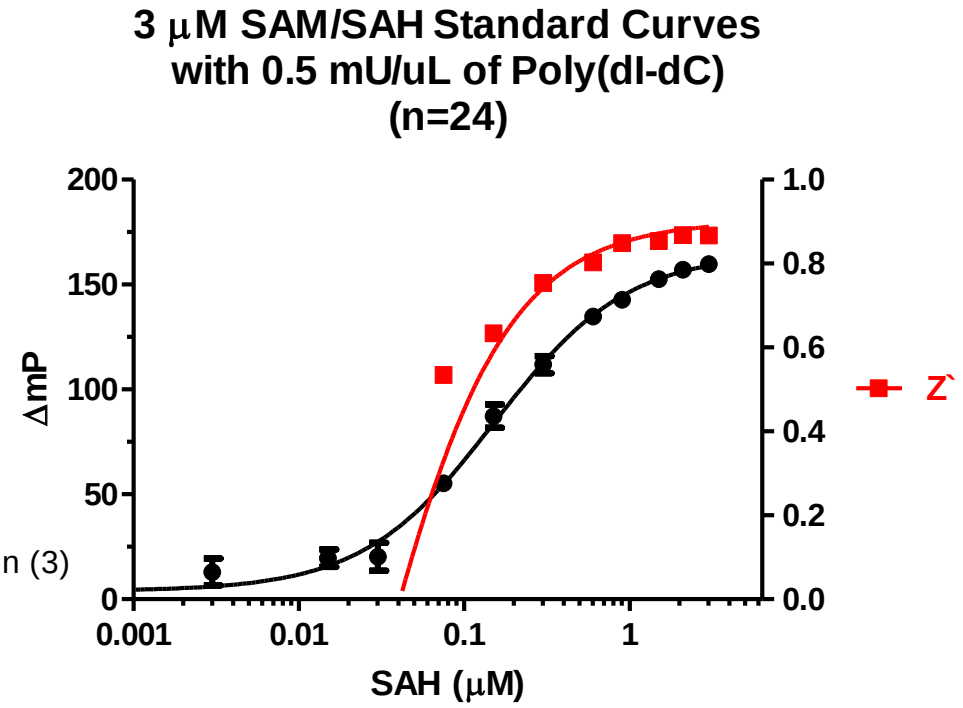
Using the optimum AMP/GMP antibody concentration, excellent Z' values are achieved at < 10% SAM consumption

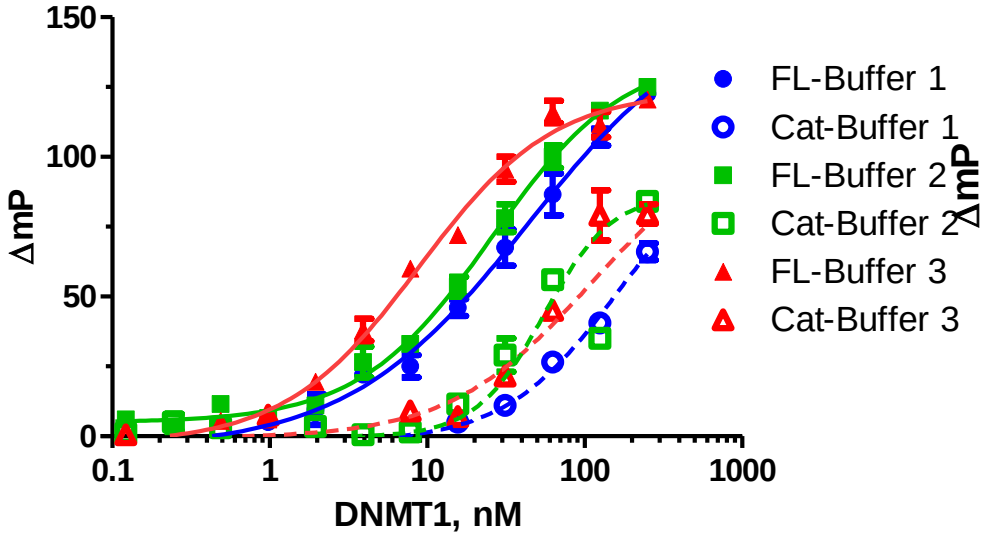


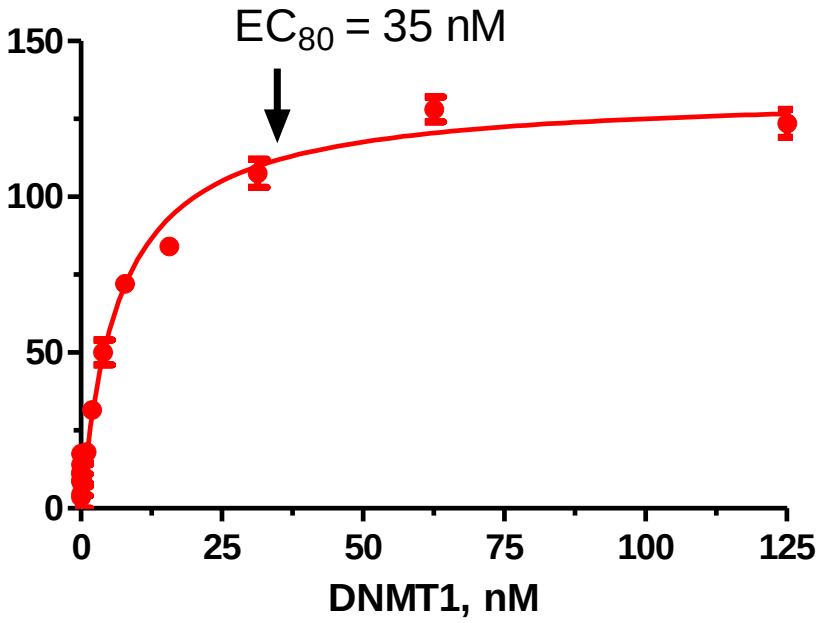


Z'= 0.5 with 2.5% SAM consumption (LLD)
Z'=0.75 with 10% SAM consumption

Simple Buffer: 50 mM Tris (pH 8.5), 1 mM DTT. Reactions were performed at RT

Increased DNMT1 activity is observed with the inclusion of EDTA and Triton X-100





- DNMT1 was titrated in the presence or absence of 3 µM SAM and 0.5 mU/µL of Poly d (I-C).
- 15 µL enzyme reactions were performed at RT for an hour and was stopped using 2.5 µL of stop reagent, followed by 2.5 µL of detection mix.

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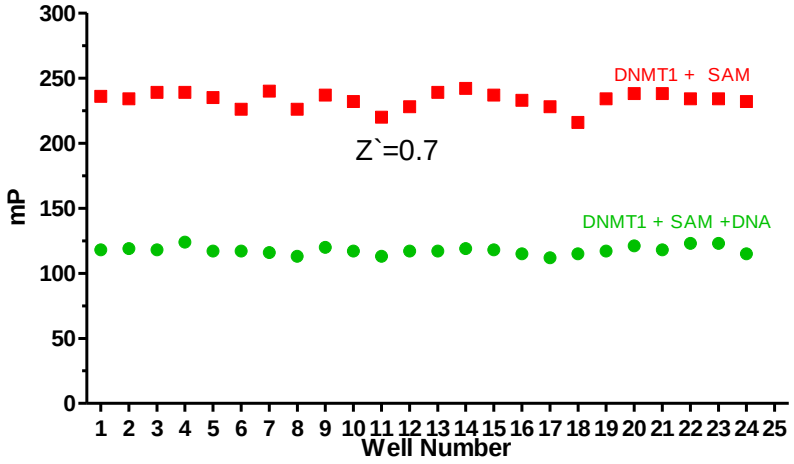
A sensitive HTS-ready assay has been developed for full length DNMT1



	EC80 [nM]	Ave mP	Δ mP	Std. Dev	Z'	Condition
DNMT1, Full Length	35	117	100	3. 8	0.68	E+ Poly(dI-dC)+ SAM
		218		7.5		No SAM
		215		8.9		No Poly (dI-dC)
DNMT1, Cat domain	220	203	37	5.3	0.34	E+ Poly(dI-dC)+ SAM
		243.		3.4		No SAM
		236		3.7		No Poly (dI-dC)

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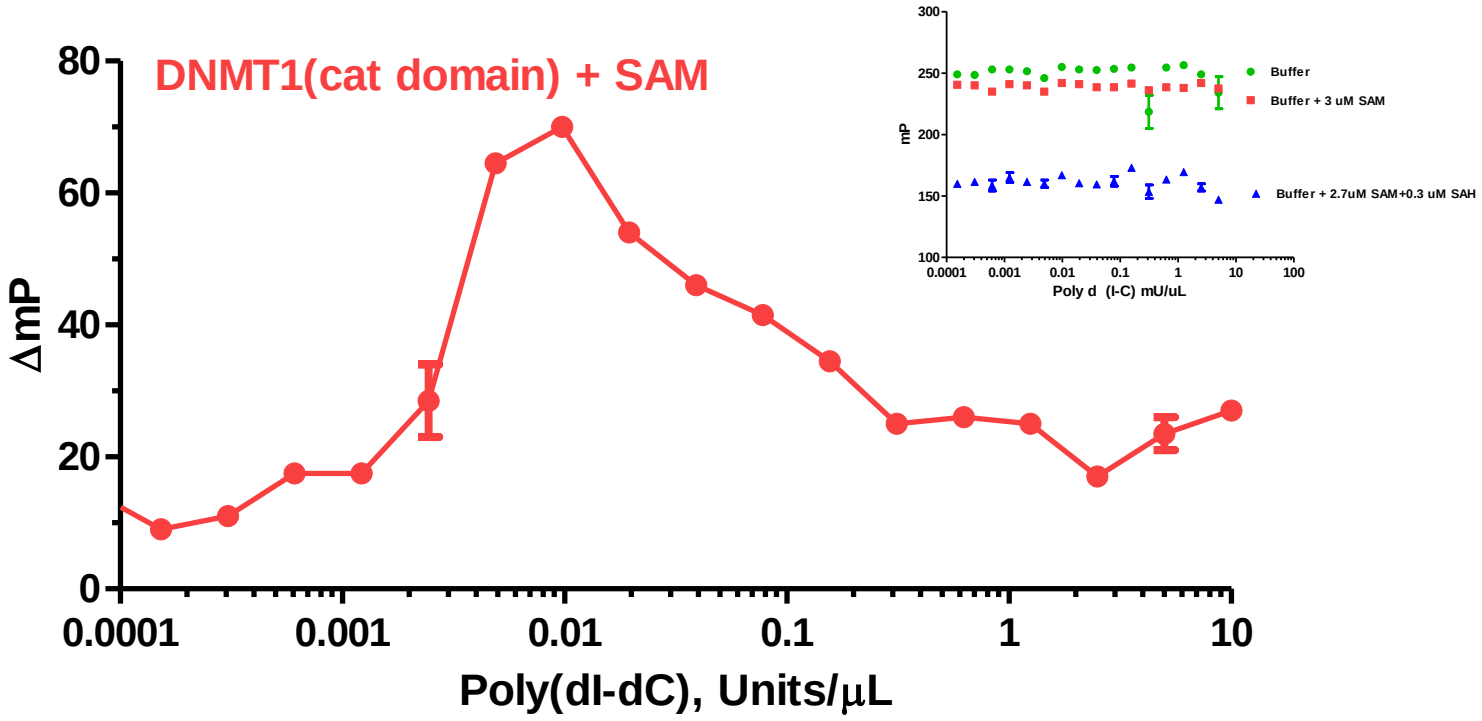
Optimization of the DNA substrate concentration improves DMNT1-catalytic domain activity.



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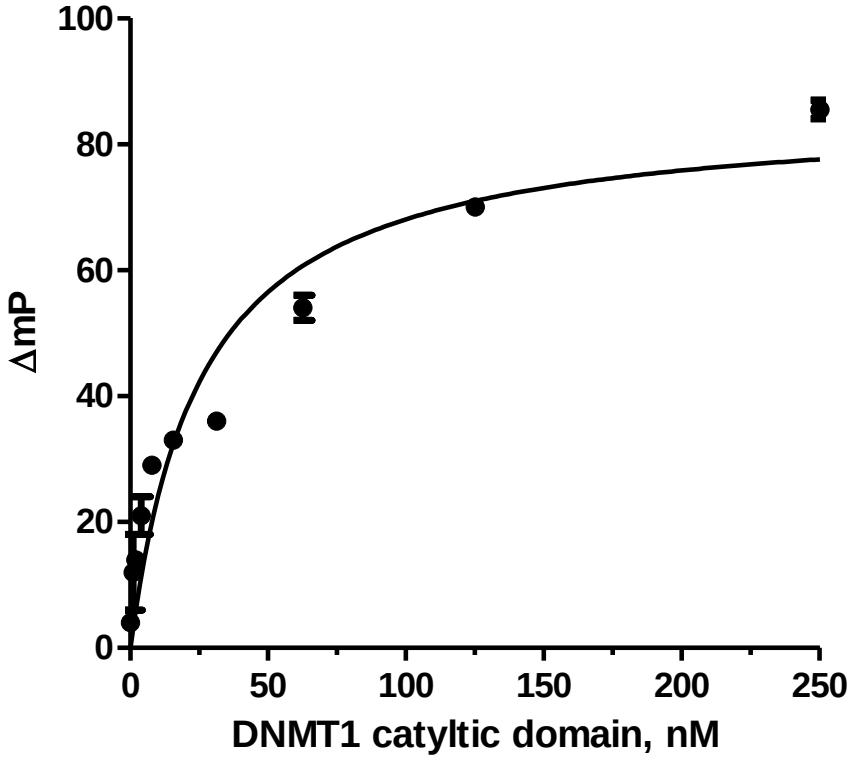
An HTS-ready assay for the catalytic domain is achievable with further assay optimization



- DNMT1 (Cat Domain) was titrated in the presence or absence of 3 µM SAM and 0.01 mU/µL of Poly d (I-C).
- 15 µL enzyme reactions were performed at RT for an hour and was stopped using 2.5 µL of stop reagent, followed by 2.5 µL of detection mix.

Case Study DNMT- Solution!

- An HTS-ready DMNT1 assay was established with the Transcreener EPIGEN Methyltransferase Assay.

- An assay window of >100 mP units and Z'= 0.7 can be achieved with 35 nM DMNT1 when incubated at room temperature for 1 hour using 3 µM SAM + 0.05 units of Poly(dI-dC).

- Inclusion of EDTA and Triton X-100 in the enzyme reaction enhanced the DMNT1 activity.

- The assay window for DMNT1(catalytic domain) was improved by optimizing the Poly(dI-dC) DNA substrate concentration making it amendable for HTS.

Reference:

J Biomol Screen. **January 2012** vol. 17 no. 1 **59-70**
Development and Validation of a Generic Fluorescent Methyltransferase Activity Assay Based on the Transcreener AMP/GMP Assay. Klink TA, Staeben M, Twesten K, Kopp AL, Kumar M, Schall Dunn R, Pinchard CA, Kleman-Leyer KM, Klumpp M, Lowery RG.

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TRANSCREENER® Assay Development Service

Available services include:

1) **Quick enzyme activity test: We will determine the Transcreener reagent concentrations, establish a standard curve and perform an enzyme titration using user-defined reaction conditions.**

Specs: Z' value > 0.6 at <30% substrate consumption

2) **Assay optimization: We will determine the optimal enzyme concentration, buffer composition, and reaction time for maximal signal under initial velocity conditions.**

Specs: Z' value > 0.6 at <30% substrate consumption

3) **IC_{50} determination: We will perform a 12 point dose response experiment, in triplicate, and determine the IC_{50} with inhibitors of your choice.**

4) **Pilot Screen: We will perform a pilot screen of 1120 small molecule drugs.**

Specs: Z value of at least 0.5.