BACKGROUND:

The randomness of X chromosome inactivation in 46,XX female tissues provides a marker system to distinguish monoclonal from polyclonal proliferations. A chemical modification of DNA, methylation, has been associated with many polymorphic genes on the inactive X chromosome. Thus, it is possible with G6PD, HPRT, and the androgen receptor to directly identify the inactivated X chromosomal DNA by methylation-specific restriction enzyme digestion.

Oligonucleotide PCR primers (Designated AR-a/b, sequence in reagent section at end) directed to the androgen receptor gene at Xcen-Xq13 flank a trinucleotide repeat element and two *Msp*1/*Hpa*II sites methylated on the inactive X chromosome. Of 8 patients evaluated thus far, 6 (75%) have a heterozygous genotype demonstrable from use of these primers and simple PAGE. Clonality is scored by comparing the PCR products of undigested, *Hha*1 digested genomic DNA. *Hha*I will selectively cut the activated allele between the primer sites, preventing amplification. If the source DNA is monoclonal, only one allele would amplify; both if polyclonal. *Hpa*II digestion is too unreliable to contribute to interpretation of results.

Since interpretation of results is based on biased amplification of specific alleles following *Hha*1 digestion, it is crucial to rule out amplification bias on undigested sample. Whenever possible amplify undigested normal and tumor DNA from the same case to ascertain bias not due to digestion process.

Citations:

1)General Method reference, incl primers: Mutter GL, Chaponot M, Fletcher J. A PCR assay for non-random X chromosome inactivation identifies monoclonal endometrial cancers and precancers. Am J Pathol 1995; 146:501-508.

2)Use of deaza GTP: Mutter GL, Boynton KA. PCR bias in amplification of androgen receptor alleles, a trinucleotide repeat marker used in clonality studies. Nucleic Acids Res 1995; 23:1411-1418.

3)Result Interpretation: Mutter GL, Boynton KA. X chromosome inactivation in the normal female genital tract: Implications for identification of neoplasia. Cancer Res 1995; 55:5080-5084.

PROTOCOL

General Comments:

Incomplete digestion caused by inappropriate electrolyte composition must be avoided by careful attention to the buffers needed for digestion. Digested DNA still in

digestion buffer (usually a high Mg++ solution) must be removed from the buffer or substantially diluted when added to the PCR reaction to avoid increasing Mg++ during PCR (increases nonspecific bands).

The sensitivity of this technique can detect DNA from a single cell, making results erroneous if a reaction is contaminated. Always wear gloves. The amplified PCR products should be kept away from the areas and pipets used to prepare stocks and the pre-PCR reaction mixtures. Opening and closing tubes, as well as running a microfuge generates substantial aerosol that can contaminate a reaction. For this reason, all stocks (esp. oligo masters) should be handled in the hood using special PCR pipet set. This is essential for any PCR detection requiring more than 40 cycles.

DNA ISOLATION:

FRESH TISSUE:

Standard Bead Beater micro scale or grind up large scale isolation by Pro-K digestion and extraction cleanup is adequate. BE SURE DNA NEVER IS HEATED ABOVE 60'C, as this will denature it and prevent digestion. High salt extraction protocol doesn't work well for unknown reasons (as per Sklar lab).

PARAFFIN TISSUES:

Avoidance of HEATING ABOVE 70'C is essential, and makes usual Crum Chelex protocol useless. That suggested below is a composite of several methods, with a special cleanup at the end with EtBr.

CUTTING SECTIONS

1) Set up the microtome with disposable blades after carefully and completely wiping down the knife area. Best to wipe with xylene impregnated wipe to remove all traces of old paraffin.

   Chill blocks to be cut by placing in freezer (-20'C) for 1 hour. Longer is OK.

2) Using GLOVES, mount the block and orient chuck so the the block face will cut evenly. orient in both the right-left axis and up-down axis.

3) Lightly face the block to remove loosely adherent contaminants from the surface. Using a clean Kimwipe, rub down block face with warm water. Rub ice cube on block face for 2-3 seconds (Repeat when needed).

4) Cut two 4um thick paraffin sections (floated on water) for H&E. This will be used to map area to be inked.

5) For UV-PCR cut 7um paraffin sections and pick up on regular glass slides (from

the box, no extra washing). 1-2 cuts per slide, depending on face size. Important to not let sections sit long in water bath, or DNA will be leached out. Use four slides per isolation tube.

6) Cut "tube" sections for PCR at 10um thickness. Cut section, then pick up ribbon or rolled paraffin with clean wooden stick or eppendorf tips. Transfer to 1.5ml screw cap microfuge tube. Use 3 sections per tube. Only consider increasing the number of sections per tube if the area of tissue cut is very small (< 0.5 cm^2) Cap and label.

6) Re-clean, and repeat above for new block.

2) Bake slides overnight at 37-55'C, or 2-3 hours at 58-62'C.

UV IRRADIATION:

This is an optional step to isolate DNA from only a part of a histologic section.

1) Under dissecting scope delineate the area desired by circling it on the H+E stained coverslipped slide with a fine felt marker. Write date of UV irradiation on circled slide so that it serves as a record of what area was selected.

2) Prepare one 1.5 ml tube containing 0.5ml of Waterproof Black Opaque ink (Perfex# Corp.: 1-609-399-6767) for each case to be processed.

3) Under darkfield dissecting microscope apply ink with a 1-200ul bevelled pipet tip to areas from which DNA is desired. This is the same area previously delineated in the H+E slide.

4) When ink is dry, Place slides in Stratalinker 1800. Set dose at maximum energy (9999 x 100 ujoules/cm2) and expose, carefully measuring the time needed for completion. Then, use the exposure time option to expose the slides for an additional 19 times the measured interval. (Total exposure 20 x 9999 x 100 ujoules/cm2, or 20 joules/cm2). Expect about 1 1/2 hours total exposure time.

5) Use a straight edge single sided razor to scrape irradiated tissue section from the slide. This will usually curl and be easy to place in a screw-top 1.5ml centrifuge tube. Don't unecessarily scrape areas that are not inked, as these DNAs are not desired anyway.) Put scraped material from about 4 slides in each tube.

LYSIS

1) The paraffin section will usually fragment in the tube, and may fly out when opened. Tap tube to bring tissue fragments into the bottom. DO NOT centrifuge, as this will pack the paraffin fragments into an undigestible mass. If needed gently push down paraffin fragments using large end of 200ul pipet tip.

2) Add 300ul Lysis buffer per tube. AGITATE by vortexing vigorously for 5-10 seconds. Tap tube to bring tissue fragments and lysis buffer droplets into the bottom. DO NOT centrifuge, as this will pack the paraffin fragments into an undigestible mass.

Lysis/Solubilization Buffer for paraffin sections

Component	Stock	Final	Vol. for 5ml	10ml
Proteinase K	10mg/ml	200ug/ml	100ul	200ul
Tris pH 8.3	1M	50mM	250ul	500ul
Carrier Glycogen	20mg/ml**	100ug/ml	25ul	
Tween 20	50%*	0.5%	50	100
Calcium Chloride	0.1M	1.5mM	75	150
Water		to vol	4500ul	9050ul

*Tween is manufactured at 100%, but should be diluted with an equal volume of water to reduce viscosity and make pipetting easier.
**Carrier mussel glycogen: Sigma Cat#G1508 (alternate is Carrier RNA= 16s+28s e.coli rRNA (Boehringer Cat.#206938)

3) Warm to 61'C to melt wax, and mix vigorously on vortexer, and tap on counter to get all fragments under fluid level.

4) Incubate tubes at 60-61'C for two days. Best to use oven to provide even heat. Spike twice with additional Pro.K (every 12-24 hours). When spiking, add 6ul of 10mg/ml ProK to each 300ul tube. Mix again.

CLEANUP

For the real connoisseur, this generates DNA of high purity for delicate enzymatic manipulations, at an efficiency reduced from the preceeding. Since no boiling is used, isolated DNA wil be double stranded and amenable to restriction digestion.

1) At the end of the digestion interval add 2 ul of 0.5M EDTA (final about 3mM EDTA) to chelate Ca++. This will chelate the Ca++, and prevent it from interfering with phenol extraction. Vortex vigorously. Let sit for 1-2 minutes before proceeding with phenol cleanup.

2) Extract with an equal volume (300ul) of Phenol, vortex and let sit for 1-2 minutes. This will break up the ink. Remove auqeous phase to new tube.

Repeat once more with another 300ul phenol (Total of two phenol extractions).

3) Add 36ul of EtBr (stock 5mg/ml in water). Mix well. Let sit for 1-2 minutes. Mix again. CAUTION: when adding EtBr, swirl pipet tip a bit to mix, and prevent precipitate.

4) Add 1/2 volume (160ul) of 7.5 M AMMONIUM Acetate. Mix well.

5) Extract with equal volumes (about 500ul) of the following:
Phenol/Chloroform x 1
Chloroform x 3

6) Remove aqueous phase to fresh tube.

7) Add 1 drop of reconstituted Chelex-100 to aqueous phase. Use 1 ml serological pipet. Vortex Gently. Let Stand 5 minutes, vortexing every few minutes.

8) Spin briefly, and remove aqueous phase to fresh tube, leaving Chelex beads behind. This is best done with a P200 tip which is too small a diameter to pick up beads. BE SURE NOT TO TRANSFER BEADS, AS THEY WILL inhibit any reactions to follow.

9) Add 2 volumes (800ul) of cold 100% ethanol.

10) Incubate overnight at -20'C (preferred, minimum of 6 hours at -20'C). Pellet DNA with 20 minute spin at 4'C in eppendorf microfuge. Decant supernatant without warming tubes. Best done by putting tubes in Stratacooler during tranfers.

11) Wash pellet in ice cold (from -20 freezer, keep on ice when working at bench) 70% ethanol. Vortex briefly but vigorously from temporary storage on ice (or Stratacooler) enough to disaggregate pellet. If pellet doesn't disaggregate after 1-2 minutes of vortexing, this is ok and you should stop trying to break it up. Re-precipitate pellet by spinning at 4'C for 10 minutes. Decant and dry briefly (residual ETHANOL INHIBITS digestion!!!).

SAMPLE RESUSPENSION PRIOR TO DIGESTION

Paraffin DNA: DNA from one block in 40ul of Hha1 buffer.
Fresh(frozen tissue) DNA: 4ug of DNA in 40ul of Hha1 buffer, as below.
ALWAYS re-precipitate DNA stored in aqueous form so it can be resuspended in optimal buffer.

1) Resuspend pellet in 40ul 1x Hha1 Buffer supplemented with glycogen (New England Biolabs buffer #4, green stripe tube), and 1ul of marker phi-X RF DNA at RT. Vortex gently, let sit, vortex again. Incubate at 37'-50'C briefly (10 min-

45 min) to solubilize completely. Vortex again and spin briefly to collect droplets.

***Hha*1 Suspension Buffer:**

Component	Stock	Desired	Vol for 500ul	Vol for 1ml
NEB Buffer#4	10x	1x	50ul	100ul
NEB Albumin	10mg/ml	100ug/ml	5ul	10ul
Glycogen	20mg/ml	500ug/ml	12.5ul	25ul
Water		to vol	432.5	865ul

2) Split samples into two tubes of 20ul each for digestion, as below.

DNA DIGESTION: May be from frozen or paraffin tissue

1) Begin with:
"D" Tube: 20ul Aliquot of solubilized DNA in a tube labelled for HhaI digestion.

"U" Tube= 20ul "undigested' This sample will be treated identical to "D" except not get endonuclease.

2) "D" = 20ul "digested" tube.
Paraffin: Add 1ul of NEB high concentration HhaI (at 20U/ul) to the "digested" tube with 20ul DNA. Mix in vortexer, pellet sidewall drops with quick spin, and incubate at 37'C. Always incubate undigested control tube at the same time.
Fresh: Add 1ul of NEB high concentration HhaI (at 20U/ul) to the "digested" tube with 20ul DNA, and an additional 1ul at 1 hour. Mix in vortexer, pellet sidewall drops with quick spin, and incubate at 37'C. Always incubate undigested control tube at the same time.

Incubation interval depends on amount of DNA:
Paraffin DNA: section DNA overnight.
Fresh DNA: (1ug/tube) overnight incubation.

Optional Digestion Check:
Check completeness of digestion by running 1 ul of cut and uncut samples on A 1% (high melting point) agarose midi-gel. When digestion is complete, expect to see no full length DNA left.

Intervention, if needed:
If some samples are incompletely digested, add another 1ul of Hha1, mix, pellet sidewall, and incubate for several more hours. Always re-check these samples for digestion completeness by running another midigel before inactivating the enzyme.

3) Inactivate enzyme as follows[@]:
   -increase volume to 220ul by adding 200ul water
   -add 100ul phenol and vortex, sit for a few minutes
   -add 100ul chloroform and vortex vigorously
   -spin in eppendorf for 5 minutes

   @:J.Singer-Sam recommends this over heat inactivation, as heating may produce transient enzyme configurational changes which alters specificity of cutting.

4. -remove aqueous phase to new tube.
   -add 25ul 3.0M SODIUM Acetate
   -add 500ul ice cold 100% ethanol and mix
   -allow to precipitate O/N at -20'C (preferred, minimum is 6-8 hrs.).

5. Recover DNA precipitate by spinning.
   Spin at 4'C for 20 min. to get pellet. Decant.

6. Wash pellet with with ice cold (from -20'C freezer) 70% ethanol. THIS IS IMPORTANT to do well. Keep tube cold on chilled rack, and vortex intermittently to knock pellet off tube tip, and solubilize residual salt. Gently dry Pellet.

7. Resuspend "digested" and "undigested" pellets in 1x PCR buffer. For paraffin DNA use 10ul buffer/tube. For 1ug fresh DNA, resuspend in 20ul/tube to yield 50ng/ul.

SET UP PCR REACTION.

Amplify DNA from each tube in a separate PCR reaction. Mg++ in digest will interfere with PCR if more than 1ul is added to a PCR reaction.

1) Determine the number of (50ul) reactions to be performed.

   Standard Controls include:

   a)Negative control: Complete reaction (with Taq, oligos) missing target template. Should yield no product.

   b)Positive Control: 100ng of biallelic female DNA

   c)Clonality Control: To check assay performance, include informative DNA from a monoclonal tumor (leiomyoma is good).

2) Change to a pair of CLEAN GLOVES and:

Make "Master Mix" for above reactions in a single tube. Mix two extra aliquots so pipetting errors won't cause shortage. Taq is expensive so don't overmix! This Master Mix is made up at 90% of final Volume, to permit addition of oligos and template without dilution.

* USE CLEAN PCR PIPET SET FOR ALL PIPETTING OF THESE STOCKS****
These pipets should NEVER be used to pipet PCR REACTION PRODUCTS.

"Master Mix"

Component	final	Stock	Final for 50ul
PCR Buffer*	x	10x	5.0
Water	to 90%		38.8
dNTP Mix	0.2mMea	2mMea	5.0
Oligo AR-a	0.3 uM	30uM	0.5
Oligo AR-b	0.3 uM	30uM	0.5
32PdTTP#	0.06uM	12.5uM	0.25

ICN #33015H: 32P dTTP; 10mCi/ml, >600Ci/mMol, 16.7uM
#NEG005A: 32P dTTP; 10mCi/ml, 800 Ci/mMol, 12.5uM

3) To above master mix add 50ng template DNA (1ul of digest or control tube above, which is at 1ug/20ul=50ng/ul), directly from digestion reaction. Do not add more than 1ul of DNA per tube! For paraffin DNA isolates, use 1ul of 10ul solubilzation buffer/tube.

4) Aliquot 45ul of Master Mix into individual tubes labelled to receive template.

5) Overlay reaction mixture with 30ul of Mineral Oil.

6) Prepare Taq for Hot start: Determine # of reactions and scale up formula below. use 0.25ul 5U/ul Taq per 50ul PCR. Dilute Taq in 1x PCR reaction buffer so that has 0.25ul Taq/5ul 1x PCR buffer.

Component	Final	Stock	Taq for 1 Rxn
Water	to vol		4.25
PCR buffer	1x	10x	0.5ul
Taq	1.25U/5ul	5U/ul	0.25ul

Final Vol per 50ul Rxn 5.0

7) Load onto PCR machine and run.

Profile for AR-a/b

Temp.	Time	Cycles
97	5 min.	1
85	10 min.	1 HOT START < Add 1.25U Taq in 5ul of 1x
95	4 min	}
55	45 secs	} 3 cycles
72	90 secs	}
95	30 secs	}
55	45 secs	} 23 (total 26 cycles) cycles (50 ng fresh DNA)
72	90 secs	} 26 (total 29 cycles) cycles paraffin DNA
72	7 minutes	1
4	Hold	1

Profile for GP-a/b

Temp.	Time	Cycles
97	5 min.	1
85	10 min.	1 HOT START < Add 1.25U Taq in 5ul of 1x
95	4 min	}
50	45 secs	} 3 cycles
72	90 secs	}
95	30 secs	}
50	45 secs	} 23 cycles (50 ng fresh DNA)
72	90 secs	} 25 cycles paraffin DNA
72	7 minutes	1
4	Hold	1

CAUTION: THESE MANIPULATIONS ARE "POST PCR"
PCR products can easily be spread around and contaminate genomic DNA or reagent stocks, causing failure of future runs. Treat PCR products as if they are infectious, and change disposable work surfaces/gloves frequently.

There is a "dirty" post-PCR 20ul Ranin pipettor under the gel boxes which can be used for these steps.

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7) Prepare sample for loading on the gel. Add 4.0ul of Bromophenol Blue/Xylene Cyanol/ Ficoll to an empty 0.5 ml tube. To this, add 20 of post-PCR reaction products. Mix by repeated aspiration/expelling of the 20 ul sample.

8) Load 4ul of sample/lane on 8% polyacrylamide gel using FLAT TIPS.

To pour 100ml 8% polyacrylamide gel:

Compound	Stock	Vol. for 100ml
Acrylamide	30%	26.6ml
TBE	5x	20ml
Water		52ml
Am.Persulfate	10%	1ml
TEMED		20ul

Size Markers: Best is Phi-X digested with HaeIII: END LABELLED with 32P

Phi X; Hae III
1,353
1,078
872
603
310
281
271
234
194
118
72

8) Run Gel in 1x TBE at Room Temperature.

Run Bromophenol blue off gel, and xylene cyanol to within 3inches of bottom. The xylene cyanol will run off gel before AR products.

Voltage	Estimated time
250V	Overnight + half a day
300V	Overnight (~18 hours)
600V	6-7 hours

9) Transfer gel to 3M paper (careful, RADIOACTIVE).

10) Dry gel in slab dryer.

11) Expose pre-flashed XAR film at -70'C.

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REFERENCES:

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2. Bartlett MH, Adra CN, Park J, Chapman VM, McBurney MW. DNA methylation of two X chromosome genes in female somatic and embryonal carcinoma cells. Somat Cell Mol Genet 1991; 17:35-47.

3. Toniolo D, Filippi M, Dono R, Lettieri T, Martini G. The CpG island in the 5' region of the G6PD gene of man and mouse. Gene 1991; 102:197-203.

4. Vogelstein B, Fearon ER, Hamilton SR, Feinberg AP. Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors. Science 1985; 227:642-645.

5. Vogelstein B, Fearon ER, Hamilton SR, Preisinger AC, Willard HF, Michelson AM, Riggs AD, Orkin SH. Clonal analysis using recombinant DNA probes from the X-chromosome. Cancer Res 1987; 47:4806-4813.

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REAGENTS

Acrylamide:

30% stock:
29g acrylamide, 1g bis-acrylamide, in 100ml water
mix and sterile filter
Store at 4'C in foil covered bottle.

Loading Buffer:

Uses Ficoll, reducing trailing effect.
Need FLAT TIPS to load
10x buffer, add 1/10 vol to sample to run

Compound	**Stock**	**10x**	**Vol.Stock for 5ml 10x**
Tris Base	solid	108g/l	0.540g
Boric Acid	solid	55g/l	0.275g
EDTA(MW372		0.5M	0.930g
Ficoll	solid	30%	1.500g
Xylene Cyanol		0.5%	0.250g
Br-Ph-Blue	solid	0.5%	0.250g

Alternate Loading Buffer:
00.5% Bromophenol blue
00.5% Xylene Cyanol
50.0% Glycerol
Water (DEP) to volume

Calcium Chloride Dihydrate; 100mM solution

MW 147.02
for 100ml of 100mM (0.1M) solution add 1.47g of CaCL2(dihydrate) to 100ml water.

Chelex 100, Biotechnology Grade: (Bio-Rad Cat.#143-2832)

Suspend in water and autoclave.
Check pH. Should be 8-9.
Note: if excessively alkaline, add 1/10 volume 1M Tris pH 8.0 to reduce pH to 8.0-8.5 (recheck with pH paper)
Store at RT as a slurry.

Deaza-GTP, 10x dNTP mix:

substitution of deaza-GTP for dGTP reduces secondary structure and improves amplification efficiency of the AR-a/b template which is GC rich and has lots of inhibitory secondary structure.

Reagent	Vendor	Cat#	Conc	Amount
dATP	Pharmacia	27-2050-01	100mM	250ul
dTTP	Pharmacia	27-2080-01	100mM	250ul
dCTP	Pharmacia	27-2060-01	100mM	250ul
deaza-GTP	Boehringer	988-537	10mM 200ul	

dNTP Mix Recipe, 10x

Compound	Stock	Final	1 ml final	2ml final
dATP	100mM	2mM	20ul	40ul
dTTP*	100mM	0.5mM	5ul	10ul
deaza-GTP	10mM	2mM	200ul	400ul
dCTP	100mM	2mM	20ul	40ul
Water			755ul	1510ul
Final Vol		to vol	1000ul	2000ul

Aliquot in 100ul volumes and store at -20'C

*For use with 32P TTP

Alternate dNTP Mix should only be used with large quantities of fresh DNA:

dATP, dTTP, dGTP, and dCTP, each at 2mM in stock purchased from Pharmacia(#27-2035-01)in neutralized form at 100mM

To Make Stock: 2mM each dNTP, 8mM total dNTP

Compound	*Stock*	*Final*	*1 ml final*	*2ml final*
dATP	*100mM*	*2mM*	*20ul*	*40ul*
*dTTP**	*100mM*	*0.5mM*	*5ul*	*10ul*
dGTP	*100mM*	*2mM*	*20ul*	*40ul*
dCTP	*100mM*	*2mM*	*20ul*	*40ul*
Water			*935ul*	*1870ul*
Final Vol		*to vol*	*1000ul*	*2000ul*

Aliquot in 100ul volumes and store at -20'C

**For use with 32P TTP*

Glycogen, carrier :

make up as 20mg/ml

Carrier mussel glycogen: Sigma Cat#G1508

Oligonucleotide Primers: Aliquot in HOOD

These are synthesized and purified according to separate protocols.

Stock solution is 100x, 30uM

Store in 50ul aliquots at -20'C

Oligonucleotides for HUMARA Assay: AR-a and AR-b

AR-a 5' CCG AGG AGC TTT CCA GAA TC 3'

AR-b 5' TAC GAT GGG CTT GGG GAG AA 3'

PCR Buffer, 10x Mix: Aliquot in Hood

Mix components in the listed order, and AUTOCLAVE to solubilize gelatin before aliquoting into 1ml. tubes for storage at -20'C.

Compound	Stock	Final	vol.20ml.	vol.50ml
Water	--	to vol	17.5ml	44ml
Tris, pH 8.4	1M	100mM	2.0ml	5.0ml
KCl MW74.6	solid	500mM	0.7456g	1.864g
Gelatin*	solid	200ug/ml	4.0 mg	10.0 mg
MgCl2 $	1M	15mM	300 ul	750 ul

*Gelatin: Sigma Cat. # G-2500

$for primers ar-a/b final 1x [MgCl] is 1.5mM. Different for other primers.

Phenol:

Solid phenol crystals from Fluka in 500ml bottle.
Add about 300ml of 1.0M Tris, pH 8.0
Let sit a few hours to solubilize
Shake occasionally to equilibrate phases.
Decant aqueous phase and replace with another 150ml 1M pH8.0 Tris
Shake, re-equilibrate for 30min or more at RT.
Decant 1M Tris and Replace with TE, pH 8.0
Store at 4'C.
WARNINGS:
DISCARD IF TURNS YELLOW
ALWAYS USE AT ROOM TEMPERATURE

Proteinase K 10 mg/ml stock:

Boehringer Cat# 161519, 25mg.

(Pro-K supplied already saturated with Ca++. Ca++ needs to be in buffer to maintain activity under adverse conditions, but not for stock storage.)

Reconstitute in 2.5 ml of water, gives 10 mg Pro K /ml
Self-digest at 37'C for 30 minutes
Store at 4'C up to 4 weeks.

RNA, carrier :

16s+28s e.coli rRNA (Boehringer Cat.#206938)
Supplied as 4ug/ul solution

Sodium Acetate:

3M stock: MW 82.03
Dissolve 24.6 g of sodium acetate into final volume of 100ml water

Stratalinker 1800: Stratagene Cat#400071
254nm bulbs

TBE Buffer
5x Recipe:

	Final Volume	
	1 L	500ml
Tris BASE	54g	27g
Boric Acid	27.5g	13.75g
EDTA,0.5M	20ml	10.0ml
Water	to volume	

TE buffer:
10 mM Tris HCl, pH 8.0
1 mM EDTA, pH 8.0

Waterproof Black Opaque ink (Perfex# Corp.: 1-609-399-6767)
Perfex Corp.
PO Box 7
252 Asbury Av.
Ocean City, NJ
08226
$19.75/pint $5.20 2 oz