August 29, 2008

This morning, Jen did:

- PCR of gata-2a-dsred using 8/19 gel isolation, tet-gata primer, dsred 3' primer, high gc, robocycler, 1:40 elongation, 58 temp. didn't work
- PCR tet by itself. gel is in blue rack, needs to be gel isolated/cleaned
- Digestion of tet-nkx-2a with BspDi? (already hit with XhoI?). After ammon acetate, no product.
- Digestion of gata-2a-red with SalI? OR NheI? , again little/no product.
- pbluescript (-) 7 colonies, aMHC construct 8 colonies (picked all 8), cmv-rtTA const, 28 colonies, picked 5. grow in 3ml LB in at 1PM
- insert check of pLentiBB with MluI? and SalI? cutting. Didn't work. Put off until later

Me: Mega PCR day

PCR gata-2a-dsred from gel iso 8/19/08

- 14.75 water, 13 betaine, 5 dntp, 5 buffer, 4.5 MgCl2? , 2.5 DMSO, 1.5 5' tet-gata, 1.5 3' bluescript oligo, 1ul gata-2a-red from 8/19 gel iso, 0.25 deaza, 1 hot, oil.
- ericomp, 58 anneal, 20-10-45, two tubes, 35 cycles each

PCR tet-nkx-2a 35 cycles new robocycler, 58 anneal 40-20-44 (two tubes at 35 cycles)

- 15.25 water, 13 betaine, 5 dntp, 5 buffer, 4 MgCl2? , 2.5 DMSO, 1.5 pblue 5', 1.5 3' gata-2a, 1ul tet-nkx-3a from gel iso, 0.25 deaza, 1 hot, oil.

PCR both versions of 2a-dsred in old robocycler, 56 anneal, 30 second elong

- 32 water, 5 dntp, 5 buffer, 3 MgSO4? , 1.5 5' either nkx-2a 5' OR gata-2a 5', 1.5 3' bluescript oligo, 1ul 2a-dsred(nkx) or 2a-dsred(gata) from gel isos, 1 hot, oil.
- best use high GC, i guess!

attach images, etc...

August 28, 2008

Quantify 0.8% gel pWPI. 10lambda, 1ul pLenti1, 2, 3

- 20, 6, 10 ng/ul

Minis of SLIC: only two colonies from the Nkx-Gata construct. None other. Grabbed them both and put each in 2ml LB and 4ul Amp each. on shaker at 2:30PM

Ammonium acetate of ligations from yesterday (the aMHC, cmv-rtTA, and pBlue negative control), add 1ul glycogen, 7 ammonium acetate, 70 100% ethanol, mix and ice ten minutes. centifuge 15 minutes. wash with 70% ethanol 5 minute cent, air dry, resuspend in 10ul water

Transform the acetated ligations into DH5A? cells. Thawed on ice five minutes, cuvettes also. Add 1ul DNA to each, xfer to cuvette, mix, wipe away moisture, 2.5kV, pulse, above 5kV good, transfer to 1.5ml tube, add 1ml LB and shake for an hour. 60 minutes later, plate on LB +Amp plate. In at 5:55PM

Restriction digest of tet-nkx-2a and gata-2a-red with XhoI?

- 2ul Buffer2, 2ul BSA, 1ul XhoI? each
  - Add 10ul tet-nkx-2a gel iso, 5ul water
  - add 10ul gata-2a-red gel iso, 5ul water
  - add 1ul pBlue mini 1, 14 ul water (control to check enzyme is cutting)
- in for 1 hour. then heat inactivated for 20 minutes at 65 degrees

Gel of pBlue 10ul mini 1 cut with XhoI? against 1ul uncut pBlue with 5ul 2log as ladder.

Amm acetate of heat inactivated ligations: +1ul glycogen, 7 ammon acetate, 70 ethanol, ice, cent, wash with 70% ethanol cent 5 min, air dry, resuspend in 10ul water

Designed and Andy ordered new oligos for inner tet for better SLIC overlap.

5’ Nkx-2a SLIC

5’ CGGGAGTGTCCACGCTGCATGGTATCCGAGCCTGG 3’

5' (nkx) 2a-Gata SLIC

5' CAGGAGACGTTGAGTCCAACCCTGGGCCCCTCGAG 3'

5' 2a-dsred SLIC

5' CTTGGCCGACAGTCACGGGGACATAATCACTGCG 3'

August 27, 2008

Quantify aMHC cut with BspDI? and NheI? , pBlue cut with with BspDi? and SalI? on 0.8% gel, respectively, is 7ng and 13ng/ul. both are gel isolations (1ul each, 10ul lambda)

SLIC: 2ul BSA, 2ul NEBuffer 2, 1ul of 0.05U/ul T4 Polymerase (first diluted 1ul enzyme at 3U/ul in 5ul nebuf2, took one of that and diluted in 9ul water) all added to the following:

- 10ul pBlue (KpnI? and SacI?), 5ul water (100ng, 3000bp)
- 10ul tet-nkx(blue), 5ul water (100ng, 1300bp)
- 12.5ul tet-gata(blue), 2.5 water (100ng, 1650bp)
- 15ul 2a-red(nkx with blue ends) (90ng, 800bp)
- 15ul 2a-red(gata with blue ends) (60ng, 800bp)
- 12.5ul tet-nkx-2a(blue), 2.5ul water (100ng, 1400bp)
- 10ul gata-2a-red(blue), 5 water (100ng, 2150)

Incubate SLIC pieces in 23 C robocycler for thirty minutes. Kill reaction with 1/10vol dCTP (2ul each), ice.

Ligation: Each gets 1ul 10x ligation buffer, 1ul recA at 20ng/ul (diluted first 1ul recA at 2000ng/ul into 10ul 10x buffer and 89ul water)

- 1.5ul tet-nkx, 2.8ul 2a-red(nkx), 2/3ul pblue, 13.03 water
- 1.2 tet-gata, 4.2 2a-red(gata), 2/3ul blue, 11.93 water
- 1.4 tet-nkx-2a, 0.9 gata-2a-red, 2/3ul pblue, 15.03 water

Incubate ligation for 30minutes in 37 degree incubator, then ice

Transform the pieces into DH5A? cells

- add 1ul of each ligation with 50ul thawed cells (careful of heat). mix, put into thawed cuvette, shake to bottom, wipe of moisture, hope for the best and shock at 2.5kV (pulse greater than 4.9...), no spark yay!
- transfer to new 1.5ml tube, add 200ul LB, shake for one hour

Plate onto LB+Amp plates. Use 100ul. (plates prewarmed to rid of moisture)

Now, non SLIC: Minis of pLenti

- First, a warning. Apparently these guys were supposed to be transformed into HP101 cells, not DH5A? , since DH5A? will undergo recombination. I will pull tons of colonies and hope for the best. Else we have to transform again into HP101. Apparently, though, they like to spit out plasmids, so we'll have to be quick with those, one day.
- pellet 1ml LB solution, resuspend in 250ul fridged RNase added P1 buffer, add 250ul P2, invert to mix, add 350ul N3, invert to mix. Ice ten minutes. Centrifuge 10 minutes, decant supernatant to spin column. Centrifuge 1 minute. Dispose wash through, add 75ul wash buffer PE, cent, dispose, cent, new catch tube. Incubate in 50ul elution buffer EB for couple minutes. Centrifuge for one minute and collect the DNA.

Ligations of aMHC and cmv-rtTA with poly A tails into pBluescript

- pblue: 3000bp, 13ng/ul
- aMHC, 2000bp, 07ng/ul, want 3.9:1, so must go 9:2.5 (don't have 9.75 alas)
- cmv_, 1413bp, 80ng/ul, want .23:1, so must go 0.58:2.5 (Hmm, I made a mistake here. I used/found the new version which has a 30ng/ul concentration. Sadly, this is now close to 1:1 ratio instead of 3:1)
- sv40: 161bp, 20ng/ul, want 0.16:1, so must go 0.26:2.5

- The following get: 2ul T4 ligase buffer, 1ul T4 ligase, 2.5ul pBlue +
  - 9ul aMHC, 0.26SV40, 4.5 water
  - 0.6cmv-rtta, 0.26SV40, 13.64 water
  - NEGATIVE CONTROL has only 14.5 water added

- Overnight ligation at 13 C (roboscyer set temp) in at 8:22PM

TO DO:

- Restriction digest with MluI? to check for the insert in pLenti. Restriction digest of pLenti backbone because recombination is our evil.
- Transform aMHC and cmv-rtta constructs after cleaning via ammonium acetate precipitation.
- Make minis from the transformed SLIC method stuff.
- Probably should redo cmv ligation reaction, can trash if the first guy worked.
- Attach gel images.

August 26, 2008

0.8% gel to quantify pBlue cut with SacI? and KpnI? . 10ul lambda-1ul cut pBlue-1ul gata-2a-red(gel iso)

- both are 10ng/ul
- attach image

gel isolated Nkx pcr product from 7-27-08: normal procedure .2g, 200ul salt, cent, 500ul wash, cent, cent 4min, 30ul elution new catch, incubate then centrifuge 2min

- problem, i didn't get a new catch tube, so ran the elution through the column a second time (in the elution buffer still)

ran a gel including Nkx gel iso, quantified at 2ng/ul... ouch!

diluted dCTP from 100mM to 10mL, 2ul of the 100mM and 18ul of water.

diluted 1ul T4 pol in 5ul Nebuffer2, then took 1 ul of that and diluted it in 9ul water for use with SLIC.

Set up SLIC (the following ALL include 2ul BSA, 2ul Nebuffer2, 1ul of above enzyme mixture (for 0.05U of enzyme)

- + then found out that we hadn't gel isolated the stuff. stopped here

gel isolate, now, those pieces that we thought were gel isolated: 5ul 2log-20 of all following in two lanes each

- 2a-red(gata), 2a-red(nkx), tet-nkx-2a, tet-gata, tet-nkx

gel isolation of above: 100ul of turbo salt per .1g, same procedure as before. this time no mistake about catch tube.

gel quantify of above: same order, 10ul lambda bst

- 2a-red(gata) 4ng/ul, 2a-red(nkx) 6ng/ul, tet-nkx-2a 8ng/ul, tet-gata 8ng/ul, tet-nkx 10ng/ul

heat inactive the digest that simina did of aMHC, 65 degrees for 20 minutes

gel isolate the pBluescript digest and the controls to check enzyme cutting.

- 2 log - uncut pblue - bspd1 cut - sali cut - cut with both - large cut with both to iso
- strange, the control uncut looked the same as these linear cuts... ???

ammonium acetate precipitate aMHC: first ice after heat inactive, then add 1ul glycogen, 7 ammonium acetate, 70 100% ethanol, ice ten minutes, spin 15 minutes, wash with 98ul 70% ethanol, centrifuge 5 minutes, air dry, resuspend in 10ul water. in blank box next to iso of pBlue

add pictures...

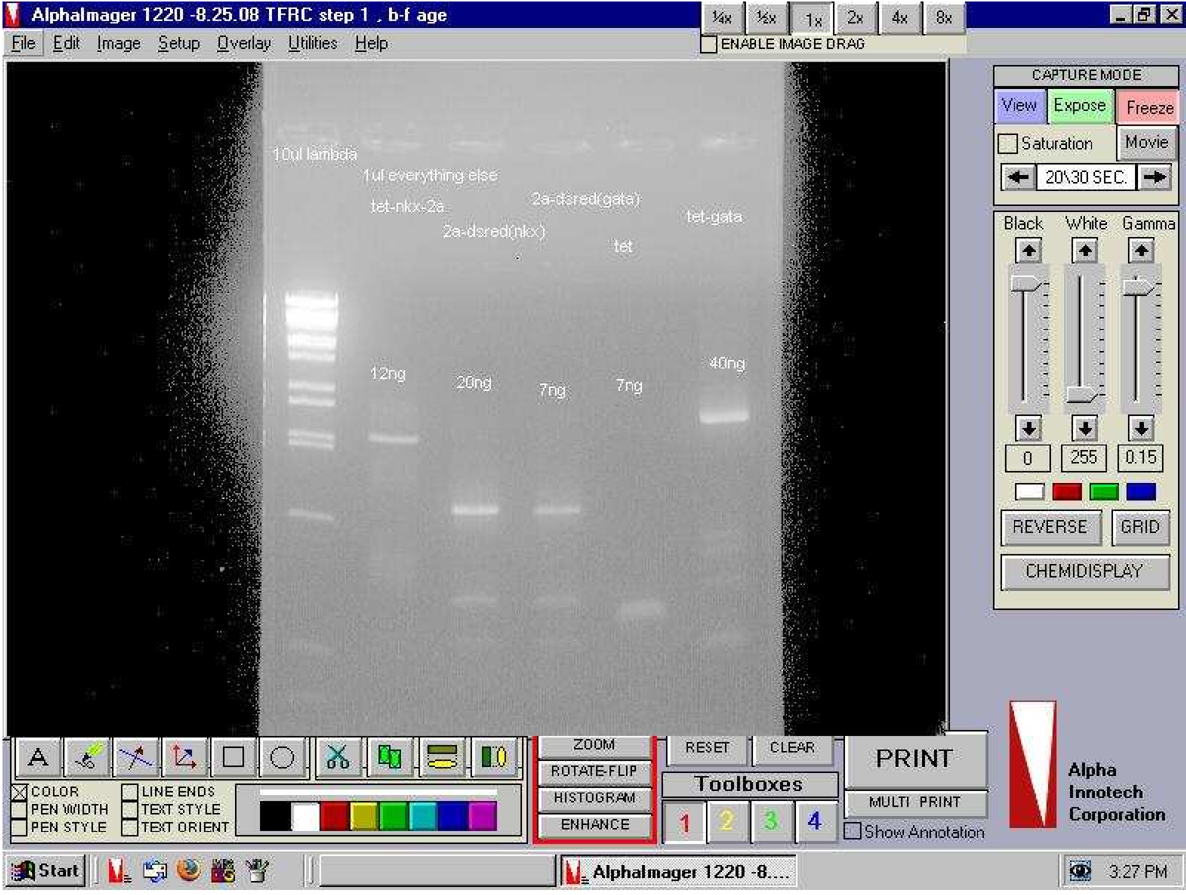
August 25, 2008

PCR of gata-2a-dsred from gel iso

- 14.75 water, 13 betaine, 5 buffer, 5 dntp, 4.5MgCl2, 2.5dmso, 1.5 5' 2a-gata oligo, 1.5 3' bluescript oligo, 1ul gata-2a-dsred from gel iso on 19 august, 0.25 deaza gtp, 1 hot, oil.
- robocycler, 58 anneal, 40-20-1:30 timing. 20-25 cycles.

Gel of 10ul lambda, 1 each of tet-nkx-2a, 2a-dsred(nkx), 2a-dsred(gata), tet, tet-gata

- want to quantify: tet-nkx is 12ng/ul
- 2a-dsred(nkx) is 20
- 2a-dsred(gata) is 7
- tet is 7
- tet-gata is 40
- 8-25-08_quantify_pieces_of_tet.jpg:



Made new lambda bst ladder. Took 50ul of lambda ladder, 50ul of 10x loading dye, and 400ul water.

Gel isolation of gata-2a-dsred: Simina made the gel, here is after the cut out.

- 8-25-08_gata-2a-red_to_gel_isolate.bmp: 8-25-08_gata-2a-red_to_gel_isolate.bmp
- weighed .6 and .42 grams, each piece. added 600ul and 420 of turbo salt respectively. melted.
- centrifuged through spin column. then used 500ul wash buffer and centrifuged short and long. (5 seconds and 4 minutes)
- then added elution buffer, 30ul, let sit five minutes. spun for 3 minutes. collected (new catch tube for this step)

Make LB/Amp plates: 17.5g LB mixture + 500ml water

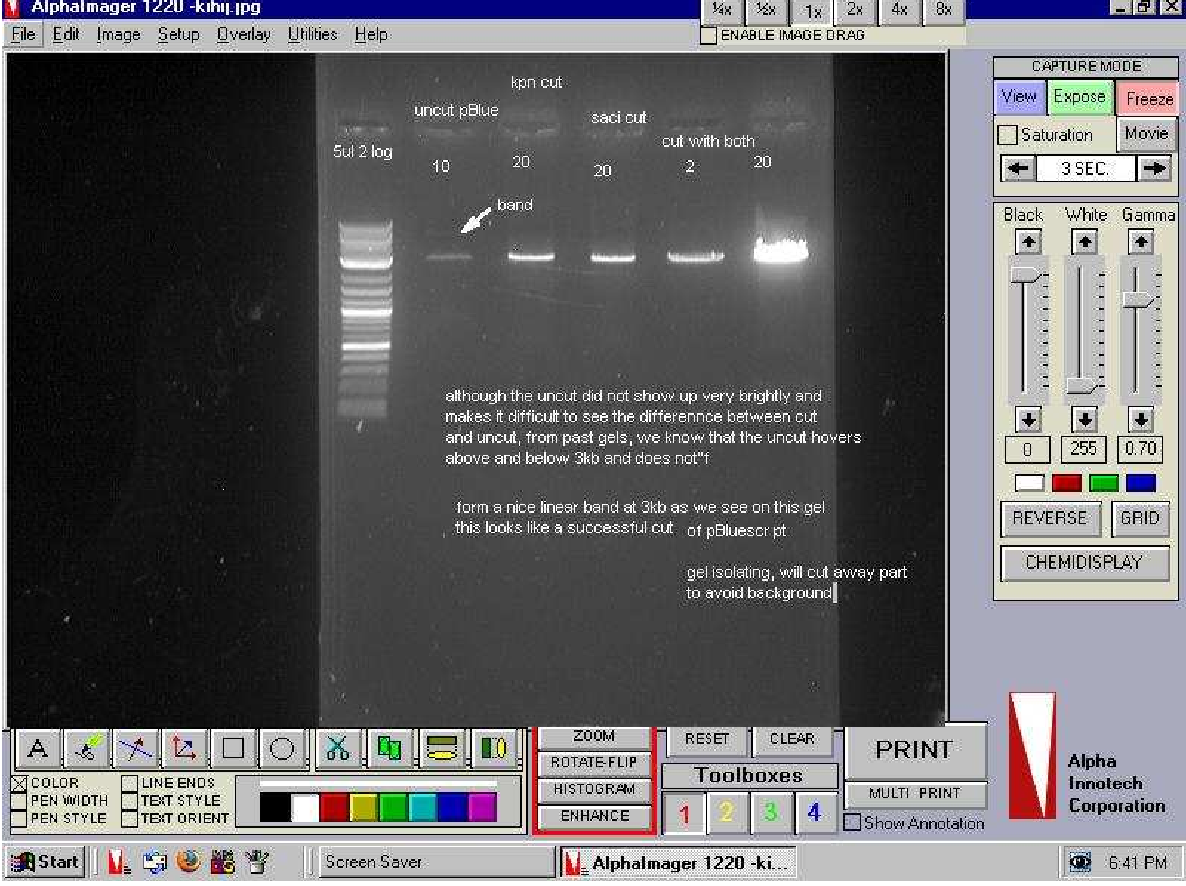
- autoclave at 121 degrees for 15minutes.
- add 1ml ampicillin. then create plates with about 10ml of the solution for each plate.
- (I screwed up royally here. First, I pipetted, which created lots of bubbles. Second, I pipetted out the solution on all plates before swooshing it around to cover the entire plate. More than half the plates were lost because the gel hardened before theentire plate was covered. When I realized what had happened. I took the rest of the mixture and just started pouring it, swooshing it, pouring, swooshing, etc. Got a bunch of plates that way. However, the last few, a few chunks fell into the plates. All in all I wasted a lot... Sorry.

Restriction Digest of pBluescript with Kpn1 and SacI? so that we can do the SLIC procedure.

- pblue cut with kpn and saci: 2ul neb1, 2 bsa, 5 pblue mini1 (100ng/ul), 9 water, 1 kpn1, 1 sac1
- same cut with only kpn: 2ul neb1, 2bsa, 0.5 pblue mini1, 14.5 water, 1 kpn1
- cut with sacI: same as above, replace kpn with sacI
- One hour digestion. 37-40 degrees.

Gel isolate the KpnI? and SacI? cut of pBluescript for use with SLIC method

- 5ul 2log - 10ul uncut pBluescript from 8/21/08 reaction - 20 ul kpnI cut - 20ul saci cut - 2ul kpn and sac cut - 20ul kpn and sac cut to gel iso. * looks good
- 8-25-08_KpnI_and_SacI_cut_of_pBluescript.jpg:



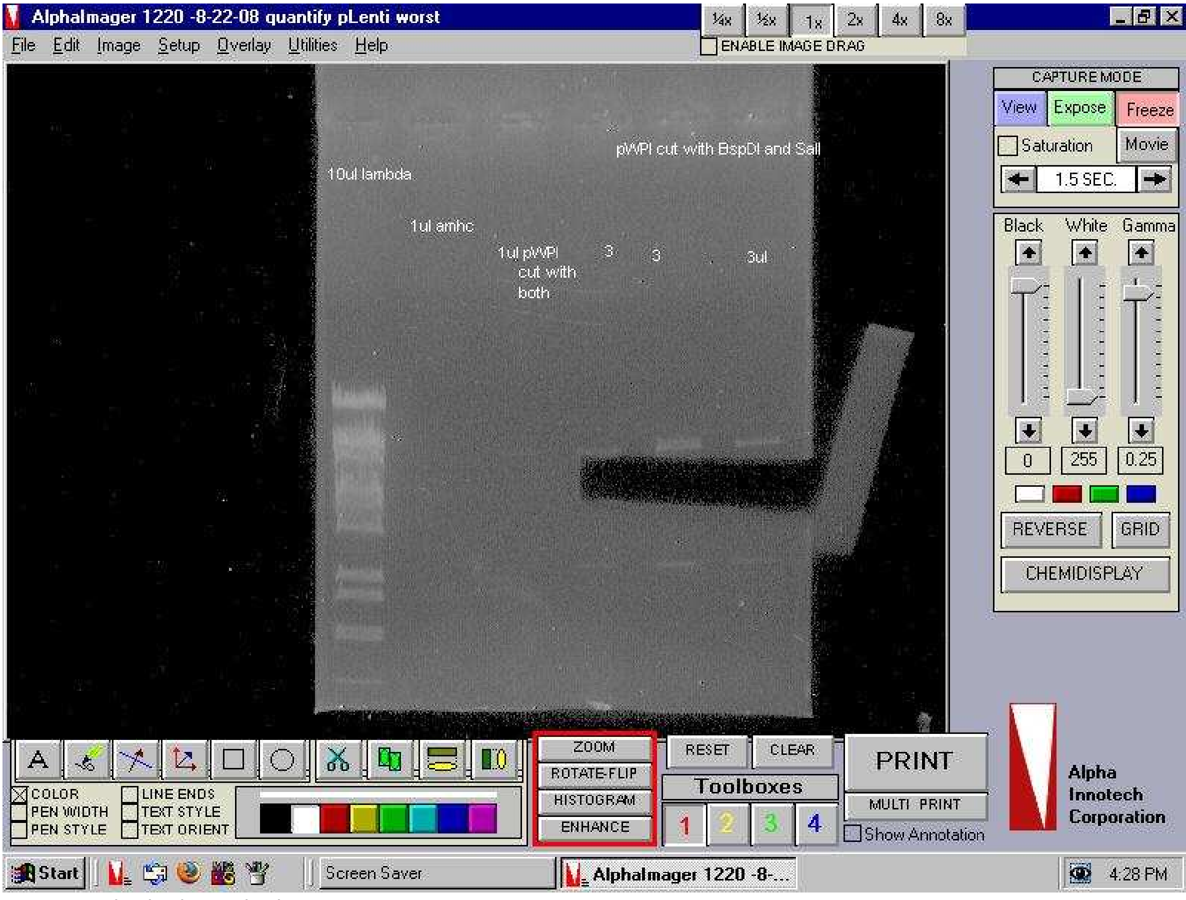
Gel isolation:

- weighs .03g, added 30ul salt, centrifuged thorugh spin column. now add 500ul wash, centrifuge short and long (5sec, 4 min)
- new catch, 30ul elution buffer, sit five minutes, centrifuge through 3 minutes. in 2008 igem box

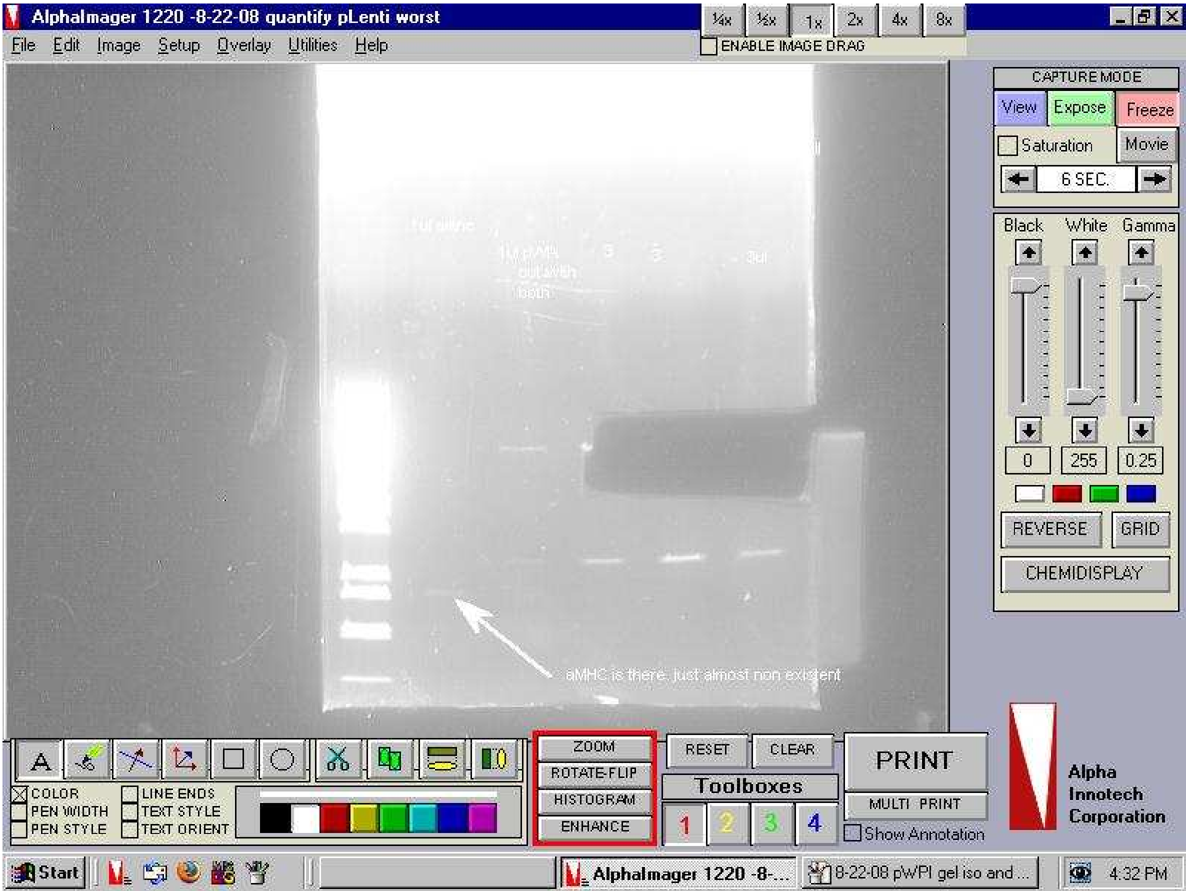
August 22, 2008

0.5% gel to quantify aMHC, then gel isolate pWPI cut with SalI? and then BspDI? . note, Simina did not heat inactivate after the cut, but she ammonium acetate ed it and put it in the freezer. Hopefully it's still good.

- 10ul lambda bst, 1amhc, 1 pwpi, 3, 3, 3 pwpi (gel isolated last three lanes)
- amhc is less than a third of a ng/ul... ammonium acetate probably bad, lost it there.
- 8-22-08_pWPI_gel_iso_and_aMHC_quant.jpg:

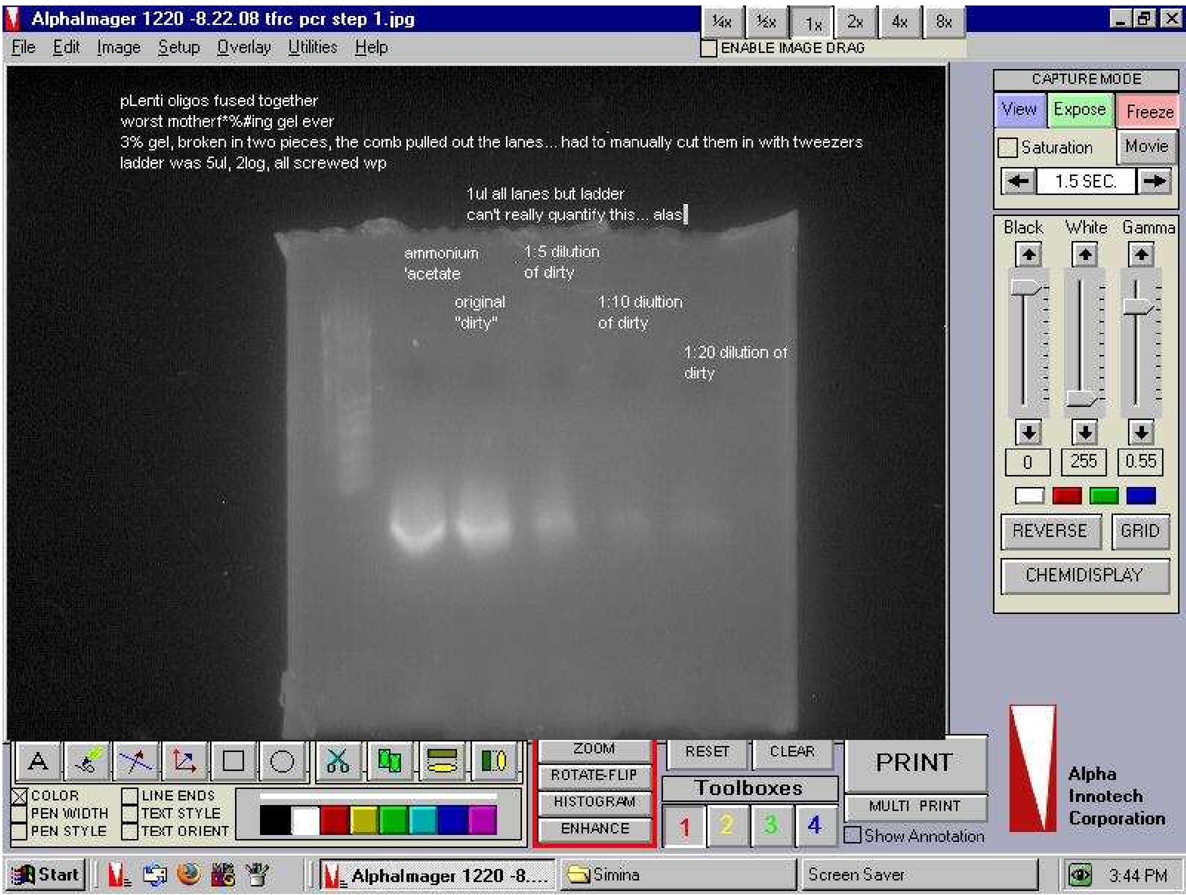


- 8-22-08_amhc_is_there,_tiny.jpg:



3% gel to quantify insert piece plenti

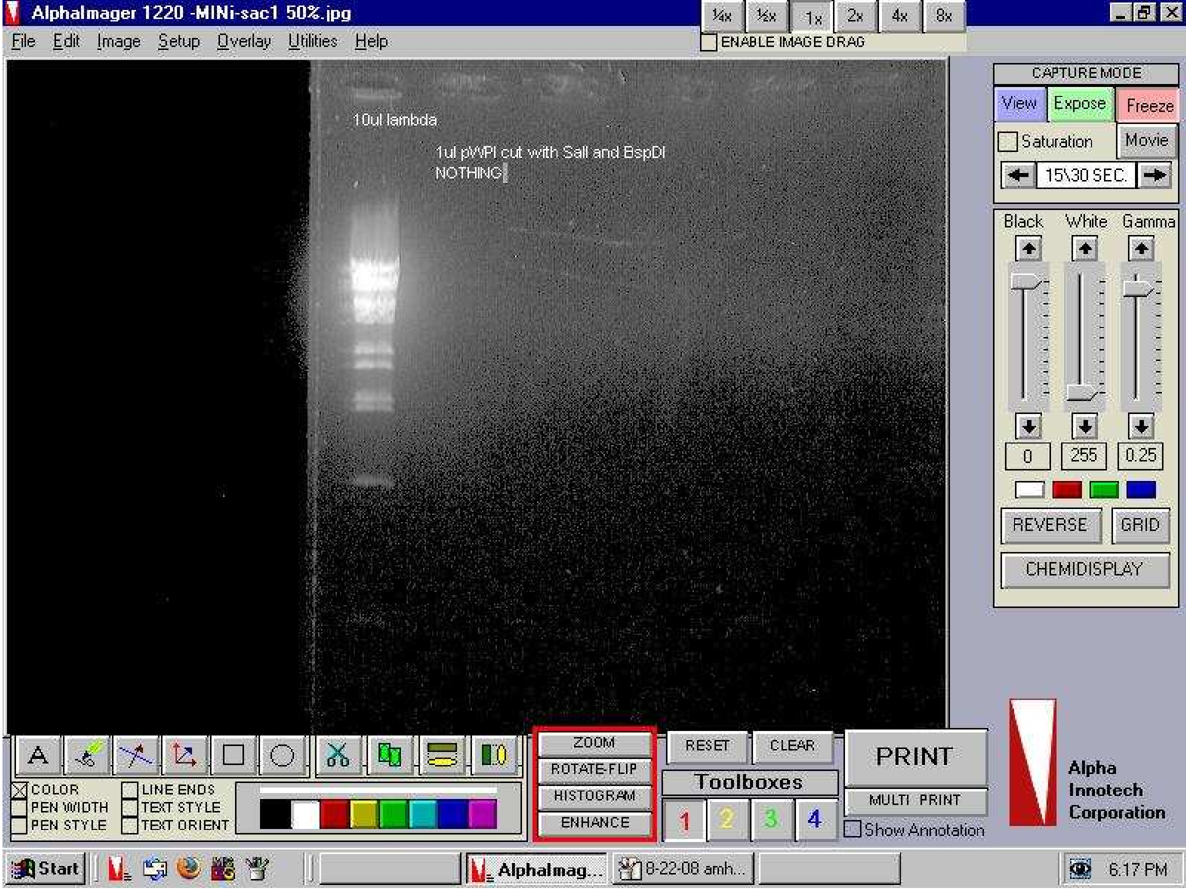
- 5ul 2log, 1 plenti ammon acetate, 1 plenti dirty, 1 1:5 plenti dirty, 1 1:10, 1 1:20
- acetate actually worked. we got it. ladder didn't seem to show, but judging by brightness, this is probably 30ng/ul or so. :/
- 8-22-08_quantify_pLenti_worst_gel_ever.jpg:



gel iso of pWPI cut with both, .41g gel, added 410ul salt sol, cent, 500ul turbo wash, cent, cent, 30 ul elution buffer, sit five minutes, then centrifuge. cleaned.

1% gel to quantify pWPI cut with SalI? and BspDI?

- 10ul lambda bst, 1ul pWPI cut with both
- 8-22-08_final_failure_pWPI_quantify_nada.jpg:



August 21, 2008

Restriction digests of cmv-rtta and aMHC-NeoR constructs with BspD1? and NheI? .

- 2ul buf 4, 2ul bsa, 1 ul bspdi, 1ul nhe1, 14ul dna for amhc (and 10ul dna for cmv + 4 ul water),
- Controls for BspD1? is pBlue uncut and cut with 2buf4, 2bsa, 1bspd1, 0.5 pBlue, 14.5water (control minus the enzyme, + water)
- Control for NheI? is Zeo uncut and cut with 2buf4, 2bsa, 1nheI, 0.5zeo, 14.5 water (control is minus enzyme, + water)
- hour digestion
- Heat inactivate for 20 minutes at 65 celsius

Restriction digest of pWPI, cut with SalI?

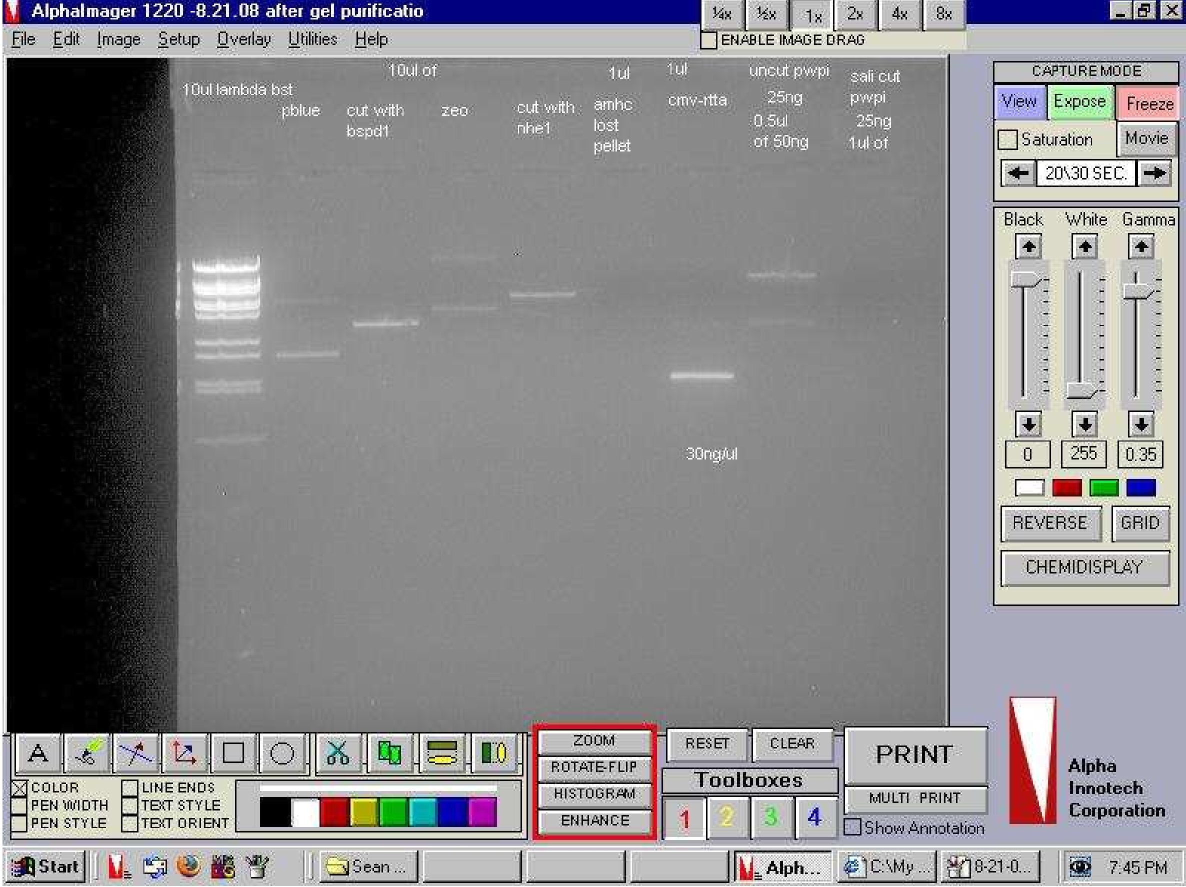
- First get a dilution of pWPI maxi 3/22/08 (i think). 5ul of 818ng/ul with 3.2ul water to get 500ng/ul concentration. Also made a 50ng dilution.
- 2ul buffer3, 2 bsa, 1ul salI, 1ul pWPI (500ng/ul), 14ul water. hour digestion
- heat inactivate 65 for 20mins

Ammoniom Acetate of those constructs:

- +1ul glycogen, +7 ul ammonium acetate (10M), +70ul 100% ethanol, ice, then spin 15minutes, then decant, washwith 70% ethanol, spin 5 minutes, decant, air dry, resuspend in 10ul water. **the aMHC the pellet may have been lost! if it is lost, redigest the rest of the aMHC gel iso from yesterday and try again**

0.8% gel of stuff

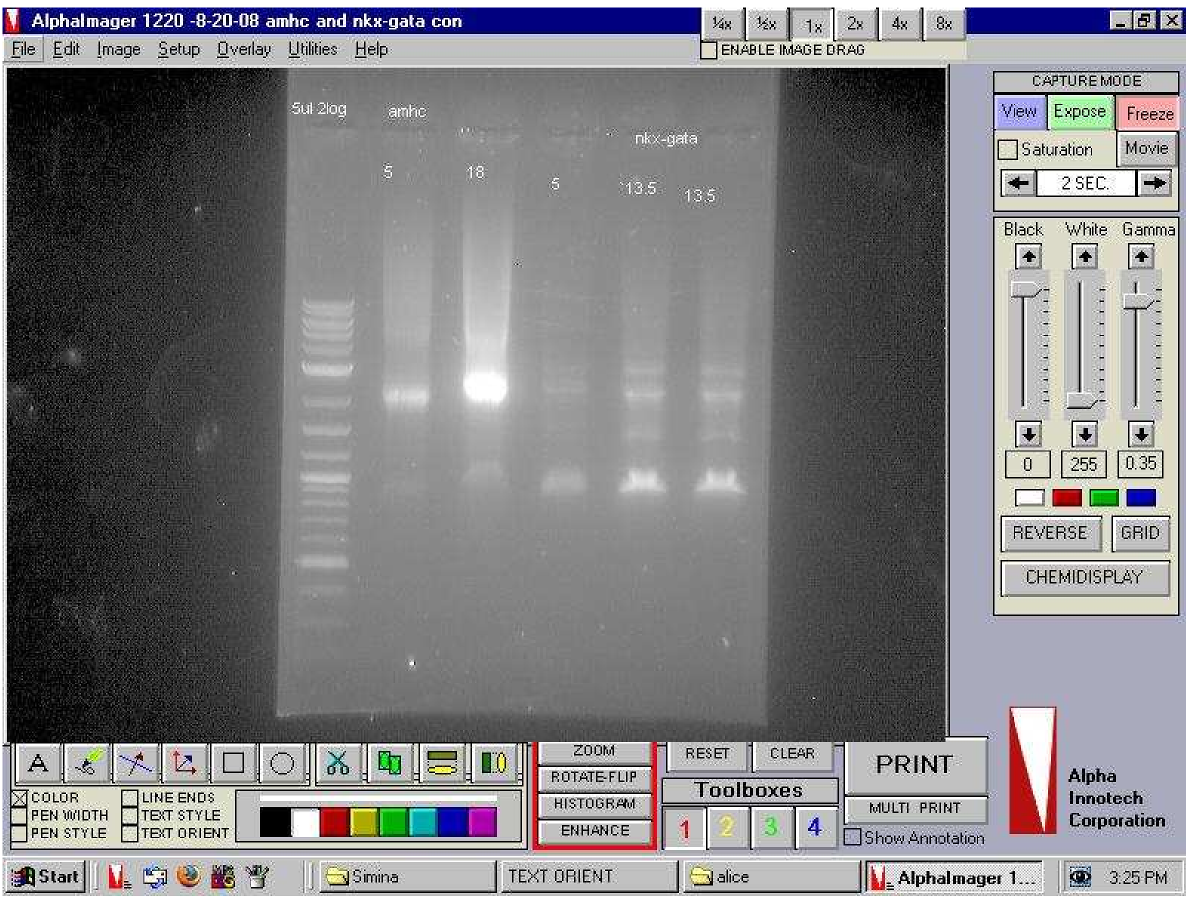
- 10ul lambda bst, 10ul each of uncut blue, bspd1 and nhei cut blue, uncut zeo, nhei cut zeo, then 1ul of aMHC ammonium acetate for quantifying, then 1ul of cmvrtta for quantifying, then 0.5ul of uncut pWPI, then 1ul of sall cut pWPI
- 8-21-08_cut_bspd1,_nhe1,_quantify_cmv_rtta,_cut_pwpi_sal.jpg:



August 20, 2008

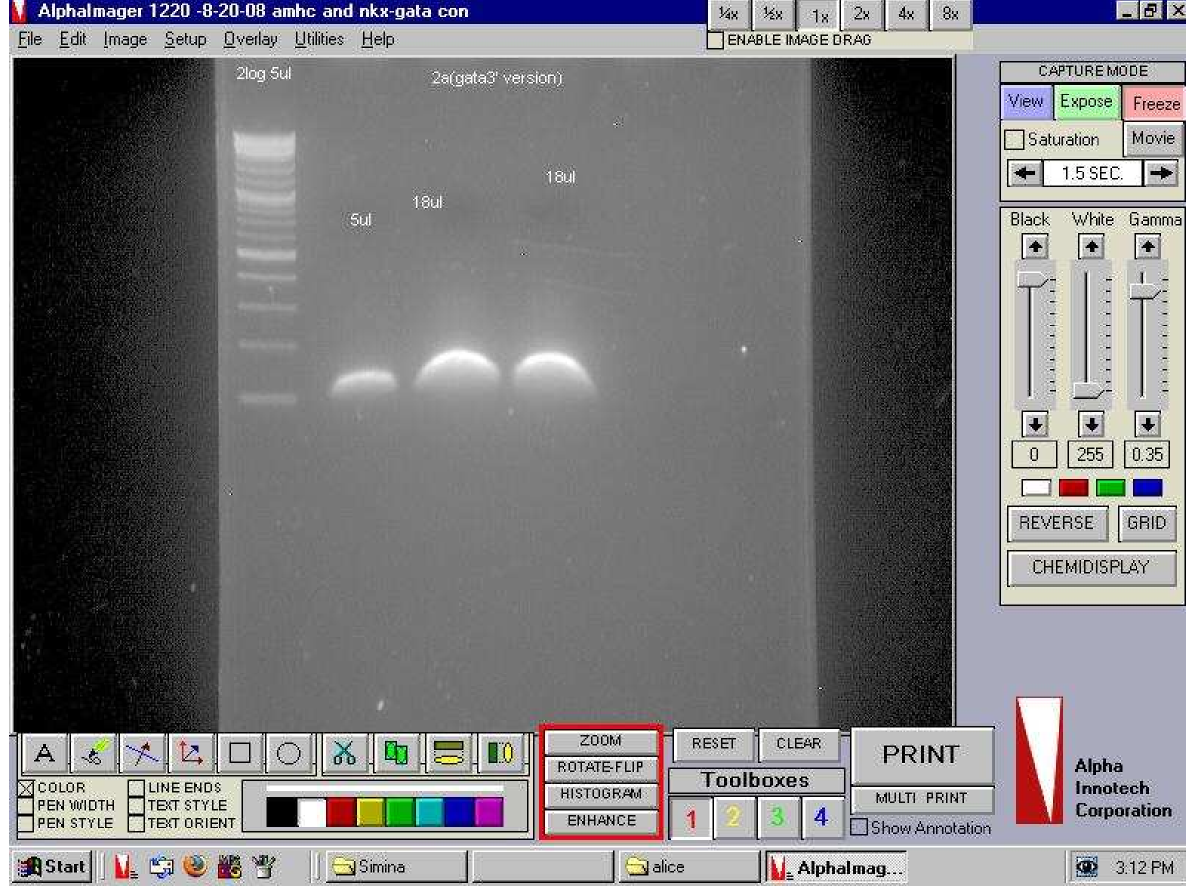
1% gel of aMHC construct and tet-nkx-gata-2a-red construct. 2log 5-amhc 5-amhc 18-nkxgata 5-nkxgata 13.5-nkxgata 13.5

- amhc looks okay, gel isolating. nkx-gata seems to have predominantly Not worked. gel is in ziploc just in case someone else has another idea.
- 8-20_amc_and_nkx-gata_constructs_longer_run_to_gel_iso.jpg:



2% gel to isolate 2a for gata 3'. 5 2log-5 2a - 18 - 18

- 8-20-08_2a_for_gata_3_side_to_gel_iso.jpg:



- gel isolated

gel isolation of 2a for gata 3' side had .325 g, so added 325ul turbo salt, heated, centrifuged through spin tube, added 500ul wash buffer, centrifuged through quickly, then for four minutes. incubated in 30ul elution buffer with new catch for 10 minutes, then centrifuged through for two. collected

gel isolation of aMHC construct. weighed .7g, so 700ul turbo salt. followed the above for the rest

PCR of 2a (gata3') and dsred off of gel isolations, used robocycler, 95, 56, 70 for 40-20-30, 27 cycles, two tubes, one off at 12 cycles
- 29 water, 5 buf (new bottle), 5ntp (new bottle), 3mgso4, 3 gata-2a 5', 3 dsred 3', 0.5 2a for gata 3' from today gel iso, 0.5 dsred gel iso from 8-11, 1 hot, oil

MAXI of TCF1, A201, and pBluescript
- resuspend pellet in 10ml P1 (from fridge), vortexed
- added 10ml P2, invert to mix, incubate 5mins or lessish
- added 10ml prechilled P3, inverted to mix, on ice for twenty minutes. prechilled the centrifuge by turning it on
- centrifuged for 30minutes at 20,000 g (13k rpm on machine)
- Simina came in and has the rest in her notebook

Gel of 2a-dsred from above should be in SiminasNotebook. However I saw it, and it failed. Nada.
-

August 19, 2008

Redo all digests from Simina's yesterday stuff. Gel was bad and there was too much dna to see clearly.
- Constructs: TCF 1, 2, 4 minis; CMV1 mini; A201,2 minis
  - 2 BSA, 2 Neb3, 1 XbaI? , 1 NheI? , 0.5 mini dna, 13.5 water
  - Also ran controls for each with 15.5 water and no enzyme. used mini 1 in each case
- Xba Control: 2 bsa, 2buf 3, 1 xbai, 1/3 pBlue, 14.67 water
  - Control with 1ul more water, no enzyme
- NheI? control: 2 bsa, 2 buffer3, 1ul nhei, 1ul pcDNAZeo3.1+, 14 water (control had no enzyme, more water)

- Ran digest for two hours at 37-40 degrees.

PCR gata-2a-red in one step on robocycler, 58 anneal, 40-20-1:30, 13, 26, and 33 cycles

- 11.25 water, 13 betaine, 5 buf, 5 dntp, 4.5mgcl2, 2.5dmso, 3 2a-gata4 5', 3 dsred 3', 0.33 gata4 mini1, 0.33 2a gata(3' side), 0.33 dsred gel iso, 0.25 deaza, 1 hot, oil

PCR amplify aMHC construct, 61 anneal, 20 and 30 cycles, COY, 40 sec elongation

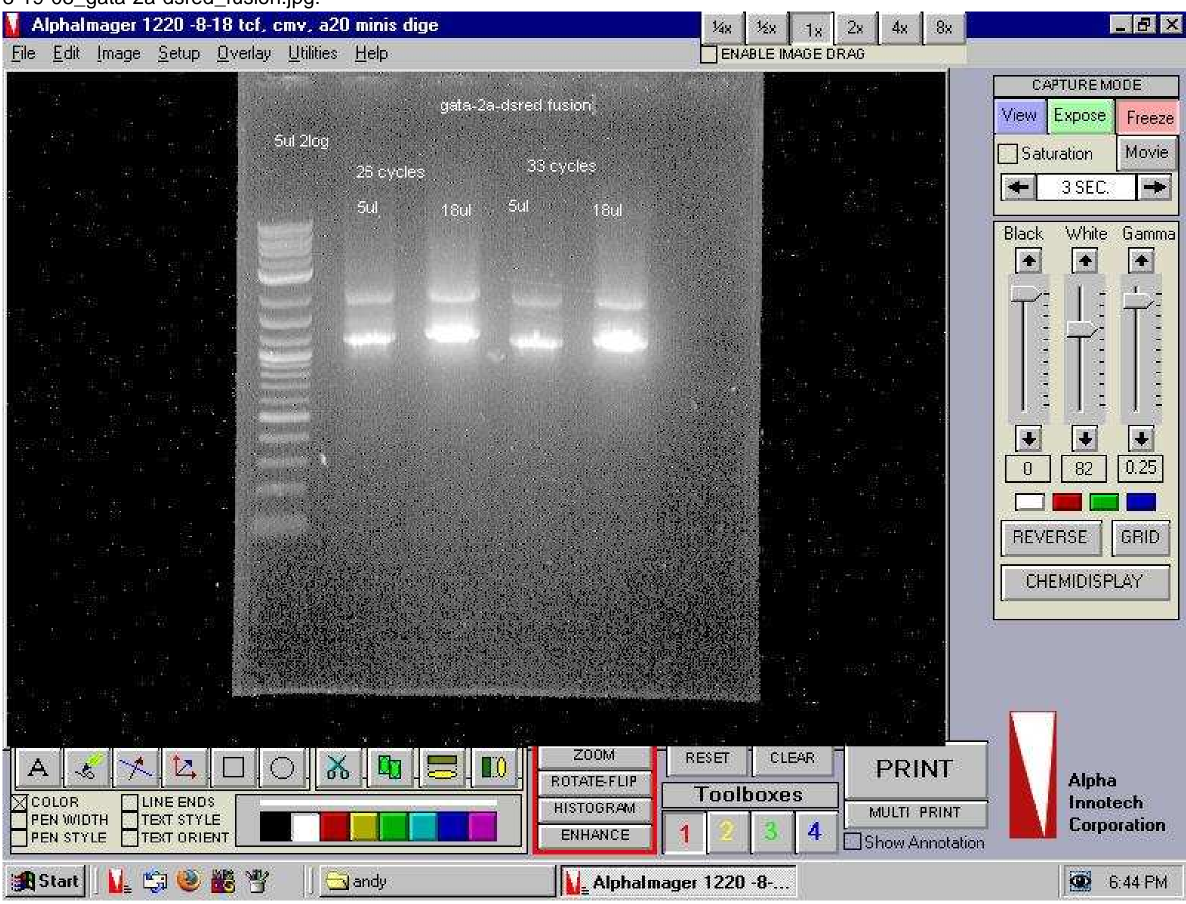
- 12.75 water, 13 betaine, 5 buffer, 5 dntp, 4.5mgcl2, 2.5 dmso, 1.5 amhc 5', 1.5 gfp 3', 3ul aMHC gel iso, 0.25 deaza, 1 hot, oil
- **Please run this on gel, both tubes, for gel isolation (365nm). I used extra DNA assuming 3ng/ul concentration**

PCR tet-gata 58 anneal, 23 second elongation (though 5 cycles first at 55 anneal), 25 cycles, other lab's pcr machine, program 555

- 12.25 water ,13betaine, 5 buf, 5dntp, 4mgcl2, 2.5dmso, 3 tet 5', 3 2a-gata 3', 0.5 tet(10), 0.5 gata for tet, 0.25 deaza, 1 hot, oil

1% gelof gata-2a-red: 2log-26 cycle 5ul-26 cycle 18ul-33 cycle 5ul-33 cycle 18 ul

- looked good, gel isolated both.
- 8-19-08_gata-2a-dsred_fusion.jpg:



gel iso of gata-2a-red had 0.185g, so 185 turbo salt added. melted. centrifuged away. added 500ul wash buffer, centrifuged away with quick, then 4minute. added 30ul elution buffer, let sit 5+mins, then centrifuged it off for two minutes

August 18, 2008

if stuff not work, can try new technique. make fusion ends with plasmid to get in one step.

make 5M betaine. 2.93g of anhydrous betaine (117.15g/mol) in total volume of 5ml with water. extra tubes in reagents box 1

tet-nkx-2a fusion pcr on robocycler, 5 cycles at 56, 20 at 58, times 40-20-44 (two tubes, one short, one full cycle)

- 13.25 water, 13 betaine, 5 buf, 5 dntps, 3mgcl2, 2.5dmso, 3 5' teto, 3 gata-2a 3', 0.5 tet-nkx, 0.5 2a(gata 5'), 0.25 deaza, 1 hot, oil

gata for tet-gata-2a-dsred construct pcr. coy machine. two tubes, one short, one long. 58 anneal, 20 second elongation

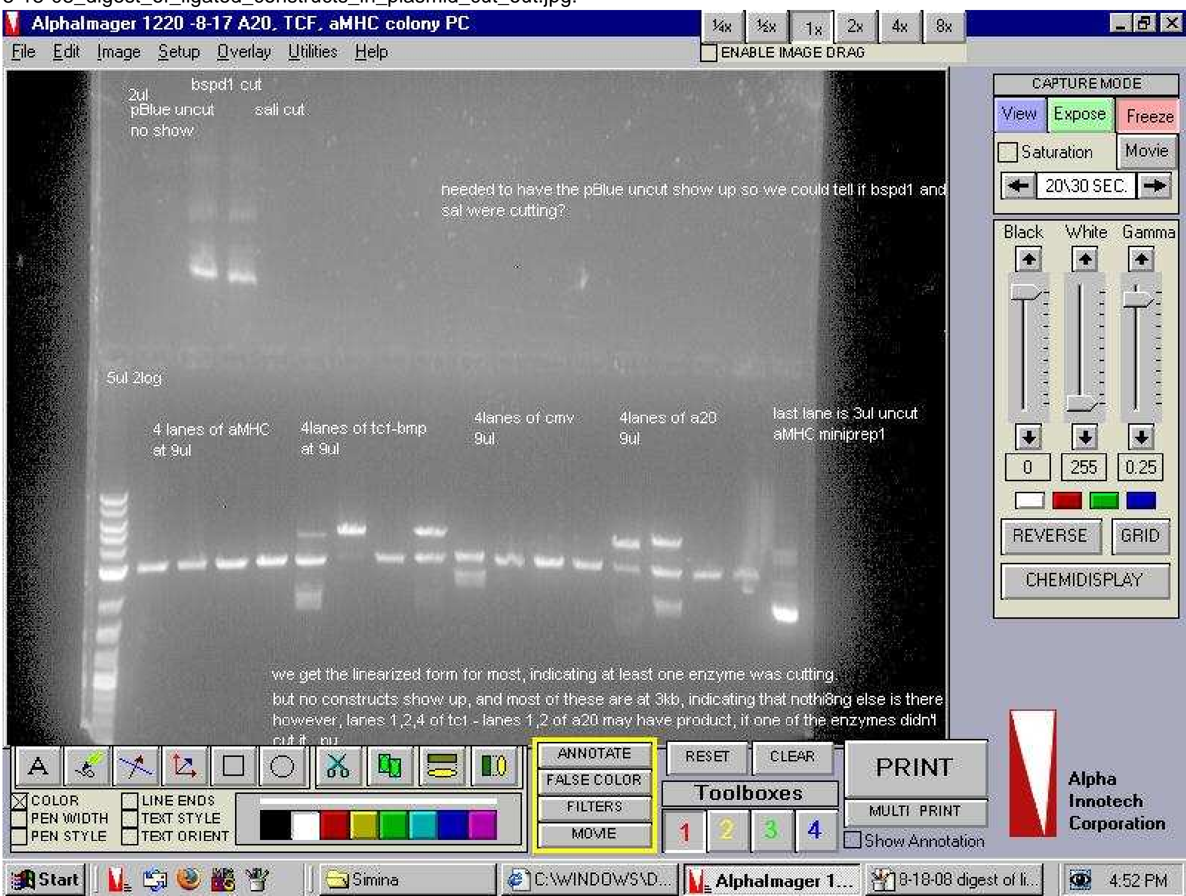
- 16.25 water, 13 betaine, 5 buf, 5dntp, 3mgcl2, 2.5dmso, 1.5 5' tet-gata, 1.5 3' 2a-gata, 1ul gata4 mini1, 0.25 deaza gtp, 1 hot, oil

gata-2a-dsred fusion pcr (attempting all three at once). 55 degree anneal, robocycler, times 40-20-1:20

- 12.75 water, 13 betaine, 5 buf, 5dntp, 3mgcl2, 2.5 dmso, 3 5' 2a-gata, 3 3' dsred, 0.5 2a(gata3'), 0.5 gata, 0.5 dsred gel iso, 0.25 deaza, 1 hot, oil

visualize sean's restriction digest: will add picture later. possibles that worked... tcf 1,2,4, cmv 1, a20 1,2? so we retried those with xba1 and nhe1.

- 8-18-08_digest_of_ligated_constructs_in_plasmid_cut_out.jpg:



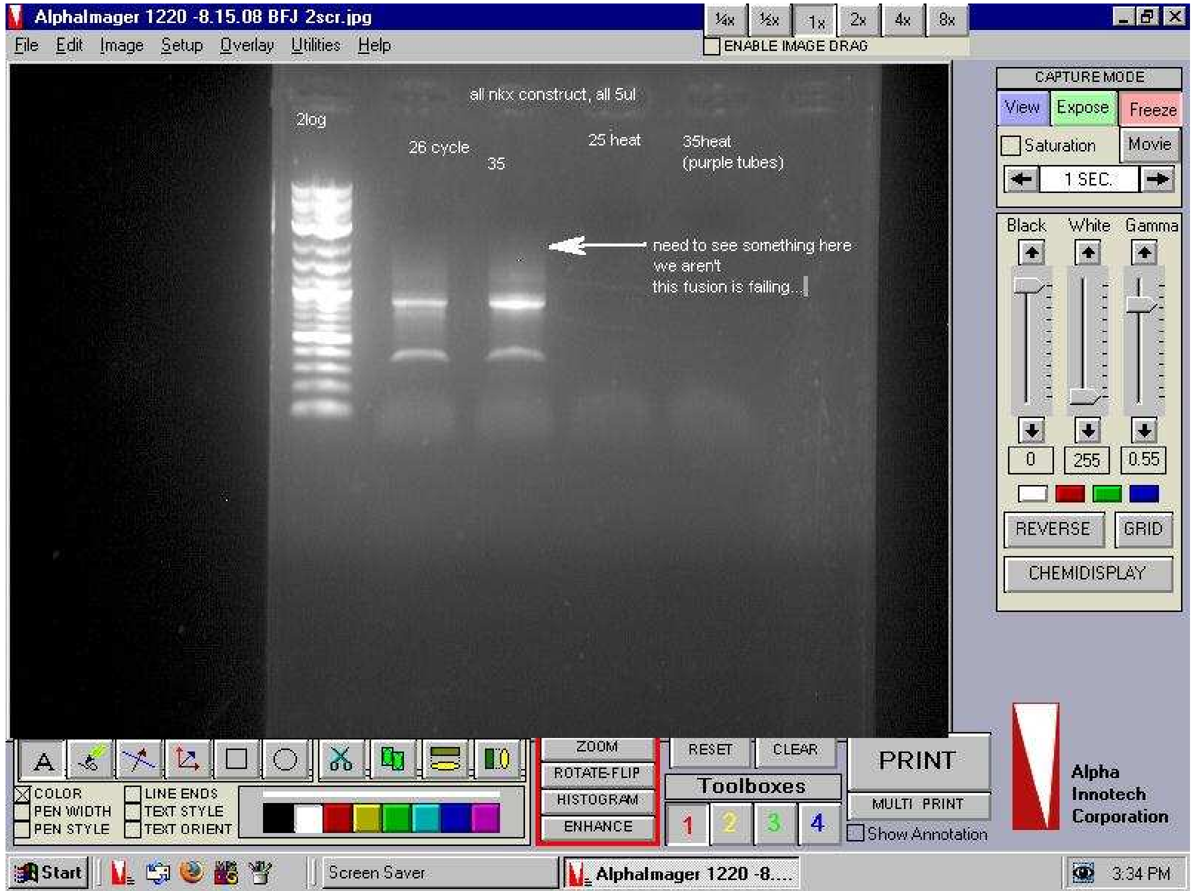
August 15, 2008

Grab DNA from minis

- pellet 1ml LB sol'n from each of two tubes of pBluescript (from shaker tubes) into 1.5ml tube (centrifuged in fridge, iced). resuspend in 250ul P1 buffer from fridge.
- add 258l P2, inverted mixed
- add 350ul N3, mix, ice ten minutes, centrifuge in fridge 10mins, decant supernatant to spin column, cent 1 min, dispose wash through. add 75ul PE wash buffer, centrifuge 1min. discard flow thorugh and centrifuge again
- add 50ul elution buffer, let sit 1min, centrifuge into clean tubes. 1 min

1%gel of 2log, tet25, tet35, tet25heat, tet35heat, 5ul each, all failed. nothing showed for the high heat ones, perhaps missing reagents. wrong product for the first two, should see 2kb band.

- 8-15-08_nkx_construct_fusion_failed_again.jpg:



minis of new gata4 plasmid from openbiosystems. 4ml LB and 8ul Ampicillin each with a touch of the glycerol stock they sent us. put in shaker at 2:40PM

streak LB+amp plate with the same gata4 clycerol stock. touch with pipet tip, streak, then new tip steak of that, etc. put in 37degree incubator at 3:15pm

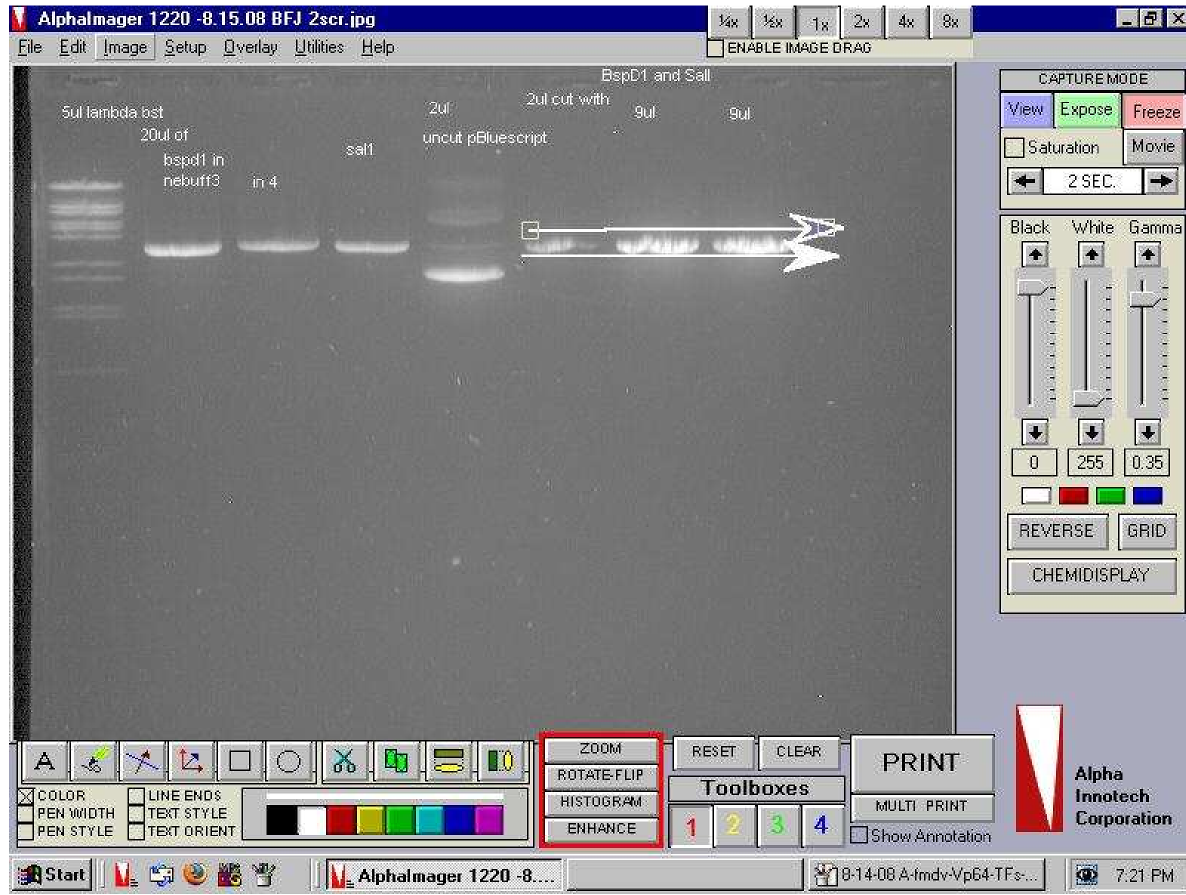
gel of pBluescript minis to quantify plasmid. 5ul 2log - 1 ul mini1 - 1ul mini2 ~100ng/ul or greater

restriction digest off plasmid with controls. cut for ~one hour from 4:50PM to 6PM in 37 degree incubator

- plasmid cut with 2ul NEBuffer3 (B3), 2ul BSA, 1 BspD1? , 1 SalI? , 5ul pBluescript mini 1, 9 ul water
- BspD1? control: 2ul B3, 2BSA, 1BspD1, 0.5 pBlue 1, 14.5 water
- BspD1? second control with Buffer 4 to check the strange result of yesterday: 2ul B4, 1ul BspD1? , 0.5 pBlue 1, 16.5 water
- SalI? control: 2ul B3, 2ul BSA, 1ul SalI? , 0.5 pBlue 1, 14.5 wter

All of the above were then loaded into a .8% gel to end the digest and check

- 5ul lambda bst, 20ul bspd1, 20ul bspd1 (b4), 20ul sali, 2ul uncut pBlue, 2ul cut pBlue with BspD1? and SalI? , 9ul of cut, 9ul of cut (last two lanes for gel iso)
- 8-15-08_pBluescript_digestion_checks.jpg:



At same time, digest of cmv-rtta costruct with bspd1 and nhe1

- 2ul B4, 2ul BSA, 1ul BspD1? , 1ul NheI? , 14ul cmv-rtta construct
  - heat inactivated at 65 degrees for twenty minutes

Ammonium acetate precipitation of above heat inactivated construct:

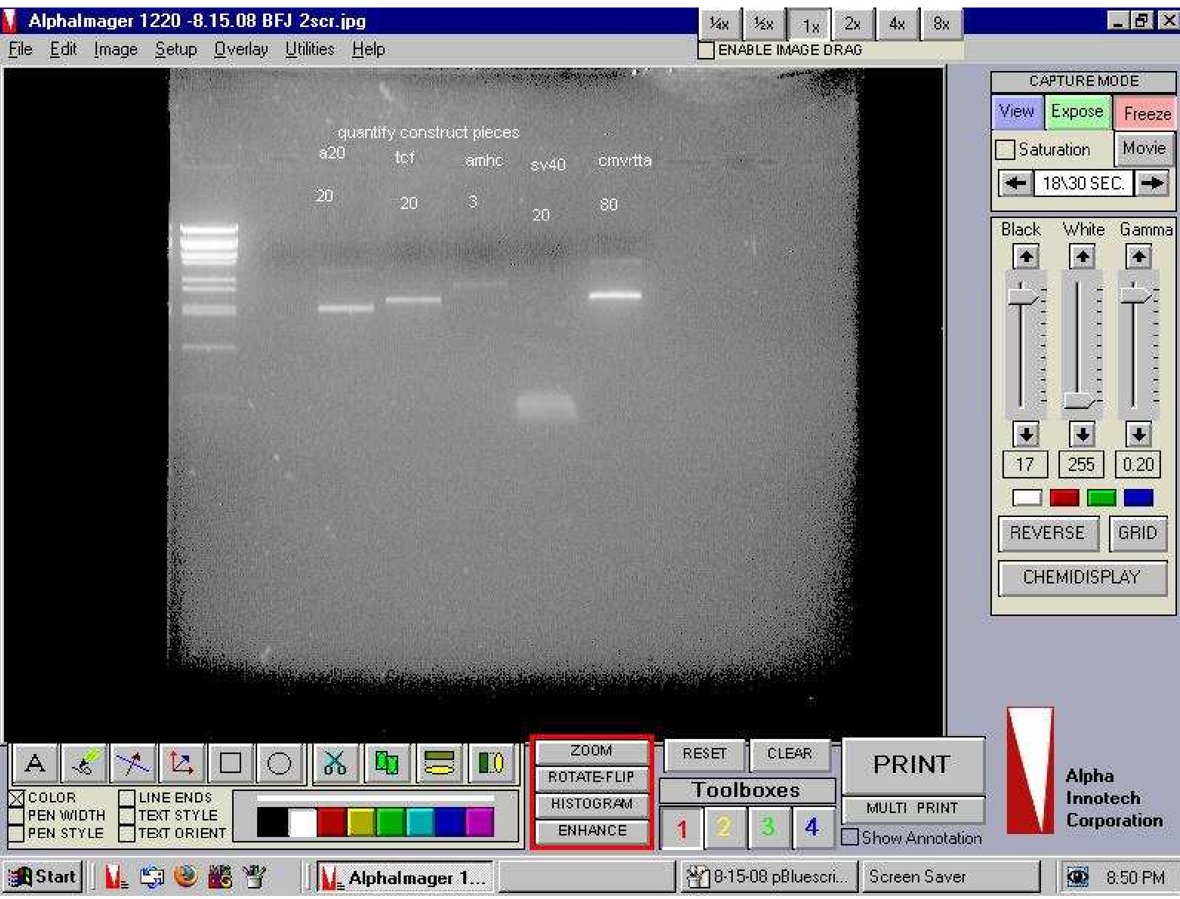
- add 1ul glycogen, 7 ammonium acetate (10M), 70 100% ethanol, ice 10 mins, spin in fridge 15 minutes, decant, wash with 70% ethanol, air try for 5ish minutes, resuspend in 10ul water

Gel isolate the [couple] above pBluescript cut with sal and bspd1

- two tubes weighed .25 and .65g. got 250ul and 650 of turbo salt respectively, in heat block to melt at 55degrees
- transferred to spin column and centrifuged through till everything is on column.
- add 500ul turbo wash, centrifuge 5 secs, dump, cent 4 minutes
- incubate in 30ul of elution buffer for 5 min in new catch tube, then centrifuged for clean dna

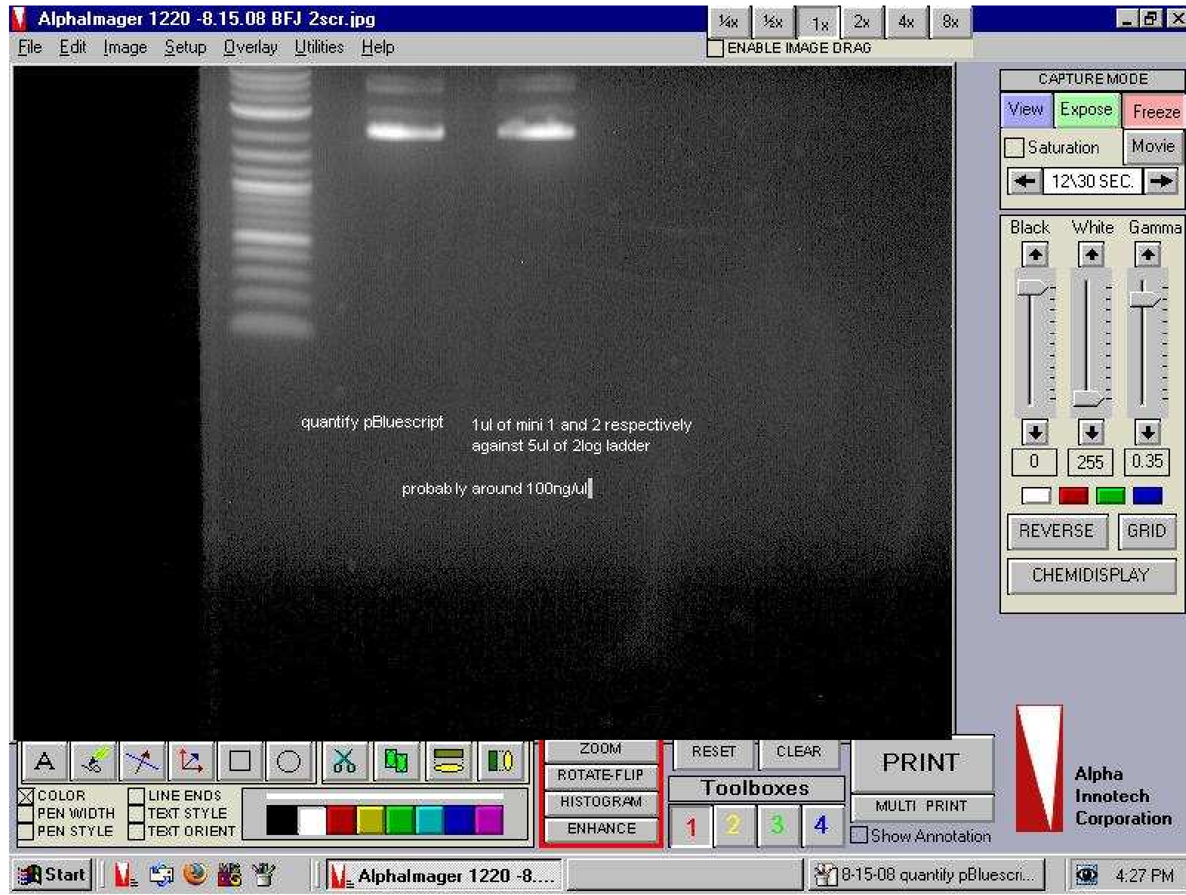
quantify dna concentrations of all constructs on 0.8% gel

- 10ul lambda bst - 1ul a20bmp - 1ul tcfbmp - 1ul aMHC - 1ul sv40 - 1ul cmvrtta
- yielded results of 20,20,3,20, 80 respectively (ng/ul) estimate
- 8-15-08_quantify_construcst.jpg:



quantify dna concentration of pBluescript in extra lanes

- 10ul lambda, 1ul pBlue, yielded about 15ng/ul concentration
- 8-15-08_quantify_pBluescript.jpg:



prepare ligation math of each insert with sv40 and into the plasmid

- INSERT TABLE HERE!!! (note that I did... but the wysiwyg editor always screws up... now you get this...
- pbluescript: 2958 bp, 15ng/ul,
- sv40: 161bp, 20ng/ul, 18.4 ratio, 0.16 3:1 ratio, 0.32:2.5 corrects for concentrations
- cmv-rtta: 1413 bp, 80ng/ul, 2.1 ratio, 1.4 3:1 ratio, 0.7:2.5 corrects
- a20-bmp4: 1400, 20ng, 2.1, 1.4, 3.4:2.5 corrects
- tcf: 1600, 20ng, 1.8, 1.7, 3.4:2.5 corrects
- amhc: 2054bp, 3ng/ul, 1.4, 2.1, 10.5:1 corrects, will use 9:1 as corrected value

Ligtions: overnight at 13 degrees set (really 15 degrees with error) on robocycler block 4. started around 9:30PM. every tube got 2ul 10x buffer, 1ul tf dna ligase, 0.32ul sv40 poly a tail piece. in addition, specific reactions got...

- 0.7ul cmv, 2.5 pblue, 13.48 water
- 3.4 a20, 2.5pBlue, 10.78 water
- 3.4 tcf, 2.5 pblue, 10.78 water
- 9 aMHC, 1 pBlue, 8,68 water

Note, i forgot the negative control of cut plasmid by itself. Jen did this for me later. Sorry.

Note that pBluescript mini2 looks like it has phage infection (white crap at bottom). Mini 1 looks cleaner.

Made glycerol stock of pBluescript SK+ from pBluescript 1 bacterial tube (the one on the shaker). 130ul glycerol (set, stabbing at 200). 500ul of the LB from the bacteria. In -80

August 14, 2008

Picked two colonies from the plate, each placed in 4ml LB and 8ul Amp. Put on shaker at 1:25PM

PCR of cmv promoter

- 32 water, 5 buf, 5dntp, 3mgso4, 1.5 5' cmv, 1.5 3' cmv-rtta, 1 pcDNA 3.1zeo+ at 10ng/ul (diluted first from 100ng/ul), 1 hot, oil
- 55 anneal, 15 second elongation, ericomp small

PCR of tet-nkx-2a-dsred off of Simina's gel isolation of this very same construct (i.e. amplifying faintness)

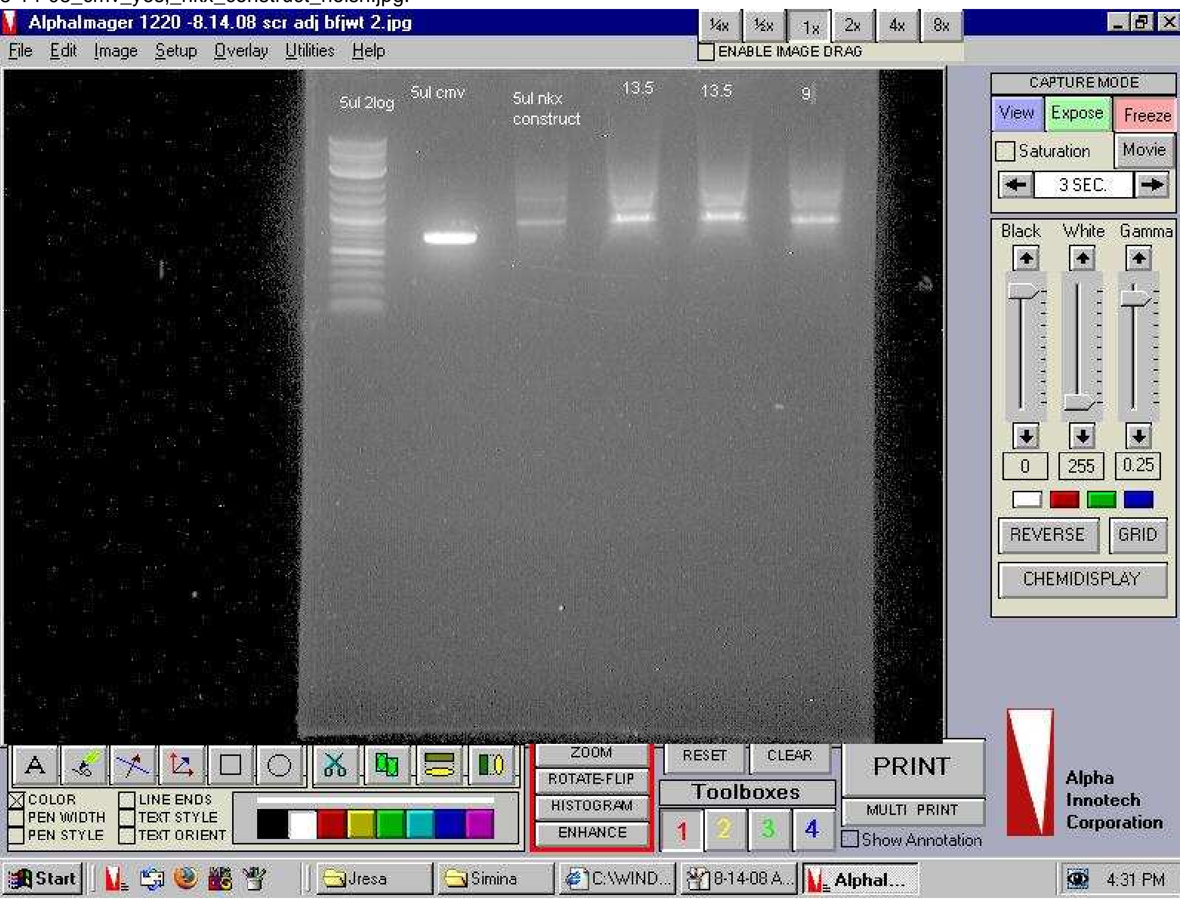
- 15.25 water, 13 betaine, 5buf, 5dntp, 4mgcl2, 2.5dmso, 1.5 5' tet, 1.5 dsred 3', 1 ul of the gel isolated nkx construct, 0.25 deaza gtp, 1 hot, oil
- robocycler, 40-20-60, 57 anneal

Noted that BspDI? may not have cut the pBluescript. We didn't have an uncut control on the gel to compare. Wanted to see if we could get what we thought was the cut with BspDi? and cut it again with something else to check if BspDI? cut or not. Chose ScaI? because it would give two distinct bands if BspDI? worked, but only the linearized form if not. However, the old reaction had loading dye in it. Tried ammonium acetate precipitation.

- Added 10ul water to bring volume to 20ul. Added 1ul glycogen at 35ug/ul. Added 7 ul ammonium acetate at 10M, added 70ul 100% ethanol, mixed, iced for ten minutes. No precipitate forming. Centrifuged in fridge at 13000 gs, 15minutes. see no precipitate. Stopped here. This wasn't working.

Gel of CMV and nkx construct.

- 5ul 2log - 5ul cmv - 5ul nkx - 13.5ul nkx - 13.5ul nkx - 9ul nkx
- cmv worked, 700 band. nkx failed, no gel isolation
- 8-14-08_cmv_yes,_nkx_construct_noish.jpg:

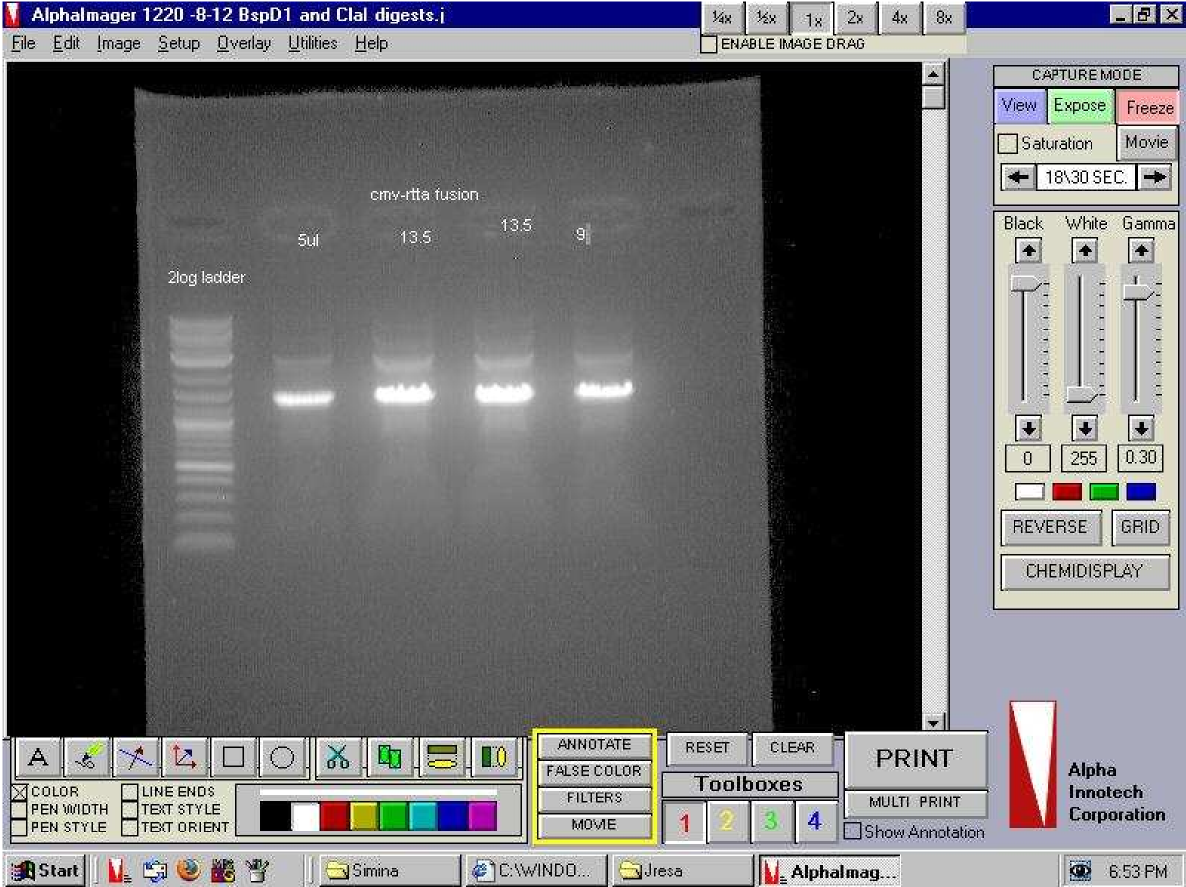


PCR of cmv-rtTA fusion

- 29 water, 5 buf, 5dntp, 3mgso4, 3 5' cmv, 3 3' rtTA, 0.5ul of cmv(19), 0.5ul of rtTA(10), 1 hot, oil
- small ericomp, 55 anneal, 25 cycles, 15 second elongation

Gel of above:

- 5ul 2log - 5ul cmv-rtta - 13.5ul - 13.5ul - 9ul
- 8-14-08_cmv-rtta_fusion_success.jpg:



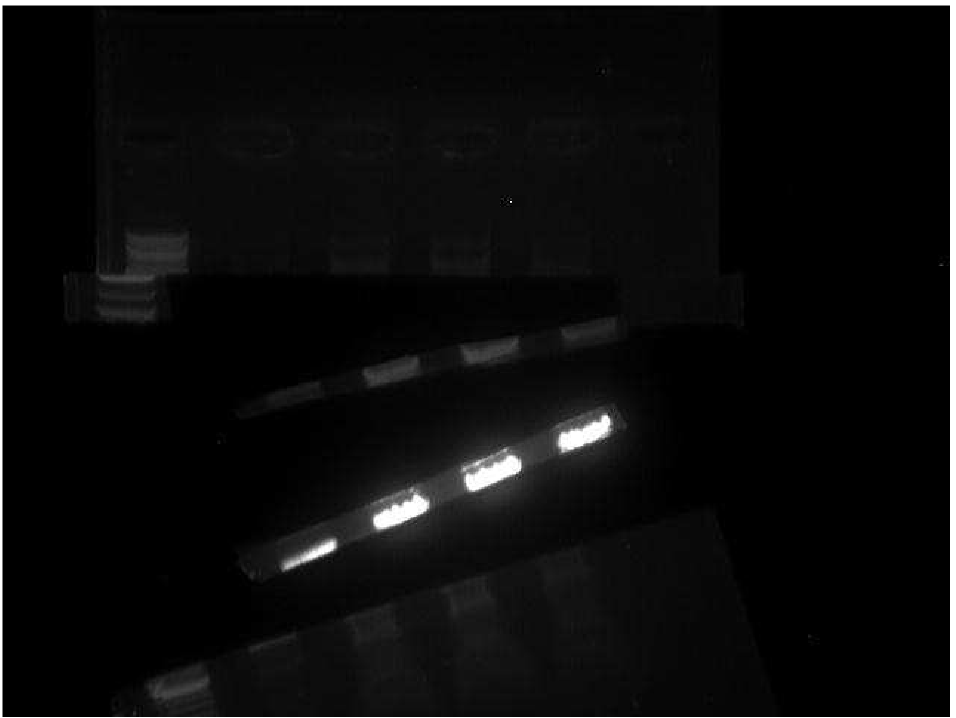
- This to be gel isolated. Ready to go.

PCR of tet-nkx-2a-dsred fusion off of a 4 cycle non-oligo fusion.

- 13.25 water, 13 betaine, 5 dntp, 5 buf, 3 mgcl2, 2.5dmso, 3 5' tet, 3 3' dsred rediluted today (95:5), 1 aliquot of the 4 cycle reaction, 0.25 deaza gtp, 1 hot, oil
- 40-60-1:00 at 56 anneal 25 and 35 cycles hopefully.

Simina's gel work...

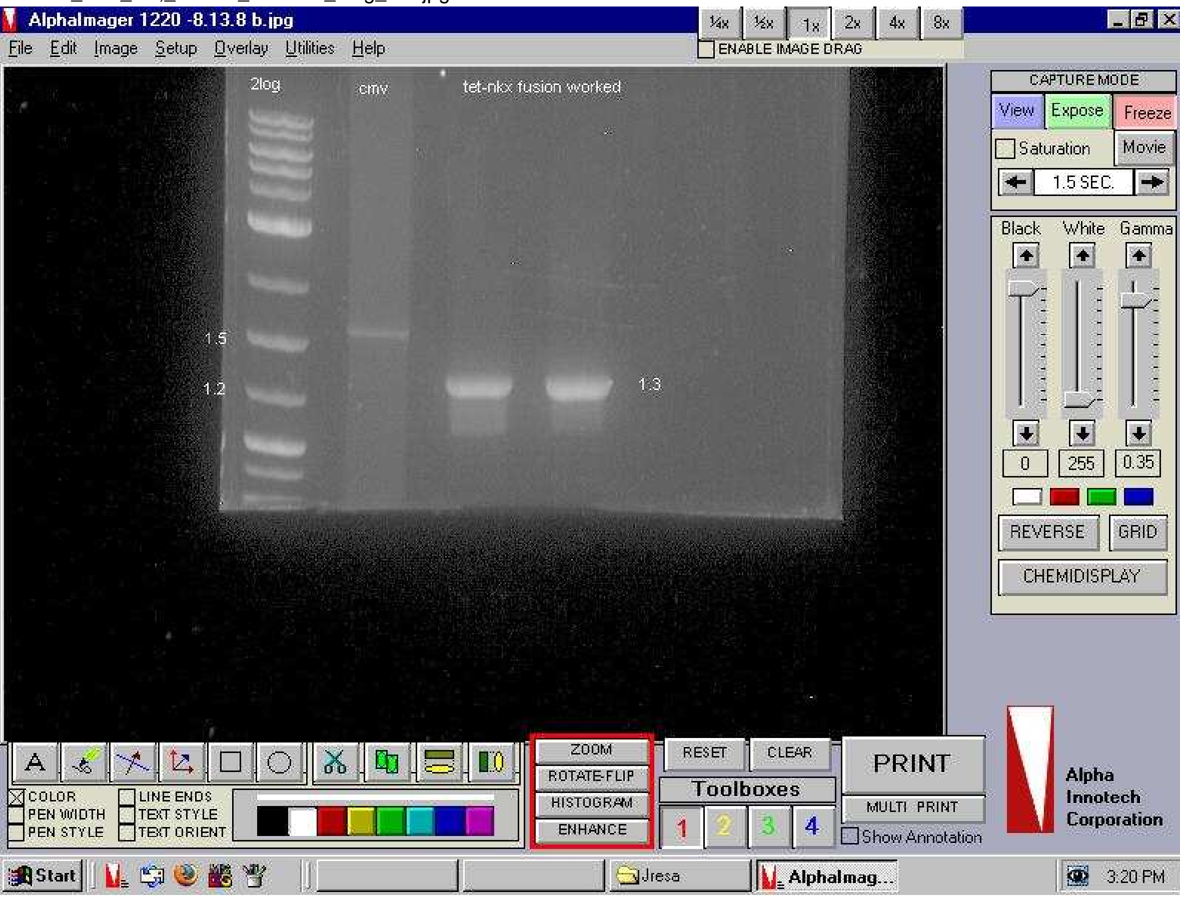
- 8-14-08_siminas_gel_work.jpg:



August 13, 2008

1% gel of tet-nkx and cmv promoter. lanes 2log - cmv - tet-nkx27 then 35 all 5 ul loaded

- tet-nkx worked!!! cmv did not, band is at 1.5kb, should be 700ish
- 8-13-08_cmv_fail,_tet-nkx_success_long_run.jpg:



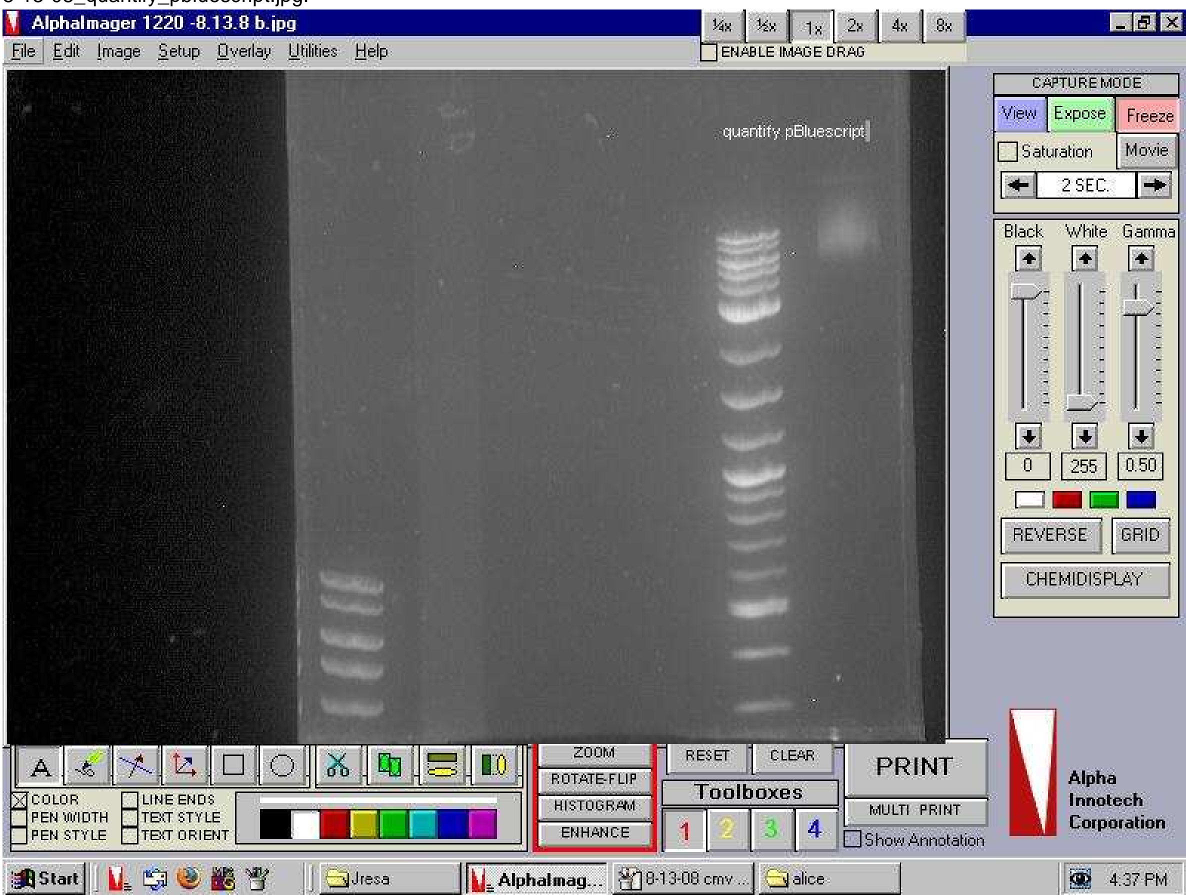
pcr entire nkx construct on small ericomp at 25 cycles. 56 anneal, 30sec elongation

- 12water, 13 betaine, 5 buf, 5 dntp, 4mgcl2, 3 tet5', 3 dsred 3', 2.5dmso, 0.5 tet-nkx(10), 0.5 2a-dsred(14), 0.25 deazagtp, 1hot, oil

Got the plasmid dna for pBluescript, suspended in 50ul water.

Quantify with 1ul of pBluescript and 4ul 2log. We determined that the plasmid looked like the 57 ng band of 2log, and that the concentration was 20-30ng/ul... However this looks like crap... Not a good looking gel.

- 8-13-08_quantify_pbluescript.jpg:



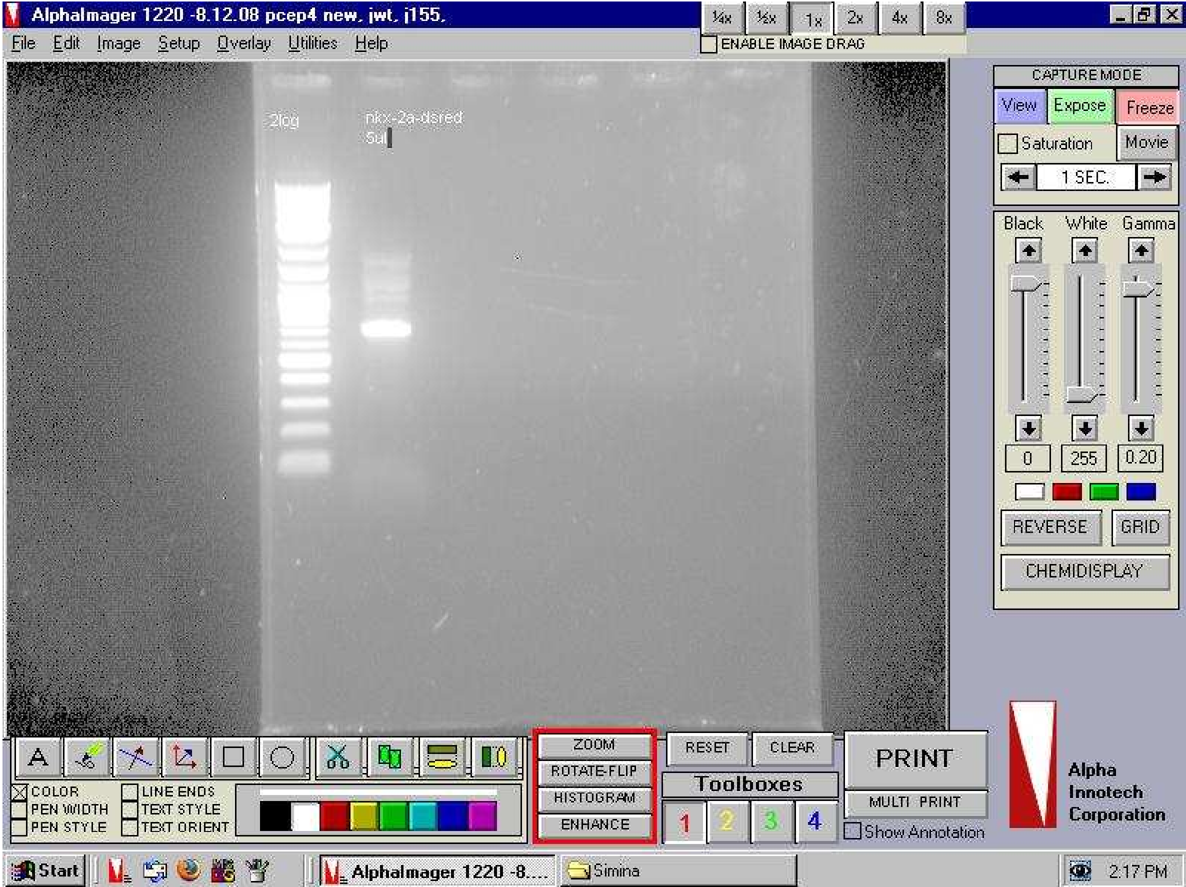
Transform plasmid pBluescript into DH5 Alpha cells.

- added 1ul of the plasmid dna with prethawed on ice cells, mixed by tapping
- transfer to cuvette, prechilled on ice for 5mins. shake to mix and settle to bottom.
- place into sample chamber set at 2.5kV, pulse both buttons, press actual volts to make sure over 5
- add 1ml of LB, shake on 37 degree for an hour
- preheat lb plate to remove moisture at 37.
- plate 20ul of cells with 80ul of LB. in 37 incubator **starting at 4:25 PM**

August 12, 2008

gel of Nkx-2a-dsred on 1%, 2log - nkx-2a-red - lambda bst (5ul of each)

- 8-12-08_nkx-2a-dsred_fusion_fail.jpg:



new enzymes arrived (cla and bspd1, time to check the plasmid pNEB193 new)

- BspD1? cuts
  - 15.5ul water, 2ul buffer 4, 2ul dna, 0.5ul bspd1. controls are uncut KRAB, cut KRAB, uncut pNEB193 new. then cuts are Klenow 1-6, Pfu 1-4 of 50ng and 100ng each
- ClaI? cuts
  - 13.5ul water, 2ul buffer4, 2ul BSA, 2ul dna, 0.5ul ClaI? . controls are uncut KRAB, cut KRAB (10ng/ul is why these don't show up on the gel...), uncut pNEB193 new, and same as above.
- BamHI? cuts
  - 13.5ul water, 2ul nebuffer3, 2ul bsa, 2ul dna, 0.5ul BamHI? . controls are uncut and cut pNEB193 old, uncut pNEB193 new. then cuts are as above.
- digested for two hours at ~37 degrees.

Brainstormed ways to get tet-nkx fusion.

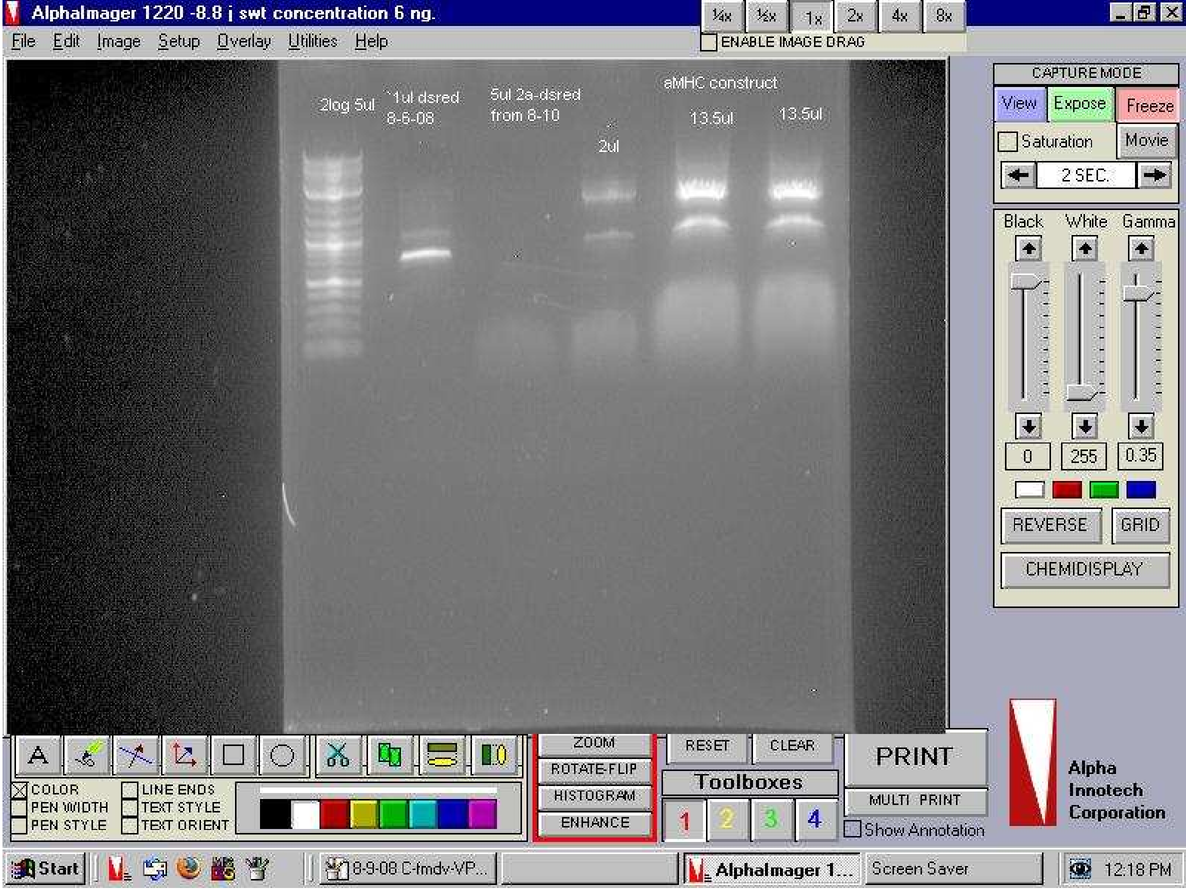
- try phusion and la taq polymerases
- redilute oligos, double check...
- **gel isolate tet and nkx, then try the fusion!**
- can try more cycles for the amplification of tet-nkx from the gel iso because the small amount of dna that might be present
- can raise temperatures to attempt to avoid any hairpins in the fusion region

Gels of digests in SiminasNotebook show that the enzymes work, but we didn't get any cutting of our plasmid. Must switch plasmids.

August 11, 2008

Gel of dsred and aMHC isolate on 1%

- lanes: 1-5ul 2log, 2-1ul dsred, 3-5ul 2a-dsred fusion from yesterday, 4-2ul aMHC construct, 5 and 6-13.5ul aMHC construct for gel isolation
- 2a-dsred failed again, got nothing. Cut out the good aMHC construct band at 2kb for gel isolation
- 8-11-08_2a-dsred_and_aMHC_run.jpg:



PCR tet-nkx fusion with new attempt at much higher concentration of nkx to tet. used nkx(25 tube and 1ul of it

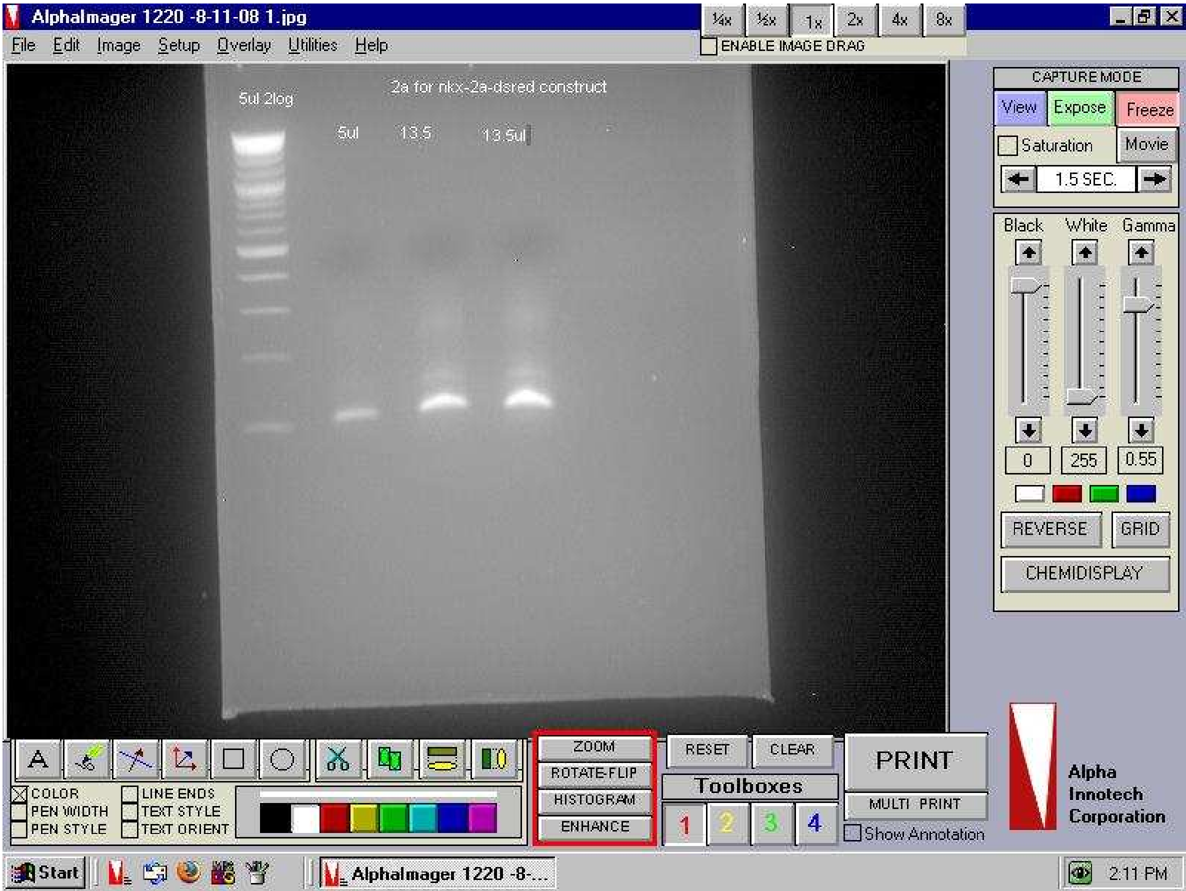
- 13 water, 13 betaine, 5 buffer, 5 dntp, 3mgcl2, 2.5 dmso, 3 5'tet oligo, 3 3' nkx-2a oligo, 1ul nkx(25) 7-27-08, 0.25ul tet(10), 0.25deazaGTP, 1ul hot start, oil
- used small machine. 5 cycles at 55 degrees, 20 cycles at 57 degrees.

aMHC construct gel isolation was 0.5g of gel, added 500ul turbo salt sol'n, put at 55 degrees for 5 minutes.

- added to catch tube thing, 5 second centrifuge. then 500ul turbo wash, 5 sec centrifuge, then 4 minutes
- in new catch, 30ul elution buffer, incubated at room temp (hot today, lol!) for 5minutes, and 1 minute centrifuge.
- placed in 1.5ml tube in the 2008igem box.

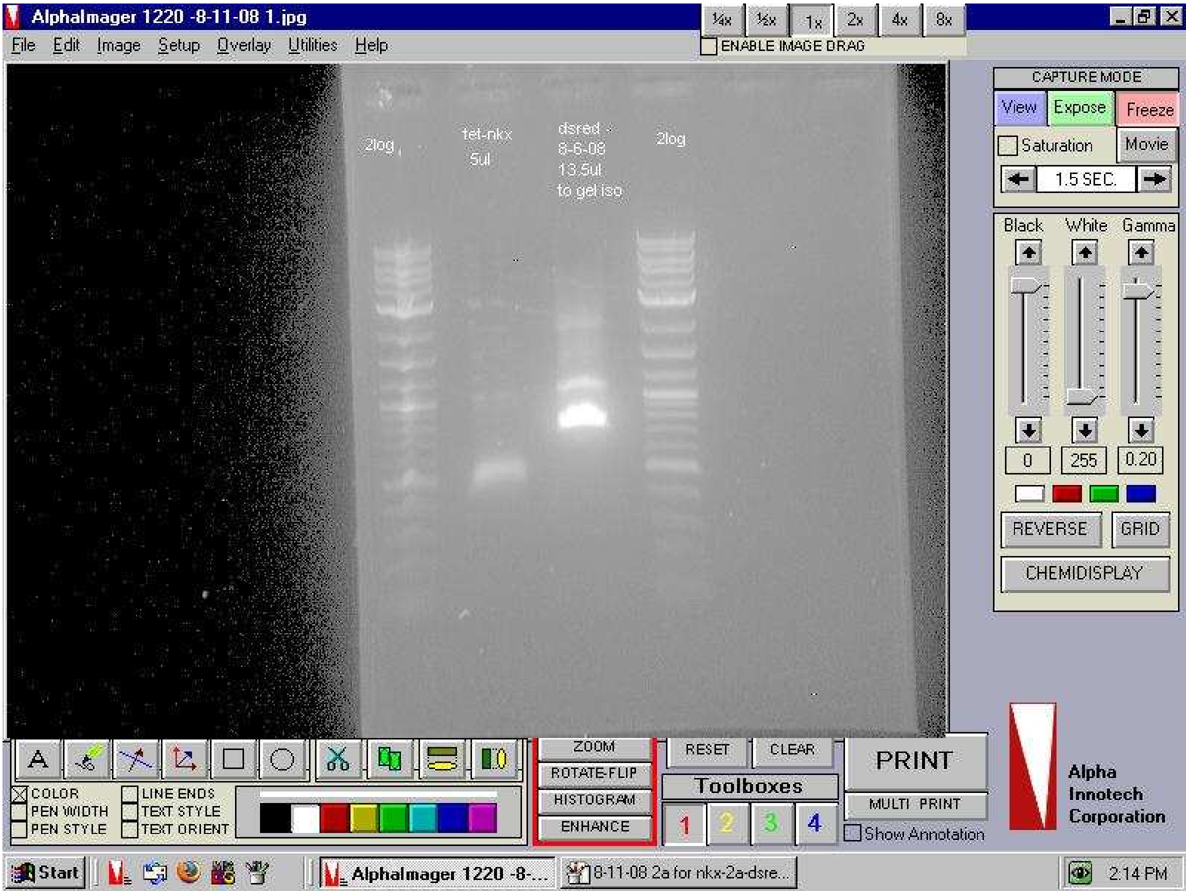
gel of 2% for 2a isolation (2a for nkx-2a-dsred)

- 5ul 2log - 5ul 2a, two lanes of 13.25ul for isolation
- fine, cut out
- 8-11-08_2a_for_nkx-2a-dsred_gel_iso.jpg:



gel of 1% dsred and tet-nkx visual

- 5ul 2log, 5ul tet-nkx, 13-15ul dsred to cut out
- tet-nkx failed, but some very faint bands... maybe with more cycles we can get a better stuff up there, or just ligate the sucker?
- cut out the dsred for gel iso
- 8-11-08_tet-nkx_fuse_and_dsred_gel_iso.jpg:



gel iso of 2a and dsred

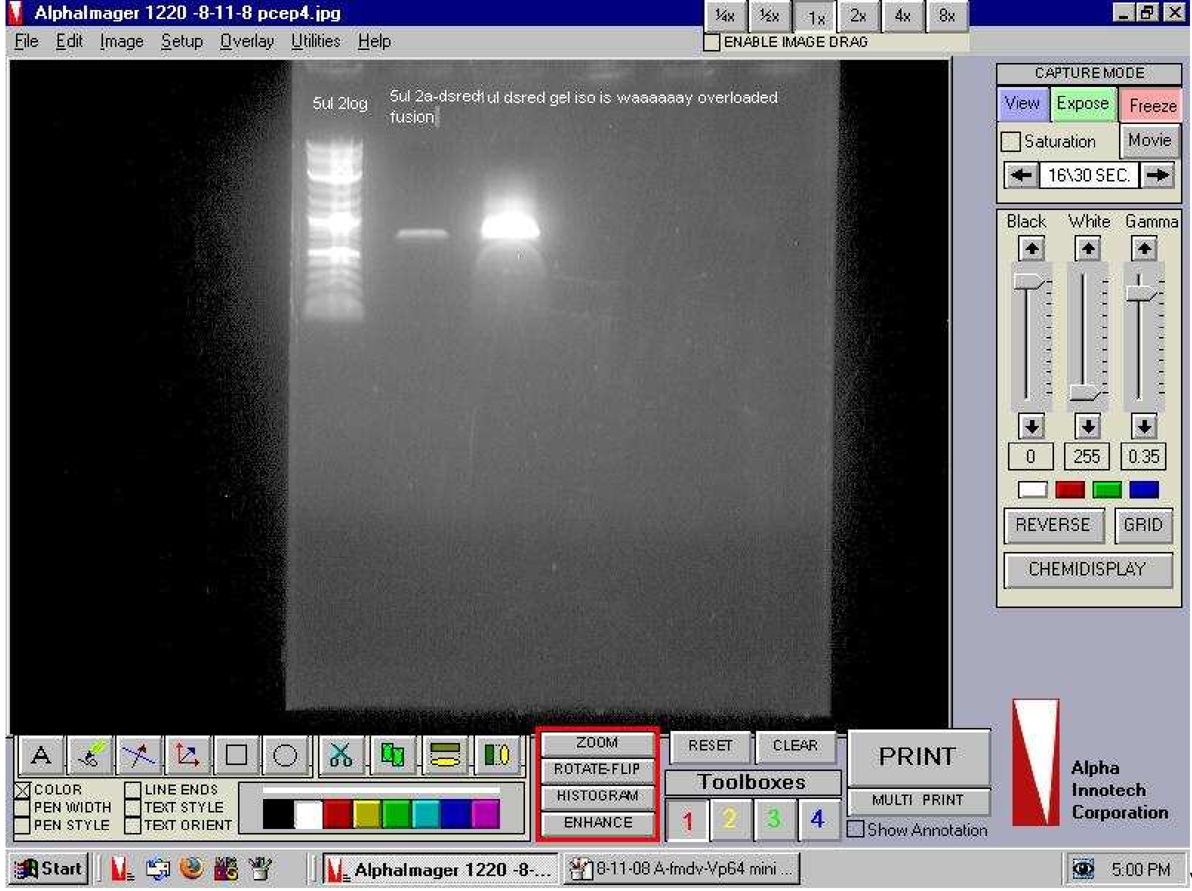
- .40g of 2a and .07 of dsred, so added 400ul salt sol'n to former and 70ul to latter.
- incubate at 55 degrees for a bit.
- transfer to mini catch tube stuff, 5 second centrifuge
- add 500ul turbo wash, 5 sec centr, then 4 minute
- xfer to new tube, add 30ul elution buffer, 5 minute incubate, then 2 minute centrifuge. collected and stored in 2008 igem box.

now attempt PCR of 2a-dsred from the gel isolates

- 29water, 5 buf, 5dntps, 3mgso4, 2.0 5' nkx-2a, 3 3' dsred, 0.5 dsred gel iso, 0.5 2a for nkx-2a-dsred gel iso, 1ul hot start, oil
- small pcr machine. 56 anneal, 15 second elongation, 27 cycles.

gel of 2a-dsred on 1% worked.

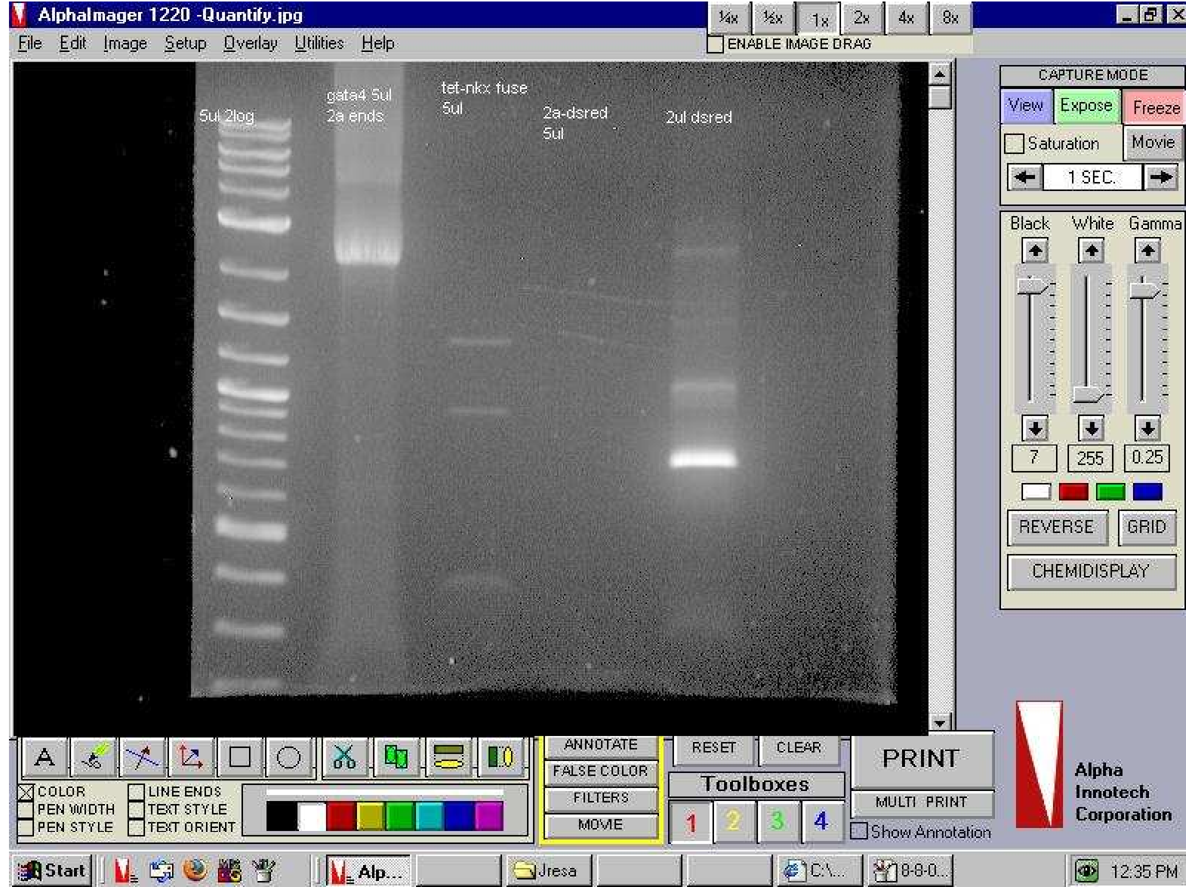
- 5ul 2log, 1ul dsred, 5ul 2a-dsred
- 8-11-08_2a-dsred_fusion_from_gel_iso.jpg:



August 8, 2008

Gel of 5ul 2log, 5ul gata4, 5ul tet-nkx fusion, 5ul 2a-red that simina did, 2ul dsred

- see image. tet-nkx worked. gata was greater than 2kb, no good. 2a-red didn't work, so redoing it.
- 8-8-08_45min_gata,_2a-red,_tet0nkx.jpg:

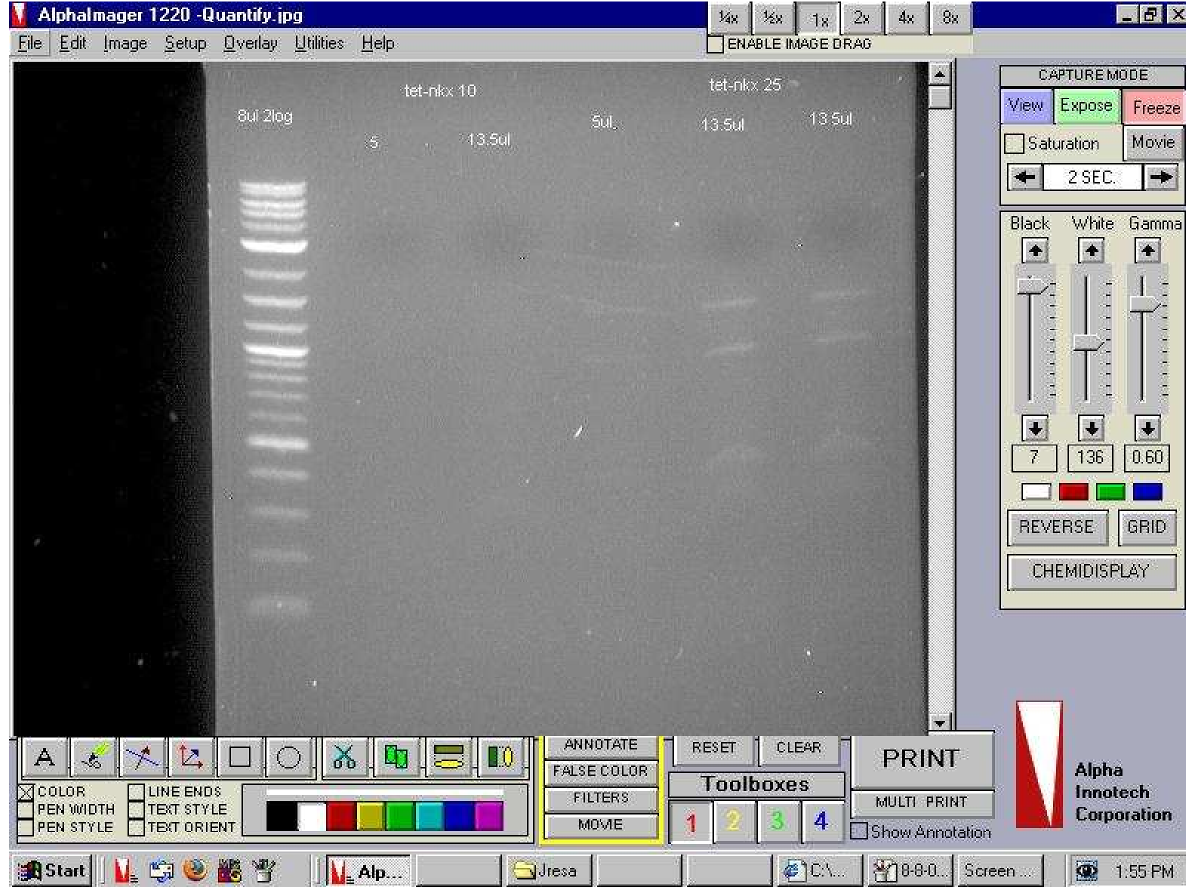


redo amhc entire construct, 4 cycles no oligos and 55, then 30 cycles with oligos at 61 degrees.

- 34 water, 5buf, 5dntp, 4mgso4, 0.5amhc-neor14, 0.5 2a-gfp, 1hot, oil
- 28 water, 5buf, 5dntp, 4mgso4, 3.0 5' aMHC, 3.0 3' gfp, 1 aliquot of above, 1hot, oil

ran tet-nkx gel of 5ul 2log, 5ul tet-nkx(10), 13.5ul 10, 5ul 25cycle, two lanes of 13.5ul 25 cycle. cut and isolated the latter two lanes.

- 8-8-08_30min_tet-nkx_fusion.jpg:



- 0.42g gel, added 420ul turbo salt, 5min at 55 degree.
- now in little tube. centrifuge 5sec. add 500ul turbo wash, cent 5 sec, then 4 minutes.
- new catch tube, 30ul elution, incubate 5mins, then 1 min centrifuge. in 1.5ml tube, labeled as 8-8-08 tet-nkx gel iso. in 2008 igem box

2a-dsred pcr retry

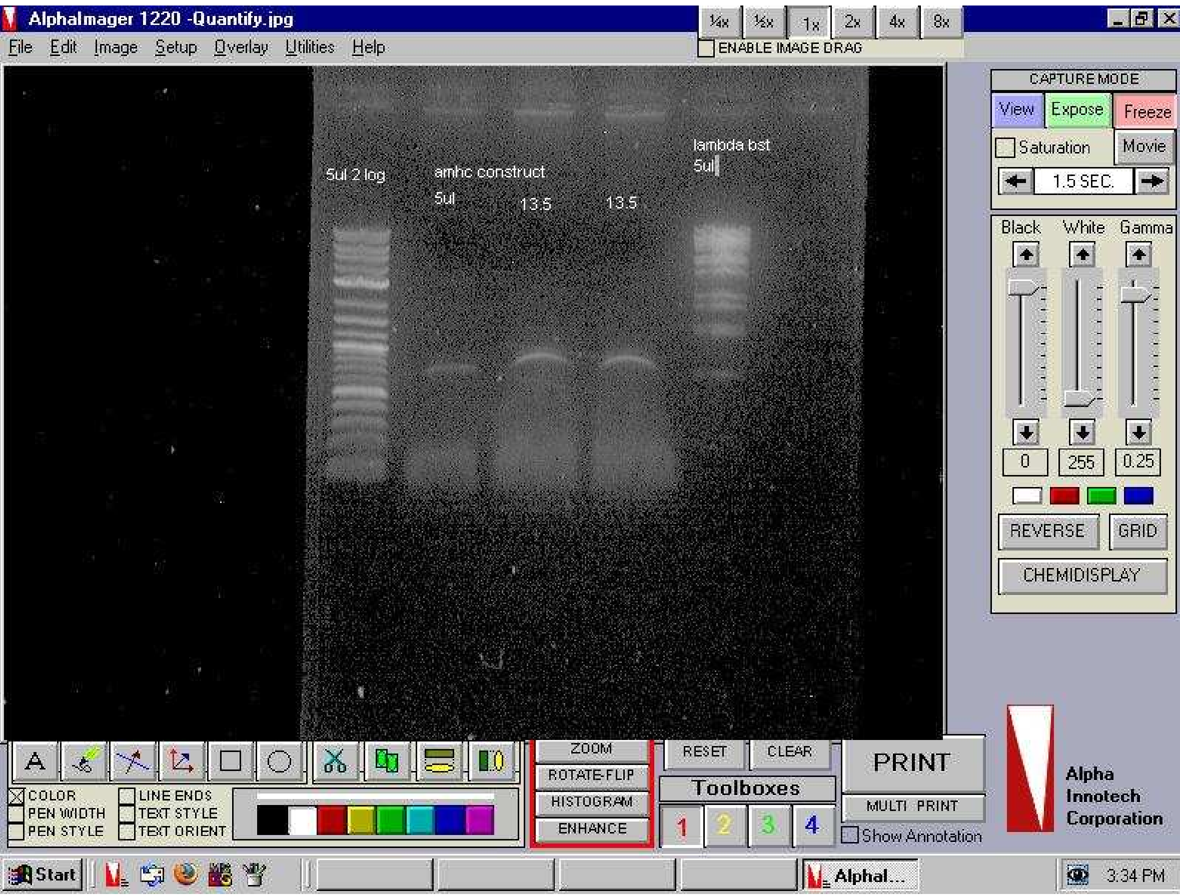
- 29 water, 5buf, 5dntp, 3 mgso4, 3 dsred 3', 3 nkx-2a 5', 0.5 2a-dsred15 8-7-08, 0.5 dsred 8-6-08, 1hot, oil
- 56 anneal, 27 cycles, ericomp small

amplify tet-nkx gel iso

- 32 water, 5buf, 5dntp, 3mgso4, 1.5 5' tet, 1.5 3' nkx-2a, 1ul of gel iso tet-nkx fusion, 1hot, oil, 58 anneal, 15 sec elong. small ericomp

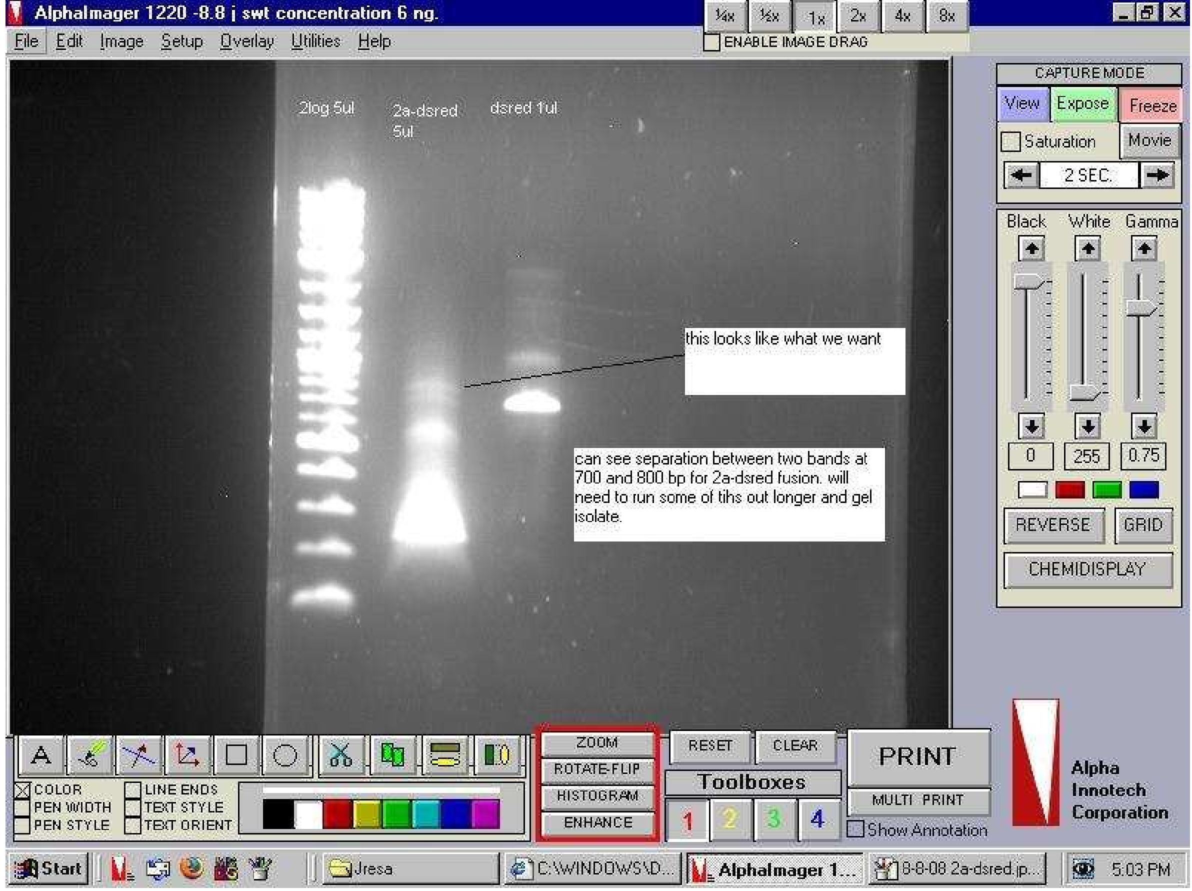
gel of aMHC construct. 5ul 2log, 5ul aMHC, 13.5aMHC, 13.5aMHC, 5ul lambdabst

- failure, banding at 1kb, not 2kb where we want it.
- 8-8-08_aMHC_construct_failed.jpg:



gel of 2a-dsred fusion that i did today

- 5ul 2log, 5ul 2a-dsred, 1ul dsred 8-6-08
- looks like we may have it. two bands, 700 and 800 bp. i think we can run this out long and gel isolate to use. then we can attempt making the nkx construct.
- 8-8-08_2a-dsred_longer_run.jpg:



August 7, 2008

Alright, so aMHC-NeoR worked. Great. Redoing it with fewer cycles though.

- 13.25 water, 13 betaine, 5 buf, 5dntp, 3mgcl2, 2.5dmso, 3 amhc5', 3 neor-2a3', 0.5 aMHC 10x gel isolated, 0.5 NeoR? , 0.25 deaza gtp, 1hot, oil.
- robo machine. 55 anneal, 40 20 40 timing. first tube out at cycle 14, second at 25 (visual)
  - worked, see gel

2a-dsred fusion (now that we got dsred! whoo!)

- 29 water, 5 buf, 5dntp, 3mgso4, 3 nkx-2a 5', 3 dsred 3', 0.5 2a for nkx, 0.5 dsred(10), 1 hot, oil
- small ericomp machine. first tube out at cycle 11. second out after 25 cycles.
- 56 anneal, 18 second elongation (i was torn between 15 and 20)
- OOPS: I don't think I was using the 2a with the correct ends to fuse with dsred! stupid...

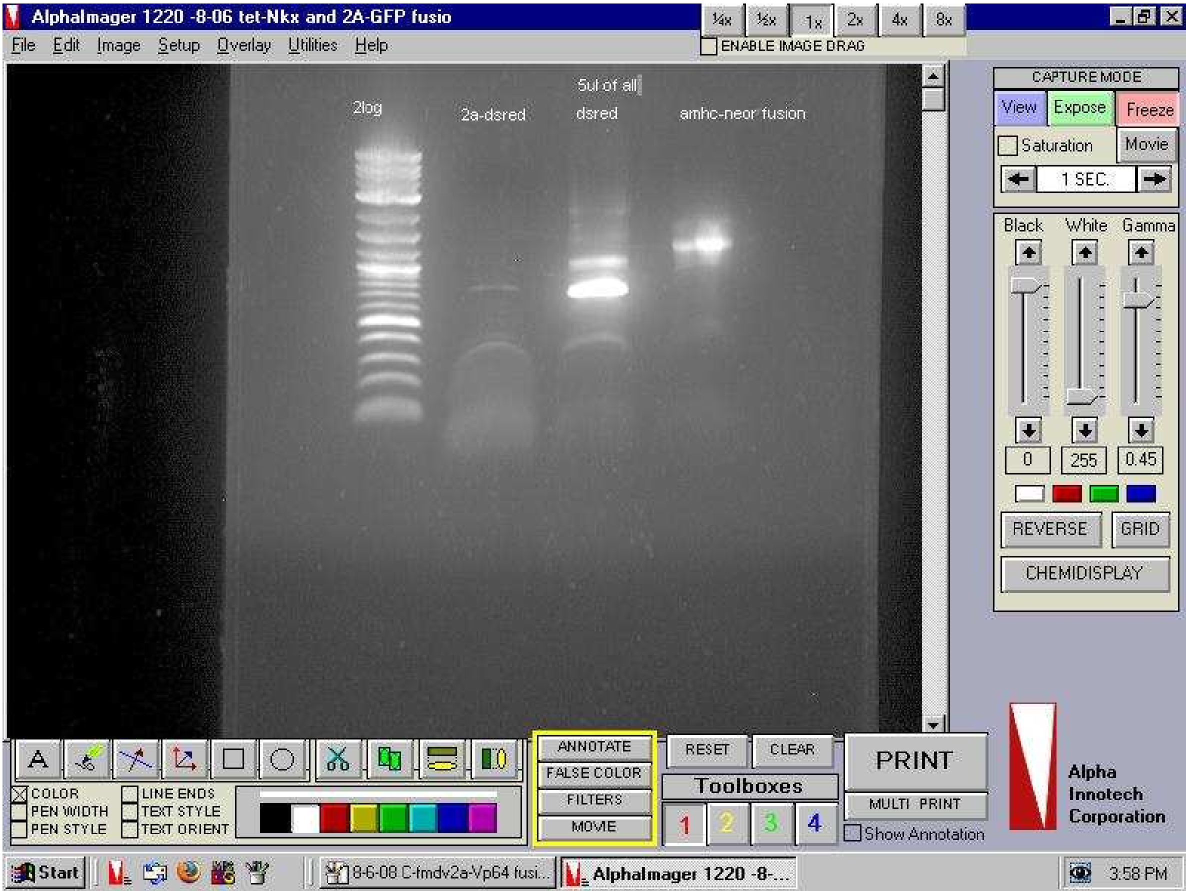
gata4. hey, all of a sudden everything is going our way. going to try it again now that our pcr machines work again. 33 cycles on coy. 55 anneal, 20sec elongation

- 16.25 water ,13 betaine, 5buf, 5dntp, 3 mgcl2, 2.5 dmso, 1.5 2a-gata 5', 1.5 gata-2a 3', 1 gata midi, 1hot, oil
- note that i'm only trying the gata that goes in the large nkx construct, assuming it doesn't work, so not wasting reagents on all versions.

I'm pretty darn tired of that mineral oil, especially for fusion-pcr where we use half a microliter. From now on, i'm transferring all purple tubes to 1.5ml tubes and i'm rolling off the oil on parafilm every time. so from now on... and any others i'll probably do if i notice it.

Gel on 1% for 2a-dred fusion and amhc-neor new

- Lanes: 5ul of all
  - 2log, 2a-dsred, dsred, amhc-neor
- 8-7-08_2a-dsred_amhc-neor_fusions.jpg:



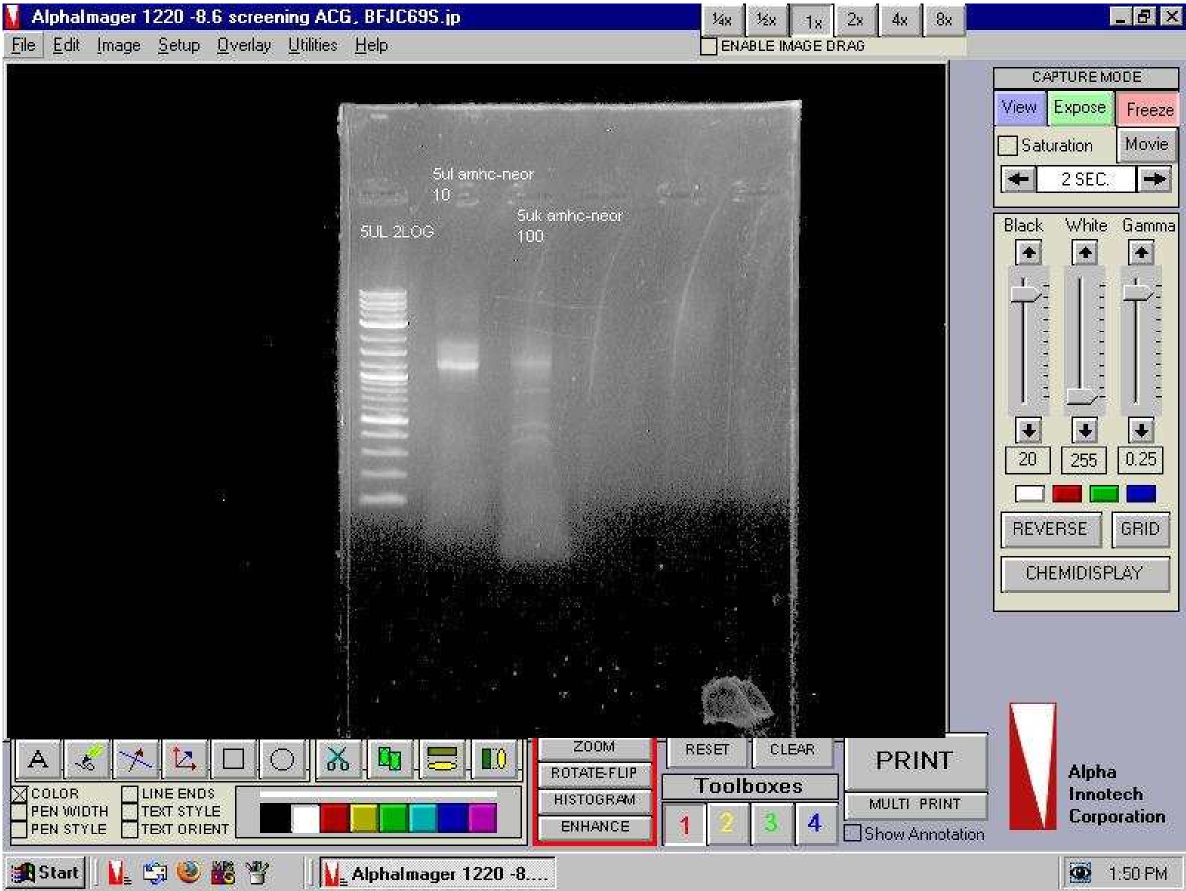
entire aMHC-NeoR-2a-GFP construct! Here we go:

- did the 3 cycle without primers first thing.
- 34 water, 5buf, 5dntp, 4mgso4, 0.5 aMHC-neoR(14), 0.5 2a-gfp (13 cycle), 1hot, oil. small ericomp, 55 anneal. 3 cycles,
- 28 water, 5buf, 5dntp, 4mgso4, 3 5'amhc, 3 3'gfp, 1 aliquot of above reaction, 1hot, oil. small ericomp, 60 anneal, 30 cycles. 20 second elongation

August 6, 2008

First, some good news. aMHC-NeoR fusion worked. Too many cycles though. We need another with fewer cycles (I thought we already had that tube, alas!)

- 8-6-08_amhc-neor_fusion_success.jpg:



Spent some time trying to figure out how to fix stuff that was going wrong.

2a-gfp fusion pcr: first put 2a from gel isolated and gfp 30 into pcr reaction without oligos. let go for 6 cycles. 55 degree melt. small machine

- 35 water, 5 buf, 5dntp, 3mgso4, 0.5 2a, 0.5gfp, 1 hot start, oil

Continue now by doing a normal fusion reaction, only putting back the flanking primers and going at the high melt temp of 61. 61 anneal temp and 30 cycles. small machine

- 32 water, 5 buf, 5dntps, 3mg, 3 5' neor-2a, 3 gfp 3', 1 aliquot of above reaction, 1 hot start, oil
- two tubes, one out at 13 or so cycles.

dsred. ours and jen's oligos as positive control. off plvtrkrab 10ng/ul dilution 12/26/01. 27 cycles at 56 anneal. two tubes, one out at 12 cycles, coy machine

- 32 water, 5 buff, 5 dntp, 3mg, 1.5 5'dsred, 1.5 3' dsred (or 1.5 jen 5', 1.5 jen 3'), 1ul krab, 1 hot, oil

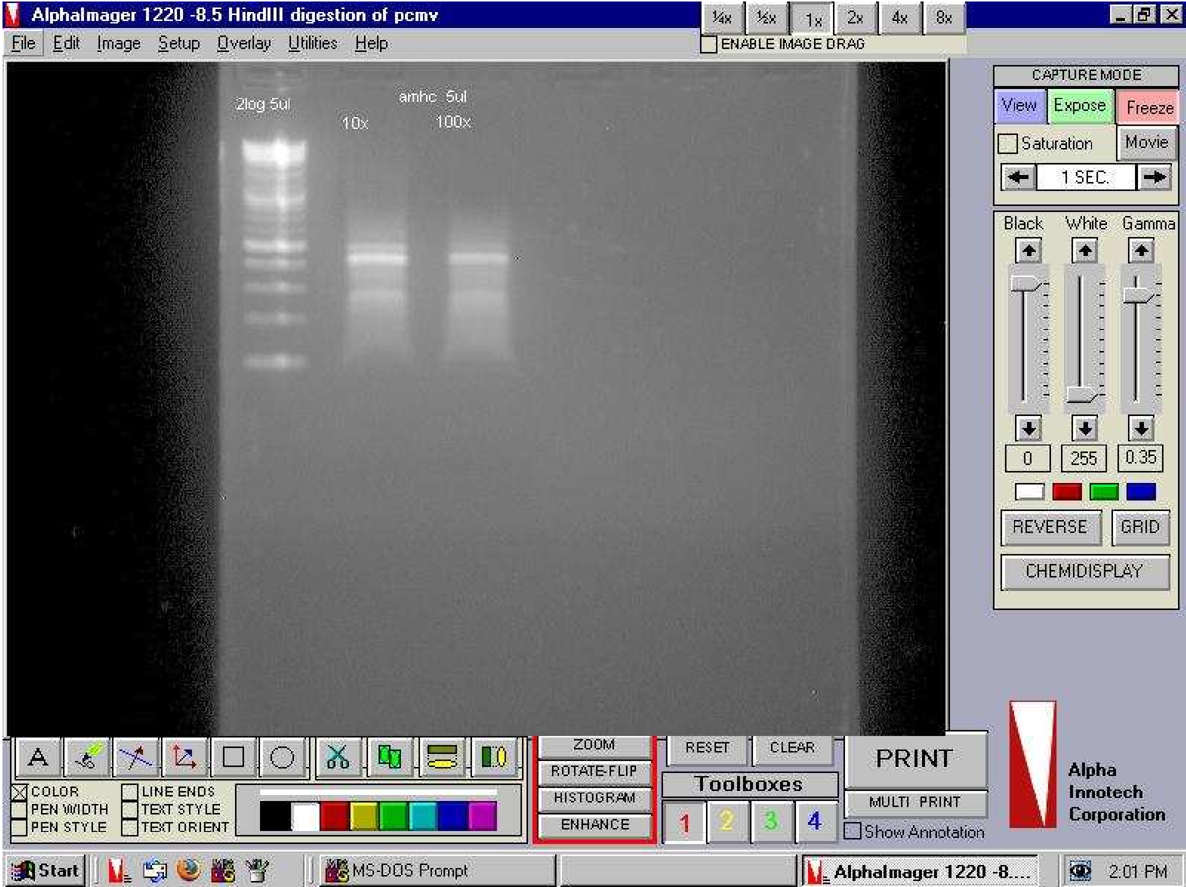
tet-nkx fusion: try to avoid the bad 500bp product we've been getting. that may be a result of the seven tet0 repeats. so want to avoid melt temperatures in that range. instead use full melt temp of flanking primers, even though that's way above the fusion temp. 30 cycles at 62 anneal temp. robocycle

- high gc content. fusion
- 13.25 water, 13 betaine, 5 buf, 5 dntp, 3mgcl2, 2.5dmso, 3.0 tet 5', 3 nkx-2a 3', 0.5 tet(10), 0.5 nkx25(7/27), 0.25 deazagtp, 1hot, oil
- two tubes, one out around cycle 13.

August 5, 2008

2% gel for aMHC. 5ul 2log ladder and 10x and 100x derivatives of aMHC. got product, but also banding.

- 8-5-08_amhc_visual.jpg:

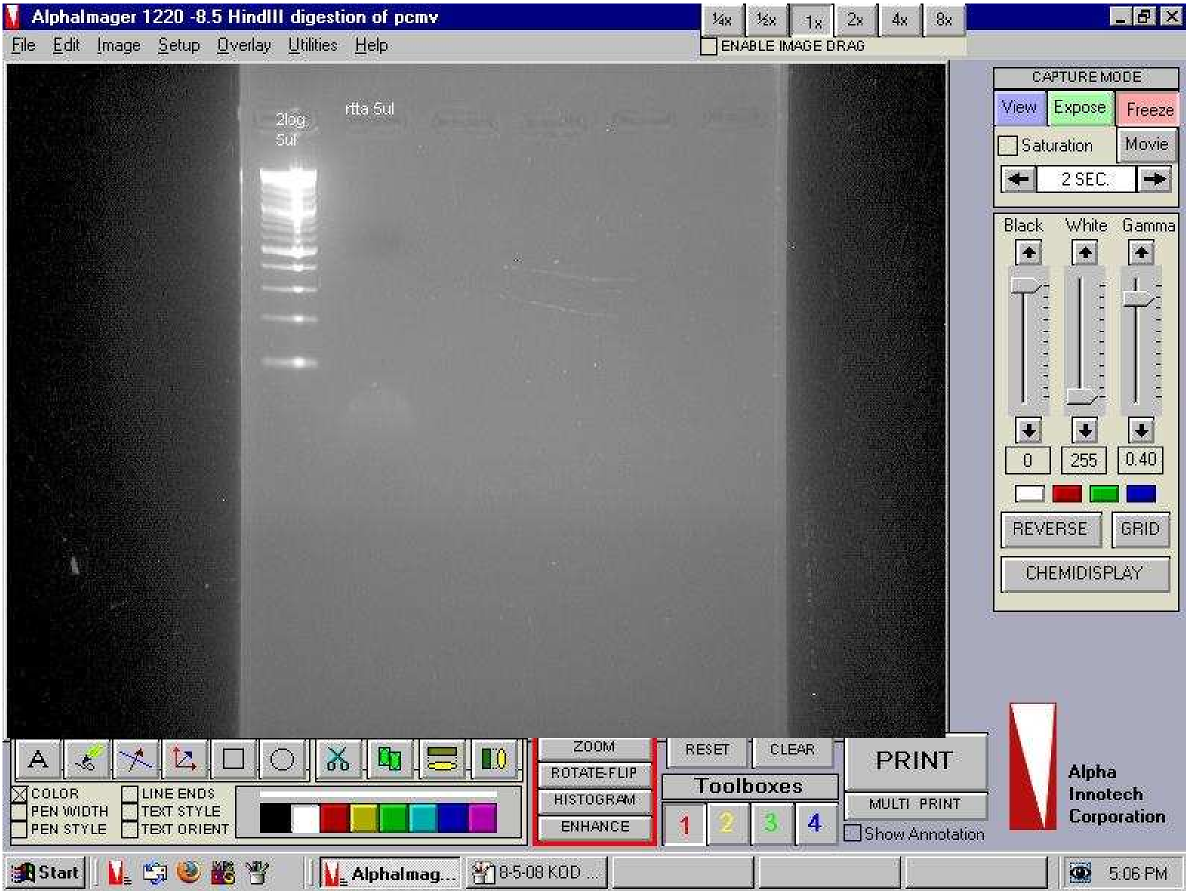


diluted rtTA oligos 5ul oligos, 95ul water. only three of four ordered.

pcr of rtTA. 32water, 5 buffer, 5 dntp, 3 mgso4, 1.5 5' cmv-rtta oligo, 1.5 rtta 3' oligo, 1 hot start, 1 ptripz A1, oil

- wanted times of 20, 10, 15. used robocycler so used times of 20, 15, and 23. anneal temp of 56 degrees.

- 8-5-08_rtTA_failure.jpg:



ran 8 lanes of aMHC on 2.5% gel, 10 ul loaded in each. cut out the band near 450 bp (rather than the 400 bp band) to gel isolate. (Simina cleaned the dna). Chose 450 band because aMHC is itself about 380bp, the oligos add 20bp flanking, and the fusion with neor adds 20bp from restriction sites and 20bp from neor fusion overhang. the 400 band is also in a tube should we find that's somehow what we want instead. can then fuse neor with amhc using this stuff.

2a-gfp pcr 28 water, 5 buf, 5dntp, 3mgso4, 3 5' neor-2a oligo, 3 3' gfp oligo. 0.5 2a gel isolated, 0.5 gfp 13. 1 hot start. oil

- 6 cycles of 55 anneal, 30 cycles of 61 anneal. robocycler, times of 20, 15, 23.

August 1, 2008

tet-nkx fusion pcr first try, 7-30 protocol, used nkx(25) instead of (10), from 7-27

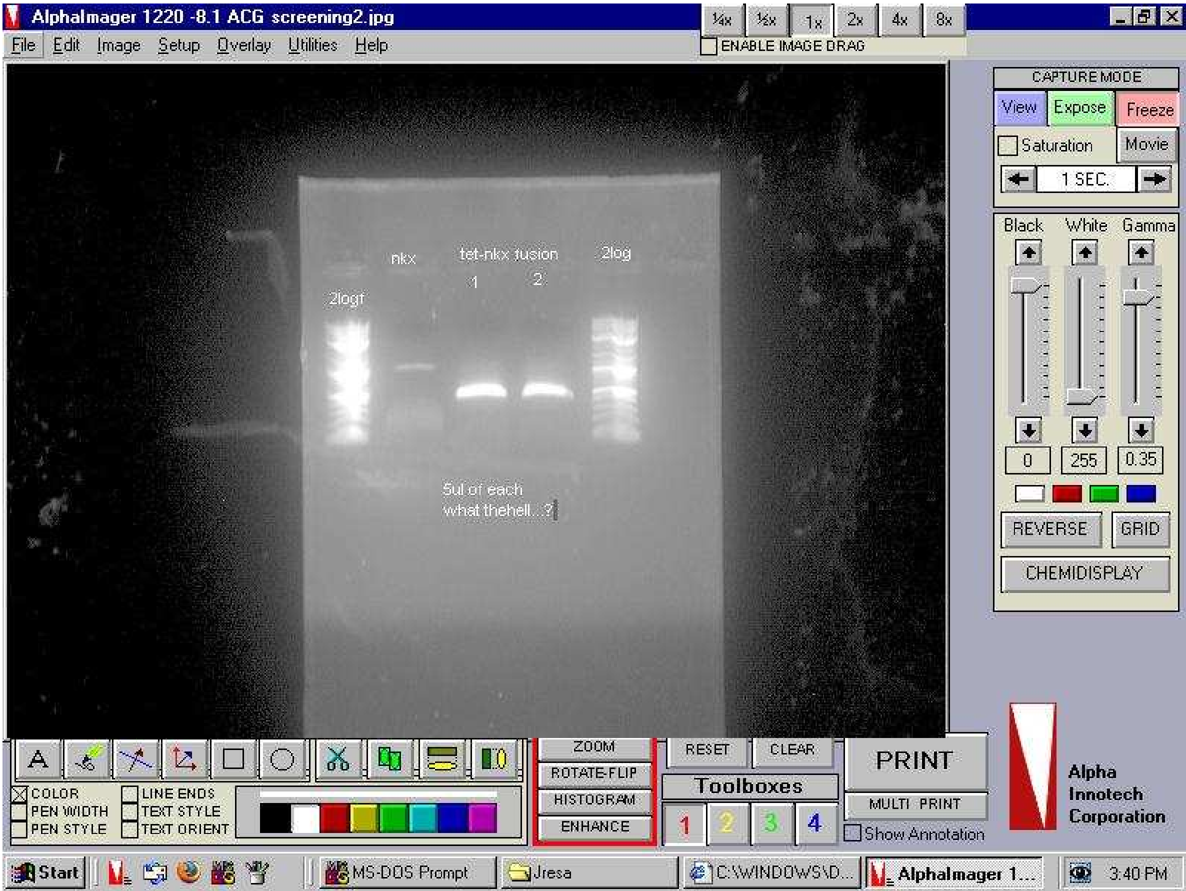
- 13.25 water, 13 betaine, 5 buffer, 5 dntp, 3mgcl2, 2.5 dmso, 3 5'tet, 3 3'nkx-2a, 0.5 teto(10) 7-24-08, .5 Nkx(25) 7-27-08, .25 deaza GTP, 1 hot start, oil
- five cycles of 55 melt, 20 cycles at 58 degrees. elongation time of 20. small machine

tet-nkx fusion second attempt (before checking whether the first thing worked)

- 13.25 water, 13 betaine, 5 buffer, 5 dntp, 3mgcl2, 2.5 dmso, 3 5'tet, 3 3'nkx-2a, 0.5 teto(10) 7-24-08, .5 Nkx(125) 7-27-08, .25 deaza GTP, 1 hot start, oil
- five cycles of 51 melt, 20 cycles at 56 degrees. elongation time of 20. small machine

gel of both above attempts: 1%, 115-120V, 2log ladder

- lanes: 5ul of everything
  - 2log - tet-nkx 1st - tet-nkx 2nd
- Failed: got bands at 500bp for both procedures. something's wrong here. we're amplifying something, just not what we want.
- 8-1-08_tet-nkx_fusion_failure_at_500bp.jpg:



GEL MACHINE REMOTE CONTROL WORKS! 192.168.0.198

July 31, 2008

News I can use:

- From SiminasNotebook: gata4 is 700ng/ul about.

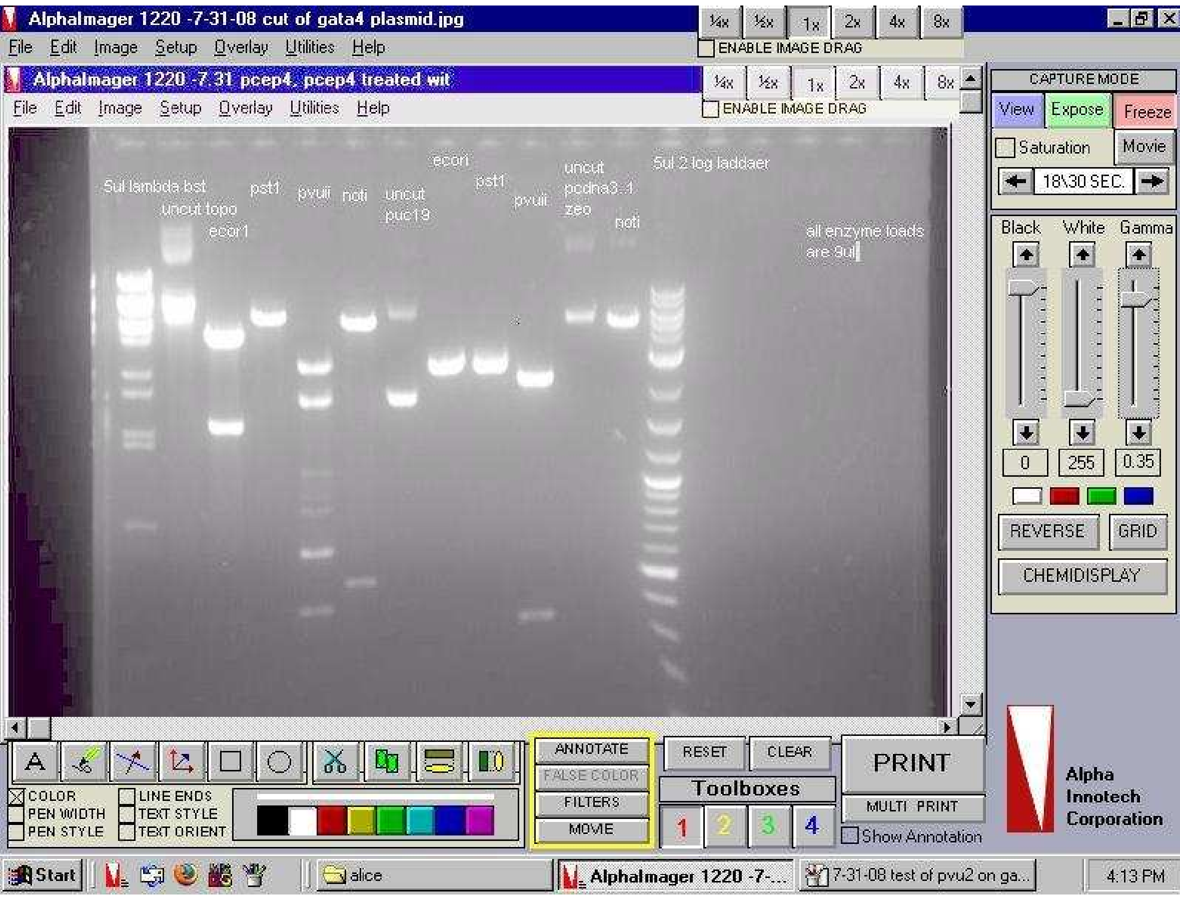
Restriction Digest of pneb with BspDI?

- Each tube had 2ul NEBuffer4, 16ul water, 1ul of DNA. The cutting tubes had also 1ul BspDI? .
- Cut Klenow 1-6, Pfu 50 1-4, and Pfu 100 1-4. Used controls of uncut Klenow2, uncut plvtrKRAB-red, and BspDI? cut plvtrKRAB
- Ran the digest for 2 hours and 20 minutes.
    - The gel needs to be run.

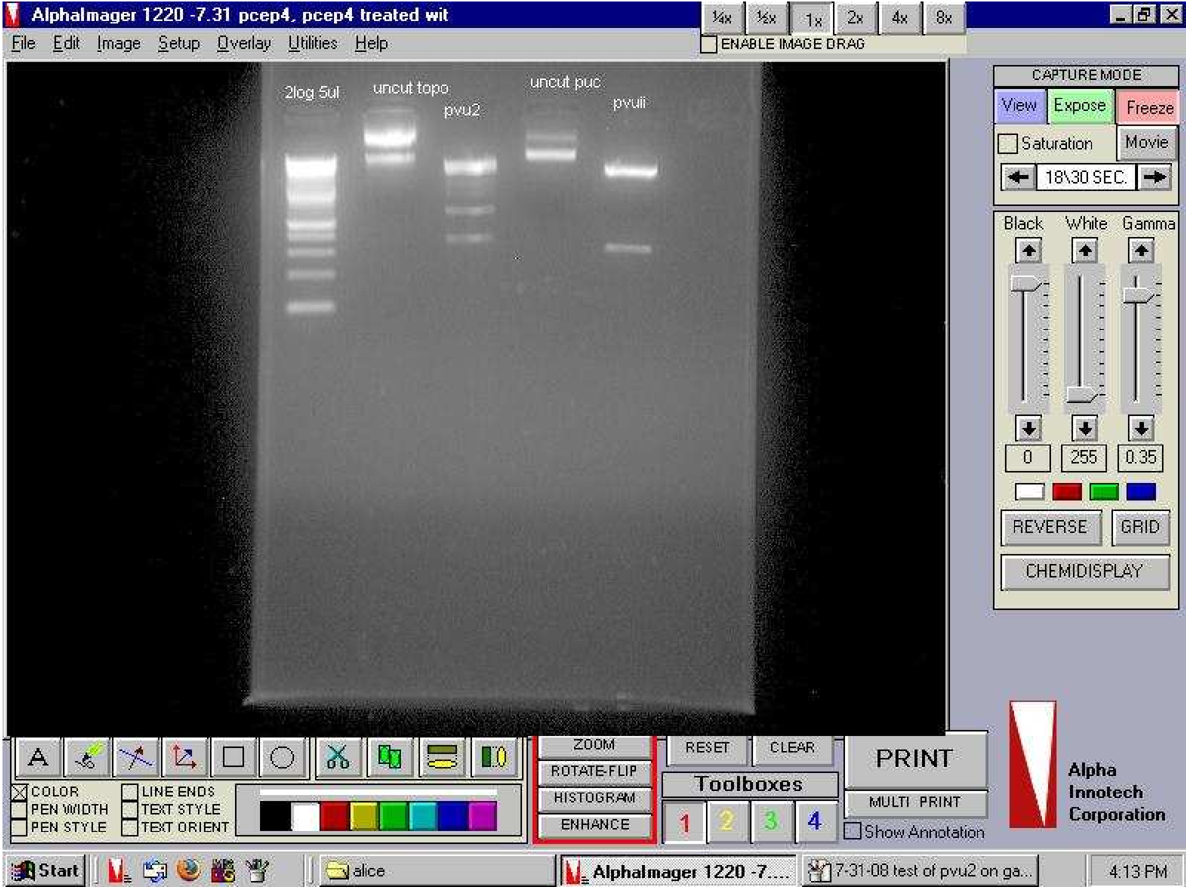
Ran the gel for the gata4 plasmid (gata4 midi), pCR4-topo digests to check that the plasmid was good.

- Lanes: ladders have 5ul, all cuts have 9ul
    - lambdabst - uncut topo - ecori - psti - pvuii - noti - uncut puc19 - ecori - psti - pvuii - uncut pcdna3.1zeo+ - noti - 2log ladder
- The gata4 looks good. i see what look like the appropriate bands.
    - eco should have 3939, 1344.
    - psti should have 5283
    - pvuii should have 2619, 1711, 593, 360
    - noti should have 4296, 987
- I think Jen meant I should use puc18, not puc19... i don't have the puc19 map.
- I looked at my copy of zeo, and it doesn't look like noti even cuts...
- in any case, these were the positive controls, and it looks like the enzymes worked anyway, from topo, and because the controls show that the enzymes change the banding...

- 7-31-08_cut_of_gata_v2.jpg:



- 7-31-08_test_of_pvu2_on_gata.jpg:



PCR of gata4 from NT2. 55 cycles at 55 anneal at 20 second elongation

- high GC content protocol. 16.25, 13, 5, 5, 3, 2.5, 1.5 tet 5', 1.5 gata-2a 3', 1ul NT2, .25 deaza, 1 hot start, oil
- robot machine, so made anneal 15seconds, made 30 second elongation

PCR of dsred

- normal protocol ingredients: used jen's oligos for dsred: pacjtz17dsredin 5' and dsred 3'. 1ul KRAB (10ng/ul).
- COY machine: 27 cycles, 56 anneal, 15 second elongation

Gel in small box hardening. For use with gata4 (in pcr box), to see if we got anything.

July 30, 2008

Sean had set up PCR for dsred off of Jen's dsred. I put it in the machine, going for 61 degrees at 50 cycles.

Visualized the tet-o-nkx gel that simina did yesterday. Saw that she used tet 5' and tet fusion 3' oligos, which only amplify tet. On the gel, that's what we saw, amplification of tet. I crossed off Nkx on the tube so we have another tube of tet if we need it.

I set up PCR of tet-Nkx fusion, using high GC content protocol, two tubes.

- 13.25 water, 13 betaine, 5 buffer, 5 dntp, 3mgcl2, 2.5 dmso, 3 5'tet, 3 3'nkx-2a, 0.5 teto(10) 7-24-08, .5 Nkx(10) 7-27-08, .25 deaza GTP, 1 hot start, oil
- five cycles of 55 melt, 20 cycles at 58 degrees. elongation time of 15.
    - However, big PCR machine stuck on 97... so switched to COY machine.

repeated my procedure for aMHC that worked when I got it for the wrong ends... standard PCR reaction. used 10x and 100x rat genomic.

- 32 water, 5 buf, 5 dntps, 3 mgso4, 1.5 amhc 5', 1.5 amhc-neor-3', 1 rat genomic, 1 hot start, oil
- 50 cycles, 55 degrees. ten second elongation.

determined that we should cut pCR4-topo (gata midi) with EcoRI? (1344, 3939), PstI? (5283), PvuII? (2619, 1711, 593, 360), NotI? (987, 4296).

- want lanes to look like: ladder, uncut topo, eco, pst, pvu, not, uncut puc19, eco, pst, pvu, uncut pcdnazeo, not1 as the controls

try pcr from NT2 cdna, 45 cycle, 55 anneal

- 32 water, 5 buf, 5 dntp, 3 mgso4, 1.5 tet-gata 5', 1.5 gata-2a 3', 1 nt2, 1 hot start, oil

July 29, 2008

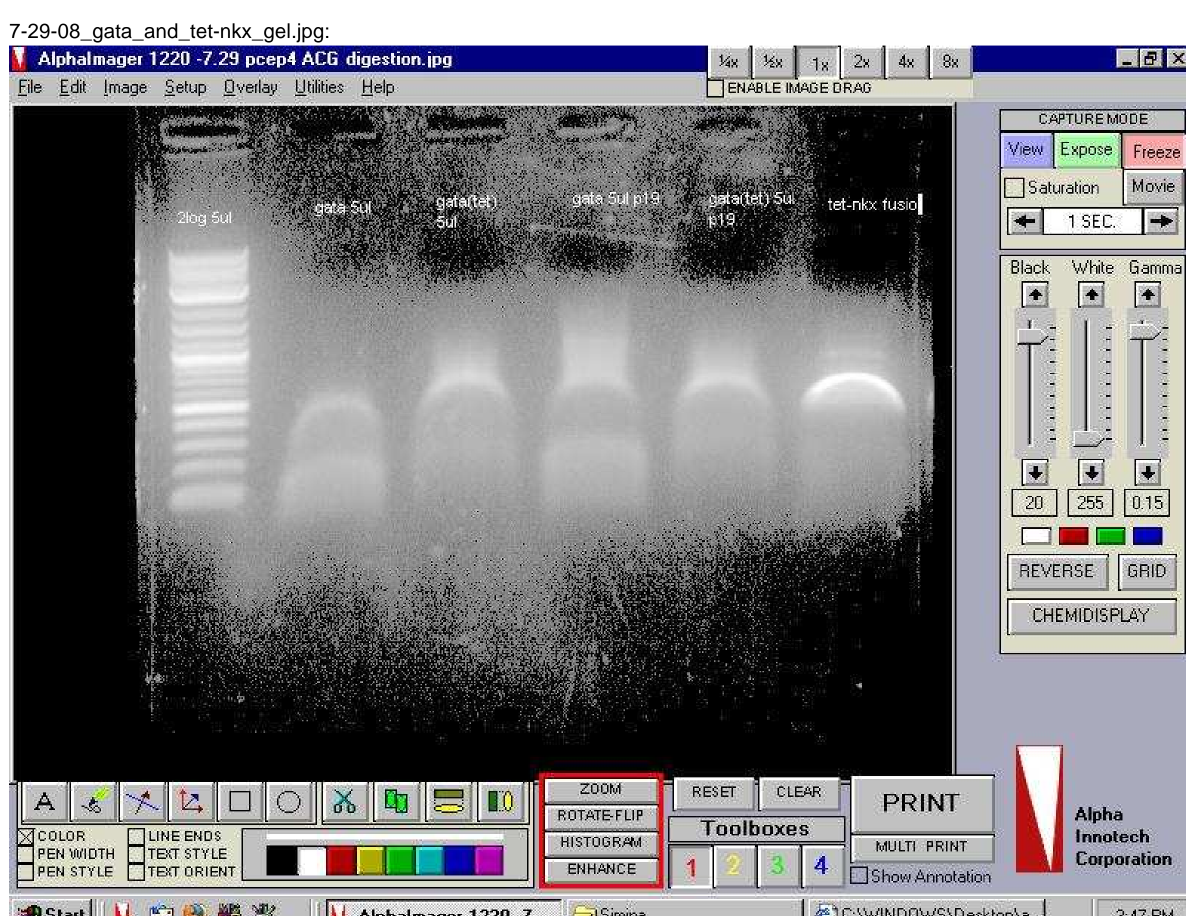
Organized PCR tubes in new box

- by construct, by date, oldest left, by use, fusion or visual with top and bottom rows.

Ran gels for gata, tet-nkx fusion, 1% gel, 115V, fifteen minutes

- 5ul 2log ladder - 5ul gata - 5ul gata(tet) - 5ul gata p19 - 5ul gatap19(tet) - 5ul tet-nkx
- all failed, semicircles. possibly bad kod hot buffer. or need to shake buffer before pcr rxn. the ladder was good looking, so it's not the running buffer.

- 7-29-08_gata_and_tet-nkx_gel.jpg:



PCR of dsred at higher melt temp, more cycles, two tubes

- 32water, 5buf, 5dntp, 3mgso4, 1.5 5'amhc, 1.5 3' amhc-neor, 1ul plvtrKRAB-red, 1ul hot start, oil
- 95-2 (95-20,58-15,70-25) 70-2 robot machine. took one tube out at 25 cycles. took other out at 50 cycles

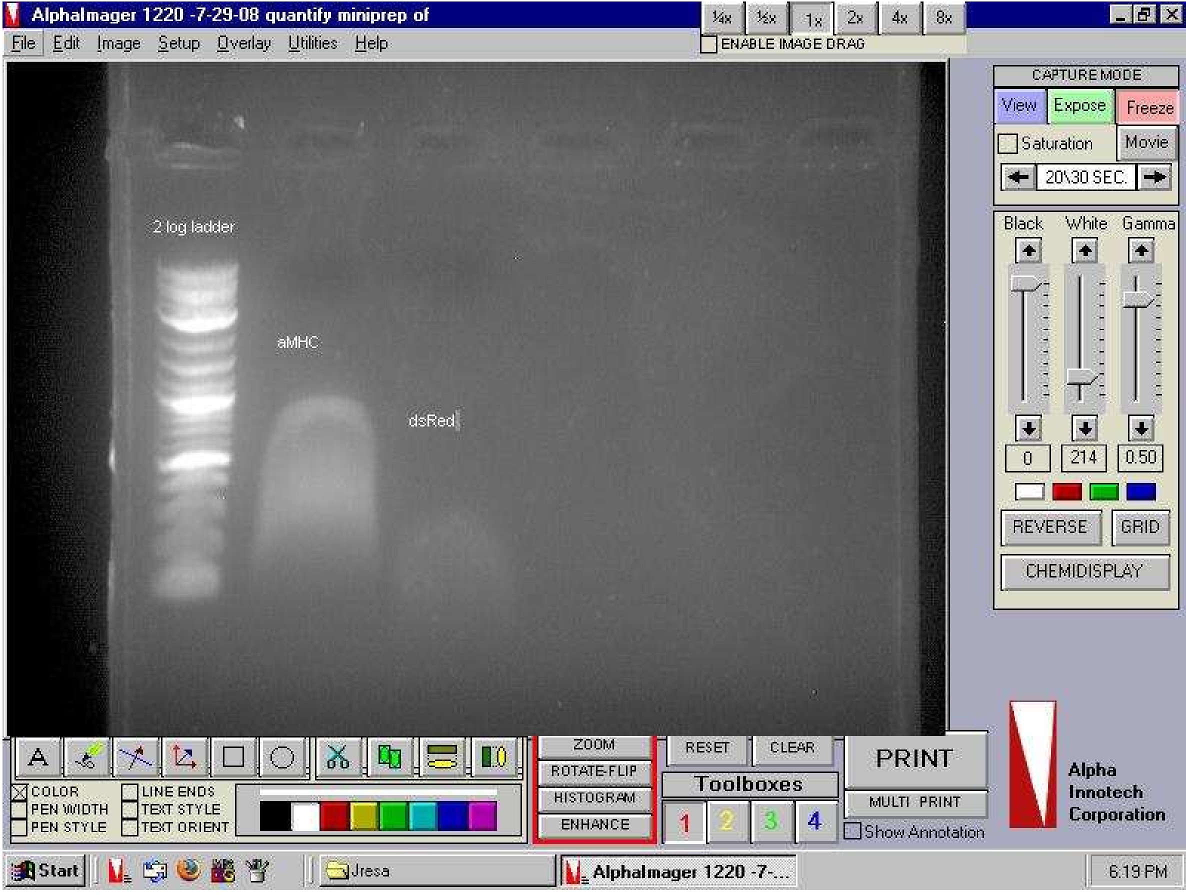
Restriction Digest of 1-6 pNEB193 Klenow, 1-4 pNEB193 Pfu 50, 1-4 pNEB193 Pfu 100.

- BspD1? : 2ul NEBuffer 4, 0.2ul 2ul of dna from above^, 15.8ul water.
  - Controls are plvtrKRAB-red (100ng/ul from 4/8/08 maxi) cut and uncut with rest of reaction mixture except cutter. and uncut pNEB193 Klenow1
- BamHI? : 2ul NEBuffer 3, 2ul BSA, 0.2ul BamHI? , 2ul DNA^, 13.8ul water.
  - Controls are pNEB193 50ng/ul (the tube with big bold handwriting) uncut and cut as well as uncut PNEB193 Klenow 1

Gel of aMHC and dsred 1% at 120V for 18minutes

- 5ul 2log, 5ul aMHC, 5ul dsred (with 4 wtaer, one dye)

- 7-29-08_aMHC_and_dsRed_gel.jpg:



July 28, 2008

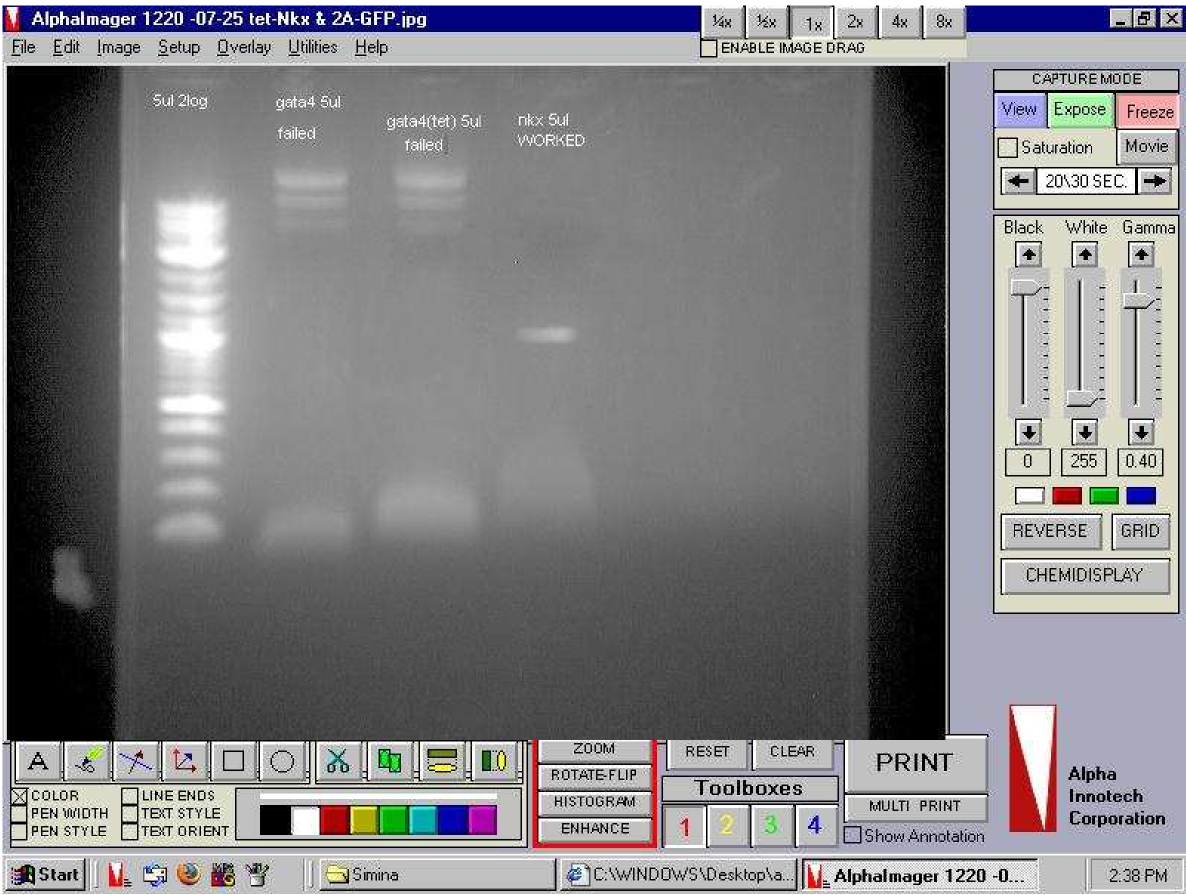
PCR of aMHC, 40 cycles

- 32 water, 5 buf, 5 dntp, 3mgso4, 1.5 5' aMHC oligo, 1.5 3' ahc-neor oligo, 1 10x genomic, 1 hot start, oil
- 95-2 (95-20, 60-15, 70-23) for 40 cycles, 70-2, 4 forever on robot machine.

Running gel of 2log (5u) - gata (5ul, 4 water, 1 dye) - gata(tet) (5ul, 4 water, 1 dye) - nkx (5ul, 4 water, 1 dye)

- gata failed. nkx worked. right at 1kb

- 7-28-08_gata_and_nkx_run_-nkx_worked.jpg:



PCR gata4 from mouse P19 cdna, using high GC content protocol

- 16.25 water, 13 betaine, 5 buffer, 5 dntp, 3mgcl2, 2.5dmso, 1.5 tet-gata 5' (or 1.5 2a-gata5'), 1.5 gata-2a 3', 1ul P19 2/11/07 (or 1ul P19 10-24-06), 0.25ul deaza GTP, 1 hot start, oil
- ran at 95-2 (95-20,55-10,70-20) 70-2 on small machine, purple tubes.
- labels as follows: gata=2a-gata four ends from 10-24-06, gata(tet)=tet-gata ends from 10/24/06, gata p19 from 2/11/07, gata(tet) P19 from 2/11/07
- eight tubes total, 4 at 10 cycles and 4 at 25 cycles.

July 25, 2008

(Apologies, I thought I put this up on Friday...)

First, rediluted the oligos. 4ul oligo, 76ul water. All the new nkx stuff...

aMHC 40 cycle, new ends

- 32 water, 5 buffer, 5 dntp, 3 mgso4, 1.5 5' oligo aMHC 5', 1.5 aMHC-NeoR 3', 1 10x rat genomic, 1 hot start, oil
- 95-2, (95-20, 55-10, 70-15), repeat 39, 70-2

dsRed PCR, 2 tubes

- same ingredients, except oligos are now 5' 2a-red and 3' red and dna is now plvtrKRAB-red 10ng/ul
- 95-2, (95-20, 60-10, 70-15), repeat 29, 70-2
- took out a tube at 13 cycles for fusion

2a-gfp fusion pcr, 2 tubes

- 28 water, 5 buf, 5 dntp, 4 mgso4, 3 neor-2a 5', 3 gfp 3', .5 2a, .5 gfp, 1 hot, oil
- 95-2, (95-20, 60-10, 70-15), repeat 29, 70-2
- took out one tube at 13 cycles for fusion

tet-nkx fusion pcr, 2 tubes

- same procedure as above except oligos are 5' tet-0, 3' nkx-2a, and the dna is tet 10 and nkx 25 (number being cycle number)

- used robot machine, one tube out at 10 cycles, others get 25. first 5 cycles at 55, next twenty at 57 degrees.

Check on gel 1%, 2log ladder 5ul, gata 5ul, gata (tet) 5ul (the version of gata that binds directly to the tet promoter, not to 2a upstream), dsred 5ul, aMHC 5ul 115V

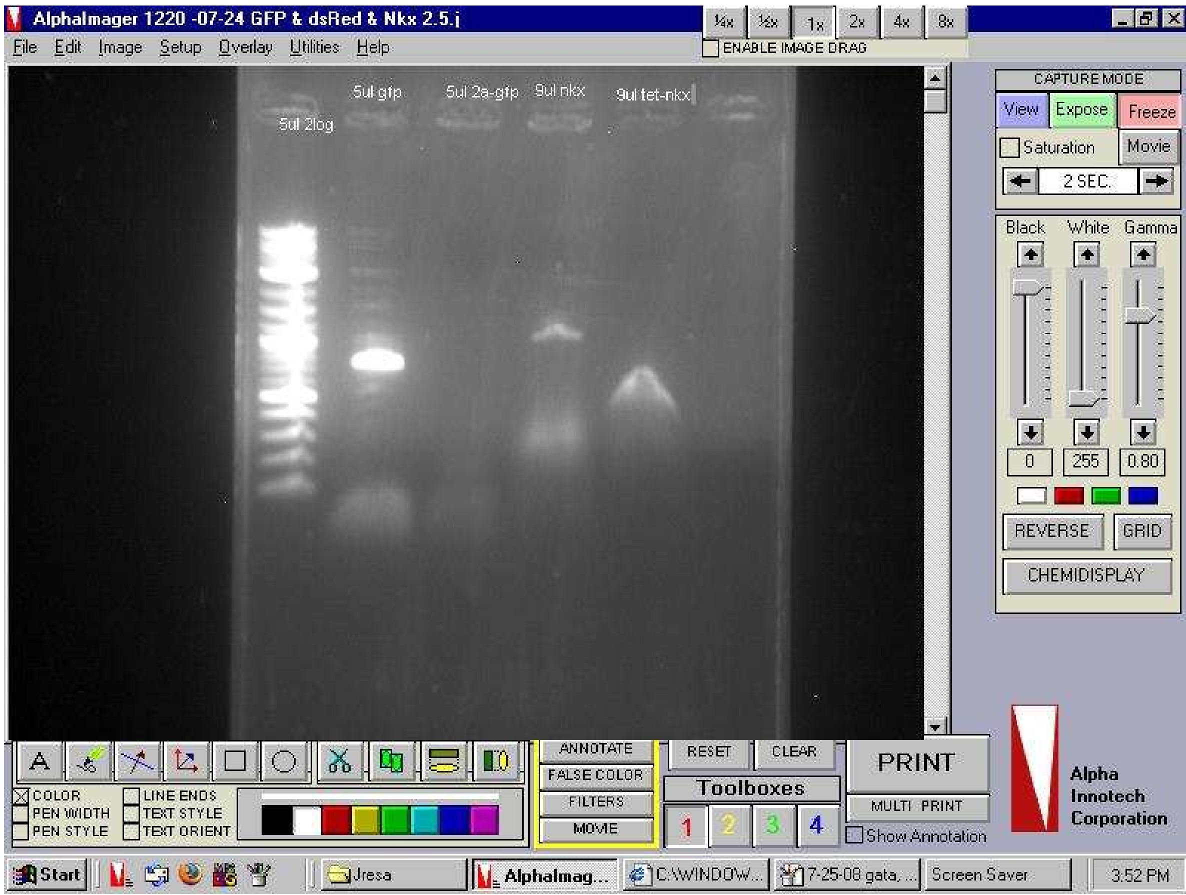
- Saw that gata failed, dsred failed, aMHC had banding indicating likely that the primers were allowing multiple targets to be hit. Indicates future aMHC PCRs should target temperatures closer to the melt temp to avoid secondary products. Main product is main band, though.

- 7-25-08_gata,_gata(tet),_dsred,_aMHC_run.jpg:

Check on gel 2%, 2log ladder 5u, gfp 5ul, 2a-gfp 5ul, nkx 25 5ul, tet-nkx 5ul 115V

- gfp is bright, correct. 2a-gfp got nada. nkx and tet-nkx are weird semicircles (replace running buffer!?). the fusion ran even faster than the non-fusion, but it is crazy bent.

* 7-25-08_2a-gfp_and_tet-nkx_fusion_visual.jpg:



Suggestions: Try 2a-gfp with 5 cycles at 55, 25 at 60.

July 24, 2008

Checked GFP, looked fine. The 3' oligo had a compatible sequence with melt temp of 47, 5' had one of 42. So reran GFP at higher anneal temp.

- 32 water
- 5 10x buffer
- 5 dNTPs
- 3 MgSO4?
- 1.5 5' oligo 2a-gfp rediluted 7/23 (2ul oligo, 38 water)
- 1.5 3' oligo gfp rediluted 7/23 (2ul oligo, 38 water)
- 1 pWPI 10ng/ul 7-12-08 Andrey's dilution from maxi
- 1 hot start
- 30 oil
    - 95-120, (95-20, 61-10, 70-15), 70-120 For 13 and 30 cycles

Cleaned Cuvettes:

- Soaked in 0.25M HCl for 15minutes
- Rinsed in distilled water, then boiled for ten minutes
- Rinsed with 95% ethanol, dried upside down.

July 23, 2008

Came in when Sean was running GFP gel. Nothing. Didn't work.

PCR of NeoR? for high GC content

- 16.25 water
- 13 betaine (5M, 10/07)
- 5 10x buffer

- 5 dNTPs
- 3 MgCl2?
- 2.5 DMSO (4/5/07)
- 1.5 5' oligo aMHC-NeoR
- 1.5 3' oligo NeoR? -2A
- 1 pCMV Tag 2A 10ng/ul
- 1 hot start
- 30 oil

(However, done at 51 anneal instead of 55 anneal for ten cycles. Then left on for 15 more cycles at 55 anneal. Will have to do again, but can check to see if this worked. 2 (20, 10, 15) 2)

GFP PCR:

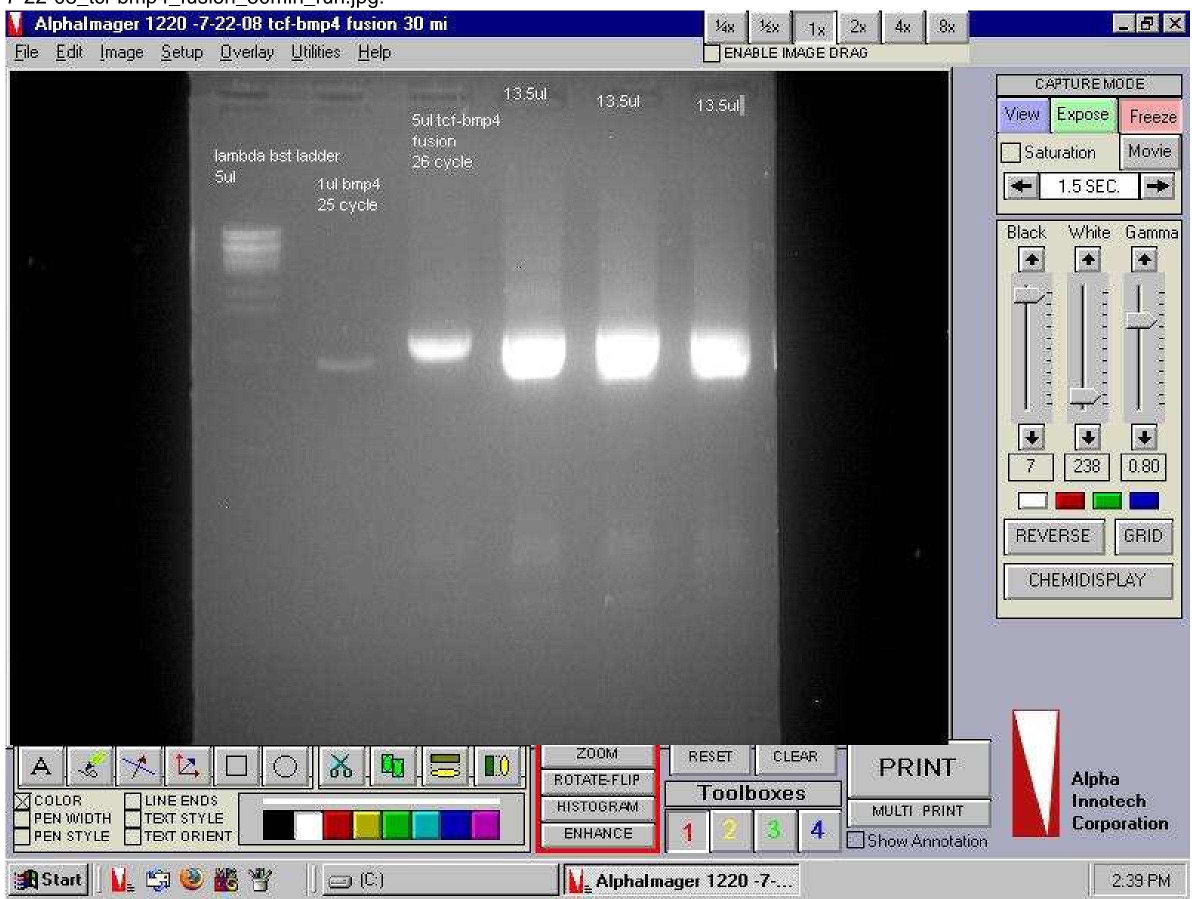
- 32 water
- 5 10x buffer
- 5 dNTPs
- 3 MgSO4?
- 1.5 5' oligo 2a-gfp rediluted 7/23 (2ul oligo, 38 water)
- 1.5 3' oligo gfp rediluted 7/23 (2ul oligo, 38 water)
- 1 pWPI 10ng/ul 7-12-08 Andrey's dilution from maxi
- 1 hot start
- 30 oil

July 22, 2008

Ran gel for TCF-BMP4 fusion (1%)

- 5ul lambda bst ladder --- 1ul bmp4 (8water, 1 dye) --- 5ul tcf-bmp4fusion (4water, 1 dye) --- three lanes of 13.5ul tcfbmpf and 1.5dye
- Good! this is the concentration to use for bmp4 control! Overloaded with 5ul though, so run again after gel isolated.

- 7-22-08_tcf-bmp4_fusion_50min_run.jpg:



Gel isolated TCF-BMP$ fusion

- three tubes, .32g, .29g, .30g get 320, 290, and 300ul respectively of turbo salt sol'n.
- heat 5min at 55degrees
- add 600ul of that to the cardtridges, centrifuge 5min
- add 500ul turbo wash, 5 second centrifuge
- empty, 4min centrifuge
- add 30ul elution sol'n, incubate at room temp for 5min, then centrifuge for 2min.

PCR of NeoR? 2min (20, 10, 15) 2min --- 95 (95, 55, 70) 70

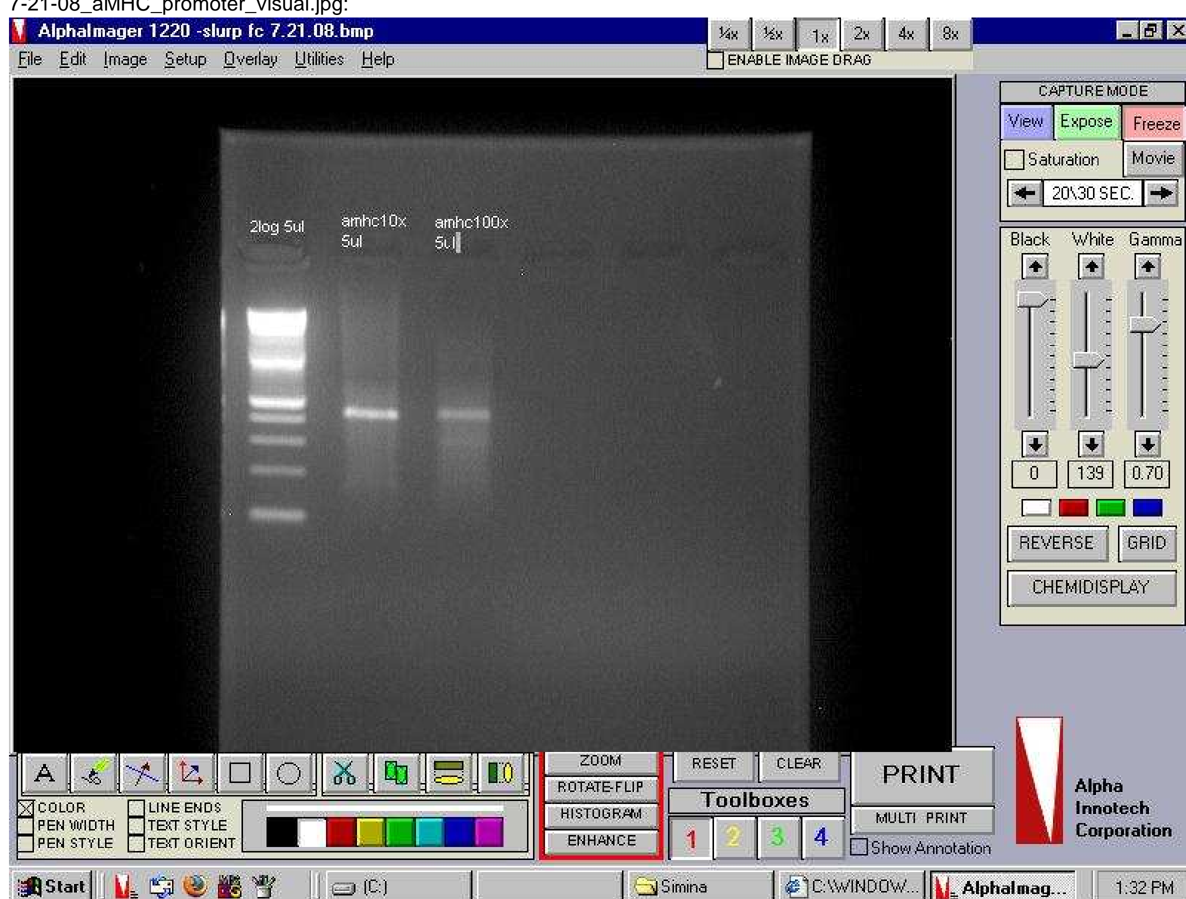
- 32 water
- 5 buffer
- 5 dntps
- 3 mgso4
- 1.5 5' oligo aMHC-NeoR
- 1.5 3' oligo NeoR? -2A
- 1ul 10ng/ul pCMV Tag 2A
- 1 hot polymerase
- 30 mineral oil

July 21, 2008

Ran gel for aMHC

- 5ul 2log ladder --- 5ul 10x dilution of 40 cycles (4 water, 1 dye) --- 5ul 100x (4 ul water and 1 dye)
- Both products look good! Finally! We have streaking though, indicating some contaminant of SDS and perhaps other. We can Probably use this product for our fusion constructs, but if that doesn't work, we probably need to run aMHC on a gel and isolate to clean it.

- 7-21-08_aMHC_promoter_visual.jpg:



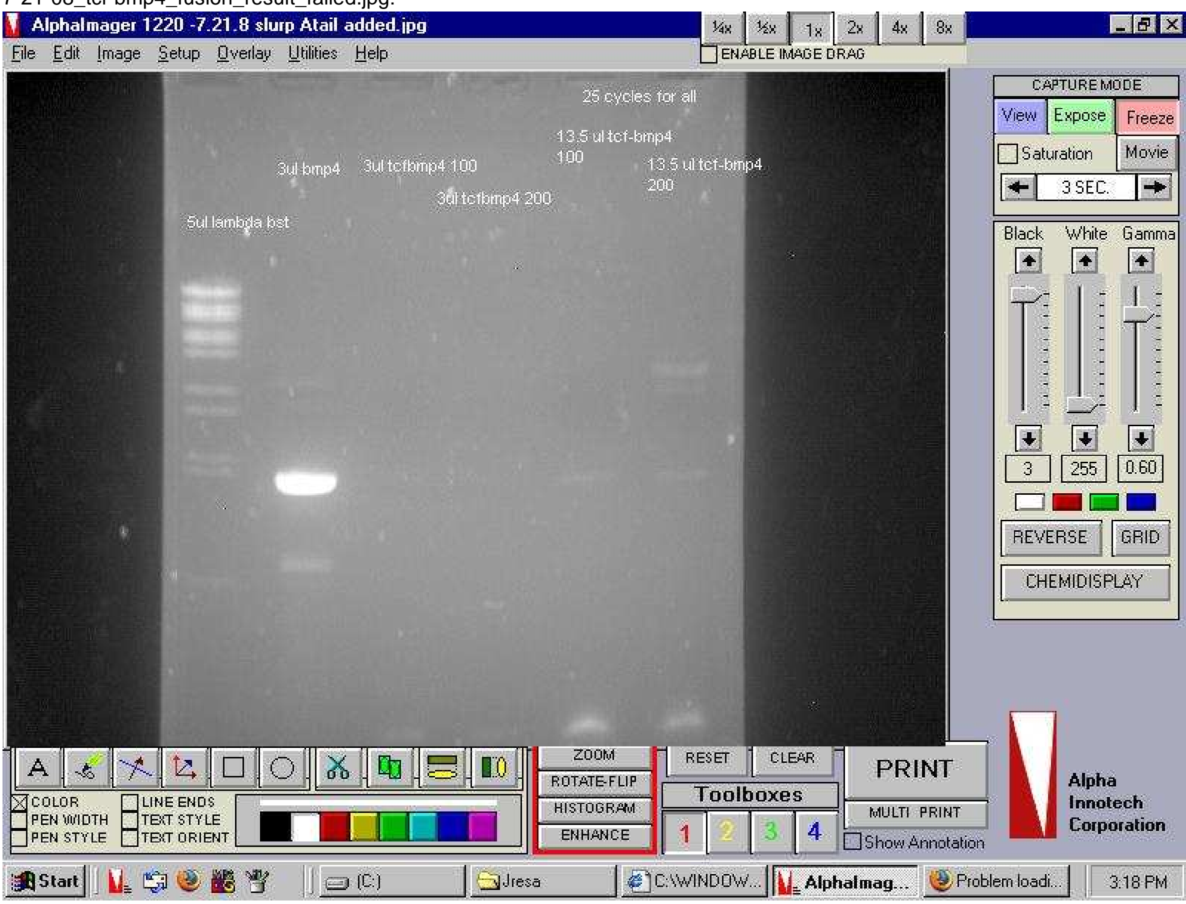
Ran gel for TCF-Bmp4 fusion from SiminasNotebook.

- 5ul lambda bst --- 3ul BMP4 from 25 cycle tube (6 wtaer, 1 dye) --- 3ul tcf-bmp4 100 (6 water, 1 dye) --- 3ul tcf-bmp4 200 (6wtaer, 1dye)

--- 13.5ul tcfbmp4100 (1.5dye) --- 13.5 tcfbmp4200 (1.5dye)

- checked at 20, 30, 45 minutes. Have only original reactants, no product. Can check my page on gel computer for image. BMP4 is still overloaded with 3ul!!! very concentrated stuff. the small 3ul reactions of fusion didn't show. the 13.5ul reactions showed small bands (likely tcf) and basically nil at the higher ends, though a very faded band appeared, probably original bmp4 reactant, just shy of the lambda ladder (so under 1300bp), pretty even with control, though overloaded. A very high band also appeared, very very faded as well.
- Realized that we never got the melt temperature of the fusion, which is really 51 degrees! Had Jen email us the rest of the constructs in case any others are like this. A20 the other day must have been a miracle, heh. Decided to redo the reaction.

- 7-21-08_tcf-bmp4_fusion_result_failed.jpg:



Redo TCF-BMP4 fusion - cycling as follows: 95 for 2min, (95 20 sec, 51 10 sec, 70 20 sec), repeat cycle four, (95 20, 55 10, 70 20), repeat cycle 20, 70 2min

- (cycle as above because the overlap is only Tm=56 or Ta=51. Want to get some product with the anneal first, then can just use the 55 anneal temp for the entire thing for the flanking primers, as the overlap is now in the construct and we are just amplifying the large pieces)
- 28 water
- 5 buffer
- 5 dntps
- 4 mgso4
- 3 5' oligo tcf 5'
- 3 3' oligo bmp4 3'
- 0.5ul tcf
- 0.5ul bmp4 dna from 10 cycle tube
- 1 hot polymerase
- 30 mineral oil

(Left in farthest PCR machine at 4 degrees)

July 18, 2008

Made 2% gel, 300ml .5x TBE + 6g agarose + 15ul ethydium bromide

Ran aMHC (65 cycles) on a 2% gel. Note that the machine was stuck on 71 degrees overnight, not 4!!! Used a 2log ladder.

- aMHC product appeared at the correct size, 400bp, but there was also banding at higher molecular weights, indicating too many cycles. Thus, redid the aMHC PCR...

Redo of aMHC pcr, now at 50 cycles times are 2min (20, 10, 10) 2min at 95, 55, 70

- 32 water
- 5 buffer
- 5 dntps
- 3mgso4
- 1.5 5' oligo aMHC
- 1.5 3' oligo aMHC
- 1ul of rat genomic, 10x
- 1 hot polymerase
- 30 mineral oil

PCR of A20-BMP4 fusion (25 cycles, 2min (20, 10, 20) 2min at 95-55-70)

- 28 water
- 5 buffer
- 5 dntps
- 4 mgso4
- 3 5' oligo a20 5'
- 3 3' oligo bmp4 3'
- 0.5ul a20
- 0.5ul bmp4 dna from 10 cycle tube
- 1 hot polymerase
- 30 mineral oil

A20-Bmp4 gel

- lambda bst ladder, 5ul --- bmp4 5ul (4 water, 1 dye) --- a20bmp4 5ul (4 wtaer, 1 dye) --- and finally three lanes of a20-bmp4 at 13.5 ul (1.5ul dye)
  - looked good, but that bmp4 25 cycle dna is very concentrated! 5ul was overloaded. can't be absolutely sure that the product isn't just bmp4 (though highly unlikely as the only oligos were the flanking oligos... so i am 95% sure the product is good)

pNEB193 ClaI? instead of BamHI? minis

- pelleted 1ml lb solution from each of ten tubes into 1.5ml tube.
- resuspended in 250ul p1 buff
- add 250ul p2. inverted many times to mix. only allowed in this state for under 5 mins
- add 350ul n3, mix
- iced for ten minutes
- centrifuged for ten minutes
- decanted supernatant into spin column
- centrifuged one min. dispose wash through
- add 75ul wash buffer, centrifuge one min
- discard flow, centrifuge again one min
- aadd 50ul elution solution. let sit. centrifuged into clean tubes to store product.
- (note to whole thing... i mixed up numbers of original large lb solution tubes with the small tubes of product, so we cannot match final product to initial tubes. also, tubes 1 and 10 of the large tubes will not be useful any longer, as they have trash in them. hopefully all minis work and thus any of the large tubes can be used to grab more if needed)

July 17, 2008

Picked pNEB193 colonies

- 10 tubes of 4ml each with LB, and 8ul Amp in each. Picked a colony from pNEB193 and placed into each.
- Put on the shaker at 2:45PM (temp reads 39.2, but i assume that's 37 based on the machine sticker.)

PCR of aMHC, three tubes (65 cycles) 2min (20, 10, 10) 2min

- 32 water
- 5 buffer
- 5 dntps

- 3mgso4
- 1.5 5' oligo
- 1.5 3' oligo
- 1ul of rat genomic, 10x rat genomic, and 100x rat genomic
- 1 hot polymerase
- 30 mineral oil

PCR of TCF (40 cycles) 2min (20, 10, 10) 2min <-- two tubes

- 32 water
- 5 buffer
- 5 dntps
- 3mgso4
- 1.5 5' oligo
- 1.5 3' oligo
- 1ul of nt2 human genomic 100ng, 1ul of 200ng
- 1 hot polymerase
- 30 mineral oil

PCR of fusion of A20-BMP4

- 29 water
- 5 buffer
- 5 dntps
- 3mgso4
- 3 5' oligo
- 3 3' oligo
- 0.5ul a20 assembly
- 0.5ul bmp4 assembly
- 1 hot polymerase
- 30 mineral oil

GELS (took pictures under jresa in desktop of the gel computer. also visualized with headgear)

Of a20-bmp4, 2 percent gel, lambda bst ladder

- lane1, ladder 5ul - 2 bmp4 5ul - 3 a20bmp4 5ul - 4, 5, 6 a20-bmp4 13.5ul
  - only thing that showed up was the ladder and the bmp4 control. saw nothing for the rest, fusion failed. maybe the 0.5ul for each dna piece grabbed mineral oil, not dna? gc content? sequence? ???

Of TCF, aMHC, 1 percent gel, 2log ladder

- lane1, 5ul ladder - 2 5ul tcf100 - 3 5ul tcf200 - 4 5ul amhc - 5 5ul amhc10x - 6 5ul amhc100x
  - tcf showed up great for both lanes. amhc failed (band too small). however 10x and 100x aMHC showed the correct band, but there were too many cycles, lots of banding above the desired band as well.

So, redoing the aMHC PCR, using the 10x dilution of rat genomic, 55 cycles. Will be in overnight. Please take out in the morning.

- 32 water
- 5 buffer
- 5 dntps
- 3mgso4
- 1.5 5' oligo
- 1.5 3' oligo
- 1ul of rat genomic, 10x dilution
- 1 hot polymerase
- 30 mineral oil

July 15, 2008

PCR of TCF from human genomic DNA (NT2)

- It was unlabeled so the concentration was not known. Performed a dot test with a known concentration of genomic DNA.

Mixed

- 1ul 2X Ethydium bromide with
  - 1ul Cos2 (400ng/ul)

    - 1ul NT2

The Nt2 appeared about twice as bright. Assumed that it was about 800ng/ul

Diluted 1:4 and 1:8 for PCR
- 12ul water
- 4ul NT2 <--- 1:4

took from above, 4ul, mixed with 4ul water for a 1:8 dilution (serial dilution)

1:4 should have 200ng, 1:8 should have 100ng, though these are very imprecise numbers, hence the use of two different dilutions

PCR of four tubes, two each of 100ng/ul and 200ng/ul. One tube got 12 cycles, the other 25.
- 32ul water
- 5ul 10xbuffer
- 5ul dNTPs
- 3ul MgSO4?
- 1.5ul 5' oligo
- 1.5ul 3' oligo
- 1ul of the diluted genomic DNA
- 1ul Hot Start Pol
- 30ul mineral oil

Did not check yet on the gel

July 14, 2008

PCR of NeoR? from pcDNA3.1myc/his
- 32ul water
- 5ul 10x buffer
- 5ul dNTPs
- 3ul MgSO4?
- 1.5ul 5' oligo (labeled 10X, i assume that was 10uM)
- 1.5ul 3' oligo (labeled 10X, i assume that was 10uM)
- 1ul pcDNA3.1myc/his
- 1ul KOD Hot Start Pol
- 30ul mineral oil
- Cycle of 95-2min, 95-20sec, 55-10sec, 70-10sec, 70-2min
- One tube for twelve cycles (for later fusion use), one tube for 25 cycles, for visualization

PCR of BMP4 from last year's differentiation box (labeled 50X)
- 32ul water
- 5ul 10x buffer
- 5ul dNTPs
- 3ul MgSO4?
- 1.5ul 5' oligo (10uM)
- 1.5ul 3' oligo
- 1ul BMP4 50X
- 1ul KOD Hot Start Pol
- 30ul mineral oil
- Cycle of 95-2min, 95-20sec, 55-10sec, 70-10sec, 70-2min
- One tube for 11 cycles (for later fusion use), one tube for 25 cycles, for visualization

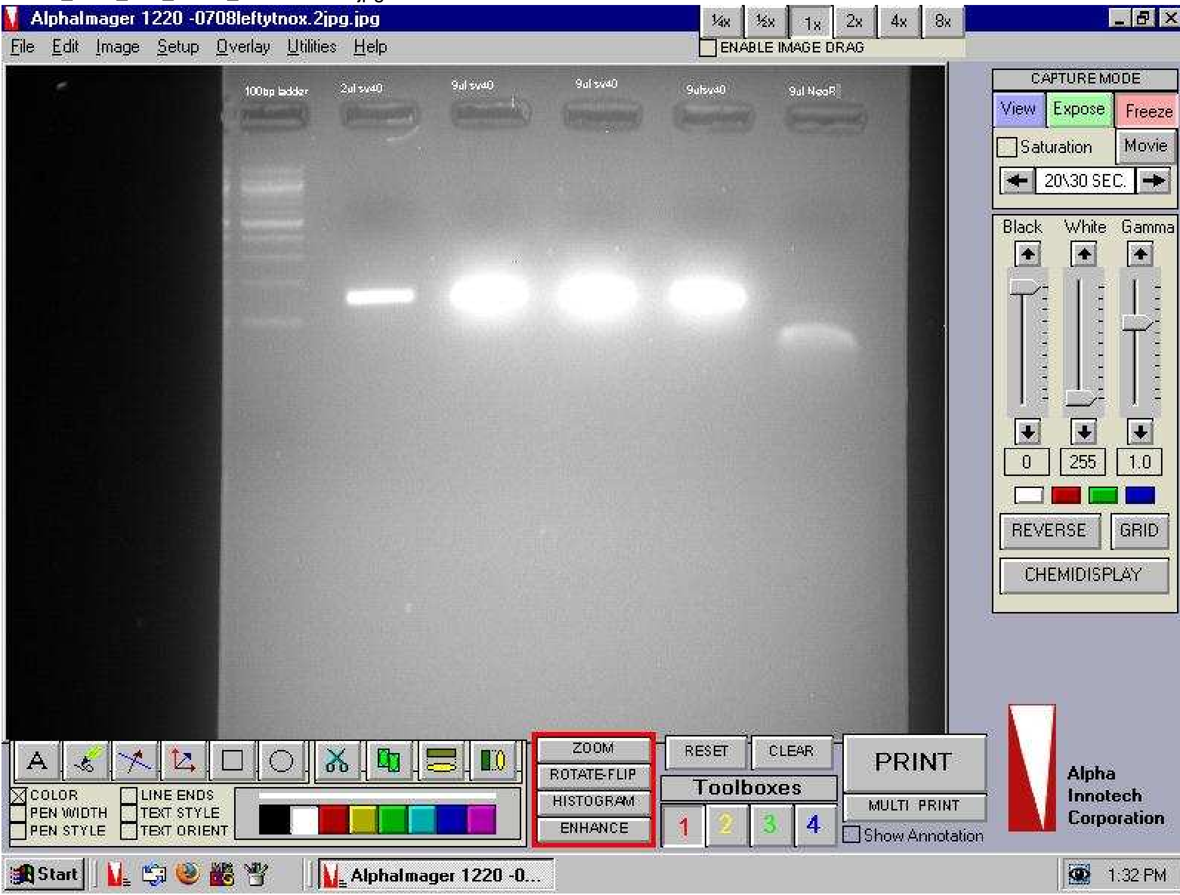
Gel of GFP product from Tuesday
- Made gel: 300ul .5x tbe solution, 3g agarose, 30ul syby visualization stuff at 10,000X (instead of ethydium bromide)
- Ran gel, but couldn't visualize. Need UV or light filter.
- Lanes: Lane 1 empty, 2-2log ladder (5ul), 3-6: GFP (9ul)

Set up gel for BMP4 and NeoR? check, but never ran, as was uncertain if I should.

July 8, 2008

Gel of SV40 polyA tail and NeoR ? :

- 2.5% gel, 100bp ladder. First lane is ladder, 10ul. second lane is 2ul sv40, third is 9ul sv40, fourth is 9ul sv40, fifth is 9ul sv40, 6th is 9ul NeoR ?
  - Got no NeoR ? .
  - 7-8-08_sv40_and_NeoR_visualization.jpg:



- Gel isolated the sv40polyA tail. Cut it out, isolated using the GENECLEAN Turbo Kit
  - Took the large cut of three bands, cut in half. Put each in a 1.5ml tube. Both gel pieces weighed .44g.
  - Added 440ul of Turbo salt sol'n to each. placed in 55 degree heat block for 5mins.
  - transferred to two cartridges and passed through, centrifuged.
  - then used wash soln. 500ul, centrifuged. 4mins
  - then elution sol'n, 30ul each tube. sat at room temp 5mins, then centrifuged for 1min
  - Placed in 1.5ml tube. labelled, in center of IGEM2008 box.

PCR of GFP. Two tubes

- 32ul water
- 5ul 10x buffer
- 5ul dNTPs
- 3ul MgSO4 ?
- 1.5ul 5' oligo
- 1.5ul 3' oligo
- 1ul pWPI plasmid (10ng)
- 1ul KOD hot start

- 30ul mineral oil on top

- Ran for 9 cycles. Then some minutes later the power went out. I assumed it had been around ten cycles and pulled one tube. I reset the machine for 15 more cycles. I am unsure when the power went out again. At that point I just grabbed that tube as well, and put it in the IGEM2008 box.

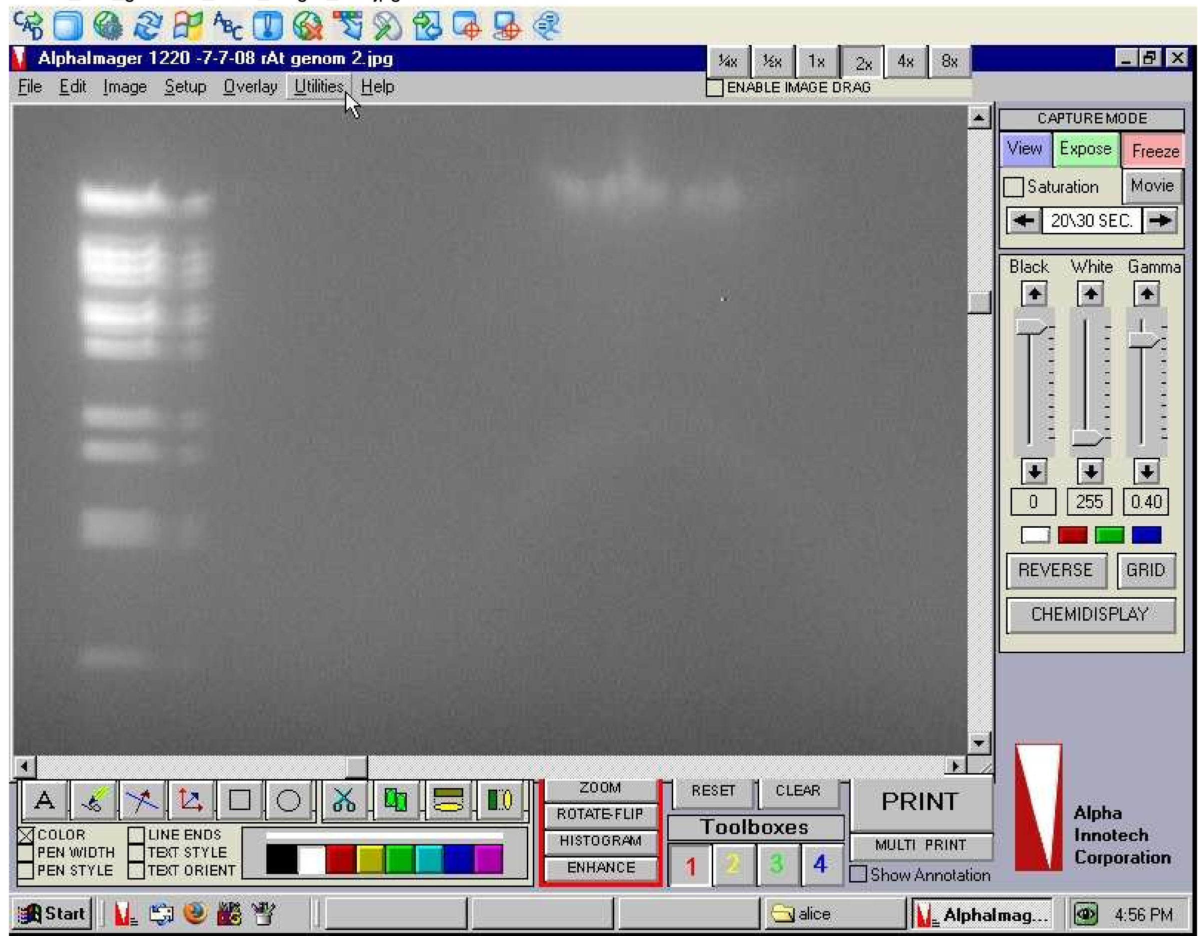
July 7, 2008

Ammoniom Acetate Precipitation

- 300ul of Simina's rat genomic DNA
- 1ul glycogen
- 100uL ammonium acetate
- 1000ul EtOH?
- mixed well
- iced for 5 min (already seeing precipitation)
- centrifuged for 15min at 13,000g in refrigerator
- decanted, washed with 70% EtOH?
- spun again for 13,000g at 5min
- decanted
- air dried
- resuspended in TE (i think 20uL)

Ran a gel at 1% (had to make more gel, (300mL TBE at .5X, 3g agarose, 15uL ethydium bromide at 10mg/ml)

- Lanes: (1: 10ul lambda bst ladder, 2: 1ul of our resuspension, 4ul h2o, 1ul dye without/rnas, 3: same but dye with/rnas, 4: 15ul simina's dna with 3ul dye, 5: same but with rnas
- 7-7-08_Rat_genomic_DNA_longer_run.jpg:



  - We think we're getting 0.5ng/ul of DNA in simina's rat genomic DNA. We got nothing from ours in the gel. (Theoretically should be 7.5ng/ul)

Were setting up for PCR until we realized we didn't get anything... Can still PCR Simina's stuff.

- 5ul KOD buffer
- 4.5ul MgSO4?
- 5ul dNTPs
- 2ul 5' aMHC oligo (last year's)
- 2ul 3' aMHC oligo (last year's)
- 29.5 ul water
- MISSING polymerase and DNA. In IGEM box if to be finished tonight.

July 3, 2008

- Recut pNEB193 with ClaI? and BamHI? , same as before, but added a positive control to test whether ClaI? was cutting at all, using plvtrKRAB-red. Also used a positive control for BamHI? , the original pNEB193 plasmid.

ClaI? Cut
- 2uL NeBuffer4?
- 2uL BSA
- 0.5uL ClaI?
- 5uL pNEB193
- 11.5uL H2O?

ClaI? Cut of KRAB
- 2uL NEBuffer4
- 2uL BSA
- 1uL ClaI?
- 1uL plv-trKRAB-red (100ng/uL, 5/11/08)
- 14uL H2O?

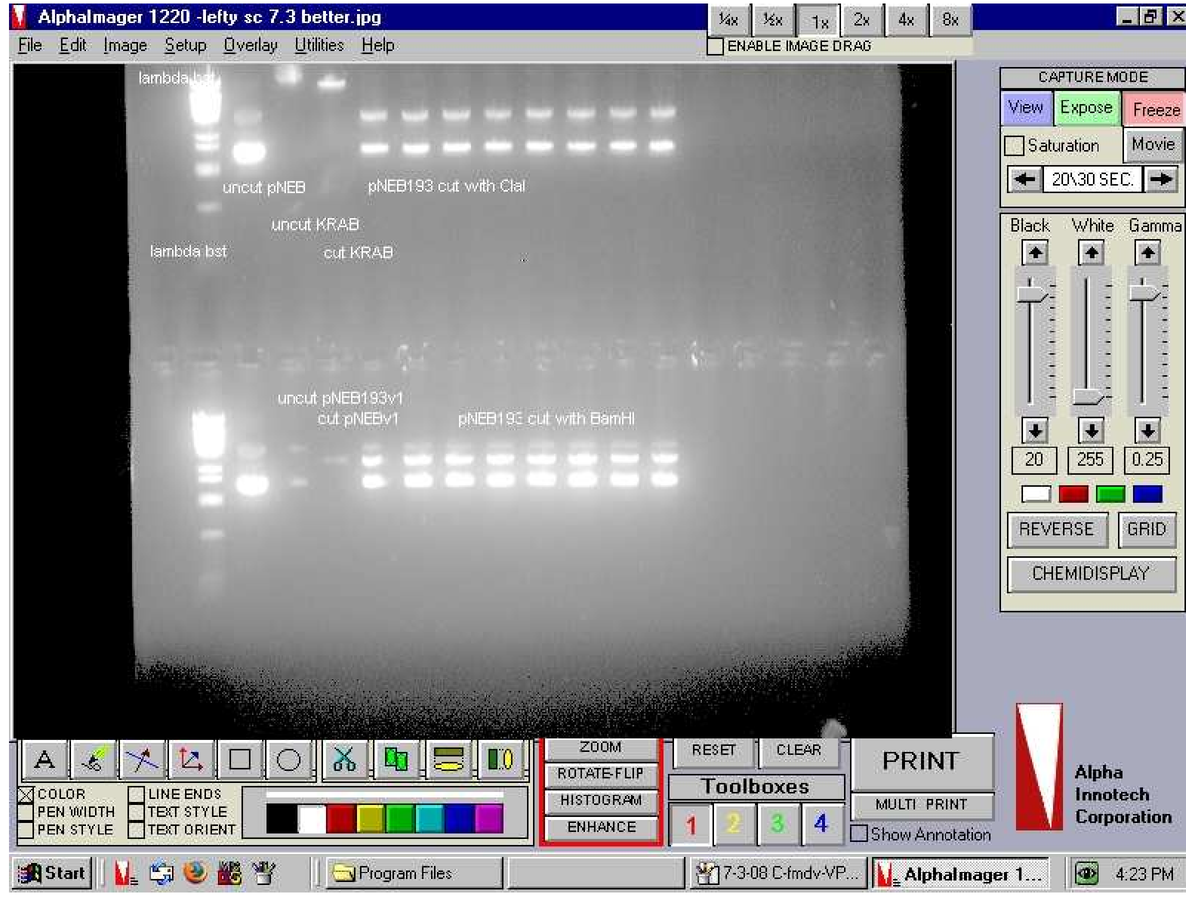
BamHI? Cut (567-604, 92680829)
- 2uL Nebuffer 3
- 2uL BSA
- 0.5uL BamHI?
- 5uL pNEB193
- 11.5uL H2O?

BamHI? Cut of original pNEB193
- 2uL Nebuffer 3
- 2uL BSA
- 1uL BamHI?
- 1uL pNEB193 (pNEB193 50ng/ul, 9/22/07)
- 14uL H2O?

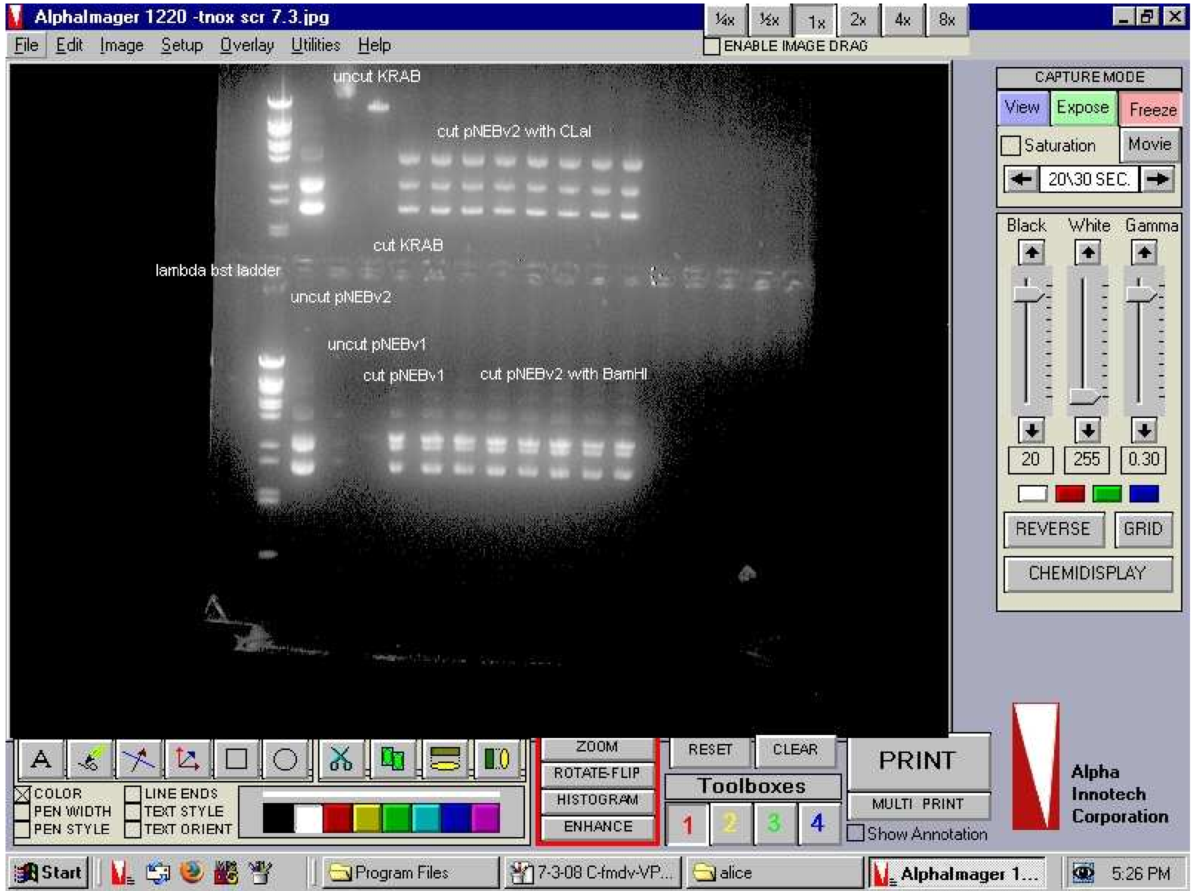
Let sit in 37 degree incubator for just over one hour.

Made more 0.8% gel.
- Added 2.4g Agarose to 300mL TBE (0.5X), and then added 15uL ethydium bromide.

Ran the gel at 120Volts. Checked at two intervals.
- 7-3-08_ClaI_and_BamHI_cut_of_pNEB193.JPG:



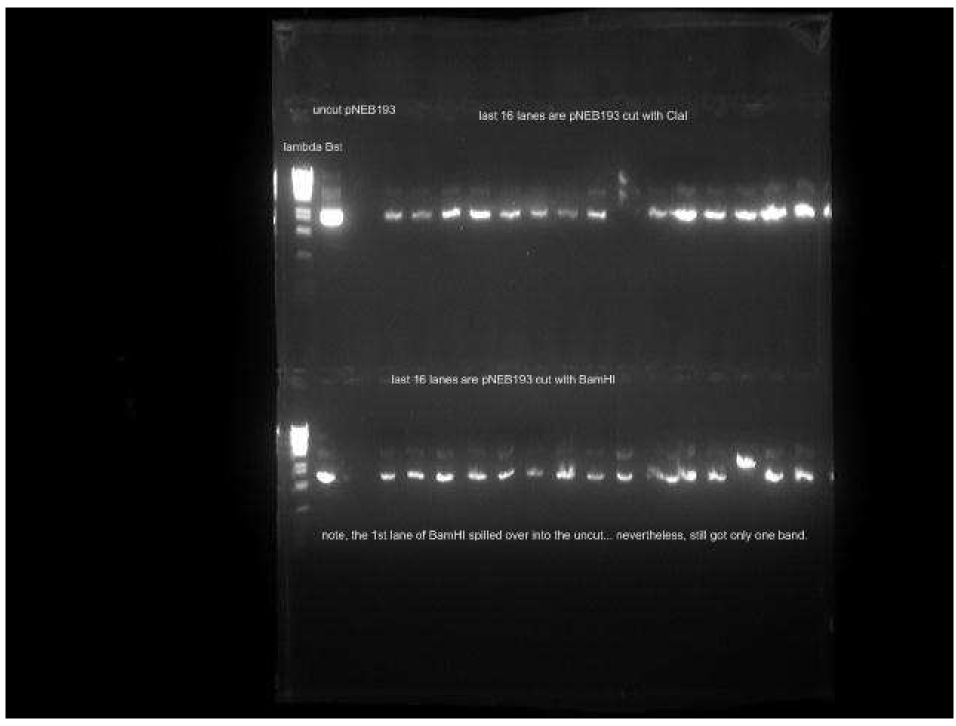
- 7-3-08_ClaI_and_BamHI_cut_of_pNEB193_longer_run.JPG:



- On this gel, it appears like BamHI? may be cutting. Note the band even with the cut control. However, I overloaded the uncut control, so those bands can't be defined for sure.
- In any case, we can see that the enzymes still work. The gels clearly show ClaI? and BamHI? cutting the controls. The problem is with pNEB193 version 2, our modified pNEB193 plasmid.

July 2, 2008

- Ran a gel for Simina's restriction digest the day before, consisting of uncut pNEB193 as a control and Clai and BamHI? as the experimentals. ClaI? and BamHI? did not cut.
  - Used 0.8% gel, ladder Lambda Bst. Used the 50ng tubes for uncut control.
- 7-2-08_pNEB193_Cla_and_Bam.jpg:



- Made four tubes with ptripz. 4mL LB in each and 8uL Amp in each. Picked one colony of ptripz and added to each tube.

-- JoshuaResa - 07 Jul 2008

I	Attachment	Action	Size	Date	Who	Comment
	7-2-08_pNEB193_Cla_and_Bam.jpg	manage	27.7 K	07 Jul 2008 - 12:15	JoshuaResa	
	7-21-08_aMHC_promoter_visual.jpg	manage	73.8 K	01 Aug 2008 - 15:02	JoshuaResa	
	7-21-08_tcf-bmp4_fusion_result_failed.jpg	manage	75.8 K	01 Aug 2008 - 15:03	JoshuaResa	
	7-22-08_tcf-bmp4_fusion_50min_run.jpg	manage	71.7 K	01 Aug 2008 - 15:04	JoshuaResa	
	7-25-08_gata,_gata(tet),_dsred,_aMHC_run.jpg	manage	77.5 K	01 Aug 2008 - 15:05	JoshuaResa	
	7-28-08_gata_and_nkx_run_-nkx_worked.jpg	manage	79.2 K	01 Aug 2008 - 15:06	JoshuaResa	
	7-29-08_aMHC_and_dsRed_gel.jpg	manage	74.2 K	01 Aug 2008 - 15:07	JoshuaResa	
	7-29-08_gata_and_tet-nkx_gel.jpg	manage	120.2 K	01 Aug 2008 - 15:08	JoshuaResa	
	7-3-08_ClaI_and_BamHI_cut_of_pNEB193.JPG	manage	299.7 K	07 Jul 2008 - 12:20	JoshuaResa	
	7-3-08_ClaI_and_BamHI_cut_of_pNEB193.bmp	manage	1406.3 K	07 Jul 2008 - 12:17	JoshuaResa	
	7-3-08_ClaI_and_BamHI_cut_of_pNEB193_longer_run.JPG	manage	261.1 K	07 Jul 2008 - 12:21	JoshuaResa	
	7-3-08_ClaI_and_BamHI_cut_of_pNEB193_longer_run.bmp	manage	1406.3 K	07 Jul 2008 - 12:17	JoshuaResa	
	7-31-08_cut_of_gata_v2.jpg	manage	86.8 K	01 Aug 2008 - 15:25	JoshuaResa	
	7-31-08_test_of_pvu2_on_gata.jpg	manage	78.5 K	01 Aug 2008 - 15:26	JoshuaResa	
	7-7-08_Rat_genomic_DNA_longer_run.jpg	manage	179.9 K	07 Jul 2008 - 17:23	JoshuaResa	
	7-8-08_sv40_and_NeoR_visualization.jpg	manage	72.6 K	08 Jul 2008 - 15:57	JoshuaResa	
	8-1-08_tet-nkx_fusion_failure_at_500bp.jpg	manage	94.3 K	06 Aug 2008 -	JoshuaResa	

				16:38	
	8-11-08_2a-dsred_and_aMHC_run.jpg	manage	91.4 K	11 Aug 2008 - 14:52	JoshuaResa
	8-11-08_2a-dsred_fusion_from_gel_iso.jpg	manage	80.1 K	11 Aug 2008 - 17:07	JoshuaResa
	8-11-08_2a_for_nkx-2a-dsred_gel_iso.jpg	manage	76.0 K	11 Aug 2008 - 14:55	JoshuaResa
	8-11-08_tet-nkx_fuse_and_dsred_gel_iso.jpg	manage	86.8 K	11 Aug 2008 - 14:56	JoshuaResa
	8-12-08_nkx-2a-dsred_fusion_fail.jpg	manage	88.7 K	14 Aug 2008 - 19:06	JoshuaResa
	8-13-08_cmv_fail,_tet-nkx_success_long_run.jpg	manage	86.0 K	14 Aug 2008 - 19:07	JoshuaResa
	8-13-08_quantify_pbluescript.jpg	manage	79.2 K	14 Aug 2008 - 19:07	JoshuaResa
	8-14-08_cmv-rtta_fusion_success.jpg	manage	81.2 K	14 Aug 2008 - 19:10	JoshuaResa
	8-14-08_cmv_yes,_nkx_construct_noish.jpg	manage	89.8 K	14 Aug 2008 - 19:08	JoshuaResa
	8-14-08_siminas_gel_work.jpg	manage	8.3 K	14 Aug 2008 - 19:11	JoshuaResa
	8-15-08_nkx_construct_fusion_failed_again.jpg	manage	78.4 K	20 Aug 2008 - 15:06	JoshuaResa
	8-15-08_pBluescript_digestion_checks.jpg	manage	76.7 K	20 Aug 2008 - 18:03	JoshuaResa
	8-15-08_quantify_construcst.jpg	manage	86.7 K	20 Aug 2008 - 18:13	JoshuaResa
	8-15-08_quantify_pBluescript.jpg	manage	95.4 K	20 Aug 2008 - 18:15	JoshuaResa
	8-18-08_digest_of_ligated_constructs_in_plasmid_cut_out.jpg	manage	99.6 K	20 Aug 2008 - 18:19	JoshuaResa
	8-19-08_gata-2a-dsred_fusion.jpg	manage	111.9 K	20 Aug 2008 - 18:20	JoshuaResa
	8-20-08_2a_for_gata_3_side_to_gel_iso.jpg	manage	88.0 K	20 Aug 2008 - 18:28	JoshuaResa
	8-20-08_amhc_and_nkx-gata_constructs.jpg	manage	87.5 K	20 Aug 2008 - 18:21	JoshuaResa
	8-20_amc_and_nkx-gata_constructs_longer_run_to_gel_iso.jpg	manage	93.0 K	20 Aug 2008 - 18:26	JoshuaResa
	8-21-08_cut_bspd1,_nhe1,_quantify_cmv_rtta,_cut_pwpi_sal.jpg	manage	76.8 K	22 Aug 2008 - 17:52	JoshuaResa
	8-22-08_amhc_is_there,_tiny.jpg	manage	72.3 K	22 Aug 2008 - 17:57	JoshuaResa
	8-22-08_final_failure_pWPI_quantify_nada.jpg	manage	104.4 K	22 Aug 2008 - 18:54	JoshuaResa
	8-22-08_pWPI_gel_iso_and_aMHC_quant.jpg	manage	84.9 K	22 Aug 2008 - 17:53	JoshuaResa
	8-22-08_quantify_pLenti_worst_gel_ever.jpg	manage	87.0 K	22 Aug 2008 - 17:57	JoshuaResa
	8-25-08_KpnI_and_SacI_cut_of_pBluescript.jpg	manage	84.0 K	25 Aug 2008 - 19:03	JoshuaResa
	8-25-08_gata-2a-red_to_gel_isolate.bmp	manage	1406.3 K	25 Aug 2008 - 19:02	JoshuaResa
	8-25-08_quantify_pieces_of_tet.jpg	manage	80.9 K	25 Aug 2008 - 19:00	JoshuaResa

	8-5-08_amhc_visual.jpg	manage	77.5 K	06 Aug 2008 - 16:40	JoshuaResa
	8-5-08_rtTA_failure.jpg	manage	84.2 K	06 Aug 2008 - 16:40	JoshuaResa
	8-6-08_amhc-neor_fusion_success.jpg	manage	78.8 K	06 Aug 2008 - 16:41	JoshuaResa
	8-7-08_2a-dsred_amhc-neor_fusions.jpg	manage	81.1 K	07 Aug 2008 - 17:13	JoshuaResa
	8-8-08_2a-dsred_longer_run.jpg	manage	81.2 K	11 Aug 2008 - 13:07	JoshuaResa
	8-8-08_30min_tet-nkx_fusion.jpg	manage	78.2 K	11 Aug 2008 - 13:10	JoshuaResa
	8-8-08_45min_gata,_2a-red,_tet0nkx.jpg	manage	90.7 K	11 Aug 2008 - 13:11	JoshuaResa
	8-8-08_aMHC_construct_failed.jpg	manage	111.5 K	11 Aug 2008 - 13:12	JoshuaResa
	9-25-08_2a-gfp_and_tet-nkx_fusion_visual.jpg	manage	74.9 K	01 Aug 2008 - 15:27	JoshuaResa

This topic: IGEM2008 > WebHome > NoteBooks > JoshuasNotebook
History: r48 - 28 Oct 2008 - 23:56:00 - AndyMendelsohn

Copyright © by the contributing authors. All material on this collaboration platform is the property of the contributing authors.
Ideas, requests, problems regarding TWiki? Send feedback