June 10 2004

Experiment: Inoculate Convostat with 100, 200, 400 µl of Enhanced Formula.
Protect was Inoculated with 10^6 CFU per mL of CPC98 N. Gonorrhoea.
Protect Formula was aged 48 hours and Inoculated directly on basis of Mutant Lot #. NO Extraction of DNA was done.
Mutant lot # B-1757
Chloramphenicol lot # 4112854.
Enhanced Formula protect Lot # B-1798 11 FEB 05.

Results

Incubated at 37°C 5% CO_2 for 48 Hours.

Transformants.

1. 100 µl TNTC.
2. 200 µl TNTC
4. 400 µl TNTC.

Oct 22 2004 — Adenine Glycylation + C.C.

Spike 1 pg C.C. DNA freshly extracted from C.C. 99 into fresh ... [illegible] H2O

PCR master mix - 10 mM Tris-(8.3) pH, 50 mM KCl (+) ... 2 mM MgCl, 50 μM deoxynucleotide triphos, 2.5 U Taq Gold, 5% Glycerol

Supplied primers S-1 - S-2 ... to master mix per reaction (100 μl) Reaction

Thermocycler 2-step profile. 25 s denaturation @ 94 C and A 25 s Annealing @ 55 °C total cycles 35.

Real-time amplification to Reaction with ... [illegible] by ... [illegible]

Results.

Hrs.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	5.0	2.1	1.4	1.0	0.9	0	0	0	0	0	0	0	0	0	Guanidine 1M
	5.0	2.6	1.8	1.9	1.0	0	0	0	0	0	0	0	0	0	Sodium Thio 2M
	5.0	3.0	2.1	1.8	0	0	0	0	0	0	0	0	0	0	0.1 M EDTA
	5.0	4.8	4.6	4.2	4.1	4.0	3.9	3.7	3.8	3.23	3.3	3.2	2.99	2.95	1M Guanidine HCl + 4M Adenine + 0.01 [illegible]
	5.0	4.23	4.5	2.99	3.8	3.99	3.7	3.68	3.6	3.5	3.29	3.1	3.0	2.79	Sodium Thio 1M 0.1M EDTA + 2M Adenine
	5.0	4.7	4.6	4.5	4.5	4.3	4.5	4.4	4.4	4.3	4.3	4.2	4.3	[illegible]	Sodium Thio + 0.1M EDTA 1M Adenine

Conclusion.

1. Adenine increases preservation.
2. Preferred combination. 1M Sodium Thio, 0.1M EDTA + 1M Adenine

Note; other reagents of similar substance may be effective in stop reagent ... [illegible] ... [illegible] to be continued.

Exp RM **21 DEC 04** Data entered [illegible] [illegible] Lab -
Survival of AF068706 (F2AD). (NM 015476)

100 ml of fresh filtered urine were spiked with: E1 - E2, E3
Ubiquitin Activating Enzyme 3 [illegible] [illegible]. 2gs protease was
additionally 2.35 protein was added 0.062 ml per 200 ml reaction.
AF068706 was added. [illegible] and [illegible] [illegible] added and
recorded [illegible]. Antibody dilution 1:1000
[illegible] [illegible] [illegible] not sure which will be responsible for
protection [illegible] [illegible] from that data base
microtitre [illegible] [illegible] to total [illegible] kg protein for urine [illegible]
with [illegible] [illegible]. Read @ 540nm.

Results

Hrs	0.5	1.0	1.5	2.0	2.5	3.0	
	4.10	3.9	3.6	3.5	3.1	3.0	F_2 Controls.
	4.12	0	0	0	0	0	Unprotected 015476
	4.10	3.8	2.1	2.83	2.70	2.46	2M Sodium [illegible] + F/[illegible]

Results - seems to be protection of NM 015476. [illegible] of microtubules.

Notes

Immunoassay would be preferable due to issues with
amplification and would give more specificity to [illegible].
Repeat experiment using [illegible] monoclonal [illegible].
to [illegible] protein.

05 FEB 05. Survival of Ubiquitin activating Enzymes Ubc2 (E-2) and Ubc3 E-2 In urine with and without Sodium [illegible] 2(M) +EDTA 0.1 M.

200 ml of Fresh collected urine was Incubated with 0.1 mg of Ubc2 (E-2) and Ubc3 -E2. [illegible] For 1 hr @ 37°C. [illegible] for each Enzyme [illegible] of [illegible]. Anti Ubc2 and Ubc3 [illegible] were used on [illegible]. [illegible] antibodies were [illegible] for 20 min [illegible] at [illegible].

Results. Dil 200 µl into 1 ml sterile PBS for Enzyme Reaction

Dil UB2 - UBC3 separately

HBS

0	1	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	
4.5	4.38	4.11	4.0	3.98	4.0	3.88	3.85	3.8	3.9	E_2 Controls 100g
4.5	3.1	1.8	2.74	1.82	1.11	096	.0310	[illegible]	[illegible]	Ubc3 + 2M sodium thio + 0.1 M EDTA
4.5	3.5	2.7	2.10	2.07	1.30	1.22	0.60	0.3	[illegible]	Ubc2 + 2M sodium thio EDTA

Results - Chemistry Has activity Against Ubc-2-3 Enzymes.

Notes.

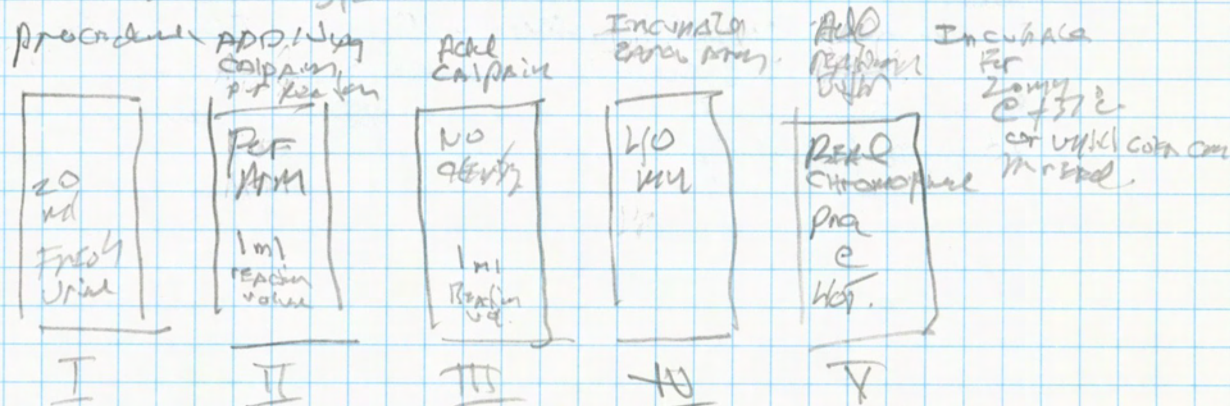
B [illegible] to ensure [illegible] need to go to Immuno [illegible] format and tagged with [illegible] HRP. Reaction [illegible].

Also need to Reduce Background of Experiment [illegible] getting to [illegible] In [illegible] Assay.

26 Nov 05

Calpain protection experiment.

1. Method — M-Calpains I and II clone P, IC Antibody. Conjugated to YVAD, pNA substrate
2. Antibody diluent PBS 10% solution.
3. Antibody dilution 1/1000 fz at -20°C + 10% glycerol
4. Calpain I - fz at -70°C stabilized with 20 Mm Imidazole, 5 Mm β-mercaptoethanol, 1 Mm EDTA + 1 Mm EGTA + 20% glycerol.
5. Reaction buffer = 10 Mm DTT per 1 ml sample 50 µl
6. 5 µl of 4 Mm of YVAD-pNA substrate (200 µM final concentration).
7. Incubated 37°C for 40 mins.
8. Blank spec with PBS @ 405 nm read background and zero spec.



Test chemistries (Arms).

1. Sodium Thiocyanate only
2. Guanidine Thiocyanate only.
3. Guanidine only
4. Sodium perchlorate only
5. EDTA only
6. Sodium Thiocyanate + EDTA
7. Guanidine Thiocyanate + EDTA
8. Guanidine + EDTA
9. Sodium Perchlorate + EDTA
10. Lithium chloride + EDTA

Read each arm at 1 hrs interval.

Results.

Bold stone plot graph

HRS 1	2	3	4	5	6	7	
5.00	0.8	⊖	⊖	⊖	⊖	⊖	Sodium Thio
5.00	1.00	⊖	⊖	⊖	⊖	⊖	Guanidine Thio
5.0	1.05	0.07	⊖	⊖	⊖	⊖	Guanidine.
5.0	1.41	⊖	⊖	⊖	⊖	⊖	Sodium perchlorate.
5.0	1.21	0.86	⊖	⊖	⊖	⊖	EDTA only
5.0	4.50	2.5	1.98	1.0	⊖	⊖	Sodium Thio/1M + EDTA
5.0	4.20	3.5	3.4	3.6	3.5	3.2	Guanidine Thio + EDTA + EDTA.
5.0	4.6	3.7	3.8	3.7	3.5	3.2	Guanidine + EDTA
5.0	4.0	3.7	3.8	3.5	3.6	3.2.	Sodium Perchlorate + EDTA.
5.0	4.3	3.3	3.7	3.2	3.4	3.1	LiCl + EDTA.