08/26/08

realized the ligations I did were missing polyA...need to redo the cmv and aMHC ligations; we didn't have enough aMHC however so I had to reamplify it from an old gel isolation:

- 14.25ul H2O? , 13ul betaine, 5ul dNTPS, 5ul KOD Buffer, 4.5ul MgCl2? , 2.5ul DMSO, 1.5ul 5'aMHC, 1.5ul GFP-3', 0.25ul deaza GTP, 1.5ul DNA (all that was left)
- in the Robocylcer: (95 61 70) for (20s, 10s, 40s)...all the times were doubled

redid tet-Nkx-2A-dsRed and tet-GATA-2A-dsRed fusions using the pbluescript 5' and 3' oligos:

- 13.25ul H2O? , 13ul betaine, 5ul dNTPS, 5ul KOD Buffer, 3ul MgCl2? , 2.5ul DMSO, 3ul 5' oligo, 3ul 3' oligo, 0.25ul de-aza GTP, 0.5ul DNA#1, 0.5ul DNA#2
- tet-Nkx fusion from 8-23, 2A-dsRed gel isolation from 8-11
- tet-GATA fusion from 8-19, 2A-dsRed for GATA from 8-23
- (95 56 70) for (20s 10s 40s)

amplified tet-Nkx from 8-23 with pbluescript oligos

- high GC content protocol (same as above) except 1.5ul 5' pbluescript, 1.5ul 3'2A-Nkx and 1ul tet-Nkx from PCR
- (95 56 70) for (20s 10s 20s)

08/25/08

Took yesterdays ligations (pWPI+pLentiBB, pbluescript+cmv, pbluescript+aMHC) and cleaned them by ammonium acetate precipitation

- add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
- mix and leave on ice for 5 mins.
- centrifuge for 15 mins at 4C (in fridge)
- decant everything except the pellet
- add 100ul 70% ethanol
- leave on ice for 1-2 mins
- spin again at 4C but only for 5 mins
- decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
- resuspend DNA in 10ul of H2O? ?

Electroporate the cells (prechill cuvettes, thaw cells on ice, 50ul bacteria (DH5alpha + 5ul of DNA (from the ammonium acetate precipitation), set electroporation machine to 2.5kV) Then added 0.5mL LB and grew them in the shaker for one hour

- for aMHC when I passed the current through the cuvette in the electroporation machine, there was a spark...I decided to plate it anyways
- Andy said the spark was probably because the ammonium acetate precipitation didn't clean out all the salt, causing it; in order to avoid that, I took 50ul bacteria and added only 2.5ul aMHC from the ammonium acetate precipitation and electroporated them--> this time everything went fine
- so now we have 2 aMHC plates

plated 50ul LB + 50ul of what was grown for one hour --> at 37C overnight

checked 2a-GFP fusion and aMHC construct PCR --> neither worked

08/24/08

I'm starting to think maybe it was the new running buffer...I made new one yesterday and it was hard to avoid getting little solid pieces. So first thing today, I made new 10X TBE: 108g Tris, 55g Boric acid, 40mL 0.5M EDTA, and ddH2O up to 1L. Then I replaced the running buffer in the small boxes only, and made new 1% gel with the new TBE

redoing the entire pWPI thing--> see yesterday for details (sorry, we're a day behind now)

ran out tet, tet-gata and gata-2A-dsRed --> tet and tet-gata looked good; gata-2A-dsRed had banding, and should be redone

ligations:

- pbluescript: 2958bp, 15ng/ul
- cmv: 1413bp, 30ng/ul
- aMHC: 2054bp, 2ng/ul
- pWPI: 8227bp, 2.5ng/ul
- pLentiBB: 50bp, 30ng/ul

reactions:

- cmv: 2.7ul pbluescript, 2ul cmv, 12.3ul H2O? , 2ul T4 Buffer, 1ul T4 Ligase
- aMHC: 1ul pbluescript, 16ul aMHC, 2ul T4 Buffer, 1ul T4 Ligase
- pLentiBB: 16.8ul plasmid, 0.2ul of 5X dilution of the pLentiBB, 2ul T4 Buffer, 1ul T4 Ligase

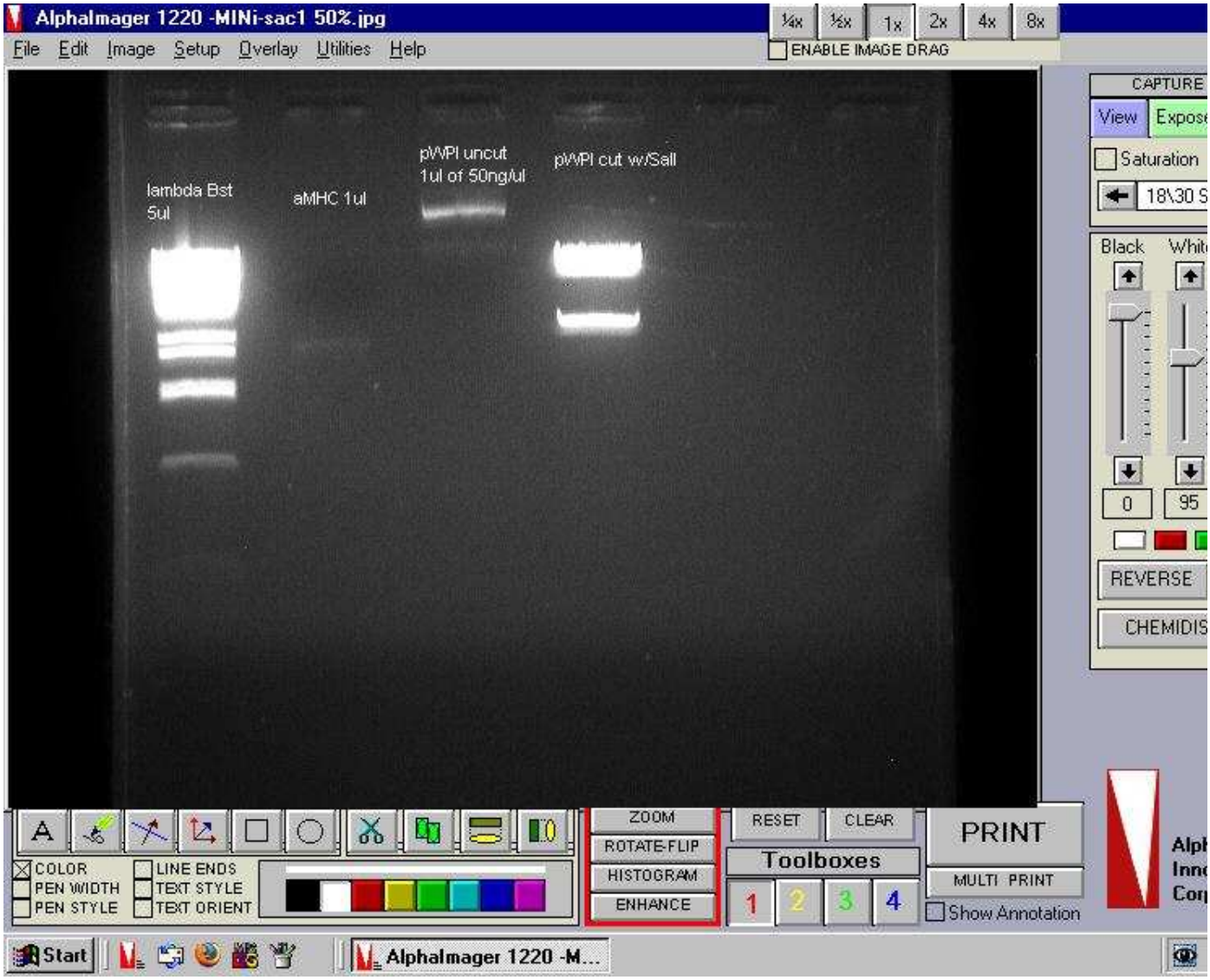
trying to figure out our whole aMHC construct again:

- the only 2A-GFP we got had a lot of banding, so I'm thinking that's why we get such bad yield...the 8-7-08 aMHC-NeoR looks good (see JoshuasNotebook)
- so i wanted to redo that fusion, but couldn't find a 2A for GFP...I think we said that the original 2A was amplified with those oligos, but just to make sure I redid "2A for GFP"
- then i redid the 2A-GFP fusion; and the aMHC construct before I left

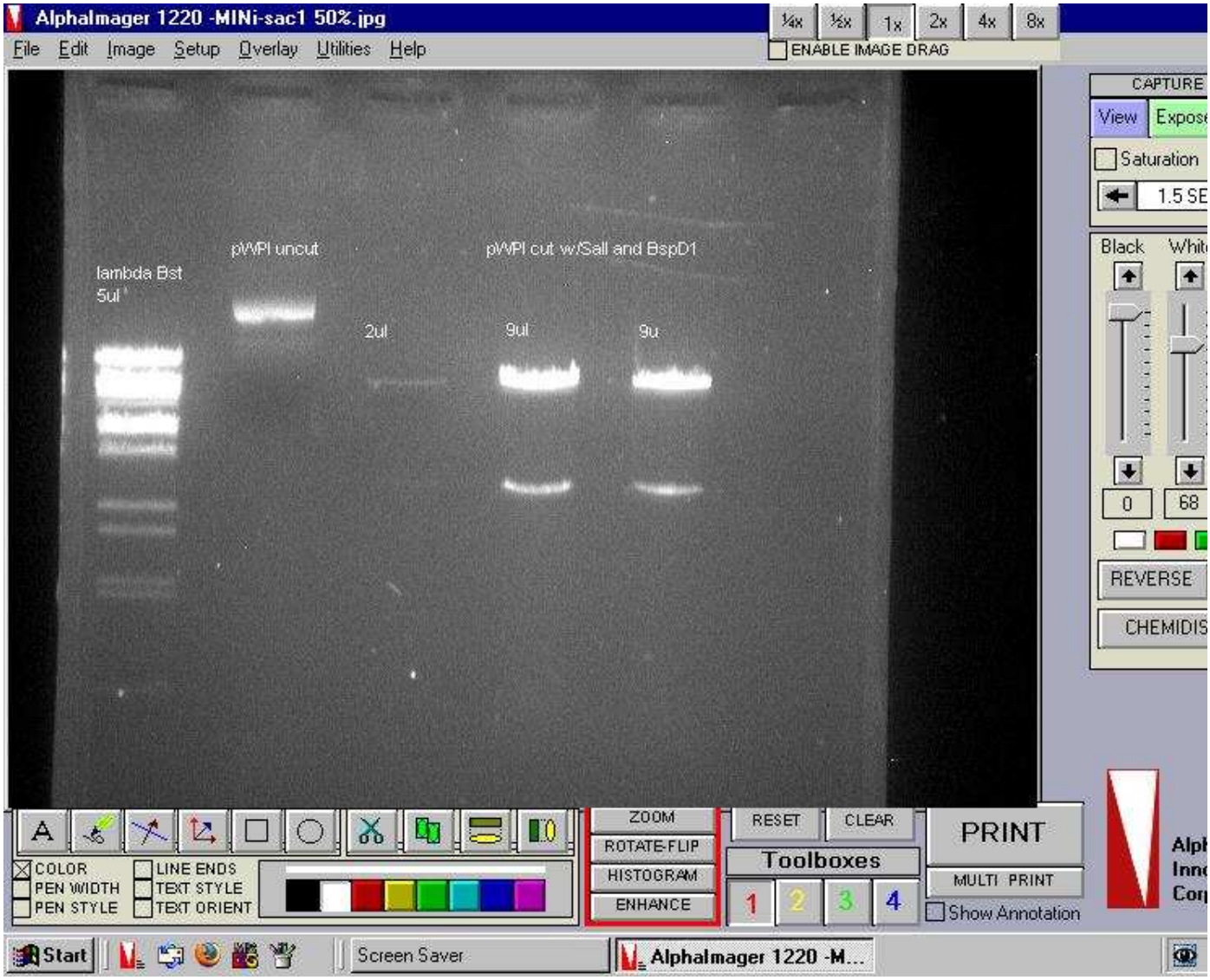
08/23/08

pWPI:

- cut with SalI? : 2ul NEB3, 2ul BSA, 1ul SalI? , 1ul PWPI (500ng/ul), 14ul H2O?
- at 37C for one hour
- heat inactivate: 65C for 20mins
- ammonium acetate precipitation:
    - add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
    - mix and leave on ice for 5 mins.
    - centrifuge for 15 mins at 4C (in fridge)
    - decant everything except the pellet
    - add 100ul 70% ethanol
    - leave on ice for 1-2 mins
    - spin again at 4C but only for 5 mins
    - decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
    - resuspend DNA in 10ul of H2O?
- cut with BspD1? : 9ul from precipitation, 2ul NEB4, 2ul BSA, 1ul BspD1? , 6ul H2O?
- ran the other 1ul out on a gel against uncut to double check that the enzyme cut



- after BspD1? digestion: ran out on a gel to gel isolate the piece that was double cut (8kb piece)



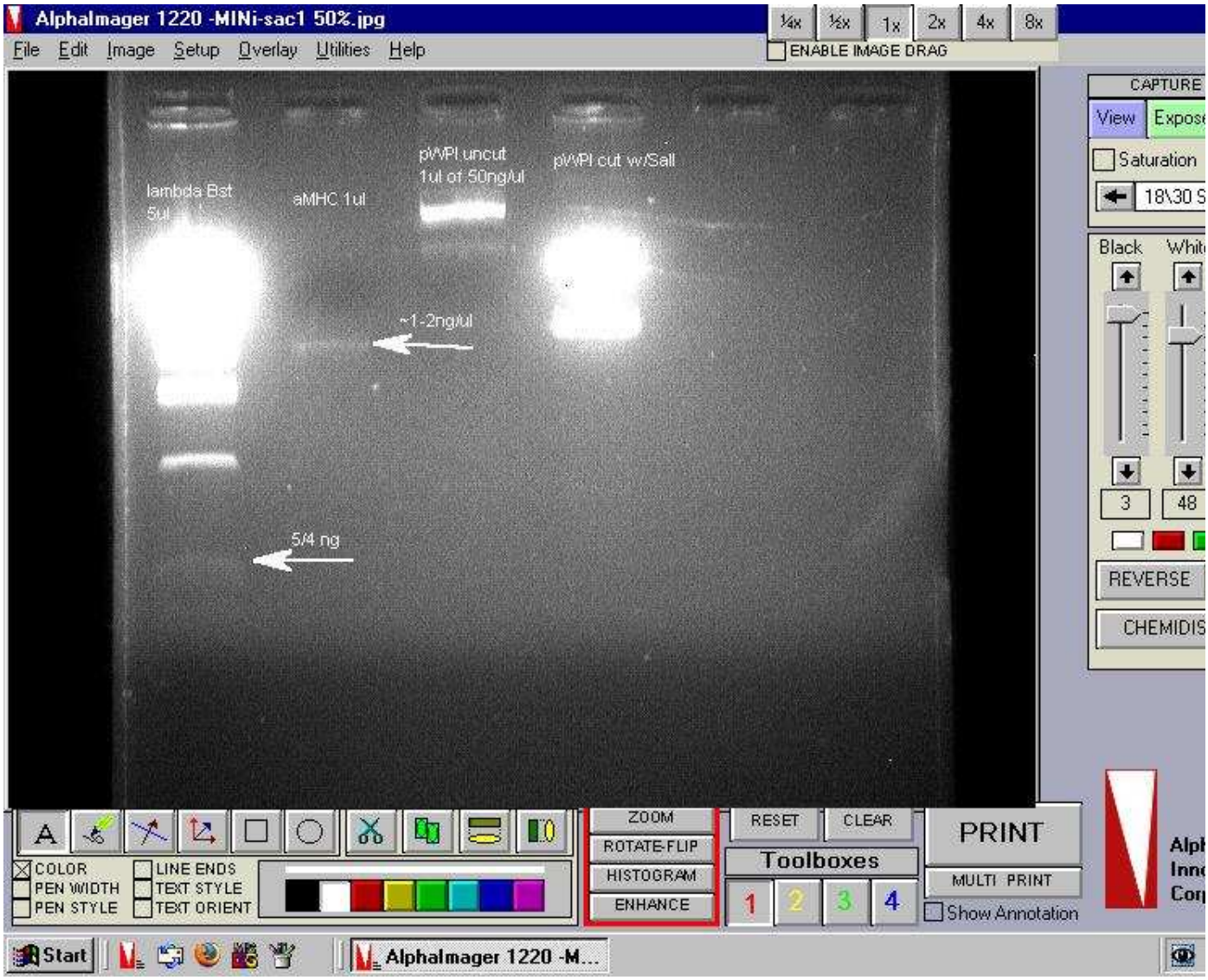
- gel isolation protocol:
    - added 220ul Turbo Salt Solution and incubated at 55C until the gel slice was completely melted
    - transferred all of it to a GENECLEAN Turbo Cartridge inside a cap-less Catch Tube
    - centrifuged it for 5 secs
    - added 500ul Turbo Wash Solution
    - centrifuged for 5 secs
    - emptied catch tube and centrifuged again for 4 mins
    - transferred filter to a clean 1.5mL tube
    - added 30ul Elution Solution and incubated at room temp for 5 min
    - centrifuged for 1 min
- ran out 1ul, 2ul, saw nothing (!!!) on the gel...i dont understand where the DNA went...i've done the gel purification protocol before...maybe it's still stuck on the column...

aMHC:

- ammomium acetate precipitation:
    - add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
    - mix and leave on ice for 5 mins.
    - centrifuge for 15 mins at 4C (in fridge)
    - decant everything except the pellet
    - add 100ul 70% ethanol
    - leave on ice for 1-2 mins

    - spin again at 4C but only for 5 mins
    - decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
    - resuspend DNA in 10ul of H2O?

- quantified it on a gel: about 1-2ng/ul (still really bad yield for this construct)

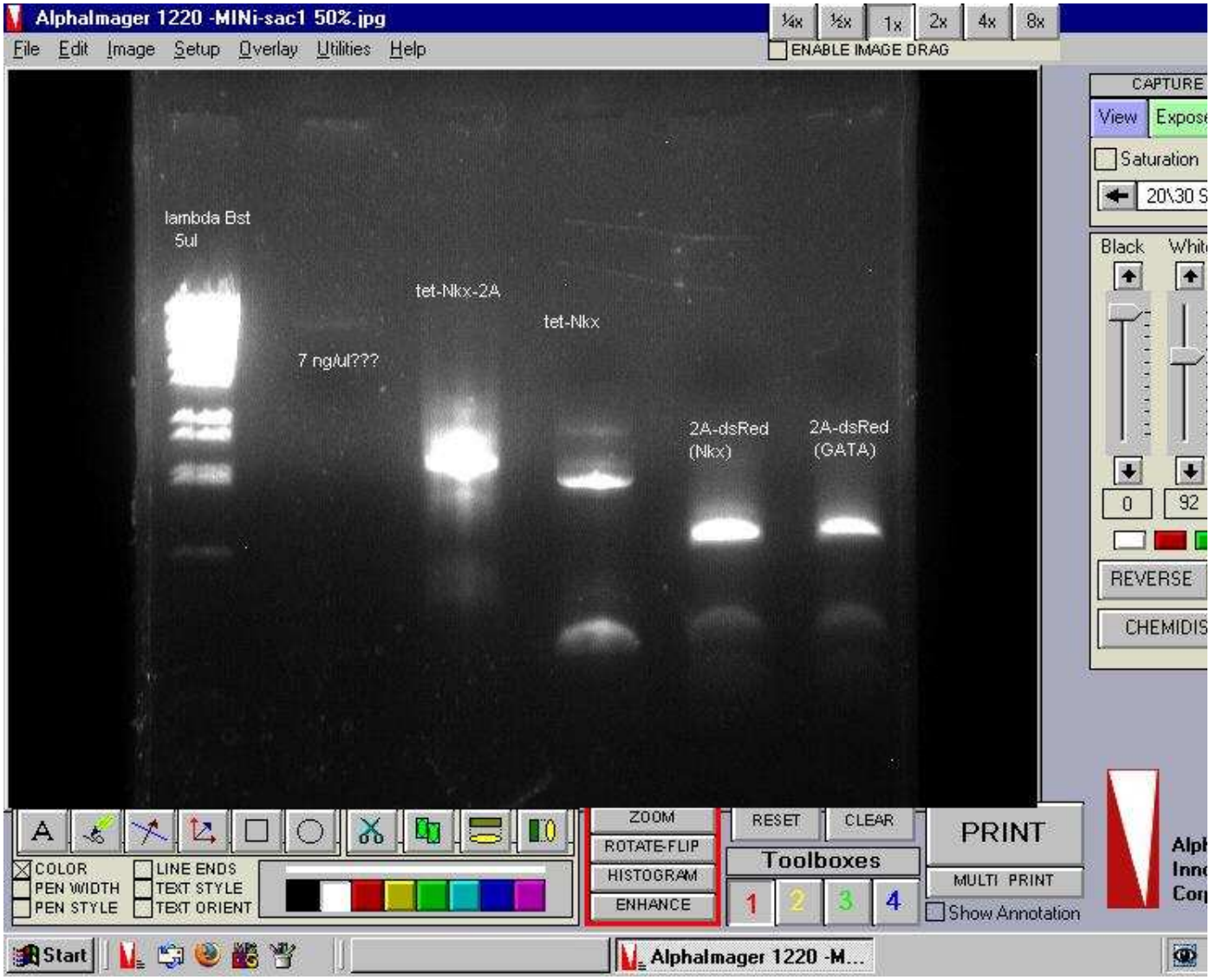


i haven't seen a convincing gel picture of the tet-Nkx fusion or 2A-GFP ...looked through the box for the most recent ones, in our pic folders and the notebooks; i think this is one of the issues with why we havent been able to get the entire constructs for these two

new oligos came: 5' and 3' pbluescript: this means we now need to amplify the nkx/gata/both constructs with the new outer 5' and 3' oligos

- i redid normal tet-Nkx fusion: 13ul betaine, 13.25ul H2O? , 5ul KOD buffer, 5ul dNTPs, 3ul MgCl2? , 2.5ul DMSO, 0.25ul deaza-GTP, 1ul KOD Polymerase, 3ul 5'tet, 3ul 3' 2A-Nkx, 0.5ul 7-24 tet 10, 0.5ul 7-27 Nkx 10 --> looked good on the gel
- amplified with 5' pbluescript oligo: tet, tet-Nkx-2A, tet-GATA
- amplified with 3' pbluescript oligo: gata-2a-dsRed, 2a-dsRed for both Nkx and GATA

Note: I did all of them at the standard temperature programs that they were done at before, for 25 cycles only, since we're going to need 1ug for the Elledge method



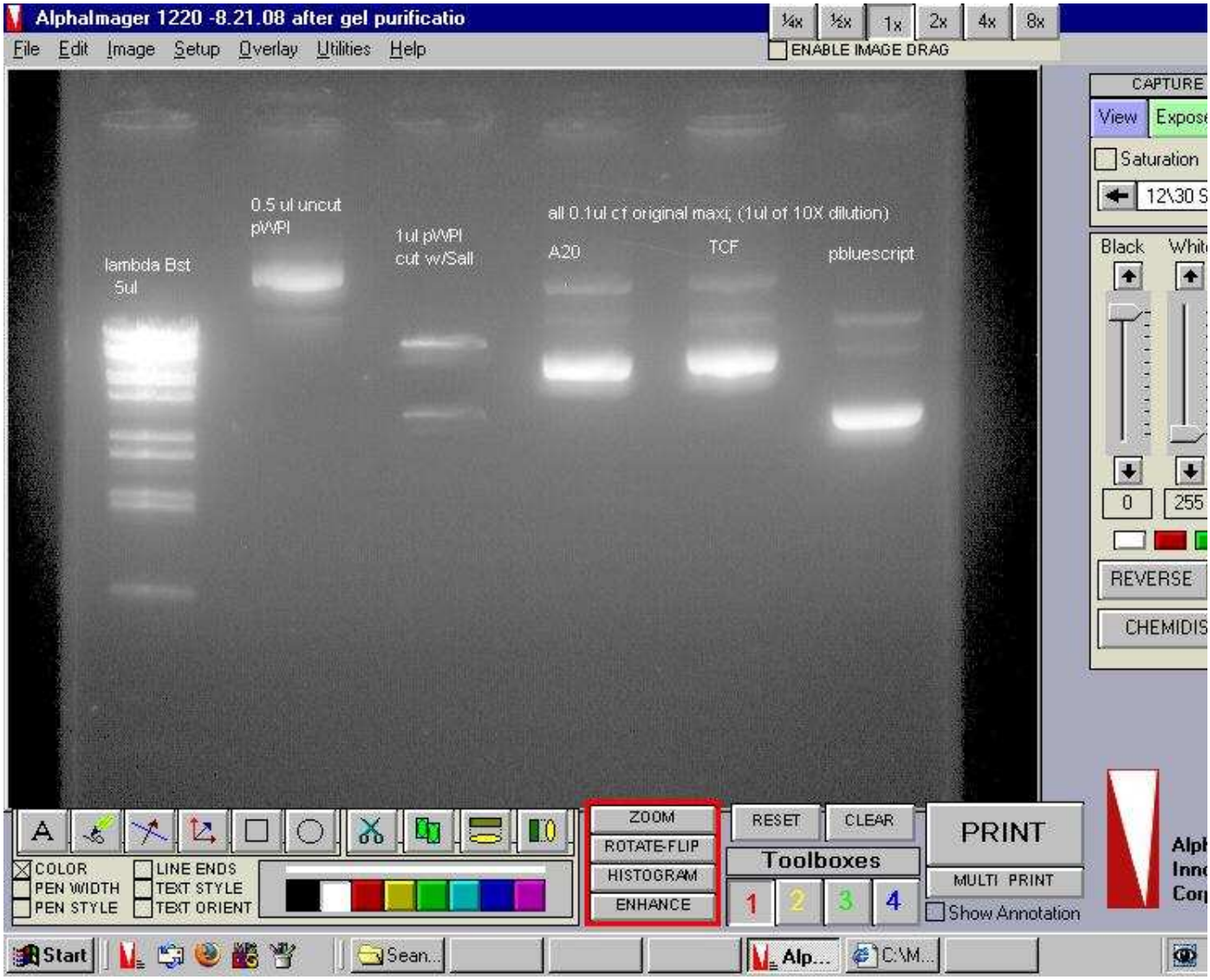
--> tet-Nkx-2A (1400bp), 2A-dsRed (800bp) for both Nkx and GATA look good on the gel

08/21/08

cleaned the pWPI that Josh had already cut with SalI? using ammonium acetate precipitation

- add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
- mix and leave on ice for 5 mins.
- centrifuge for 15 mins at 4C (in fridge)
- decant everything except the pellet
- add 100ul 70% ethanol
- leave on ice for 1-2 mins
- spin again at 4C but only for 5 mins
- decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
- resuspend DNA in 10ul of H2O?

ran it on a gel to double check; also ran 0.1ul of the maxis



pLentiBB: put the oligos together

- 1.5ul of 200uM 5' and 1.5ul of 200uM 3', 3ul 10X PNK buffer, 2ul 10mM ATP, 2ul T4 polynucleotide kinase (PNK), 20ul H2O?
- incubated at 37C for 1.5 hours
- added 4ul of 0.5M NaCl? (had to dilute it, dilution is now in our "iGEM 2008" box)
- placed in boiling water for 2 mins
- removed water from heating plate, and let it cool to room temperature
- took 20ul and ammonium acetate precipitated it (see below)

aMHC didn't work earlier...recut it:

- 9ul aMHC construct, 2ul BSA, 2ul NEB4, 1ul BspD1? , 1ul NheI? , 5ul H2O? -->at 37C for 1.5 hours

cut the cleaned pWPI that had already been cut with SalI? , with BspD1?

- took 9ul of cleaned pWPI cut with SalI? (from ammonium acetate precipitation), 2ul NEB4, 2ul BSA, 1ul BspD1? , 6ul H2O? -->at 37C for 1 hour

cleaned with ammonium acetate precipitation: pWPI cut with SalI? and BspD1? , aMHC construct cut with BspD1? and NheI? , and only 20ul of the pLentiBB fusion (Andy was worried that I might not get a pellet since the DNA is so short...there might not be enough of it, in which case we'll have to use the original, and maybe dilute the salt)

- add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
- mix and leave on ice for 5 mins.
- centrifuge for 15 mins at 4C (in fridge)

- decant everything except the pellet
- add 100ul 70% ethanol
- leave on ice for 1-2 mins
- spin again at 4C but only for 5 mins
- decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
- resuspend DNA in 10ul of H2O?

-->problems: pLentiBB did NOT precipitate at all...I left the tube in the clear box in case you guys wanted to spin it more (it spun for over 30 mins btw) and it says on the tube that it's in 100ul ethanol...if you want to do the ligation, dilute the uncleaned (ask Andy) --> we actually just did a quick approximation of the ligation...and you might need to dilute the pLentiBB insert about 4-5X in which case the salt becomes negligible and we should be able to use the unclean version...Andy's suggesting we try the ligation at different concentrations, not just the 3:1 insert:plasmid ratio

Josh quantified the cmv-rtTA after digest--> 30ng/ul --> we will need to quantify the pWPI and the aMHC and the pLentiBB (oligos that I fused together)

08/20/08

continued maxi that Josh started (TCF, A20, pbluescript), beginning with step 7

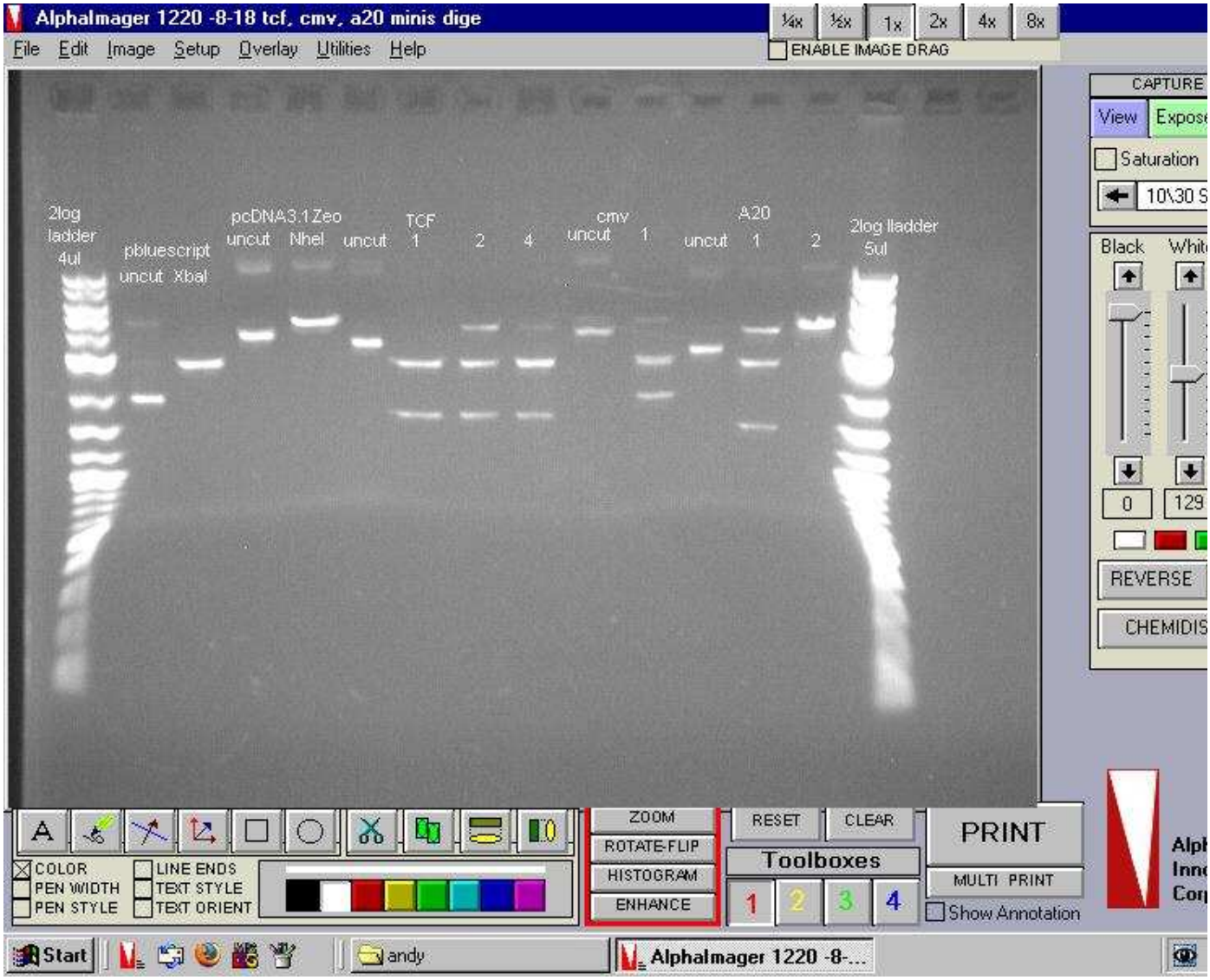
- centrifuged samples for 30mins at 4C and 13,000rpm
- removed supernatant and centrifuged it again for 15mins at 4C and 13,000rpm
- saved 120ul of sample before next step (Step 8)
- added the supernatant to QIAGEN-tip 500 columns that have been washed with 10mL Buffer QBT and emptied by gravity
- waited for supernatant to pass through
- saved 120ul of flow-through (Step 10)
- washed column twice with 30mL Buffer QC
- saved 240ul of flow-through (Step 11)
- eluted DNA with 15mL Buffer QN
- saved 60ul of elution (Step 12)
- added 10.5mL room-temperature isopropanol to the eluted DNA
- mixed and centrifuged at 11,000rpm for 30mins at 4C
- carefully decanted the supernatant
- added 5mL of room-temperature 70% ethanol and centrifuged again at 11,000rpm for 10mins at 4C
- carefully decanted the supernatant
- air-dried the pellet for 5-10mins (TCF didn't seem to have a pellet or I lost it...I highly doubt that there will be DNA in that one; the other two looked fine)
- resuspended in .5mL TE buffer

-->put it in the freezer

08/19/08

set up 2a-dsRed fusion for GATA4 construct (regular protocol, 56 anneal, 15secs elongate)

ran out Josh's restriction digests of a20, tcf, cmv --> we're sure a20 and tcf worked...we're running out cmv again (to check the size of the cut piece)



ran out tet-gata fusion, 2A-dsRed (for gata construct), gata-2A-dsRed gel isolation, and cmv again

- tet-gata worked
- 2A-dsRed didn't work...once we get this we can fuse it to tet-gata and get the gata only construct; it's a little strange it didn't work since we got the gata-2A-dsRed and this is just a shorter version...maybe we can amplify out the region we want from the gel isolation?
- gata-2A-dsRed: ran out the gel isolation, only 1ul, but it's still strange that we got 2 bands...we should probably amplify it if my next fusion didn't work (see below)
- cmv didn't work...we can probably throw away the stock (no need for a maxi) and the glycerol stock in the -70C

before I left I set up the Nkx-gata construct fusion

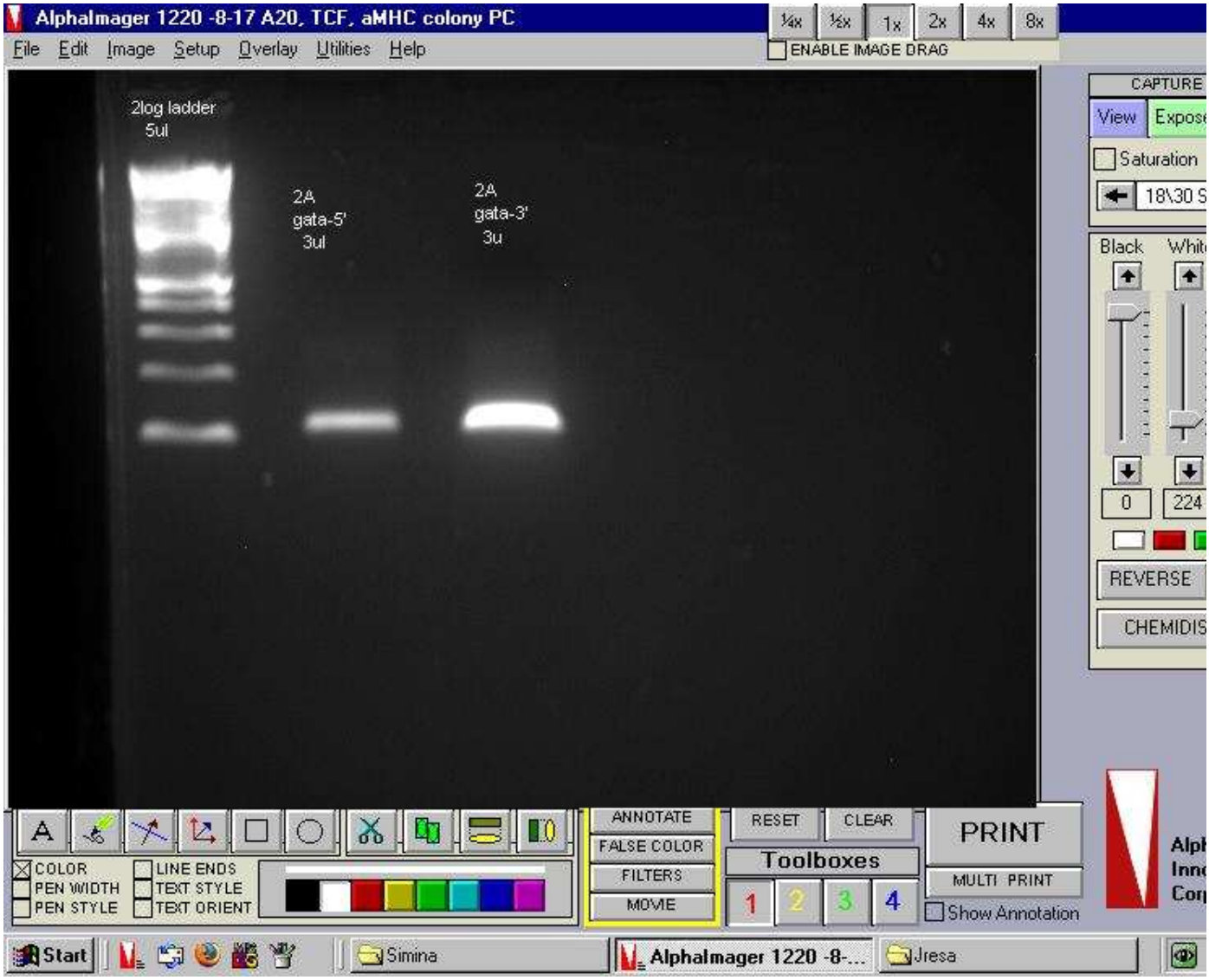
- regular high GC protocol with 1ul gata-2a-dsRed gel isolation (8-19-08) and 0.5ul tet-Nkx-2A (8-18-08) (11)
- used 3ul of each tetO-5' and 3'-dsRed
- KOD protocol says for constructs over 3kb, 25s/kb; since this construct is about 3550bp, 25s x 3.5kb = 88secs
- (95C 56C 70C) for (20s 10s 88s) for 27 cycles; since I used the Robocycler, all the times were doubled

designed oligos for pbluescript

- (5'construct)
- ATACGACTCACTATAGGGCGAATTG-AGAGAGATCGATCGAGGTTCTAG
- (3'construct)
- CCCTCACTAAAGGGAACAAAAGCTG-CTCTCTGTCGACGCTAGCCTA
- (3' construct) before reverse complement
- TAGGCTAGCGTCGACAGAGAG-CAGCTTTTGTTCCCTTTAGTGAGGG

08/18/08

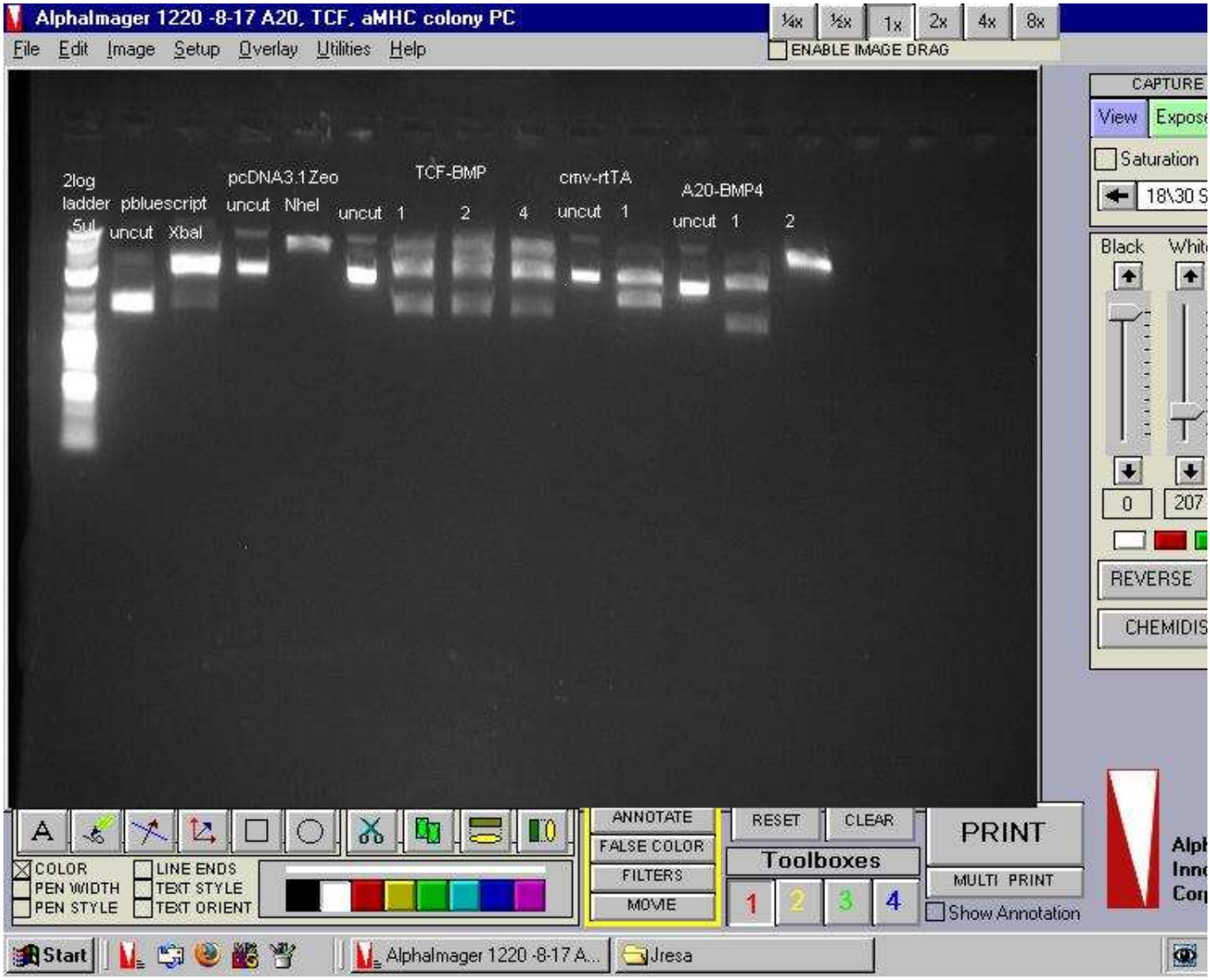
ran out the 2A for gata-5' and gata-3' (the 2As flanking gata in the Nkx construct) --> both looked good



set up restriction digests just for the minis that looked promising (TCF #1,2,4; cmv #1; A20 #1,2) and 2 controls to make sure that they worked.

- each of the mini digests had: 2ul BSA, 2ul NEB3, 1ul XbaI? , 1ul NheI? , 1ul mini DNA, 13ul H2O?
- XbaI? control: 2ul BSA, 2ul NEB3, 1ul XbaI? , 1ul pbluescript, 14ul H2O?
- NheI? control: 2ul BSA, 2ul NEB3, 1ul NheI? , 1ul pcDNA3.1 Zeo, 14ul H2Oou? guys double check? pics in my folder labeled with the date

they looked good --> hard to see some of the sizes

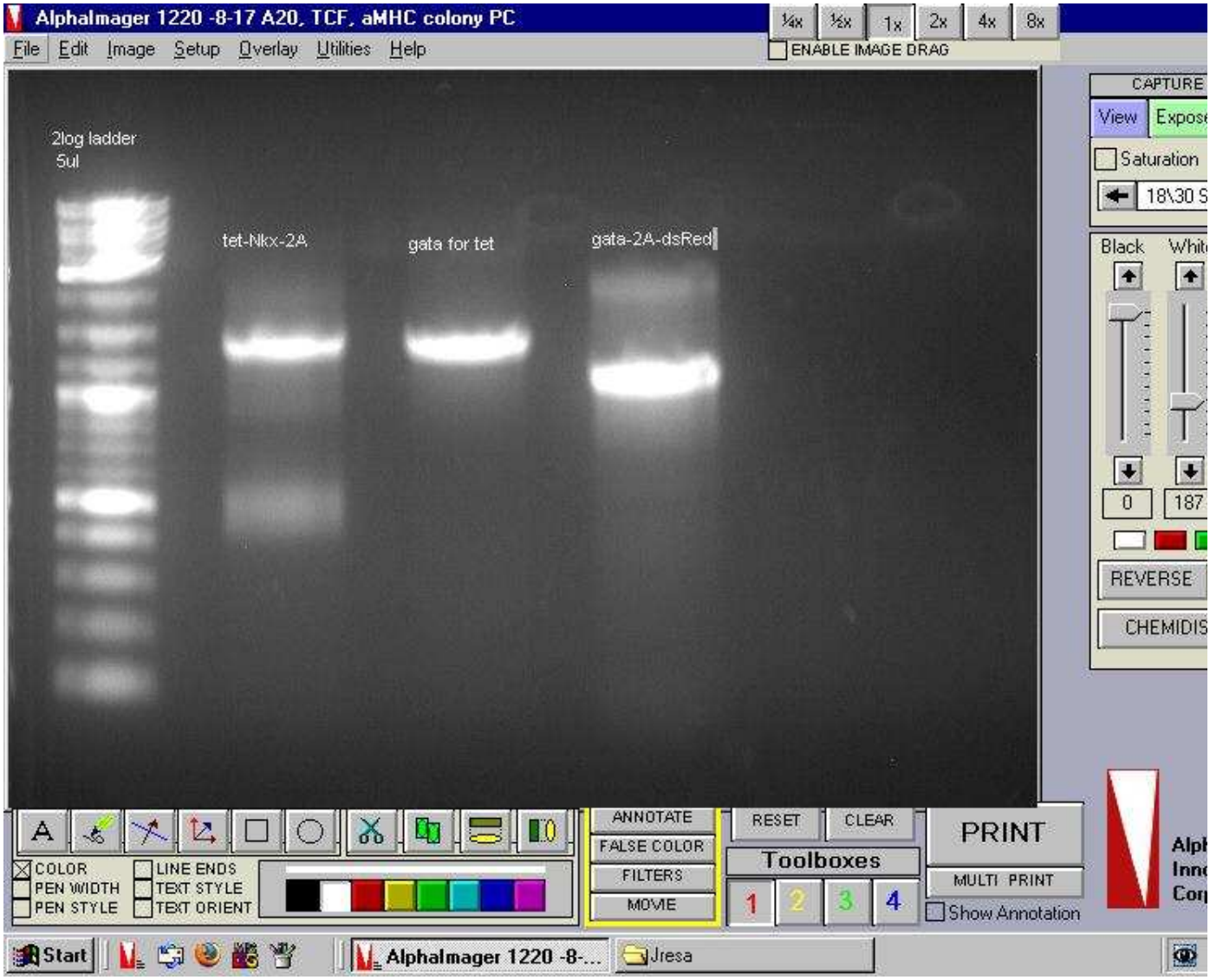


prepared maxis/glycerol stocks for TCF #1, cmv #1, A20 #1

- maxis: 200mL LB + 400ul Amp + whatever was let of the minis (range from 20ul to 80ul); in shaker at 8:30pm
- also prepared a maxi for pbluescript (for DNA used a bit of glycerol stock...swabbed with a pipette tip)
- glycerol stock: 130ul glycerol + 300ul mini DNA + 100ul LB
- also made glycerol stock of GATA4: 130ul glycerol + 400ul bacteria --> wasn't sure what to do with the tubes, so they're still on the bench

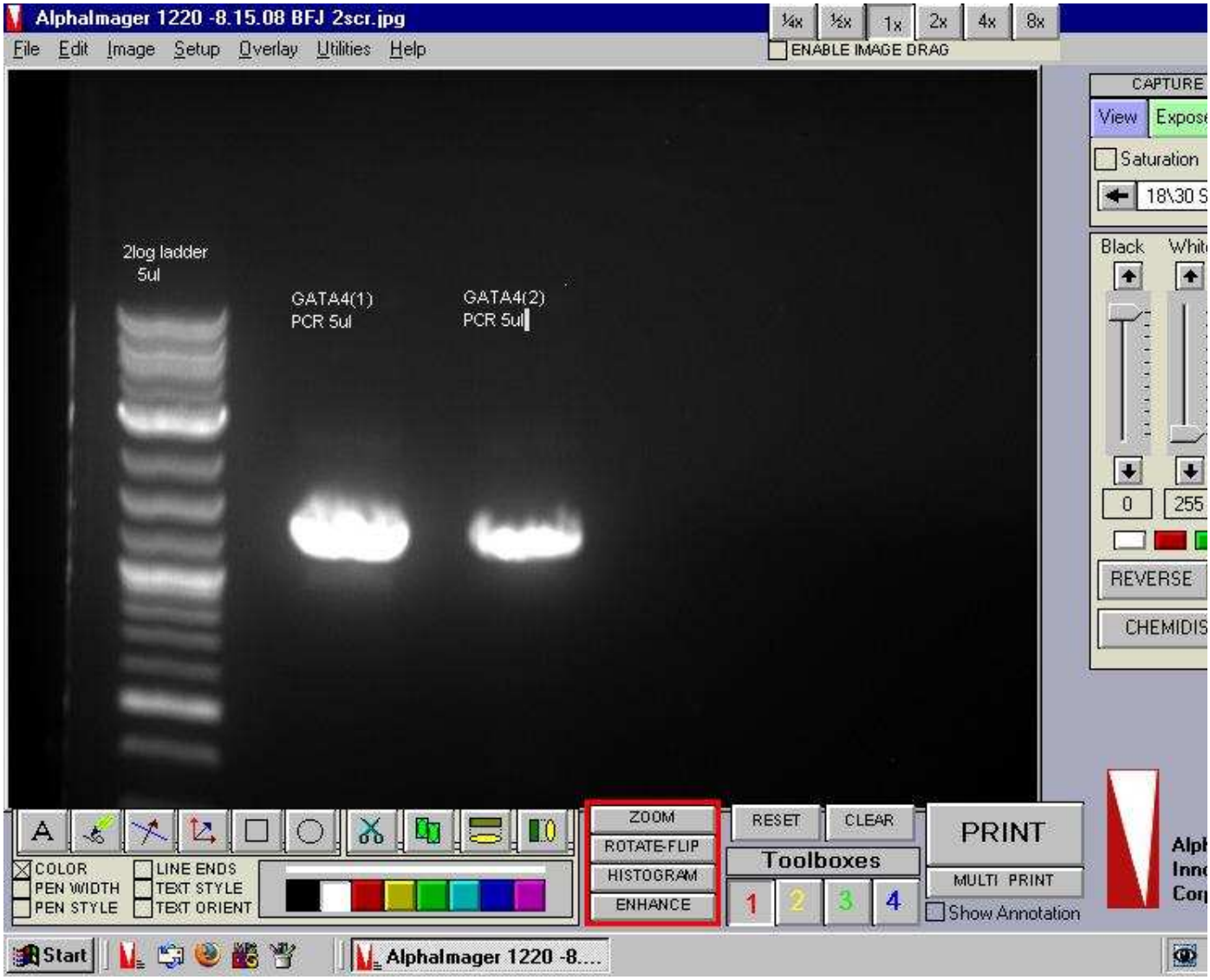
ran tet-Nkx-2A, gata for tet and gata-2a-dsRed on a 1% gel

- tet-Nkx-2A and gata for tet look about right
- gata-2a-dsRed looks too small --> check oligos?



08/17/08

redid the GATA(1) and GATA(2) PCRs using 2A-gata4-5' and 2A-gata4-3' (same protocol as yesterday, and same temperature program)



-->it worked!!! so i set up the 2A amplification for the 2A region between Nkx and GATA (called it 2A 5'gata) and the 2A region between GATA and dsRed (called it 2A 3' gata) 2A PCR:

- 32ul H2O? , 5ul dNTPs, 5ul KOD Buffer, 3ul MgSO4? , 1.5ul 5' oligo, 1.5ul 3'oligo, 1ul KOD Polymerase, 1ul fmdv2A (original, not the gel isolation)
- oligos for "2A 5' gata": Nkx-2A-5' and gata4-2A-3'
- oligos for "2A 3' gata": gata4-2A-5' and 2A-dsRed-3'
- temperature program: (95 55 70) for (20s 10s 10s) for 10/25 cycles

-->in PCR box (need to check and try fusions next)

Colony PCR for each of the constructs: A20-BMP4, TCF-BMP4, cmv-rtTA, aMHC-NeoR-2A-GFP for each of them I tried to choose the smallest region to amplify:

- A20-BMP4: 5'-A20 and 3'-A20 (to amplify A20 which is 100bp)
- TCF-BMP4: TCF oligo 5' and TCF oligo 3' (to amplify TCF which is 300bp)
- aMHC-NeoR-2A-GFP: aMHC-NeoR-3' and aMHC-5' (to amplify aMHC which is 400bp)
- cmv-rtTA: cmv-rtTA-5' and rtTA-3' (to amplify rtTA which is 700bp)

PCR reaction:

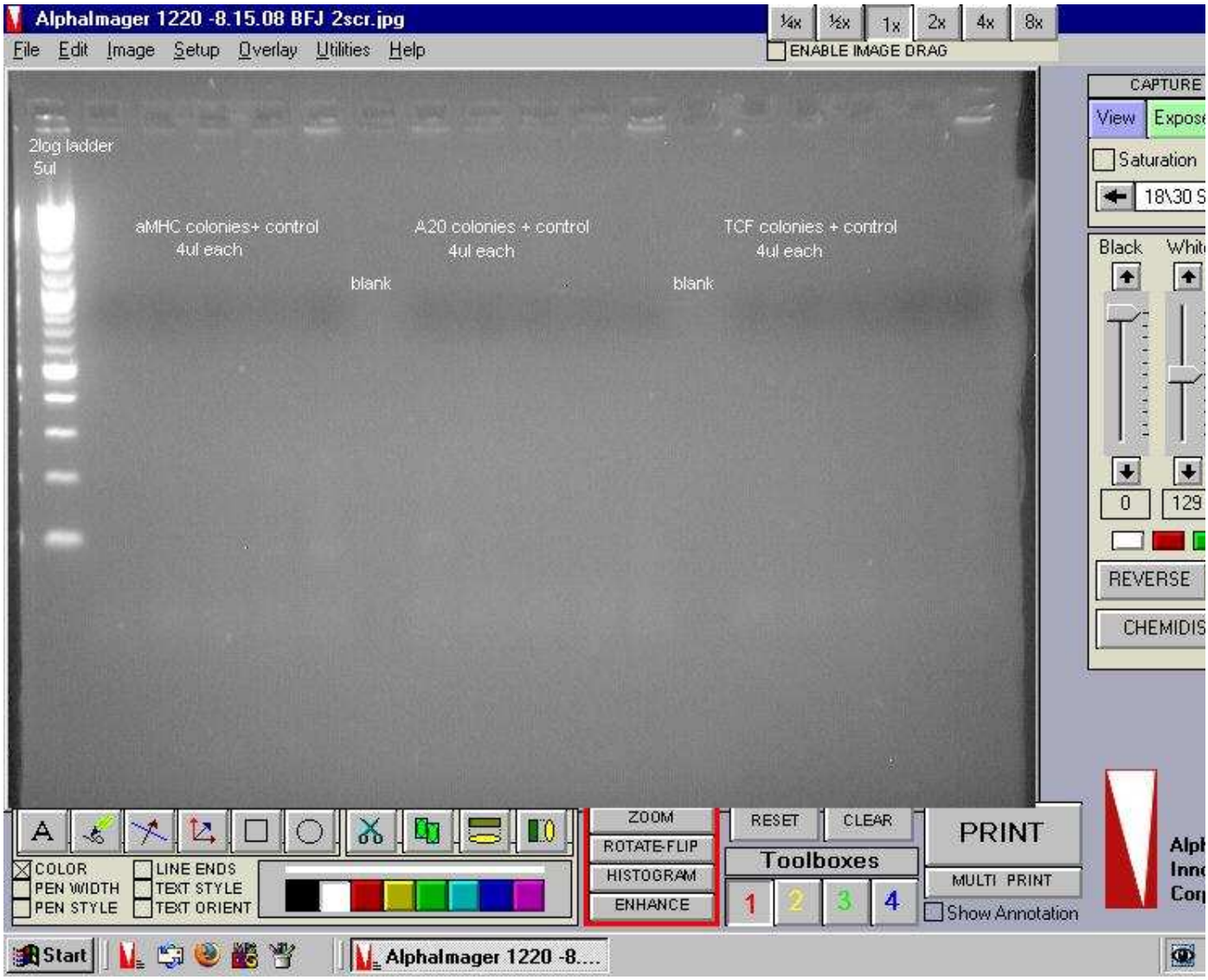
- 35.8ul H2O? , 0.2ul Pan Taq Polymerase, 5ul dNTPs, 5ul reaction buffer, 2ul 5'oligo, 2ul 3'oligo
- for each plate, I picked 4 colonies, and did one negative control (colony off the pbluescript(-) plate with the respective oligos for each of the reactions)
- temperature program: 94 for 2 mins; (94C 55C 72C) for (15 sec 30sec 45 sec) for 35 cycles...all the times here were

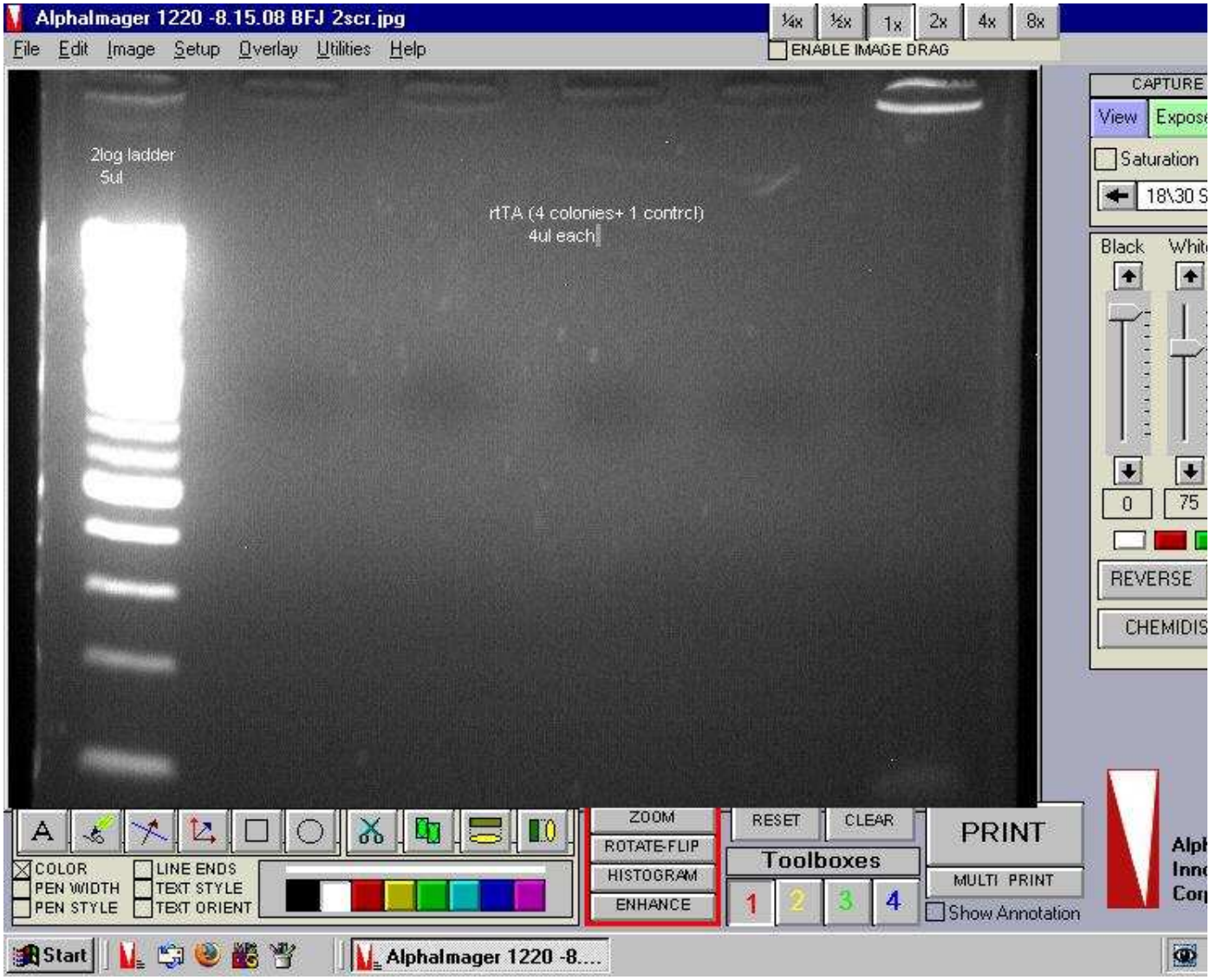
doubled in the Robocycler, and 72C for 7mins

ran them all out on 2% gel:

- rtTA showed nothing for any of the colonies (it was on a separate gel)
- aMHC, A20 and TCF also showed nothing

-->5mL tubes have 1.5mL LB, 3ul Amp --> need to be mini-ed/restriction enzyme digested





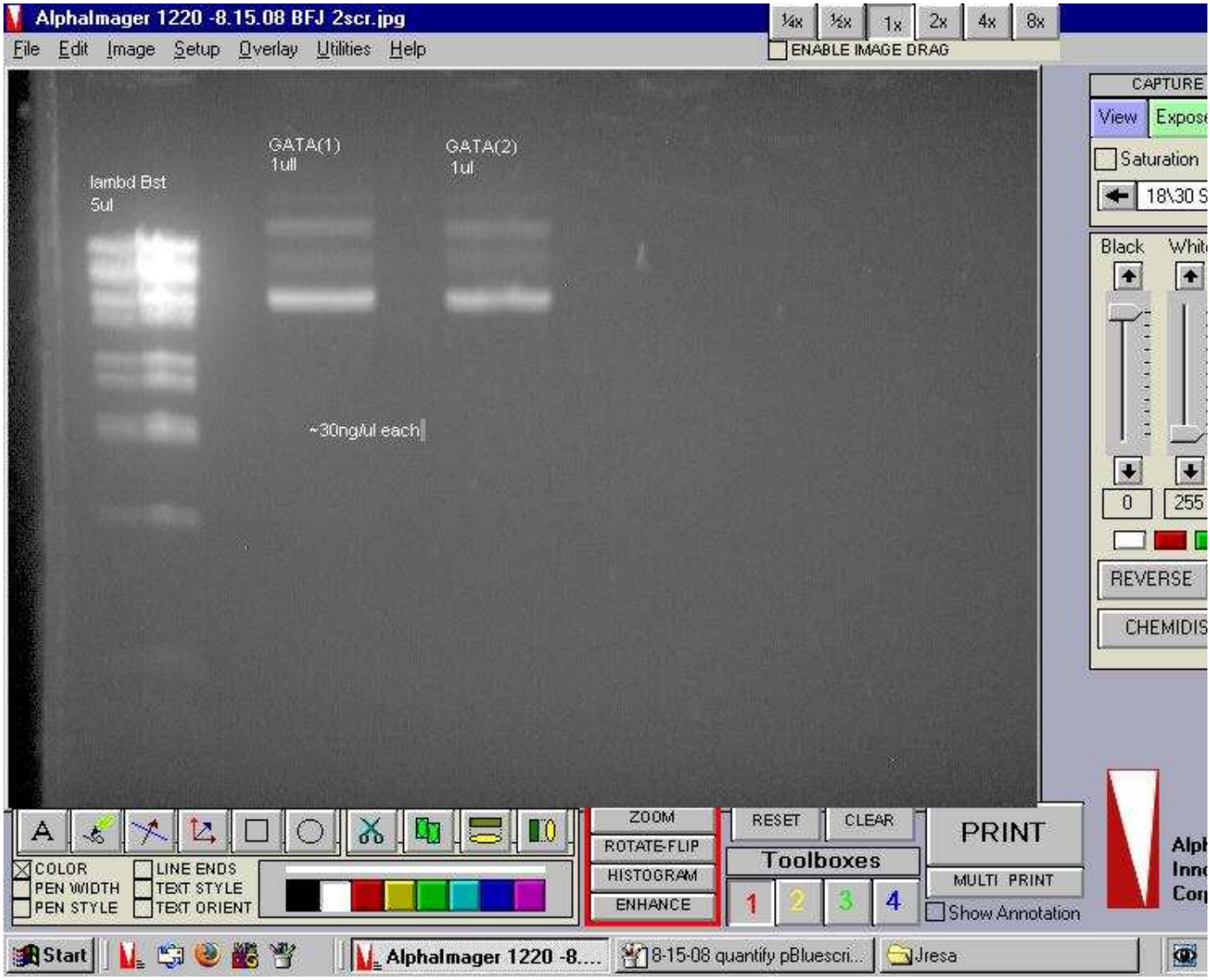
08/16/08

picked one colony off of the GATA-4 plate (8-15-08) and put it in the shaker with 4mL LB and 8ul Amp (the original plate is wrapped and in the small fridge)

Minis off of GATA(1) and GATA (2)

- took 1.5mL of each and put it in a centrifuge tube --> centrifuged it for about 10 mins
- get rid of the supernatant (now you just have a pellet of cells)
- resuspend them in 250ul Buffer P1
- add 250ul Buffer P2 and mix thoroughly by inverting the tube 4-6 times
- add 350ul Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times
- centrifuge for 10 min at 13,000 rpm
- Apply the supernatant from the previous step to the QIAprep spin column by pipetting
- centrifuge for 30-60s; discard the flow-through
- Add 750ul Buffer PE and centrifuge for 30-60s; discard the flow-through
- Centrifuge for an additional 1 min to remove residual wash buffer
- Place the QIAprep column in a clean 1.5mL microcentrifuge tube; add 50ul Buffer EB (to elute DNA)
- Let it stand for 1 min and centrifuge for 1 min

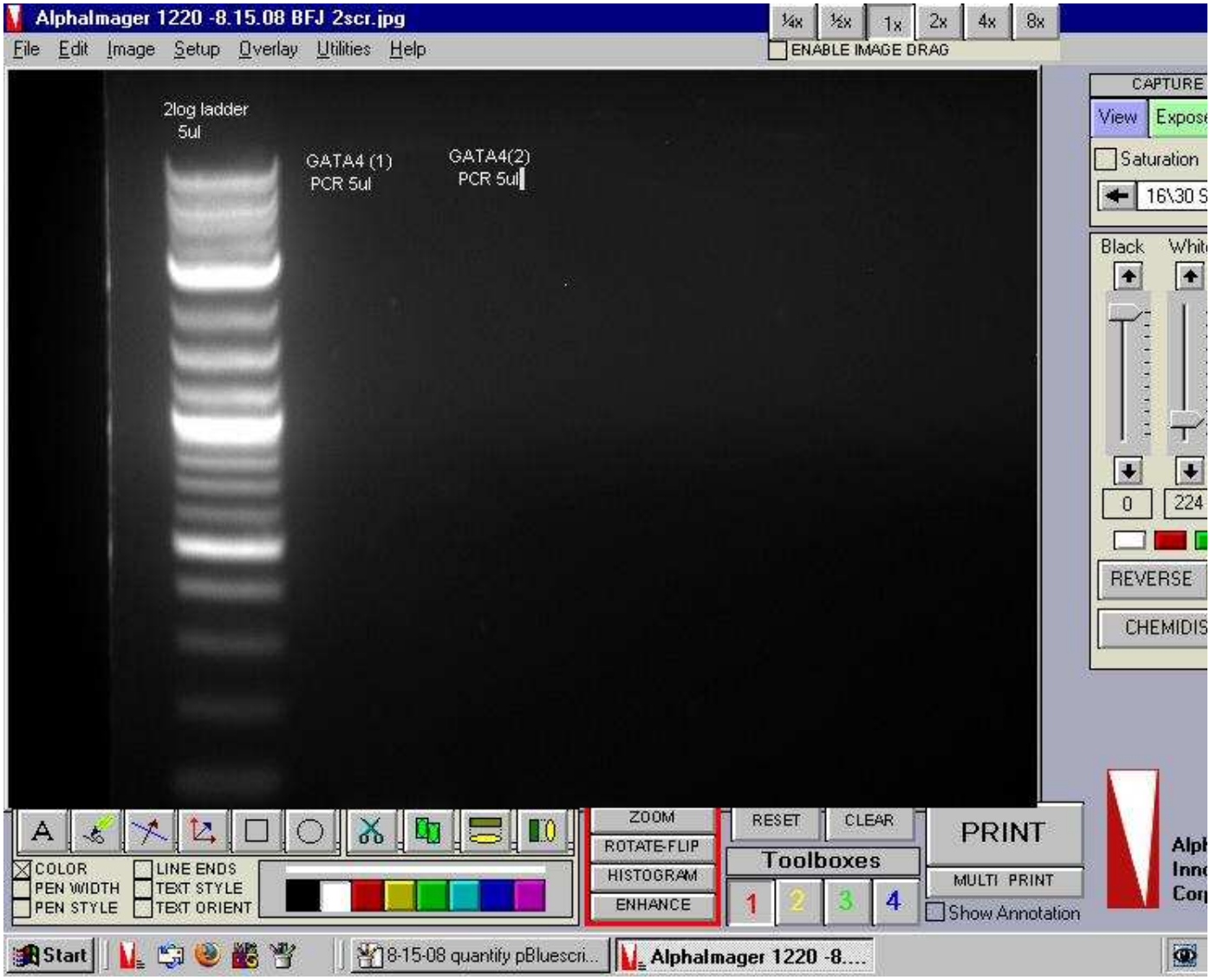
Quantified GATA minis on a 1% gel by loading 5ul lambda Bst and 1ul of each GATA(1) and GATA(2) --> they're both approximately 30ng/ul



Set up PCR reaction to get GATA4

- 16.75ul H2O? , 13ul betaine, 5ul KOD Buffer, 5ul dNTPs , 1ul KOD Polymerase, 3ul MgCl2? , 2.5ul DMSO, 0.25ul deaza-GTP, 1.5ul gata-2A-3', 1.5ul 2A-gata-5', 0.5ul GATA4 mini (both (1) and (2))
- 95 for 2 mins, 95 for 20s- 55 for 10 s- 70 for 27s (20s/kb and its 1350bp), repeat 10/25 times, 70 for 2 min

ran out on a 1% gel -->



Took yesterdays ligations that Josh left overnight and cleaned them by ammonium acetate precipitation

- add 1ul glycogen, 7ul ammonium acetate, 70ul 100% ethanol
- mix and leave on ice for 5 mins.
- centrifuge for 15 mins at 4C (in fridge)
- decant everything except the pellet
- add 100ul 70% ethanol
- leave on ice for 1-2 mins
- spin again at 4C but only for 5 mins
- decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
- resuspend DNA in 10ul of H2O?

Electroporate the cells (prechill cuvettes, thaw cells on ice, 50ul bacteria + 5ul of DNA (from the ammonium acetate precipitation), set electroporation machine to 2.5kV) Then added 0.5mL LB and grew them in the shaker for one hour

plated 100ul --> at 37C overnight

08/14/08

cleaned the pbluescript gel isolation from yesterday --> the one cut with BspD1? and SalI?

set up PCR for Nkx construct

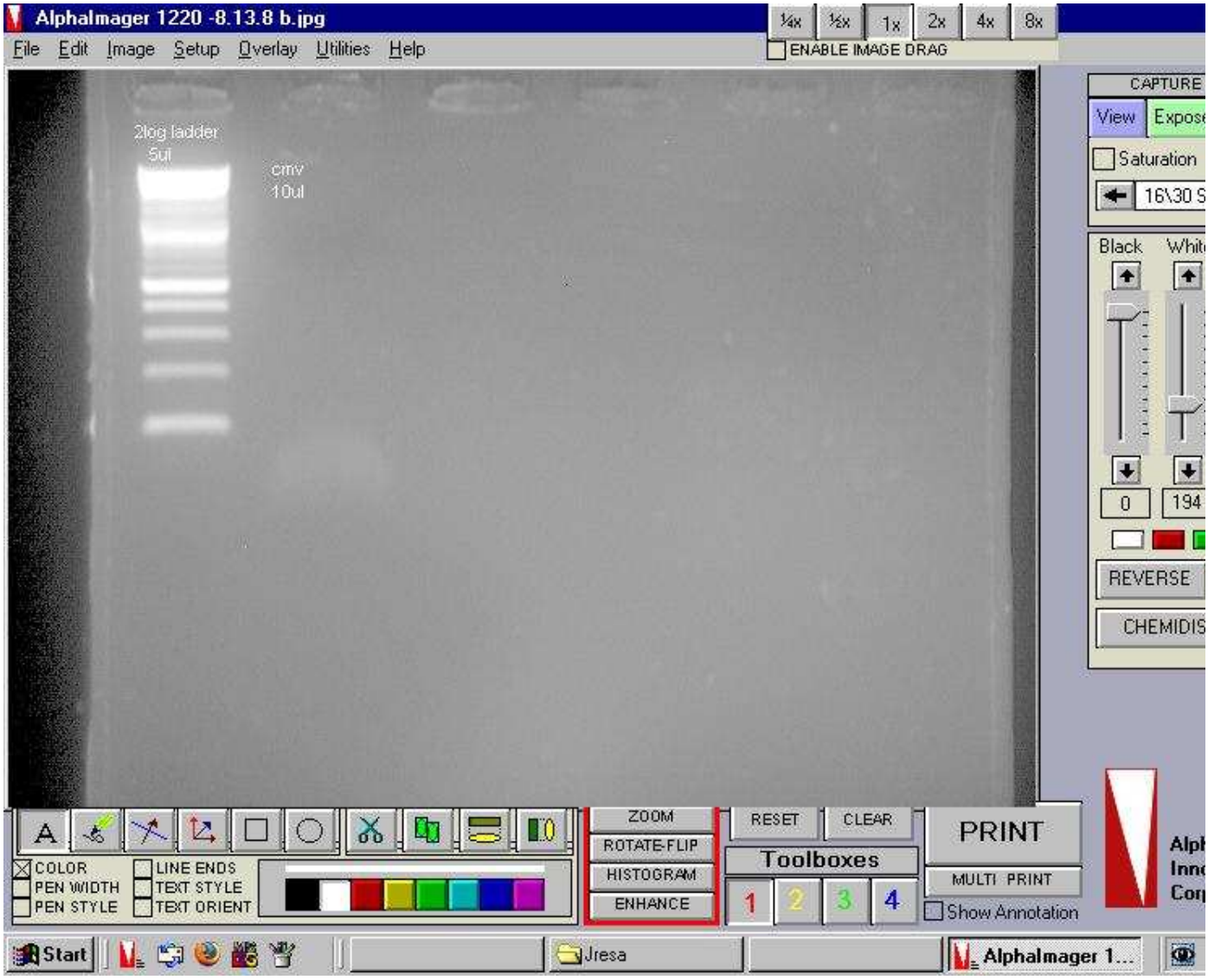
- first just with 4 cycles (regular protocol): 0.5ul tet-Nkx 10 (8-12-08) and 0.5ul 2A-dsRed 15 (8-11-08) with high GC

content protocol; (95 56 70) for (20s 10s 40s)

- then with outer oligos for 25/35 cycles (new dNTPs that are apparently really old) 3ul 5'-tet and 3ul dsRed-3' and 1ul of previous reaction; (95 60 70) for (20s 10s 40s)

08/13/08

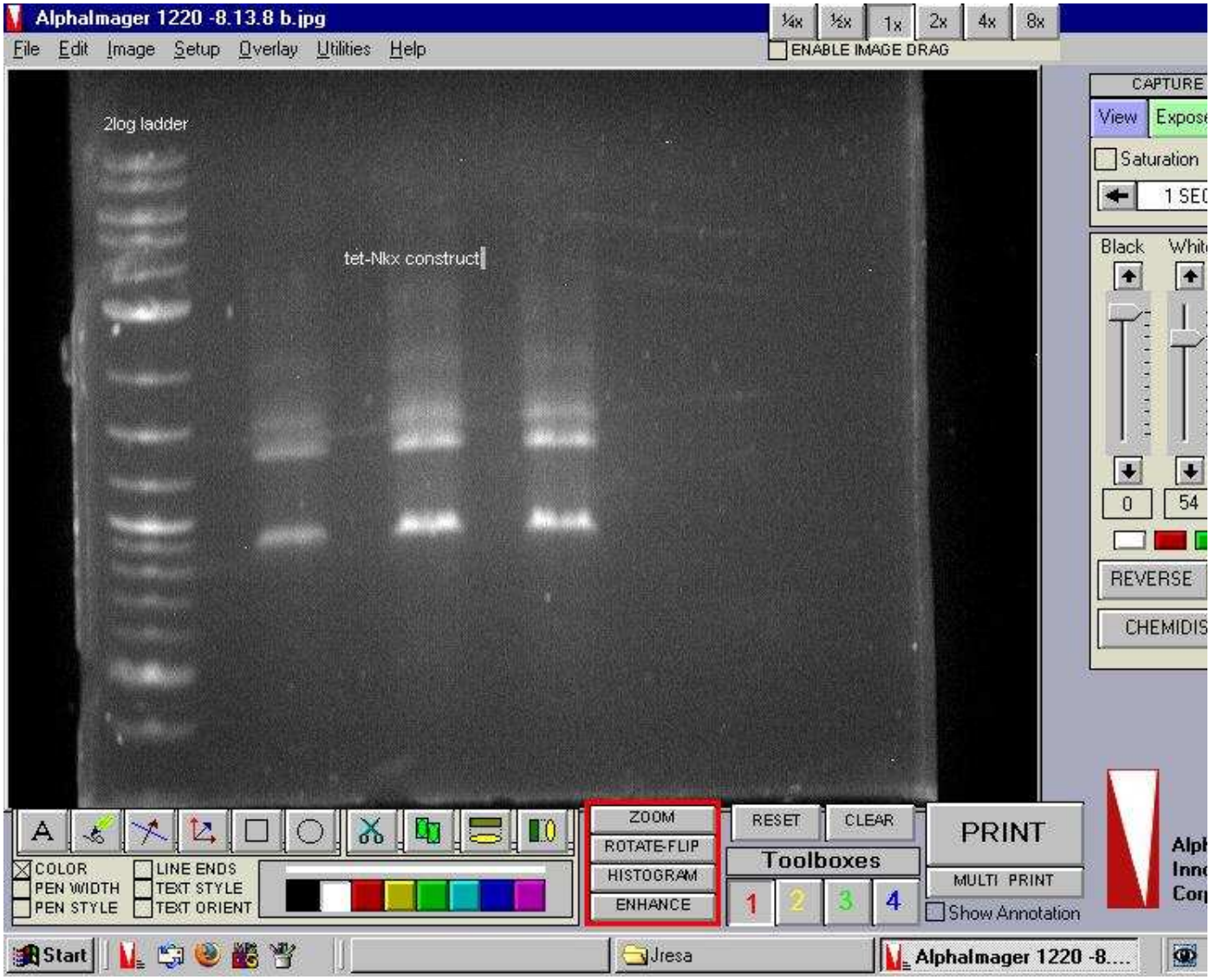
retried PCRing out cmv using high GC protocol (same program, oligos and DNA) --> didn't work



CMV PCR

- 16.25ul H2O? , 13ul betaine, 5ul KOD Buffer, 5ul dNTPs , 1ul KOD Polymerase, 3ul MgCl2? , 2.5ul DMSO, 0.25ul deaza-GTP, 1.5ul 5'-cmv, 1.5ul 3'-cmv-rtTA, 1ul ptripz
- 95 for 2 mins, 95 for 20s-55 for 10 s- 70 for 15s, repeat 10/25 times, 70 for 2 min

ran out Josh's Nkx construct --the band around 2100 was very faint, so I gel isolated it, and cleaned it...we should probably amplify it using this DNA and the outside oligos (it's in the PCR box)



restriction digest reactions:

constructs	SV40	plasmid	BspD1? control	Sal1 control	XbaI? control	NheI? control
2ul NEB4	2ul NEB3	2ul NEB3	2ul NEB3	2ul NEB3	2ul NEB3	2ul NEB4
2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA
14ul from gel isolations	14ul gel isolation	10ul pbluescript	10ul pbluescript	10ul pbluescript	10ul pbluescript	3ul pcDNA3.1 Zeo
1ul BspD1?	1ul XbaI?	1ul BspD1?	1ul BspD1?	1ul SalI?	1ul XbaI?	1ul NheI?
1ul NheI?	1ul SalI?	1ul SalI?	5ul H2O?	5ul H2O?	5ul H2O?	12ul H2O?
		4ul H2O?				

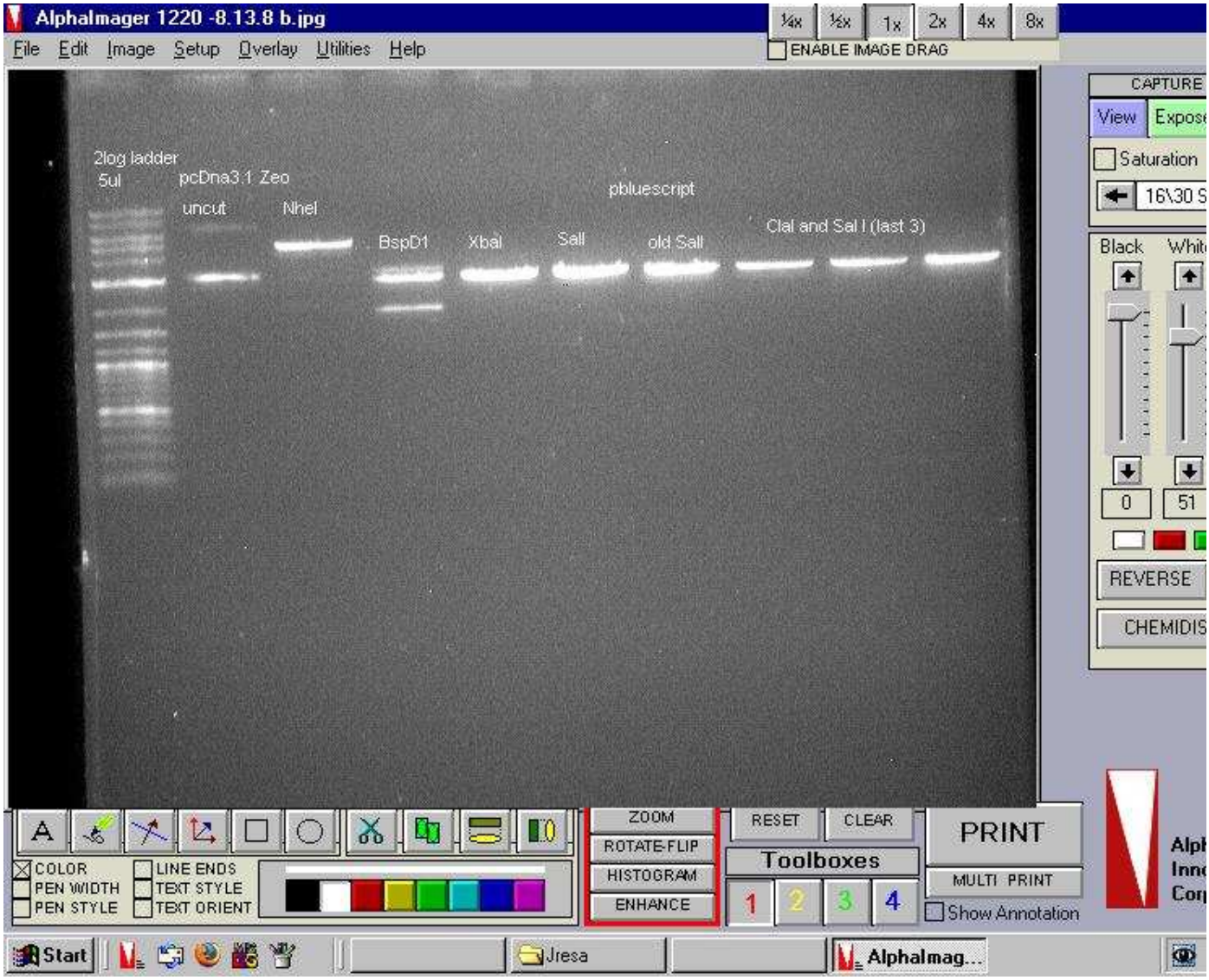
(there are 2 SalI? controls since we ran out of enzyme)

cleaned the inserts using ammonium acetate precipitation (theyre in the PCR box since there's more room)

- add 1ul glycogen
- 7ul ammonium acetate
- 70ul 100% ethanol
- mix and leave on ice for 5 mins.

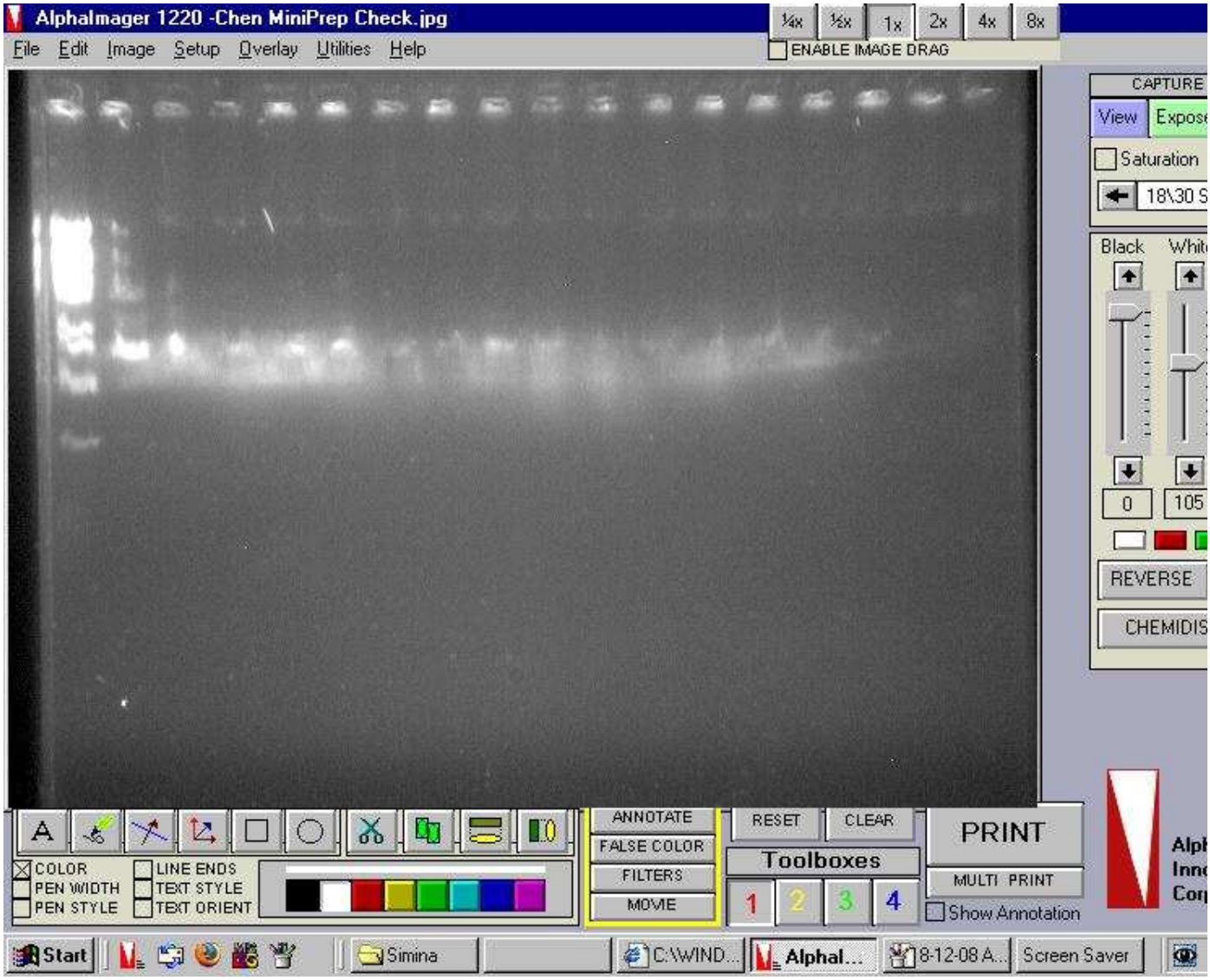
- centrifuge for 15 mins at 4C (in fridge)
- decant everything except the pellet
- add 100ul 70% ethanol
- leave on ice for 1-2 mins
- spin again at 4C but only for 5 mins
- decant everything except the pellet; wait for alcohol to evaporate (doesn't smell like alcohol anymore)
- resuspend DNA in 10ul of H2O?

ran out and cut the plasmid cut with BspD1? and SalI? ; I didn't have any more of the uncut to run, and after running it for an hour and a half, I cut out the piece and put it in a tube in the PCR box...Jen said we'll have to run it out to isolate the cut from the uncut once we get more of the plasmid...also for the restriction digest we should've cut less DNA in the controls, since we're only doing them for identification purposes and we don't need to use that much to be able to visualize them on a gel



08/12/08

visualized the BamHI? --> really blurry, needs to be rerun? ClaI? and BspD1? didn't look good...KRAB didn't show up uncut, so I'm not sure if it's the enzyme again but the uncut/cut BspD1? look the same I put them all on a rack in the freezer


AlphaImager 1220 -Chen MiniPrep Check.jpg
File Edit Image Setup Overlay Utilities Help
¼x ½x 1x 2x 4x 8x
ENABLE IMAGE DRAG
CAPTURE
View
Saturation
Black
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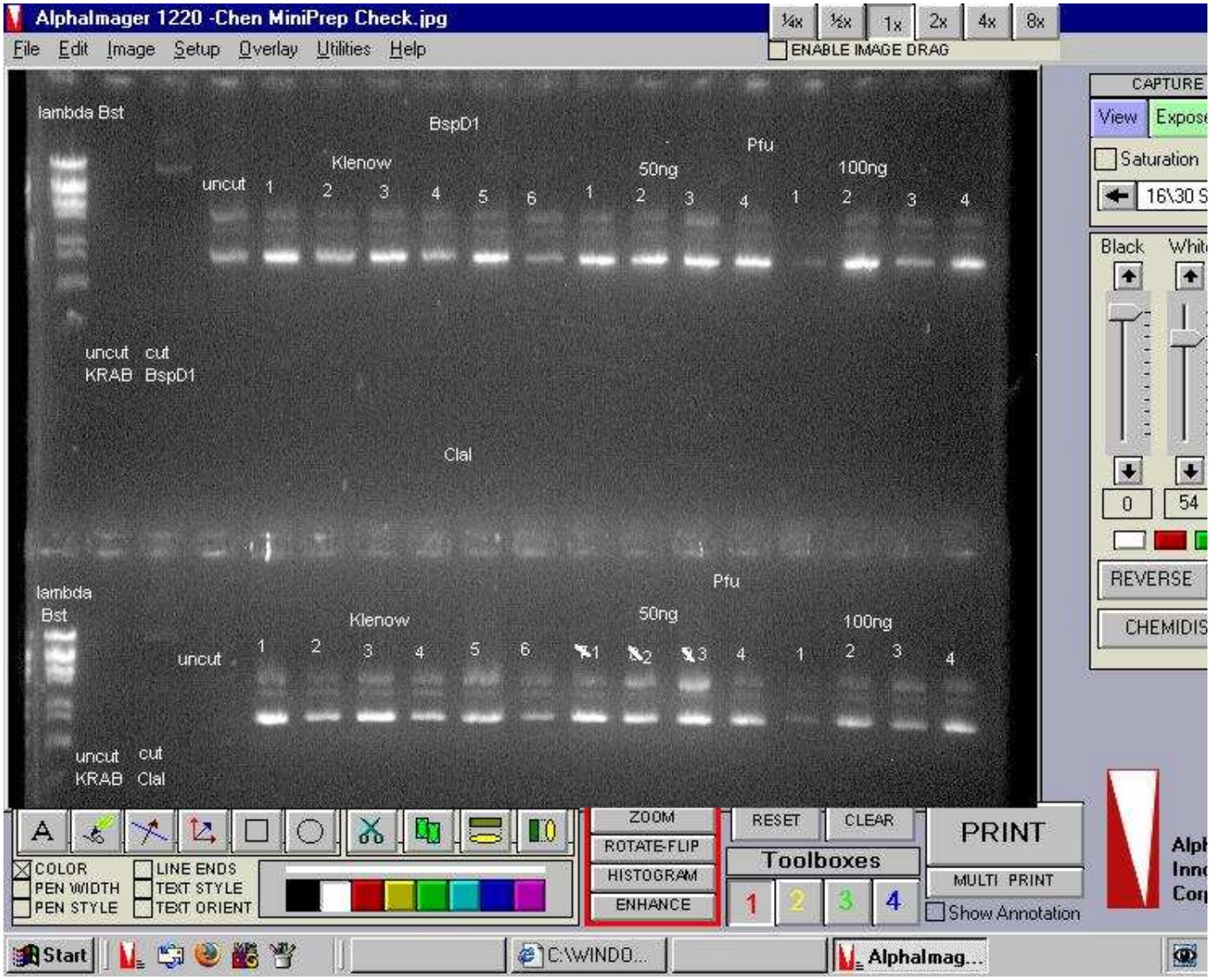
105
REVERSE
CHEMIDIS
ANNOTATE
FALSE COLOR
FILTERS
MOVIE
RESET
CLEAR
PRINT
MULTI PRINT
Toolboxes
1 2 3 4
Show Annotation
COLOR
PEN WIDTH
PEN STYLE
LINE ENDS
TEXT STYLE
TEXT ORIENT
Start
Simina
C:\WIND...
Alphal...
8-12-08 A...
Screen Saver



tet-Nkx --> set up fusion for high GC protocol (10, 27, 35 cycles...in purple tubes)....same protocol as 08/07 except used the gel isolated tet-Nkx --> needs to be run out

tet-Nkx

- 13.25ul H2O? , 13ul betaine, 5ul KOD Buffer, 5ul dNTPs , 1ul KOD Polymerase, 3ul MgCl2? , 2.5ul DMSO, 0.25ul deaza-GTP, 3ul tetO-5', 3ul Nkx-2A-3', 0.5ul tet 10 (7-24-08), 0.5ul Nkx 10 (7-27-08)

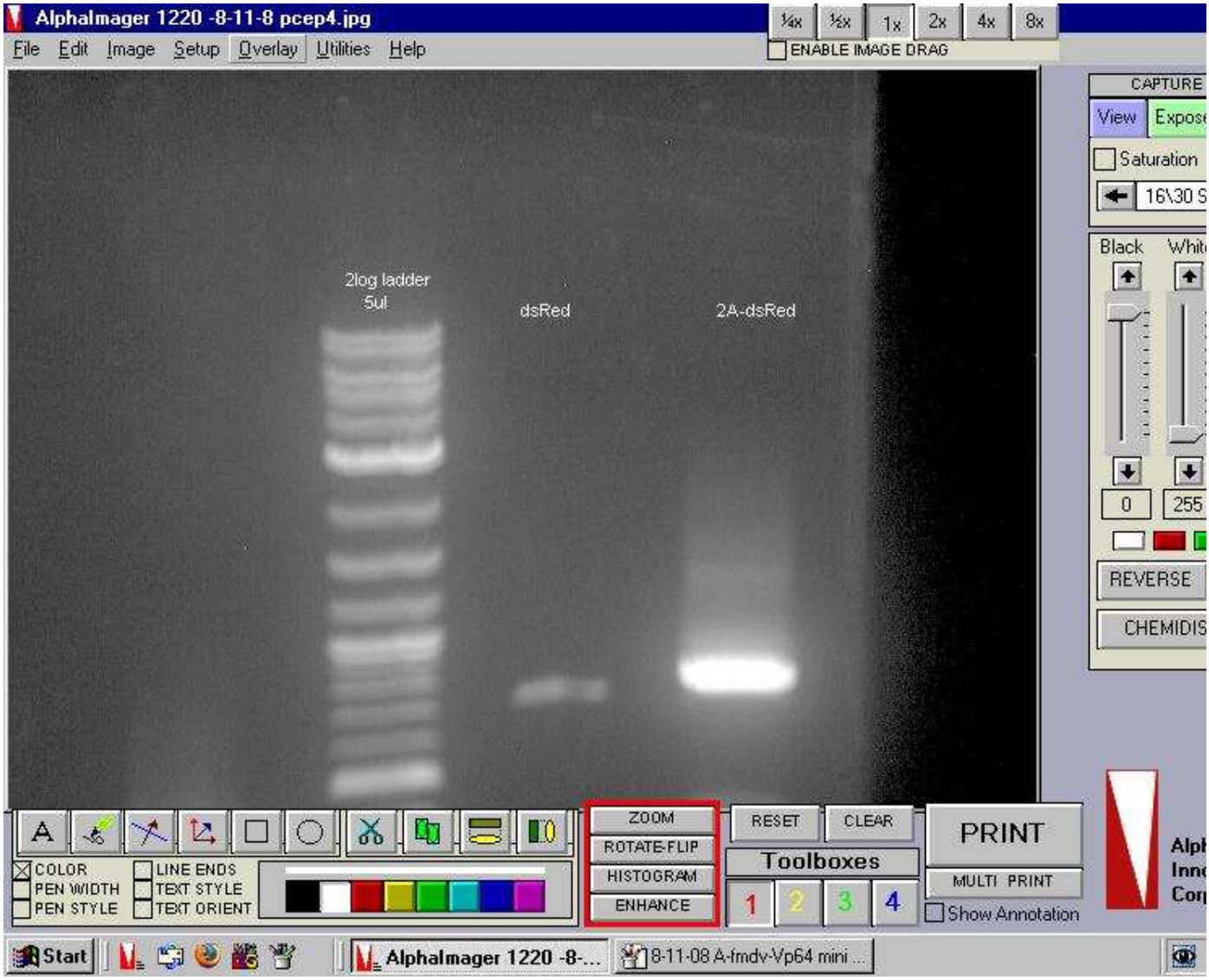
cmv: used standard protocol for KOD Polymerase (5'-cmv and 3'cmv-rtTA oligos and 1ul ptripz#1) with the temperature program (95 55 70) for (20s 10s 15s) --> needs to be run out

CMV PCR
32ulH2O
5ul KOD Buffer
5ul dNTPs
1ul KOD Polymerase
3ul MgSO4?
1.5ul 5'-cmv
1.5ul 3'-cmv-rtTA
1ul ptripz

95 for 2 mins, 95 for 20s-55 for 10 s- 70 for 15s, repeat 10/25 times, 70 for 2 min

08/11/08

ran out 2A-dsRed fusion...looks ok...



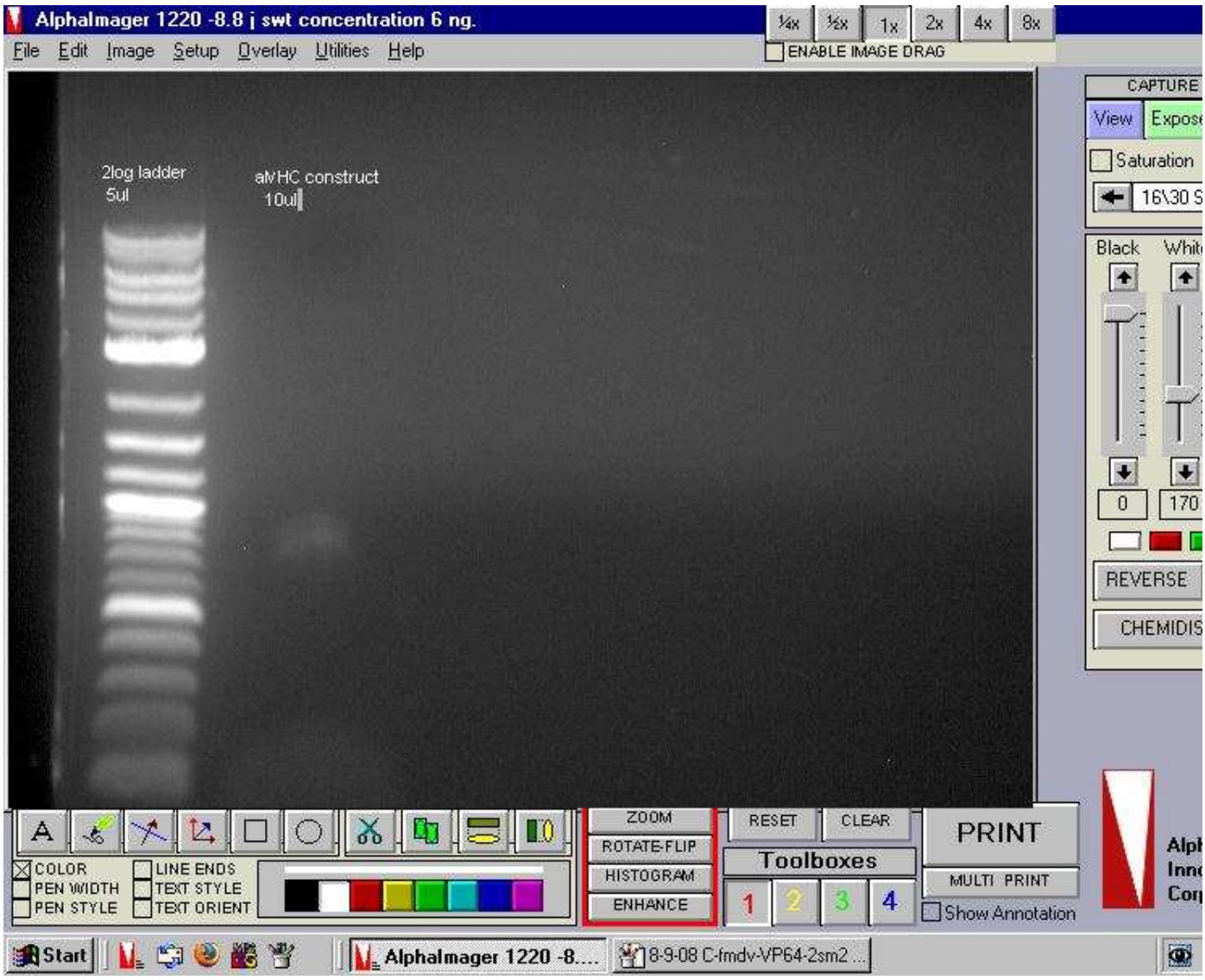
the fusion looked overloaded, but I still attempted to fuse it with Nkx, using 0.5ul of the 2A-dsRed fusion(15) and Nkx (7-27-08); 3ul each of tet-Nkx-5' and dsRed-3' temp program: (95 56 70) for (20s 10s 35s) for a total of 10/25 cycles

Nkx-2A-dsRed
13.25ul H2O?
13ul betaine
5ul KOD Buffer
5ul dNTPs
1ul KOD Polymerase
3ul MgCl2?
2.5ul DMSO
0.25ul deaza-GTP

3ul tetO-Nkx-5'
3ul dsRed-3'
0.5ul 2A-dsRed fusion
0.5ul Nkx 10 (7-27-08)

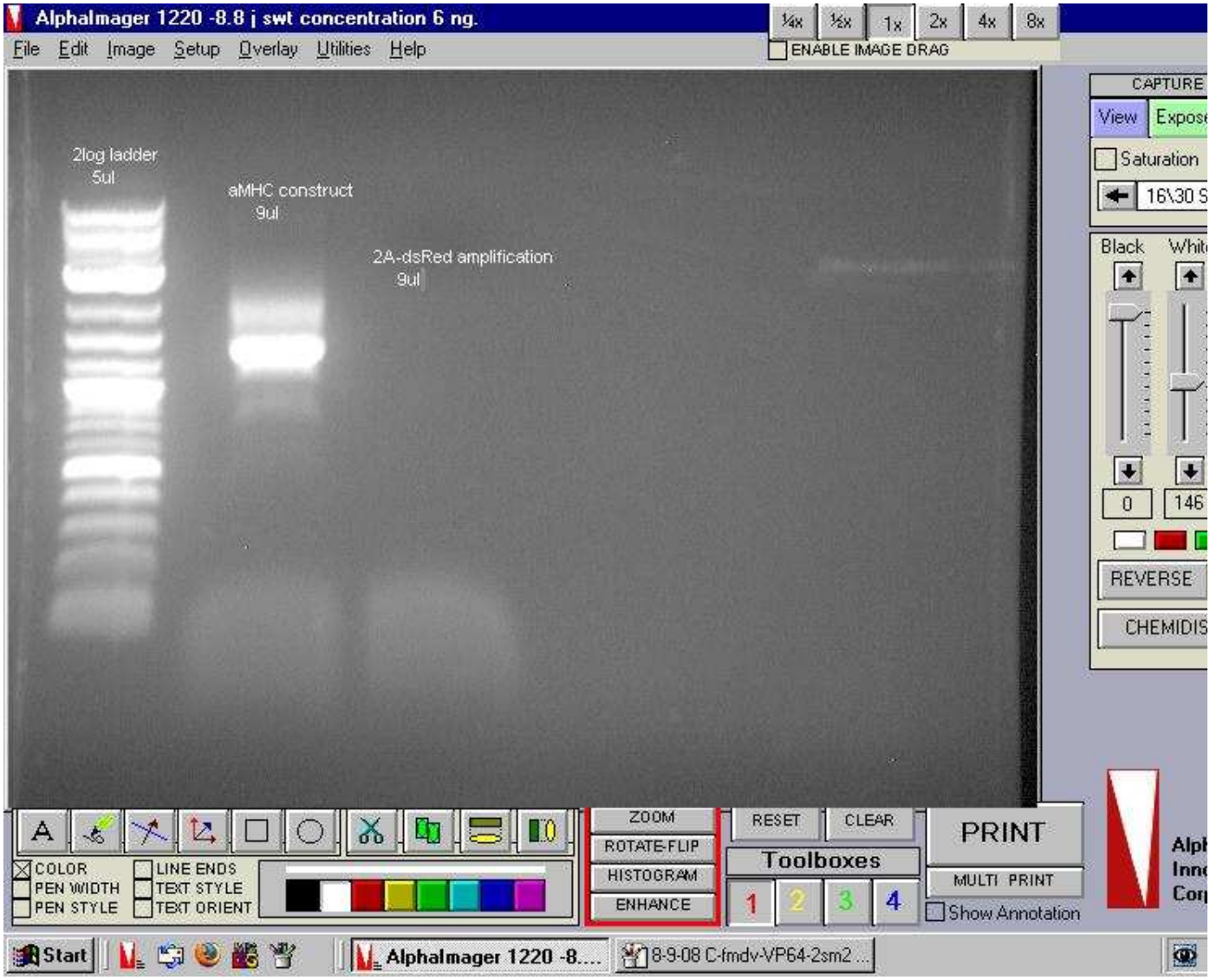
08/10/08

ran out aMHC construct PCR fusion --> got nothing...I'm pretty sure I used the wrong oligos, so I redid the whole thing --> got 2 strong bands...look at the second gel -->pretty sure it's going to need to be gel isolated



cleaned the gel piece of 2A-dsRed (new elution buffer) and amplified it -->normal protocol with 1.5ul of Nkx-2A-5' and dsRed 3' and 1ul of the cleaned DNA; program (95 56 70) for (20s 10s 15s)

ran it out-->got nothing



I'm starting to think that we should give up on the tet-Nkx fusion; we could focus on 2A-dsRed, then fuse that with Nkx and then we can ligate that to tet(?)...anyways I guess it's something to keep in mind...we need to start looking for alternative strategies to get this construct together because it doesn't seem to be working

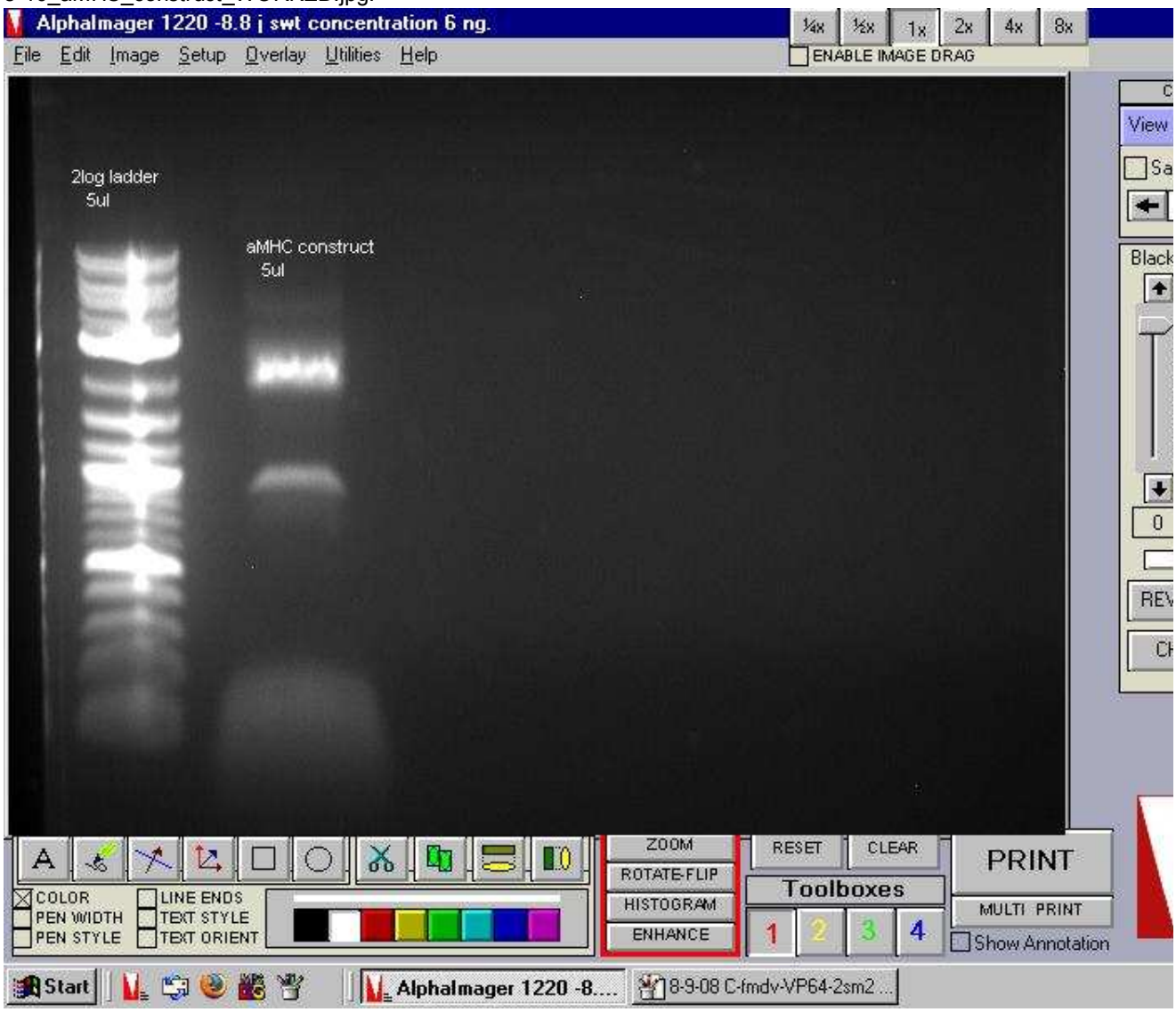
redid the 2A-dsRed fusion first doing 3 cycles without oligos, and then 10 and 25 cycles with the oligos (Nkx-2A-5' and dsRed-3') with the high GC protocol...I was looking through past fusions and it didnt seem like any used the high GC protocol...might as well try it-->left it in the RoboCycler?

2A-dsRed
13.25ul H2O?
13ul betaine
5ul KOD Buffer
5ul dNTPs
1ul KOD Polymerase
3ul MgCl2?
2.5ul DMSO
0.25ul deaza-GTP
3ul Nkx-2A-5'

3ul dsRed-3'
0.5ul 2A (8-7-08)
0.5ul dsRed (8-6-08)

not sure why this image was in my folder labeled under this date, but i'll put it up anyways

- 8-10_aMHC_construct_WORKED.jpg:

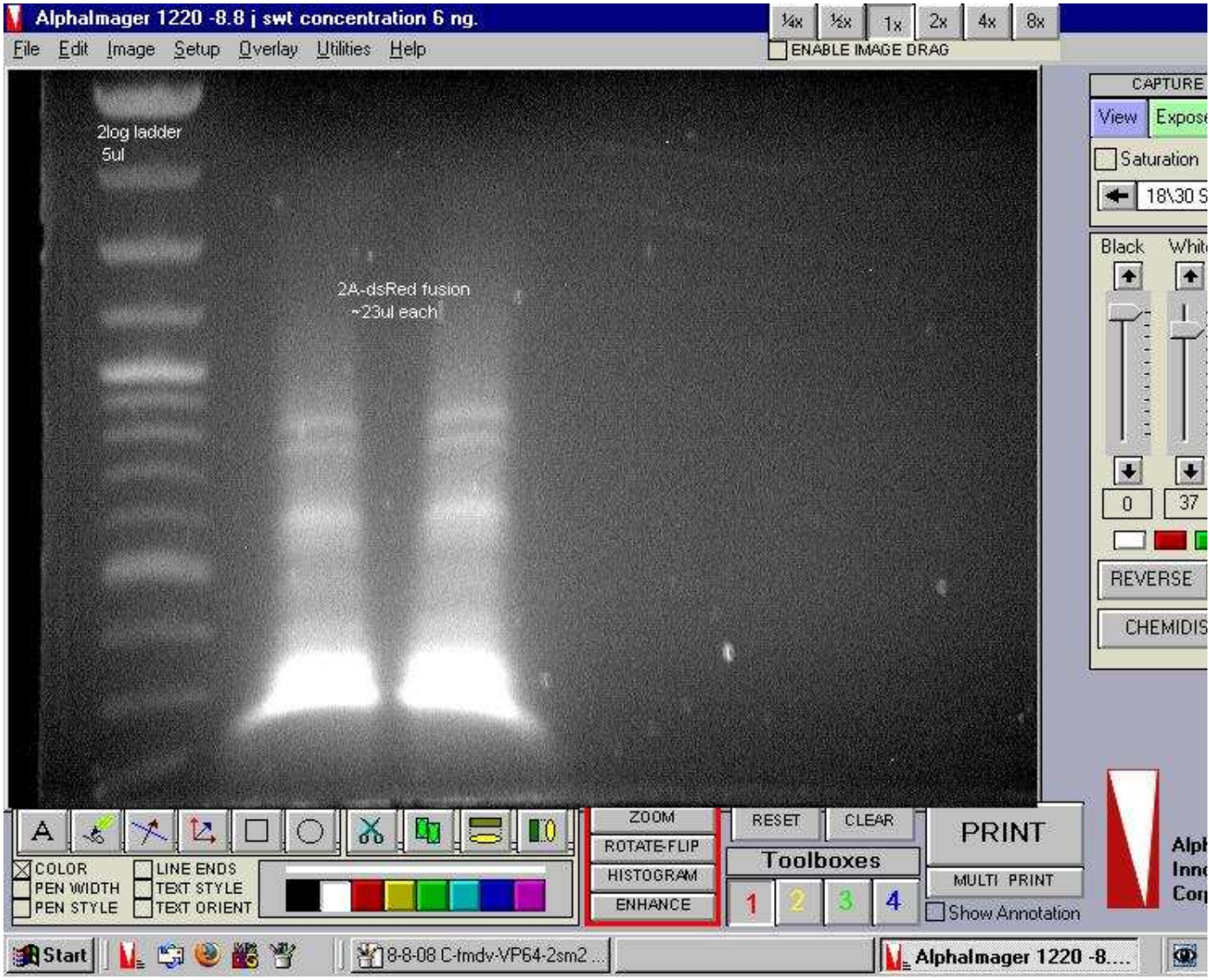


08/08/08

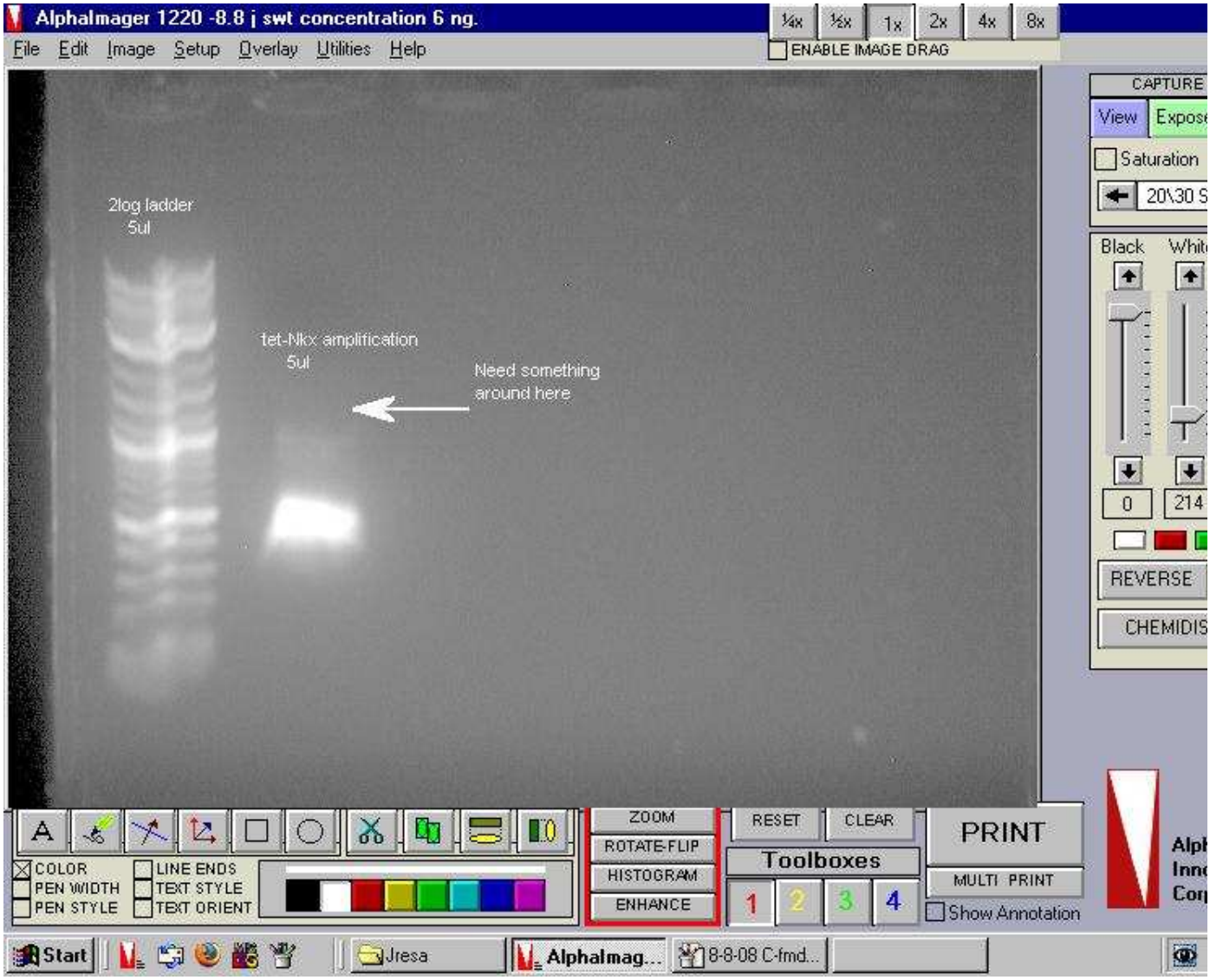
retried fusing aMHC construct

- used high GC content protocol; first did just 3 cycles at (95 56 70) for (20s 10s 40s) without the 3' and 5' oligos (just 0.5ul aMHC-NeoR and 2A-GFP)
- then used 1ul of previous PCR wuth aMHC-5' and dsRed-3' for 10 and 25 cycles at (95 55 70) for (20s 10s 40s)

ran out and isolated the 800 bp band for the 2A-dsRed fusion

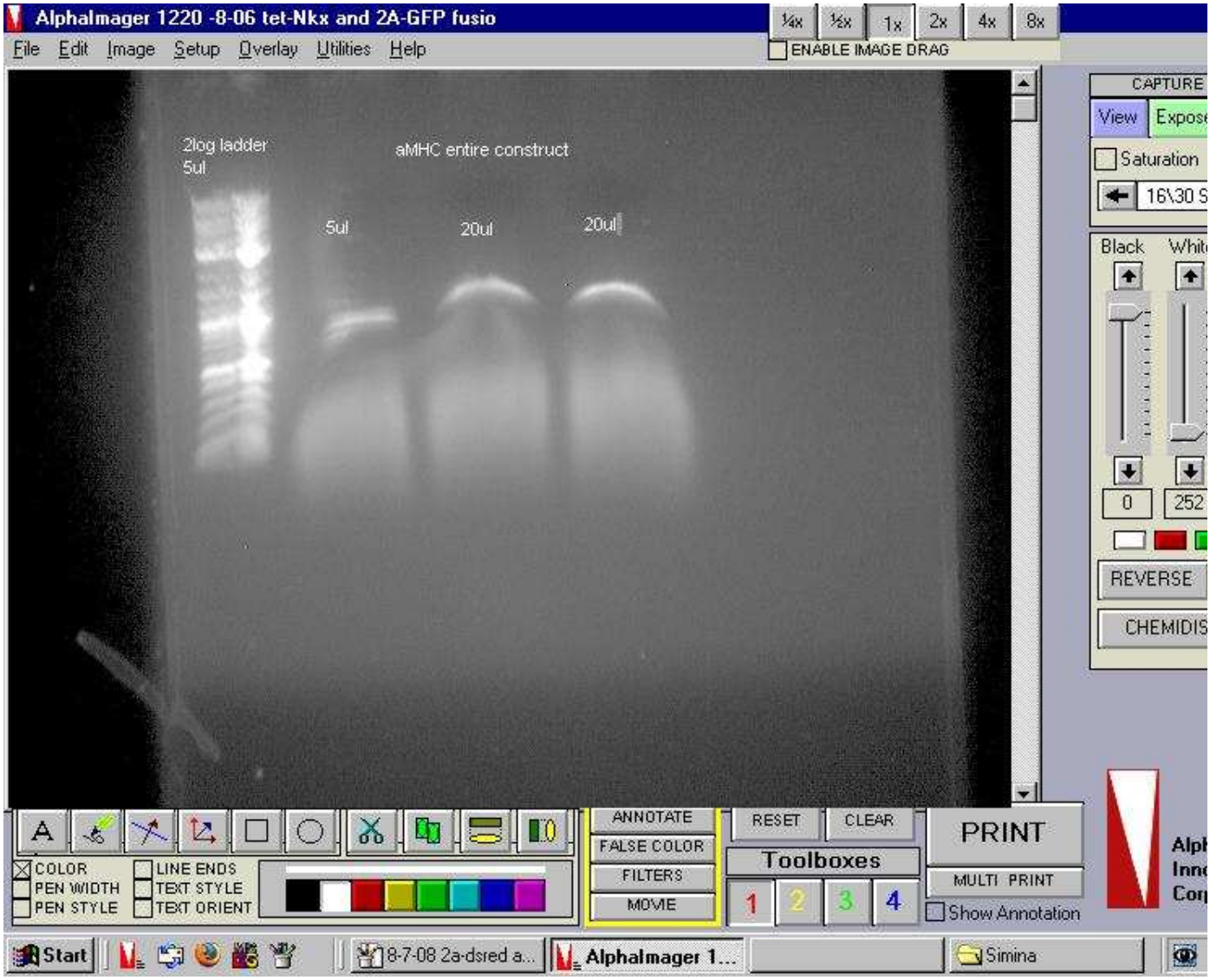


ran out the tet-Nkx amplification that Josh did of his gel isolated piece --> we're still getting a small band...there has to be something wrong with the oligos (my best guess is that they're binding somewhere else, and amplifying that piece...we should check for this, and start thinking about alternative methods of putting this construct together)

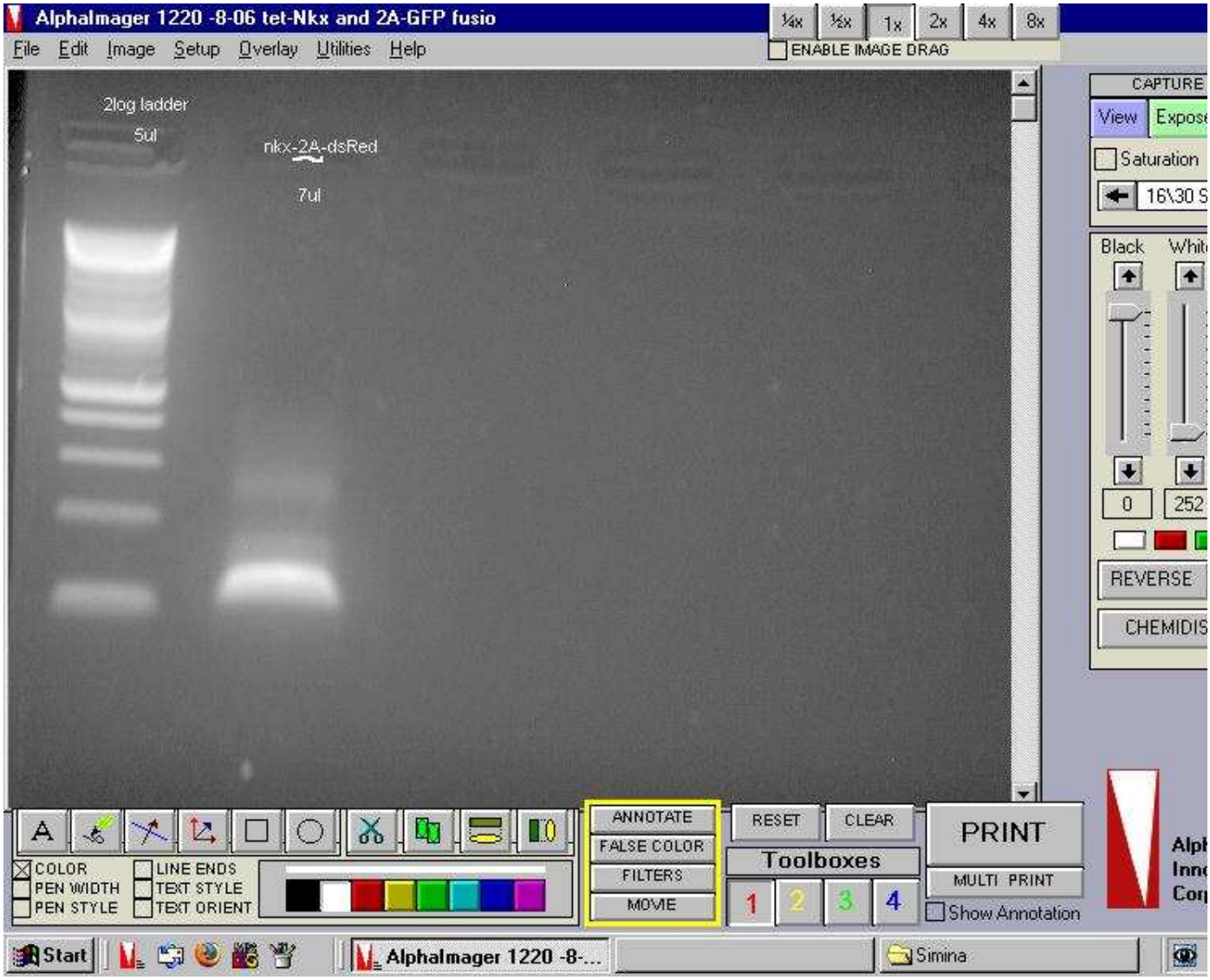


08/07/08

ran out Josh's entire aMHC construct fusion-->theres a band around 1kb but thats it



amplified 2A for the Nkx construct (ran it out; used Nkx-2A-5' and 2A-dsRed-3') on the program (95 55 70) for (20s 10s 10s) and redid the 2A-dsRed fusion using the same protocol (Nkx-2A-5' and dsRed-3') on the program (95 56 70) for (20s 10s 15s)



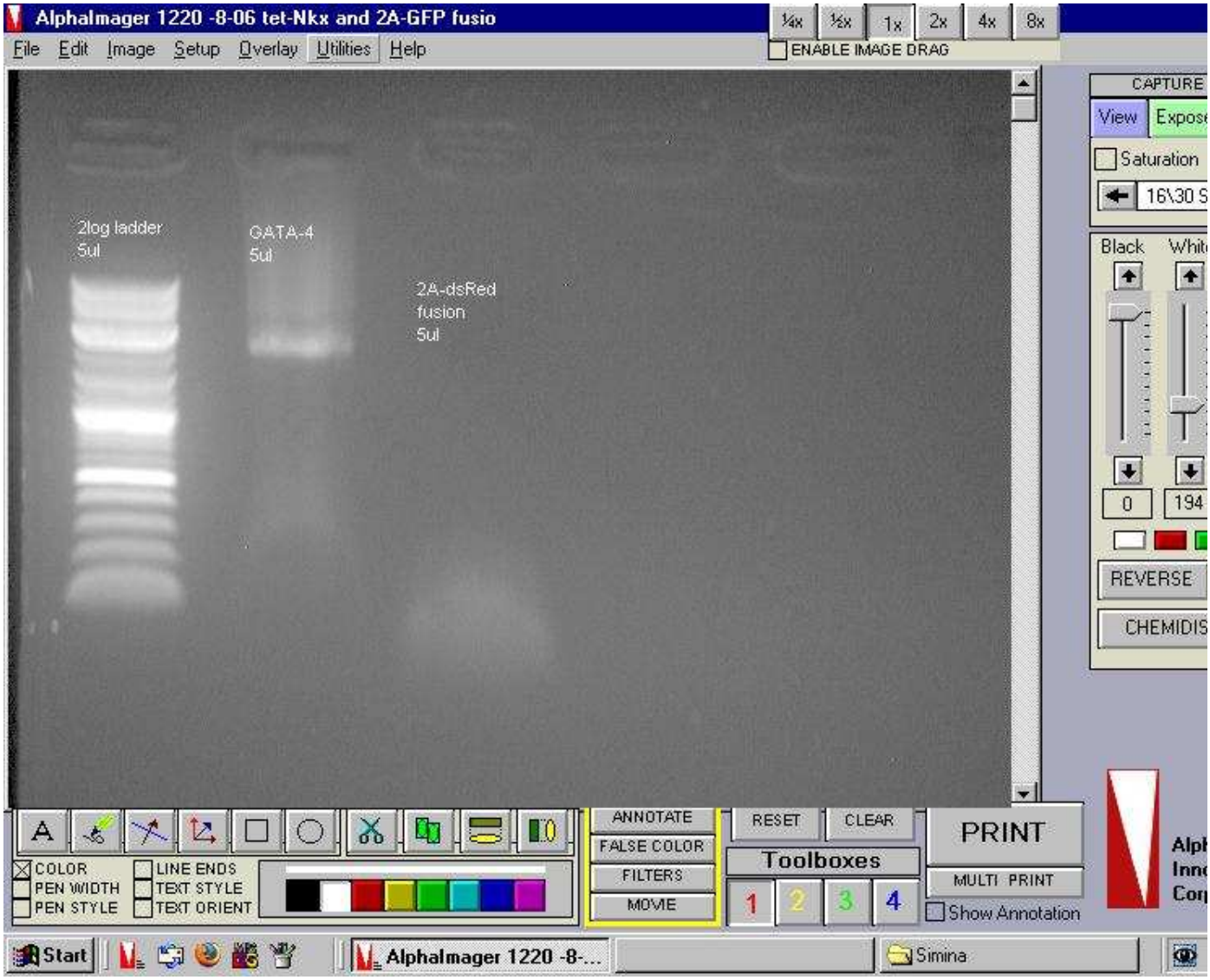
redid tet-Nkx fusion with high GC content protocol: first 3 cycles (95 55 70) for (20s 10s 20s) with no outer oligos; then for 25 cycles with tet-5' and Nkx-2A-3' (95 57 70) for (20s 10s 20s)

tet-Nkx

- 13.25ul H2O? , 13ul betaine, 5ul KOD Buffer, 5ul dNTPs , 1ul KOD Polymerase, 3ul MgCl2? , 2.5ul DMSO, 0.25ul deaza-GTP, 3ul tetO-5', 3ul Nkx-2A-3', 0.5ul tet 10 (7-24-08), 0.5ul Nkx 10 (7-27-08)

95 for 2 mins, 95 for 20s-55 for 10 s- 70 for 15s, repeat 10/25 times, 70 for 2 min

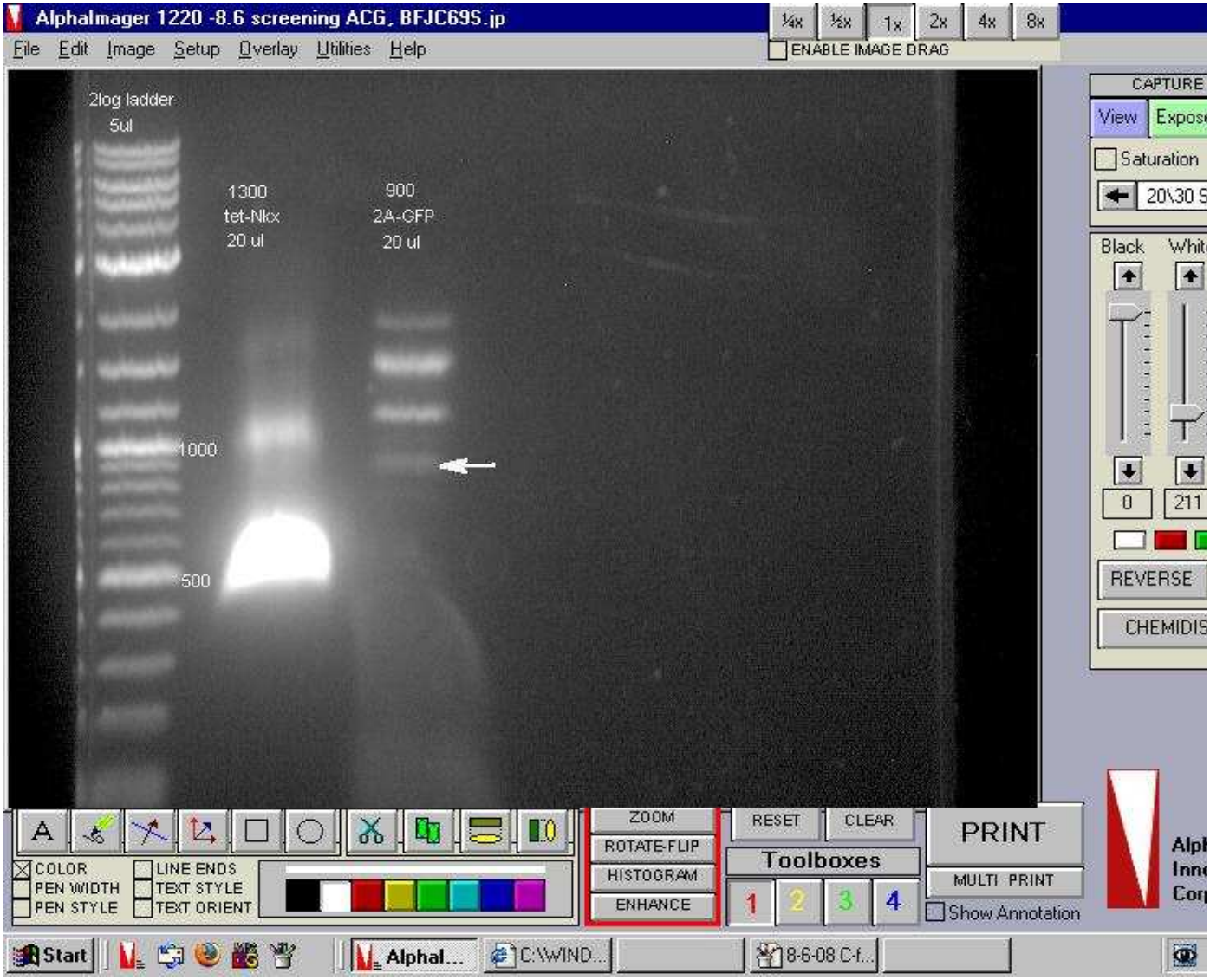
Also ran out Josh's GATA and the tet-Nkx fusion--> the GATA looks ok (is it slightly bigger than 1350?) but we got nothing for the tet-Nkx



left 2A-dsRed in COY machine

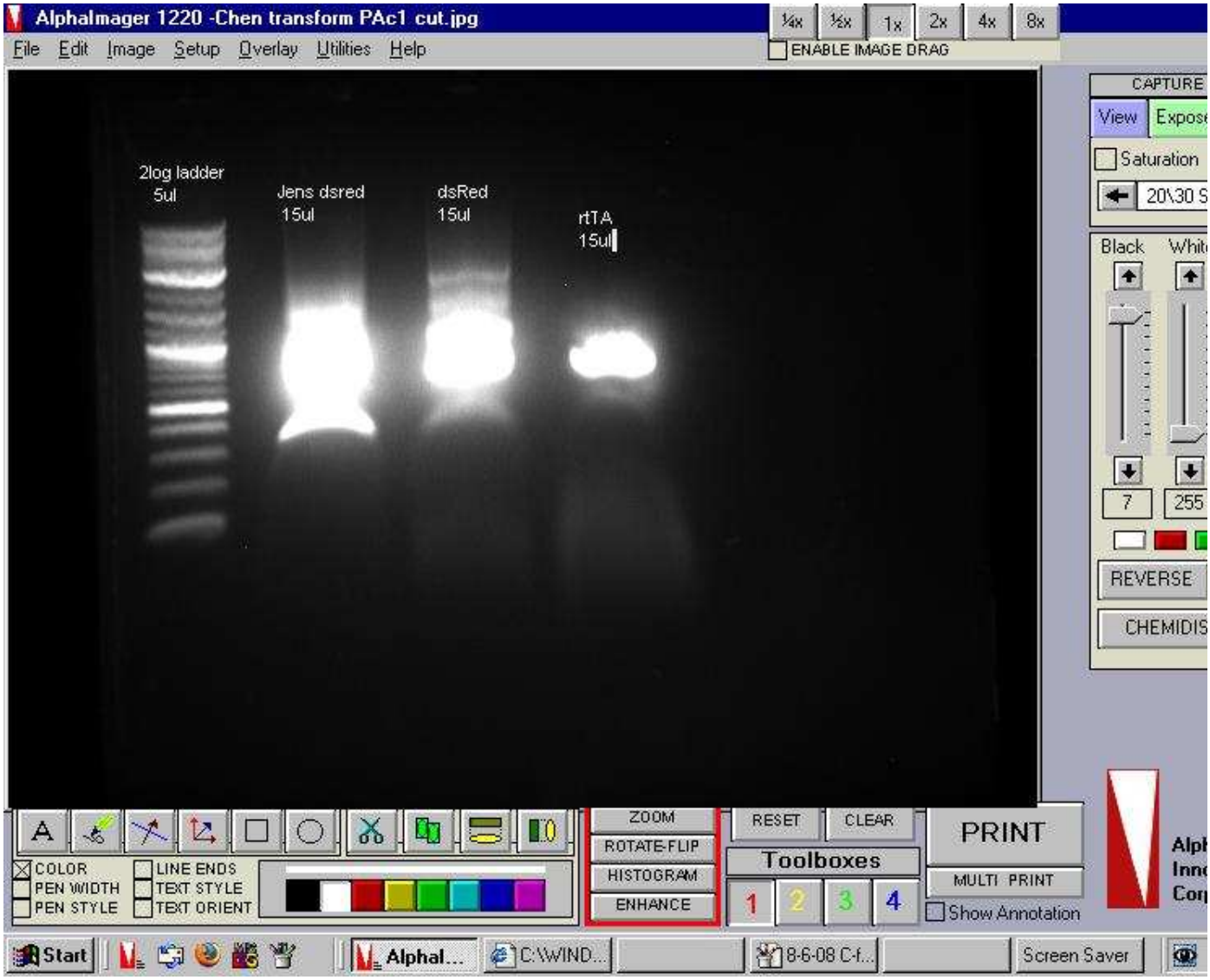
08/06/08

ran out tet-Nkx and 2A-GFP...we're definitely getting something, I'm just not sure if it's the right thing. - for 2A-GFP it looked like we were getting bands higher than what we needed so I redid what Josh did today, except the first step (fusion without oligos) only went for 3 cycles--> the 30 cycles is in the COY machine



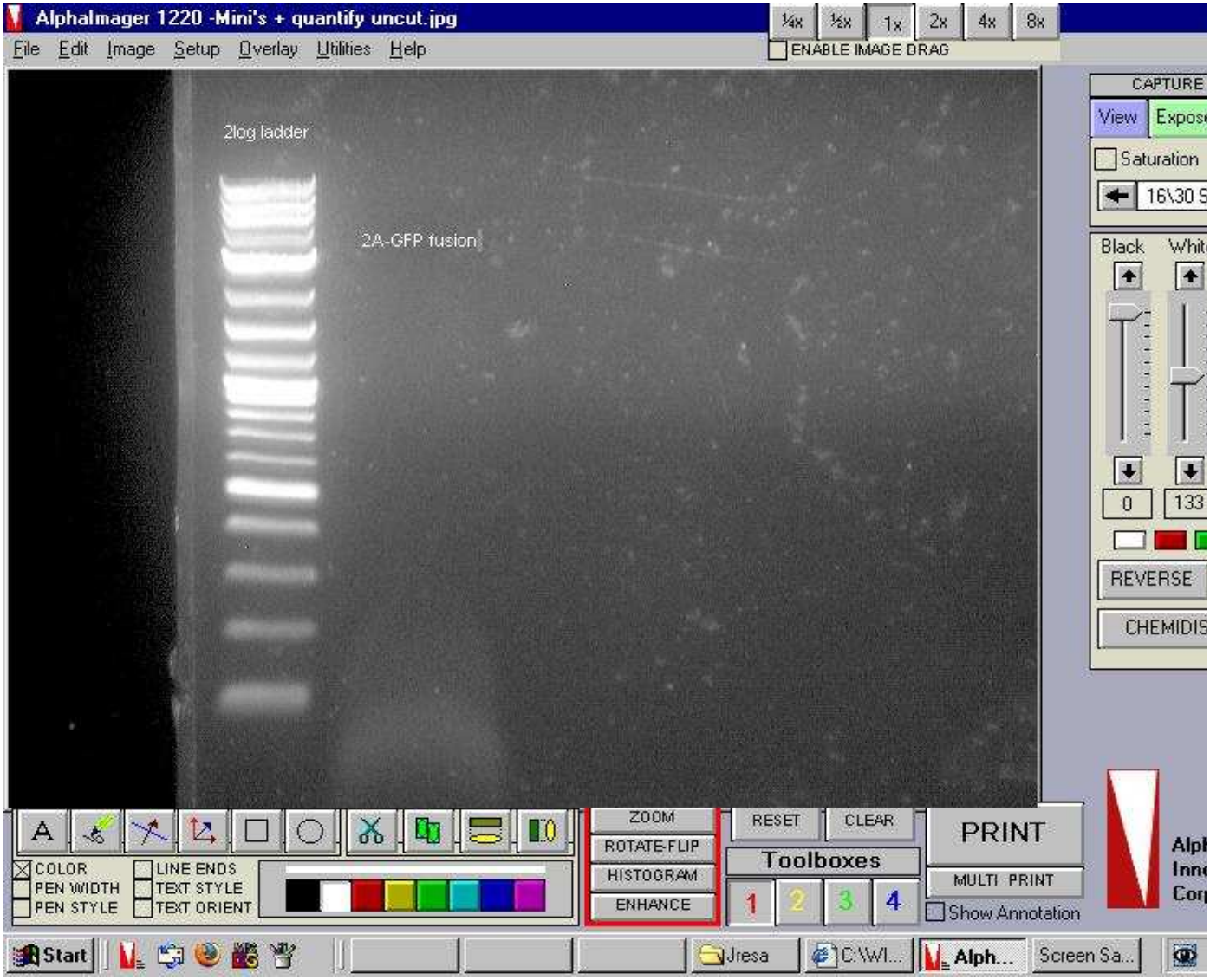
set up rtTA reaction with high GC content protocol - used 5'-cmv-rtTA and rtTA-3' oligos - (95, 56, 70) for (40s, 20s, 30s) since I used the Robocycler machine - two tubes: for 10 and 25 cycles

ran out the 2 dsRed's Josh had set up and the rtTA --> I overloaded them (since I wanted to make sure any small band would show...guess I'm getting a little pessimistic) but it actually looks like it worked!!! the rtTA might have a smaller band but other than that, I'm pretty sure they both look good --> they need to be rerun...maybe 5ul? to confirm it 100%



08/05/08

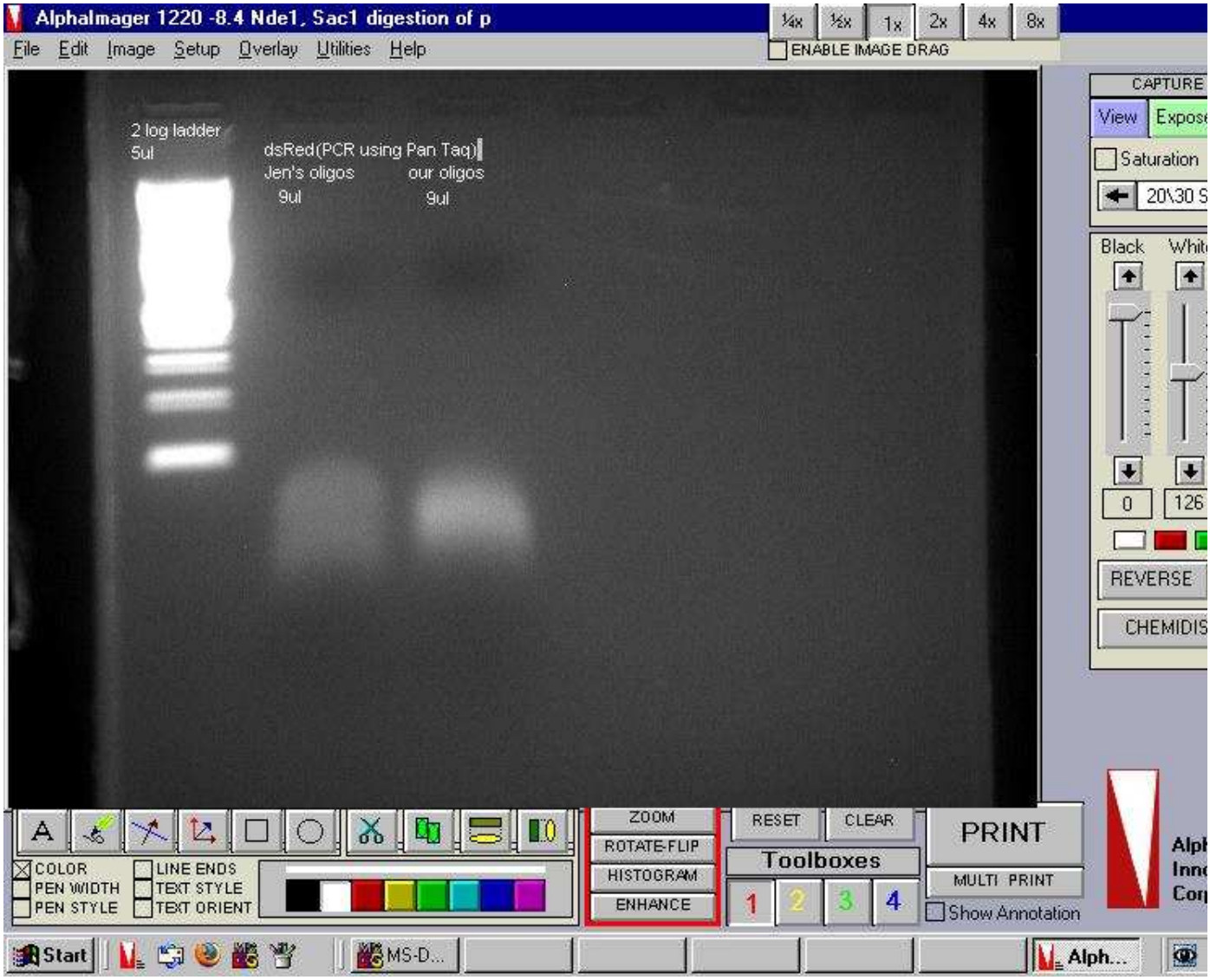
ran out 2A-GFP --> didn't work



set up aMHC-NeoR fusion in the COY machine --> used high GC content protocol (same as 08/01 protocol except now I used the gel isolated aMHC)

08/04/08

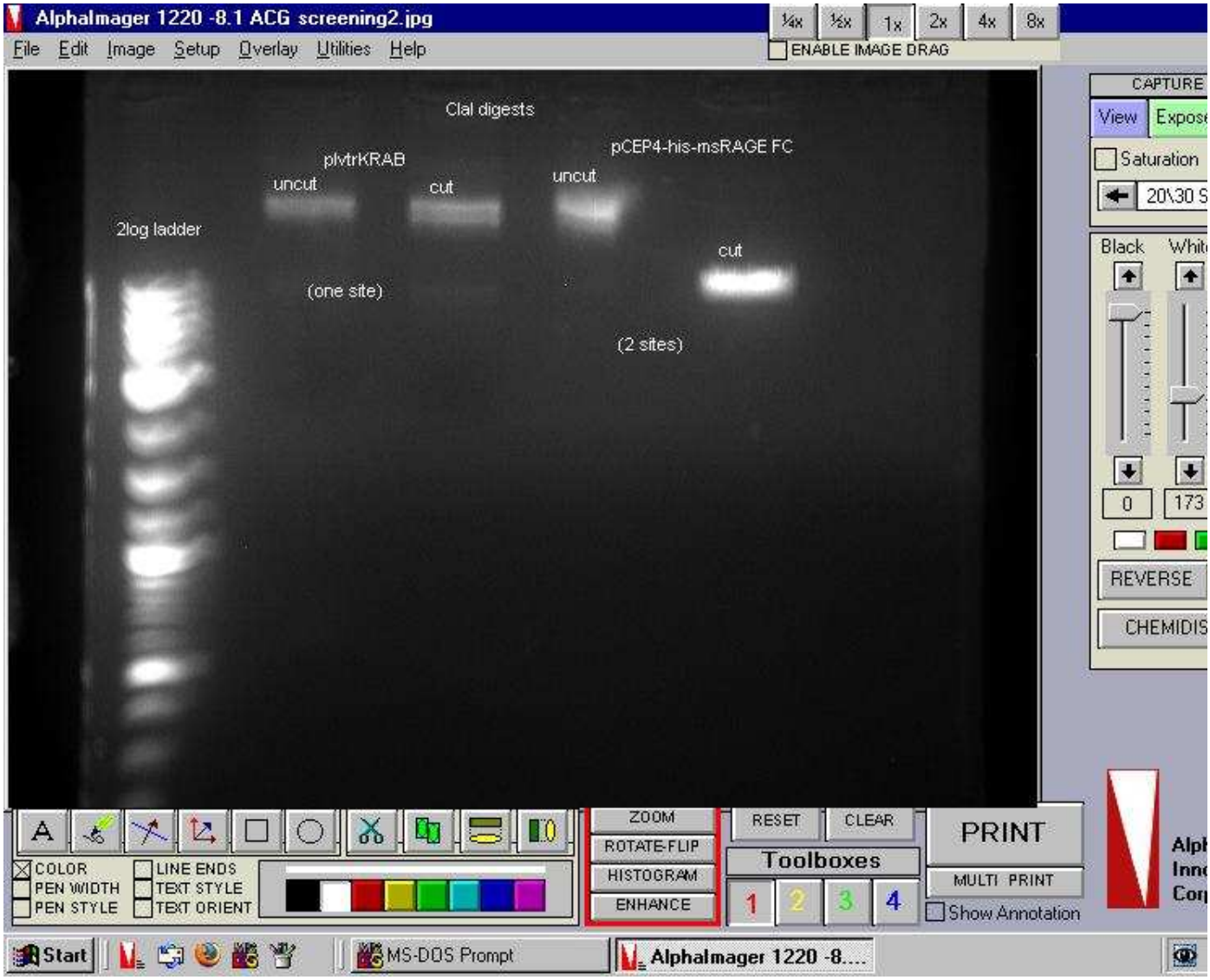
ran out the dsRed PCR using Pan Taq on a 2% gel --> got nothing



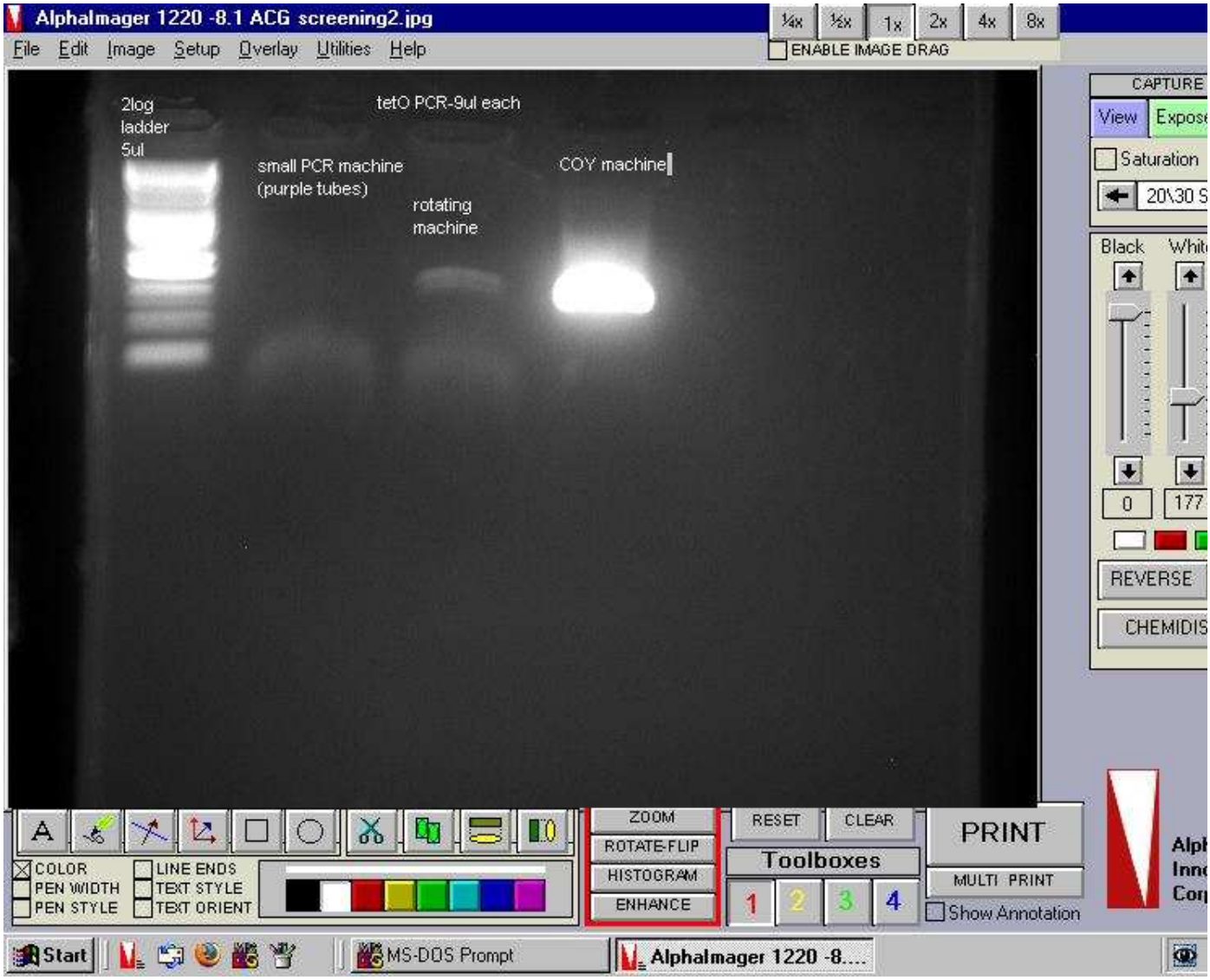
PCR reaction for aMHC (same protocol as Joshua's 7-25, since I couldn't find the tube) --> needs to be run out and gel isolated; then aMHC-NeoR fusion

08/03/08

Retried the ClaI? digests to make sure it wasn't the buffer --> new NEB4 everything still looked the same:

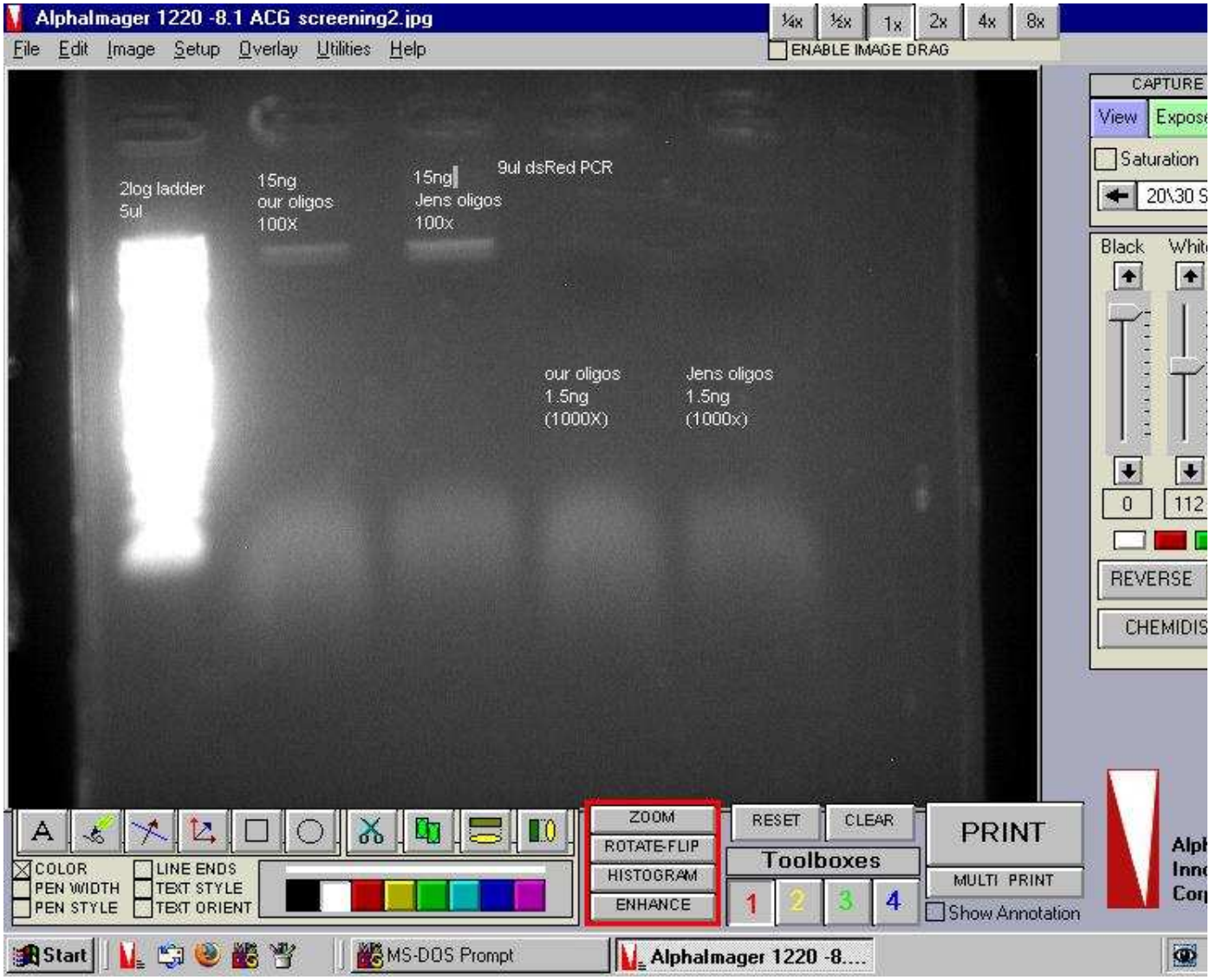


ran out the tetO PCR's from all 3 machines:



seems as though the COY machine actually works best...maybe we should also consider the small size of what we PCRed...I dunno, maybe if you guys have time you can retry this with longer pieces of DNA...for now I'm sticking to the COY which makes things take way longer, but at least it works

ran out yesterdays dsRed PCRs



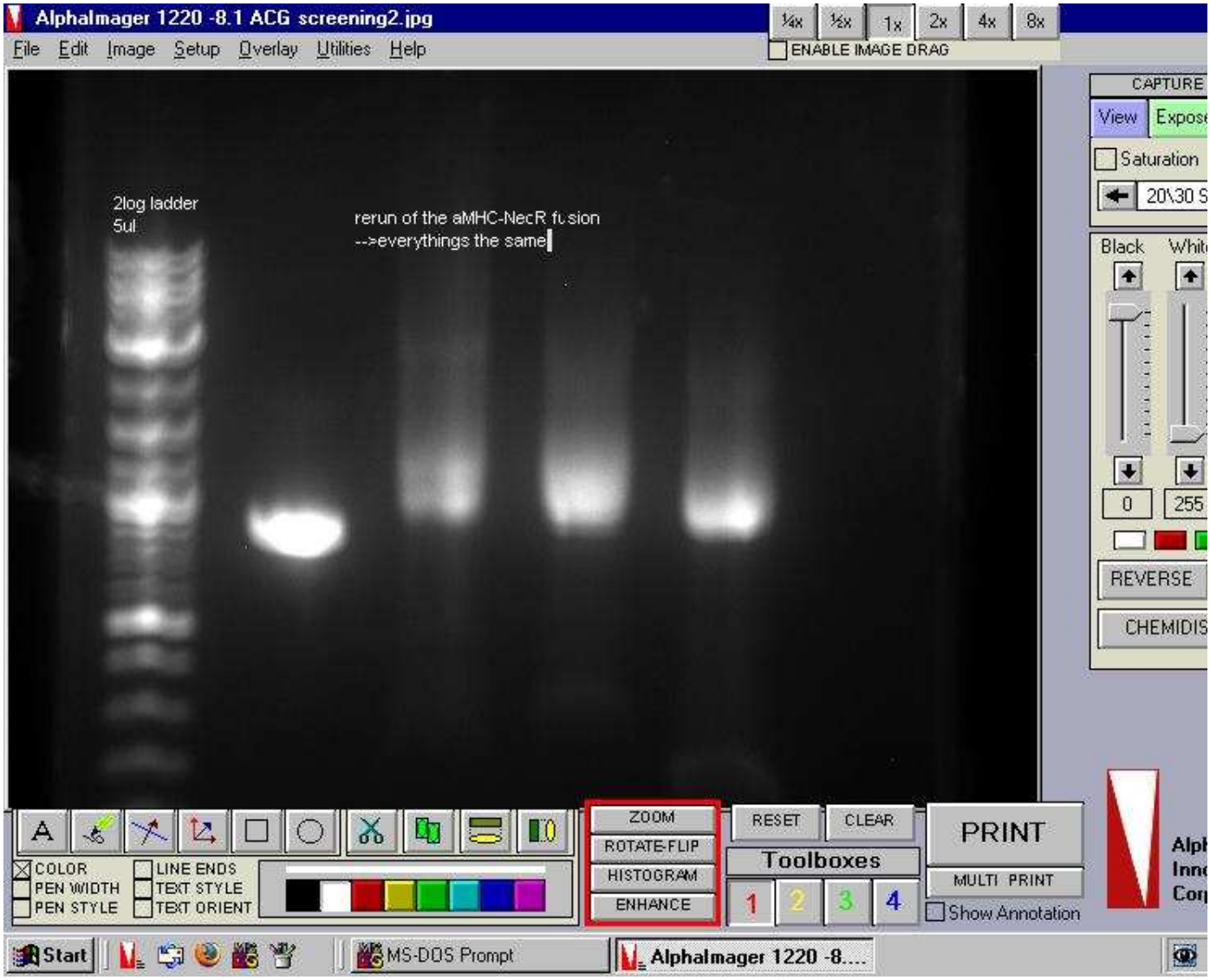
tried amplifying dsRed using Pan Taq Polymerase (Andy suggested it --> maybe it's our polymerase or maybe a different one is just more effective)

dsRED using Taq
5ul Standard Taq buffer
5ul dNTPs
2ul dsRed-3'
2ul 2A-dsRed-5'
1ul plvtrKRAB-red (10ng)
0.2ul Pan Taq Pol
34.8ul H2O?

still 27 cycles at 56C anneal --> left in COY machine

08/02/08

ran out all 3 aMHC-NeoR fusions...it almost looked like it worked, but since we got so much banding below 400 bp, I think the bands we got for the fusion are too small...meaning NeoR? is fused to one of the smaller pieces



Another option would be to amplify a larger region from the entire genome first using the following primers (I was talking to Andy about this and we looked up the rat genome and found sites outside of our region of interest)

5' outer oligo for aMHC from entire rat genomic DNA: AAC TCC TTC TTA CCT GGG ATG GAG

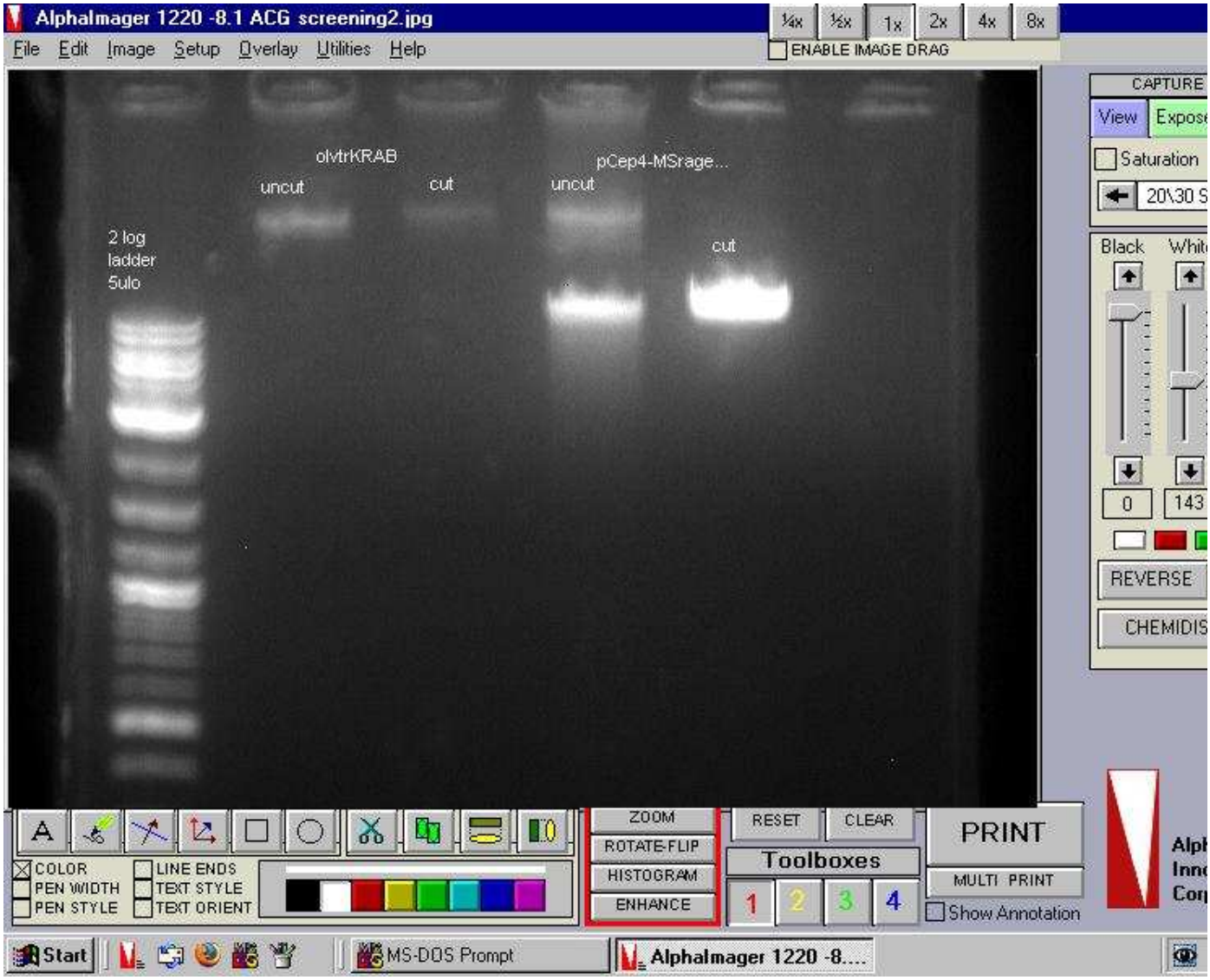
3' outer oligo for aMHC from entire rat genomic DNA: AGC TTG TGT GTT GGA GAC AGG GG

We might also want to try to first gel isolate the correct size piece and attempt the aMHC-NeoR fusion that way before using 2 sets of primers.

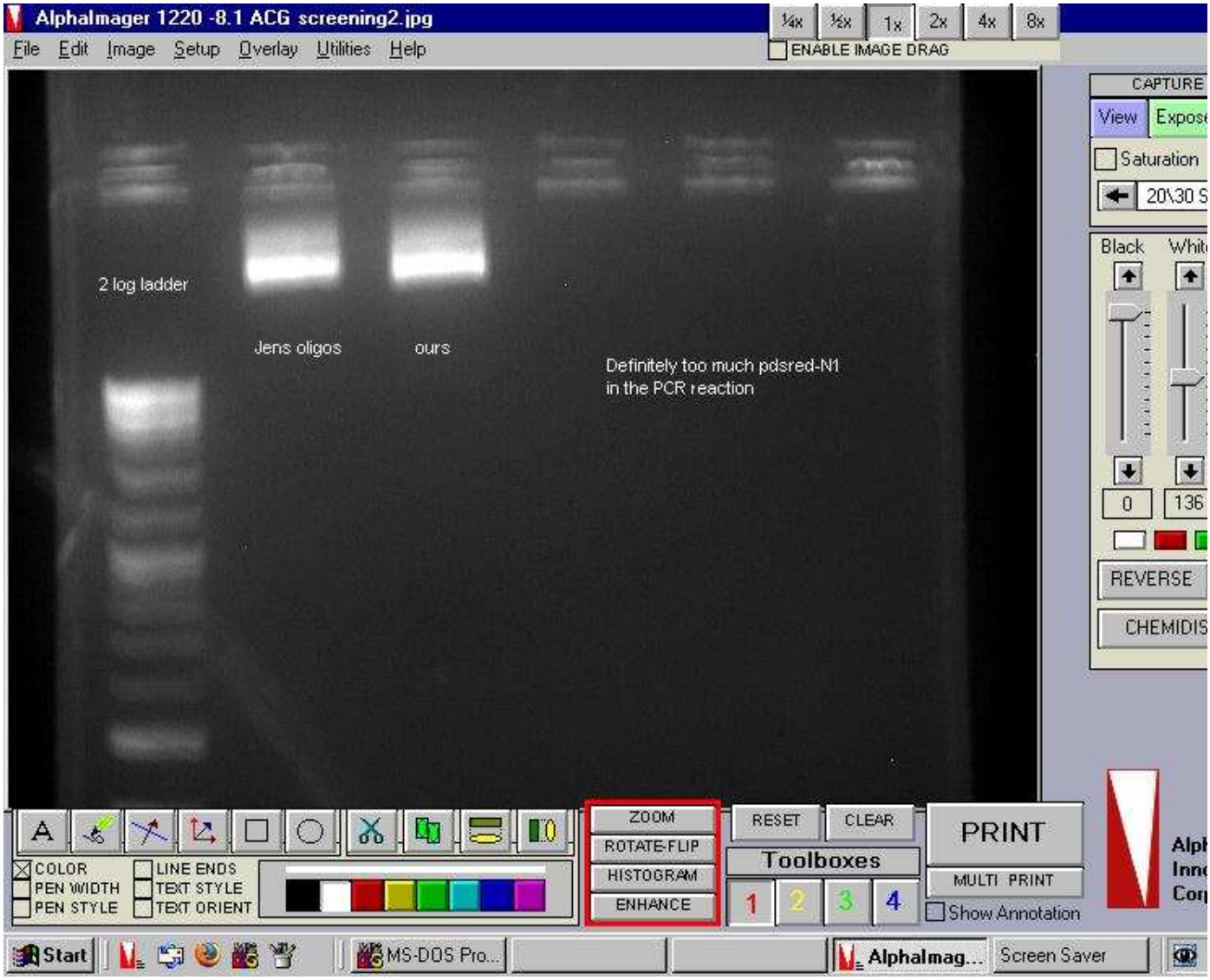
redid ClaI? digests (to check the enzyme-->make sure it's good)

uncut plvtrRAB	plvtrKRAB	uncut pCEP4-his-msRAGE FC	pCEP4-his-msRAGE FC
	1ul ClaI?		1ul ClaI?
2ul NEB 4	2ul NEB 4	2ul NEB 4	2ul NEB 4
2ul BSA	2ul BSA	2ul BSA	2ul BSA
1ul DNA	1ul DNA	1ul DNA	1ul DNA
15ul H2O?	14ul H2O?	15ul H2O?	14ul H2O?

ran them out on a 1% gel:



redid the dsRed PCR since I loaded too much plasmid:



dsRed
5ul dNTPs
5ul KOD buffer
3ul MgSO4?
1ul KOD Polymerase
1.5ul 2A-dsRed-5'
1.5ul dsRed-3'
1ul pdsred-N1 (1.5ng and 15ng)
32ul H2O?

Also, I've been talking to Andy about the PCR machines, and whether the fact that the COY takes longer or that the rotating one takes shorter affects the reaction in any way, so I reran the tetO PCR that we already know worked (from 7-24) on all 3 machines for 25 cycles

08/01/08

set up PCR of dsRed from pdsred-N1 with both ours and Jen's oligos

dsRed
5ul dNTPs
5ul KOD buffer
3ul MgSO4?
1ul KOD Polymerase
1.5ul 2A-dsRed-5'
1.5ul dsRed-3'
1ul pdsred-N1
32ul H2O?

used regular KOD protocol, 27 cycles, 56C anneal

tried aMHC-NeoR fusion

- couldn't find 7-25 aMHC, which actually looks the best

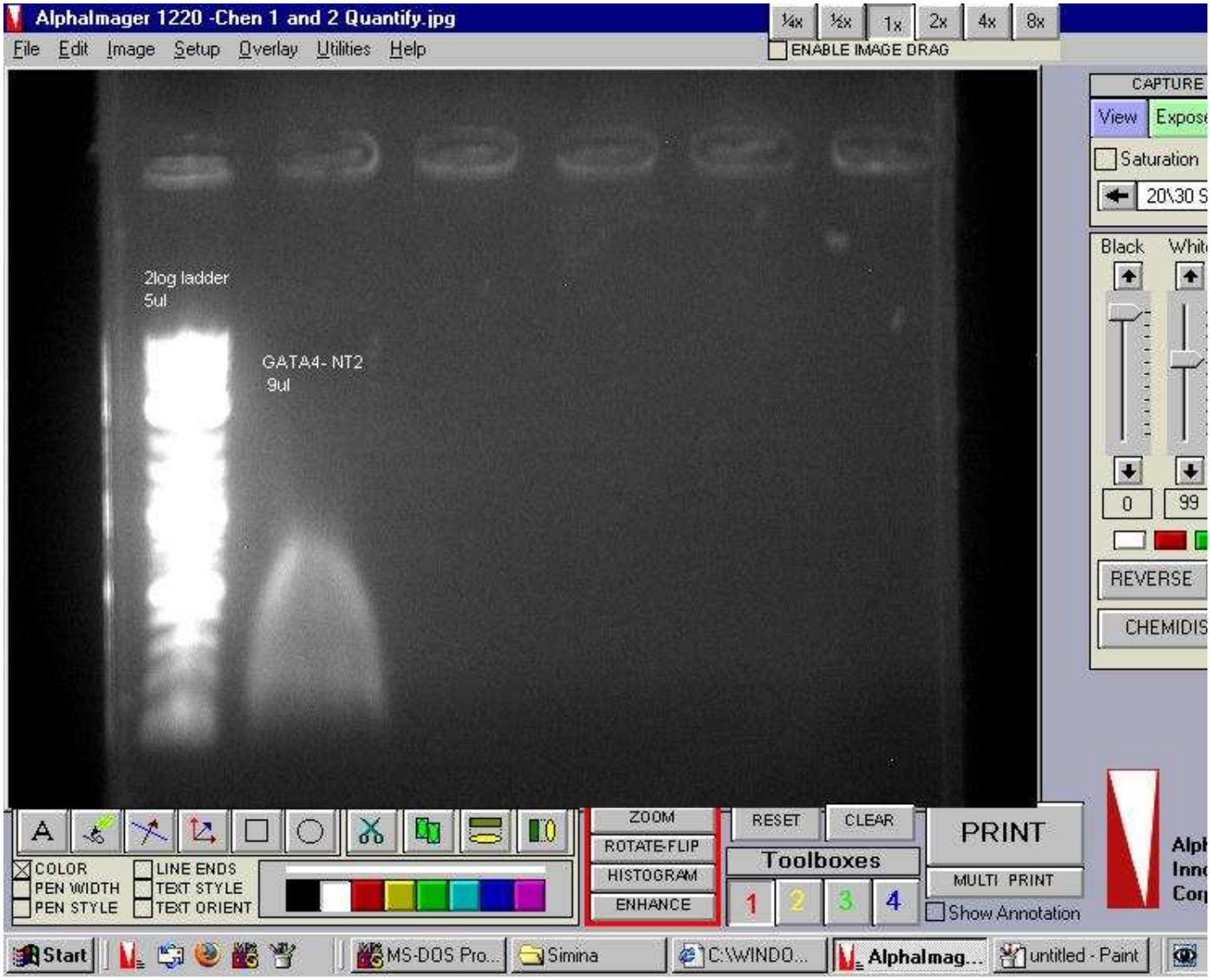
aMHC-NeoR
5ul KOD buffer
5ul dNTPs
1ul KOD Polymerase
3ul MgCl2?
13ul betaine
2.5ul DMSO
0.25ul deaza-GTP
3ul aMHC-5'
3ul NeoR? -FMDV-3'
0.5ul NeoR? (7-23)
0.5ul aMHC (1-3)

aMHC#1: aMHC 7-20-08 10X aMHC#2: aMHC 7-20-08 100X aMHC#3: aMHC 7-27-08 100X

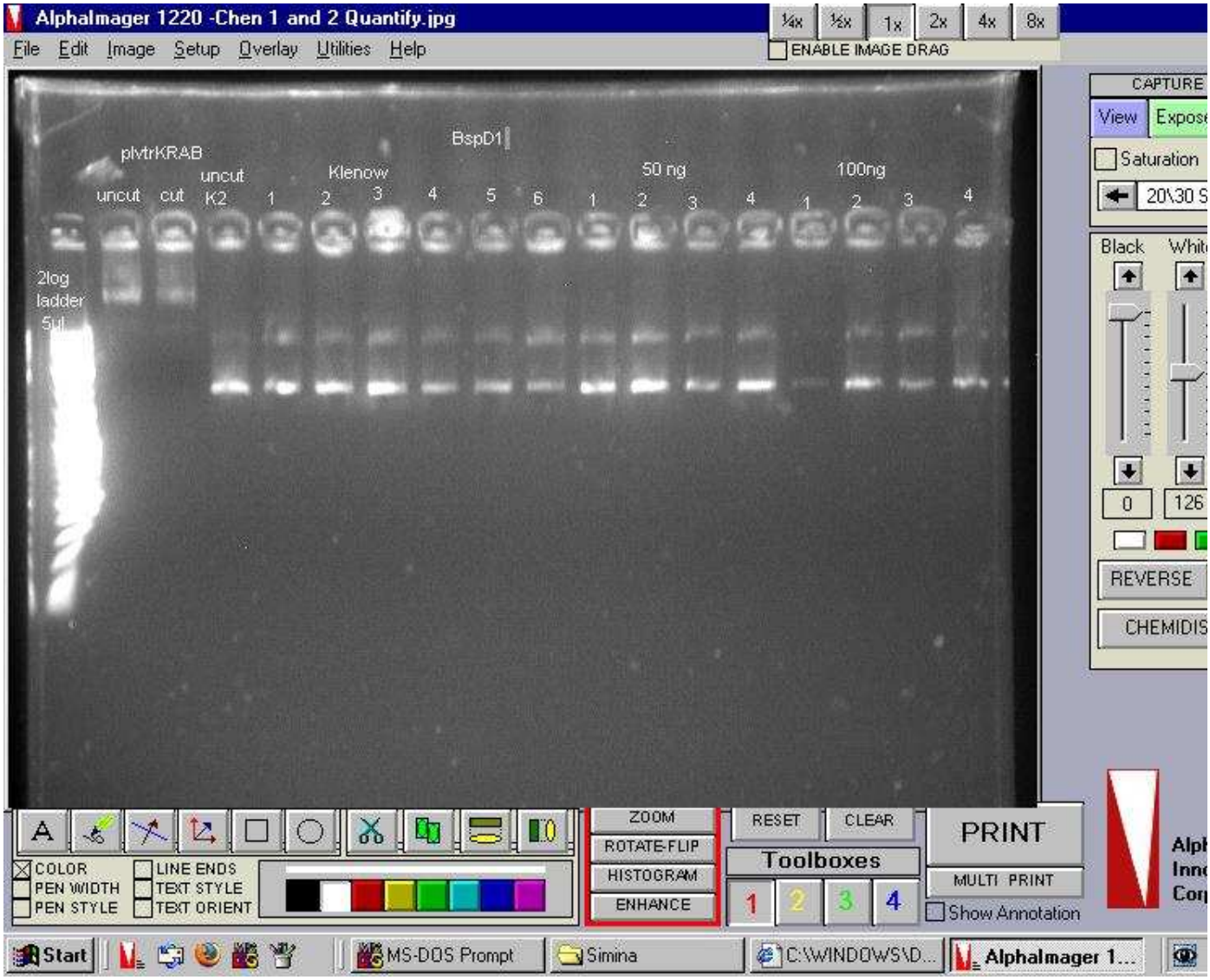
Temp program: 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 20 secs -->repeat 24X; 70C for 2 min

07/31/08

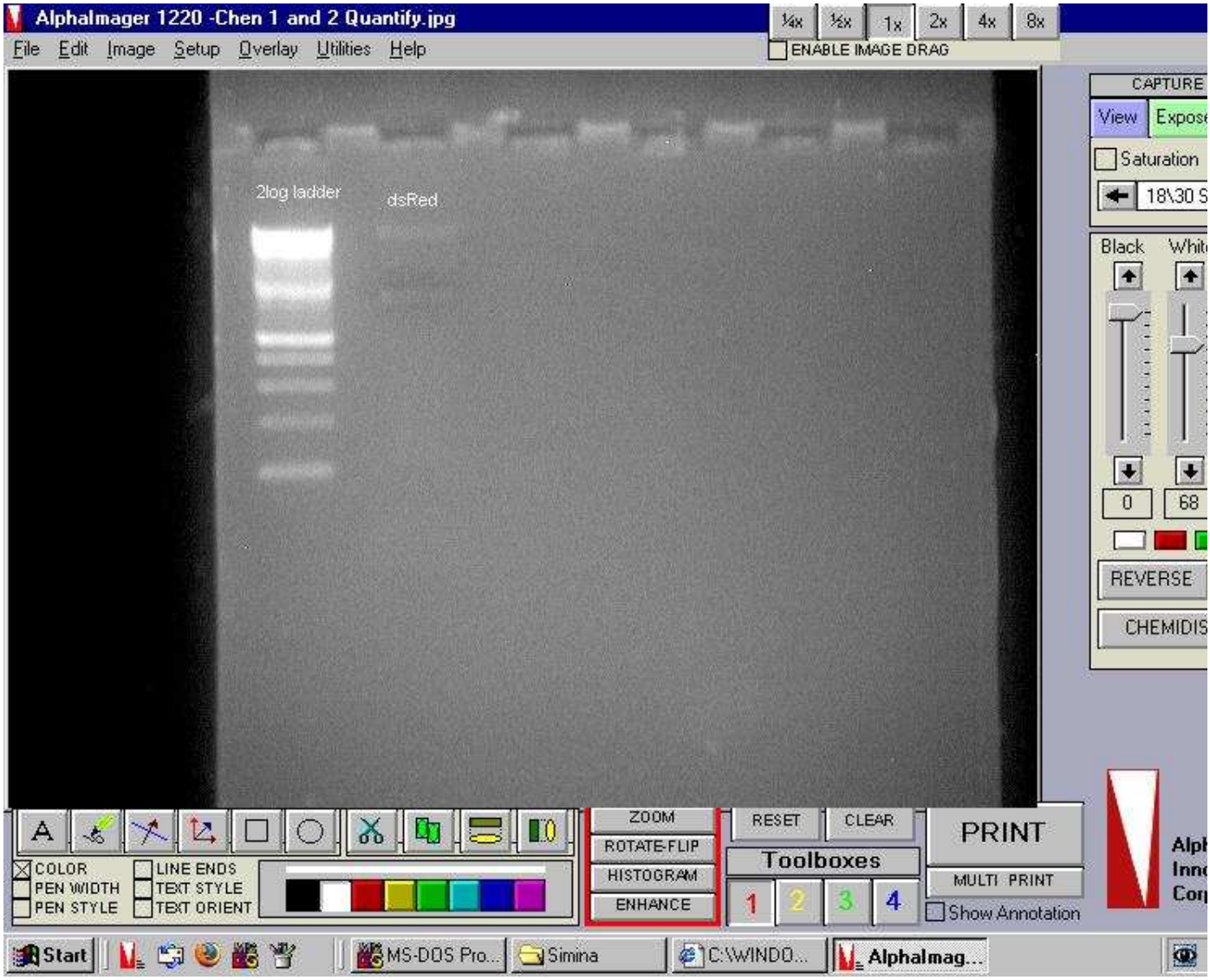
ran gel for GATA4 amplification that Josh did --> very faint spread out blob ...maybe we should try using more of the initial GATA4 (?)...also I'm not sure what Josh's temperature program is but that could be something else that might need to be changed (I know it's up but for some reason I'm confused)



ran gel for the BspD1? digests --> the control is still not cut!!! maybe try it again with ClaI? ???

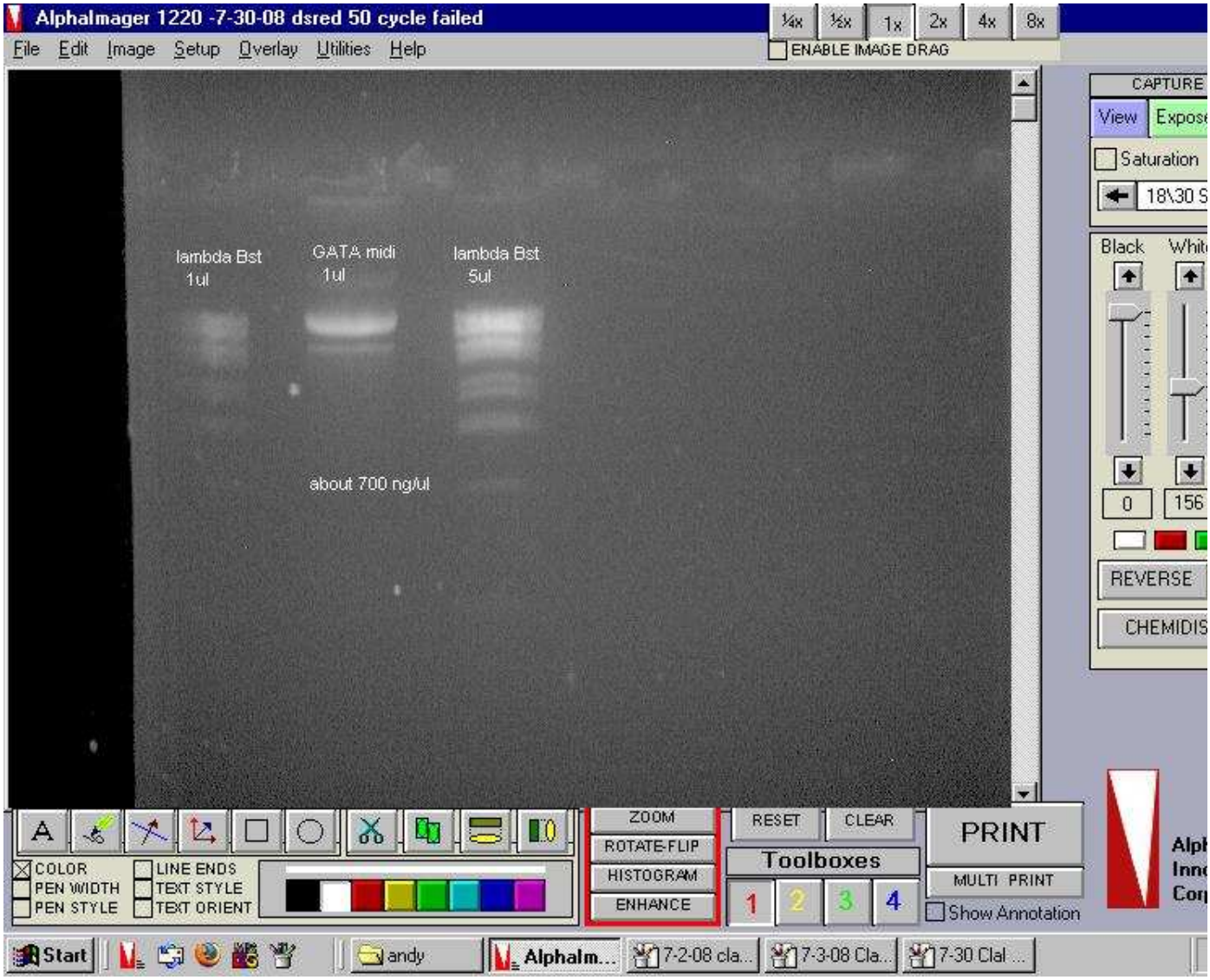


ran gel for dsRed

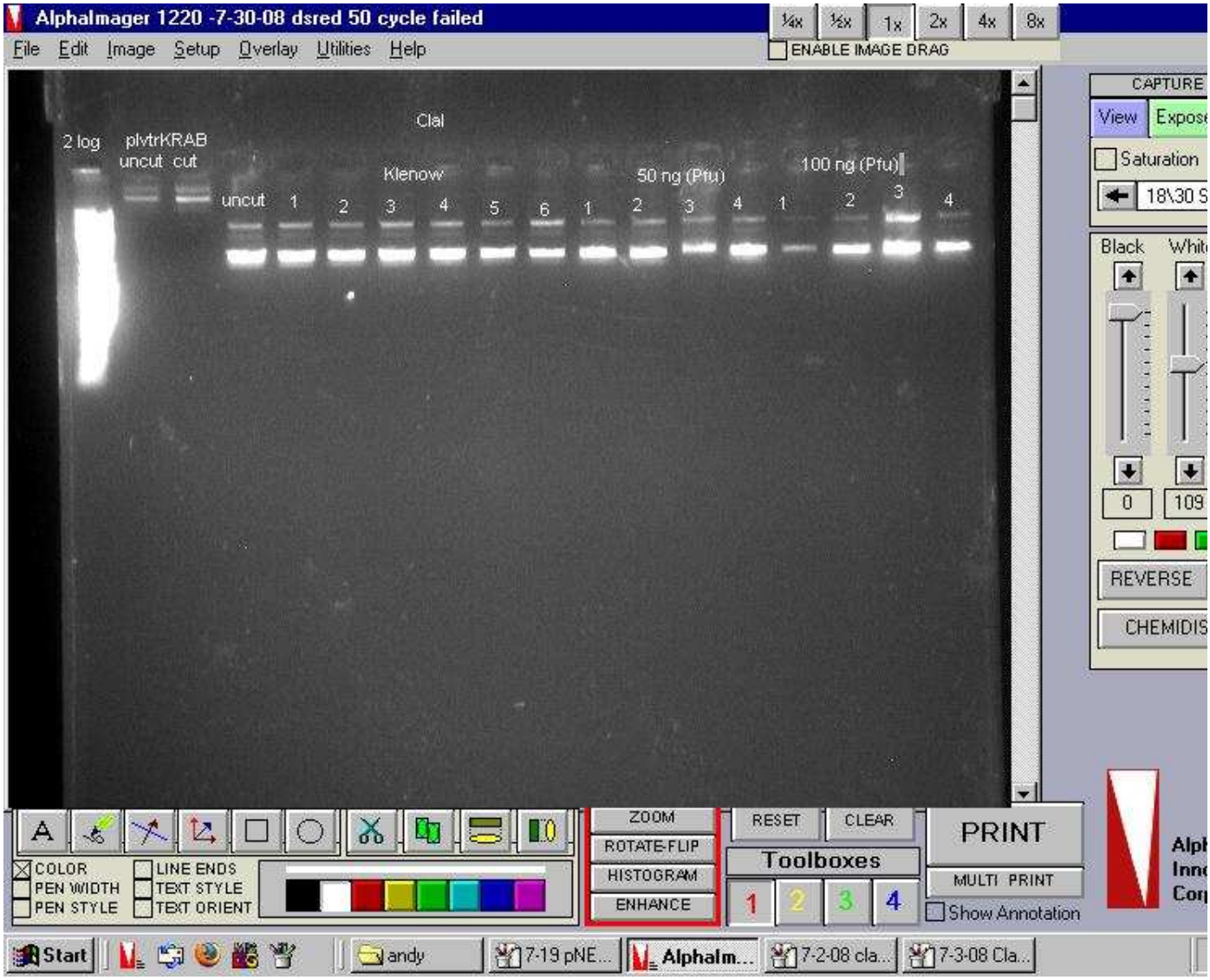


07/30/08

ran quantification gel for the GATA midi (about 700ng/ul)

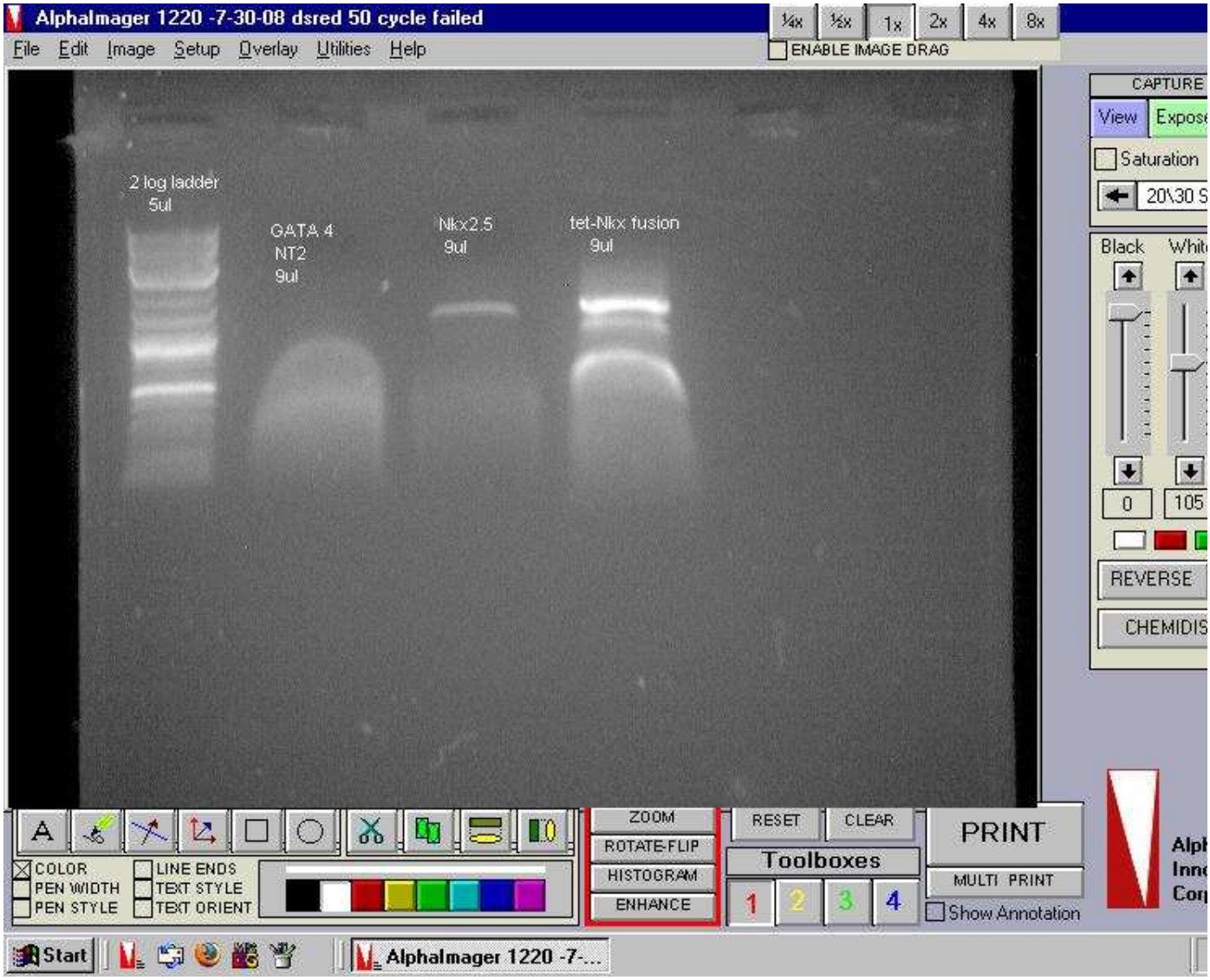


ran gel for ClaI? digests --> doesnt seem to have worked...but again the control didnt either, so either redo ClaI? or redo BspD1? with 1ul of enzyme



set up restriction digests -->in the iGEM box, need to be run out on a gel used 0.5ul GATA/puc and 2.5 of 100ng Zeo (was aiming for about 250-300ng DNA)

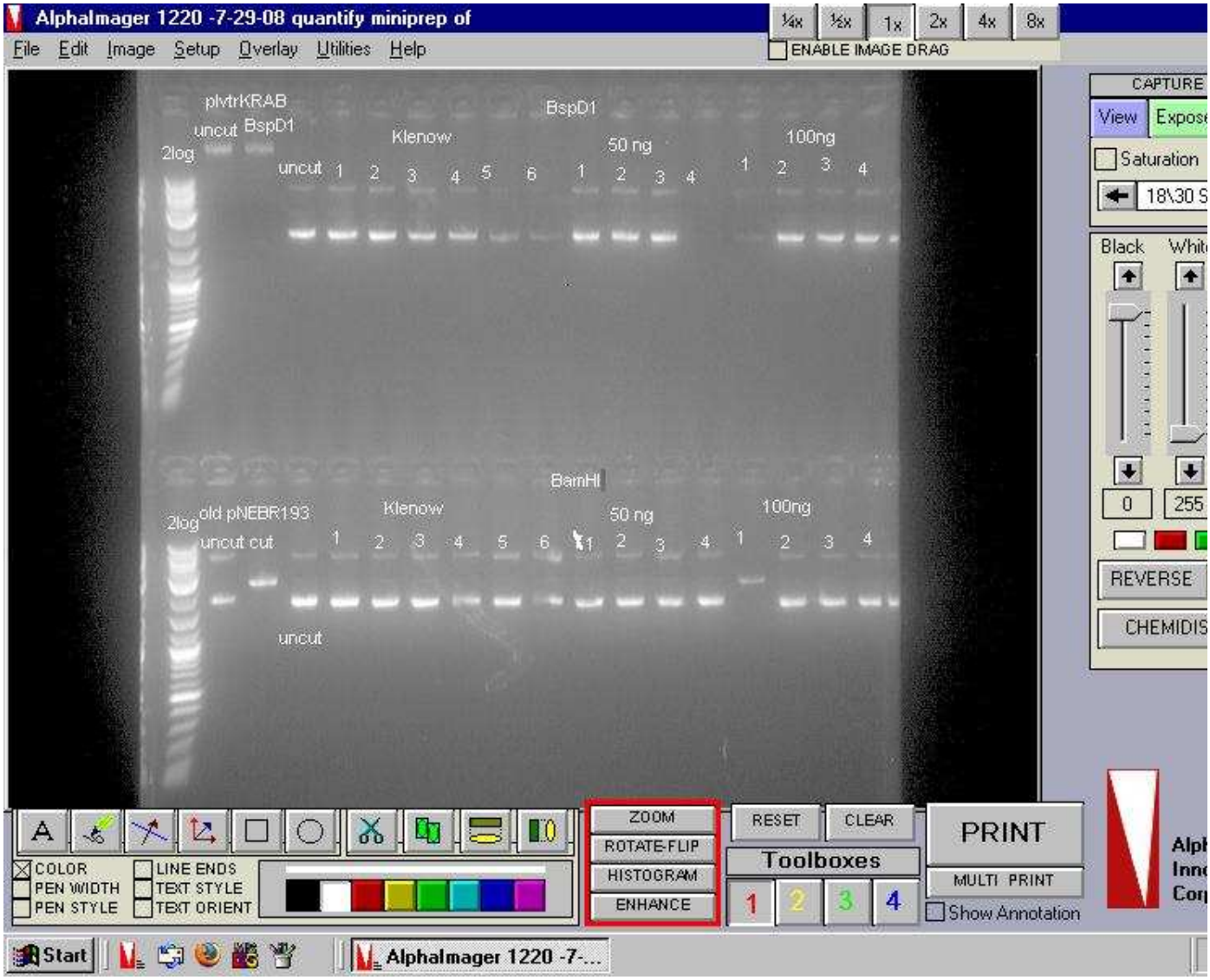
ran gel for gata and tet-Nkx fusion --> neither worked



07/29/08

ran gel for all the restriction digests for pNEB193

- looks like the BamHI? site has been eliminated, which is good
- BspD1? doesn't seem to have worked --> it didn't cut our positive control
- we might need to recut everything with Cla to double check and make sure OR recut with 1ul BamHI? (maybe thats why the control didnt work)



retried tet-Nkx fusion:

- realized that before I didn't add 0.25ul deaza GTP
- also not sure if the oligos were correct --> used tet-5' and tet fusion-3'
- just to make sure I used the most recent one Nkx (7-27) and tet (7-24)
- the 25 cycle tube will be in the PCR machine and needs to be run out on a gel

For 2A I think we should try to PCR off Jen's gel isolation. There's a tube in the iGEM 2008 box labeled "Jen's dsRed 10X 7-29-08" --> try to run the PCR off 1ul of that and the proper oligos

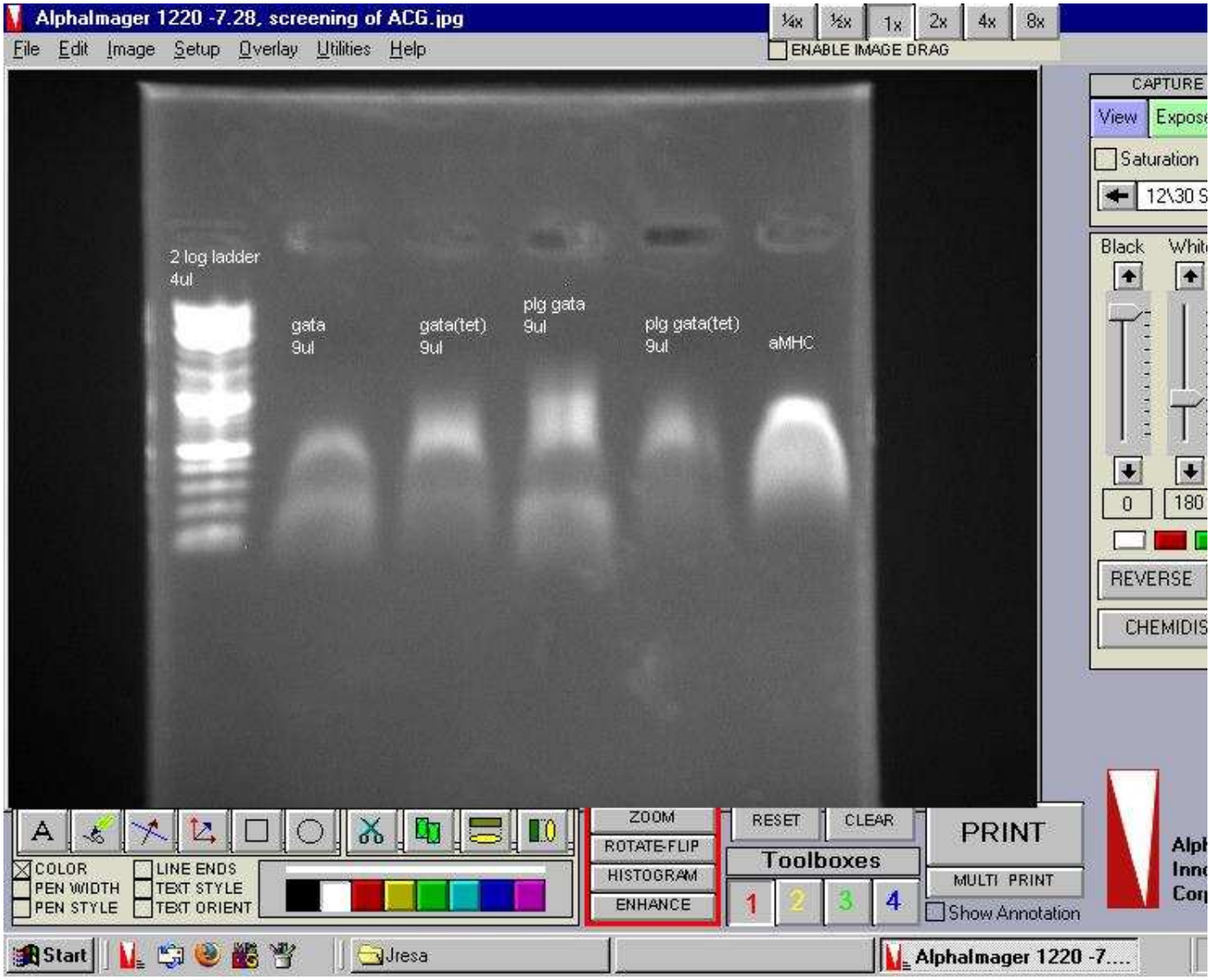
Still need to figure out what's going on with GATA/ figure out the right concentrations for aMHC since it worked before, so it should work now

07/28/08

Grew colonies from yesterday's plates:

- took 1 colony from each of the miniprep plates (Klenow) (6 total)
- took 4 colonies from 50ng/100ng ligation reactions (Pfu) (8 total)
- added to 3ml LB + 6ul Amp
- grow overnight at 37C in shaker

Ran 1% gel for Josh's GATA/GATA(tet) PCR attempts from different DNA's and aMHC -->the aMHC doesn't seem to have worked, but I think we finally have GATA



tried to do tetO-Nkx2.5 fusion using the new Nkx and the high GC content protocol

tetO-Nkx fusion
13.5ul H2O?
5ul KOD Buffer
5ul dNTPs
1ul KOD Polymerase
3ul MgCl2?
13ul betaine
2.5ul DMSO
3ul 5' tetO
3ul 3' Nkx-2A
0.5ul tet(10)
0.5ul Nkx(10)

Temp program: 55C for 5 and 57C for 20 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 15 secs -->repeat 4X; 70C for 2 mins 95C for 2 mins; 95C for 20 secs, 57Cfor 10 secs, 70C for 15 secs -->repeat 19X; 70C for 2 mins

tried to amplify 2A for GFP--> I just realized we did that for Nkx, but not for GFP which is probably why our fusion didn't work

2A(GFP)
32ul H2O?
5ul KOD buffer
5ul dNTPs
3ul MgSO4?
1ul KOD Pol
1ul 2A
1.5ul 5'oligo (NeoR? -FMDV-5')
1.5ul 3'oligo (FMDV-GFP-3')

Also I'm not sure if I used the right oligos...what I wrote down is what I meant to use but then after setting up the reaction realized that the ddilutions and the main tubes dont correspond...maybe i couldn't understand the handwriting, but if someone could just pair those up, that would be great; also if it doesn't work, we might want to assume I took the wrong oligos and redo the reaction

07/27/08

Transformed XL1-Blue MR Supercompetent Cells:

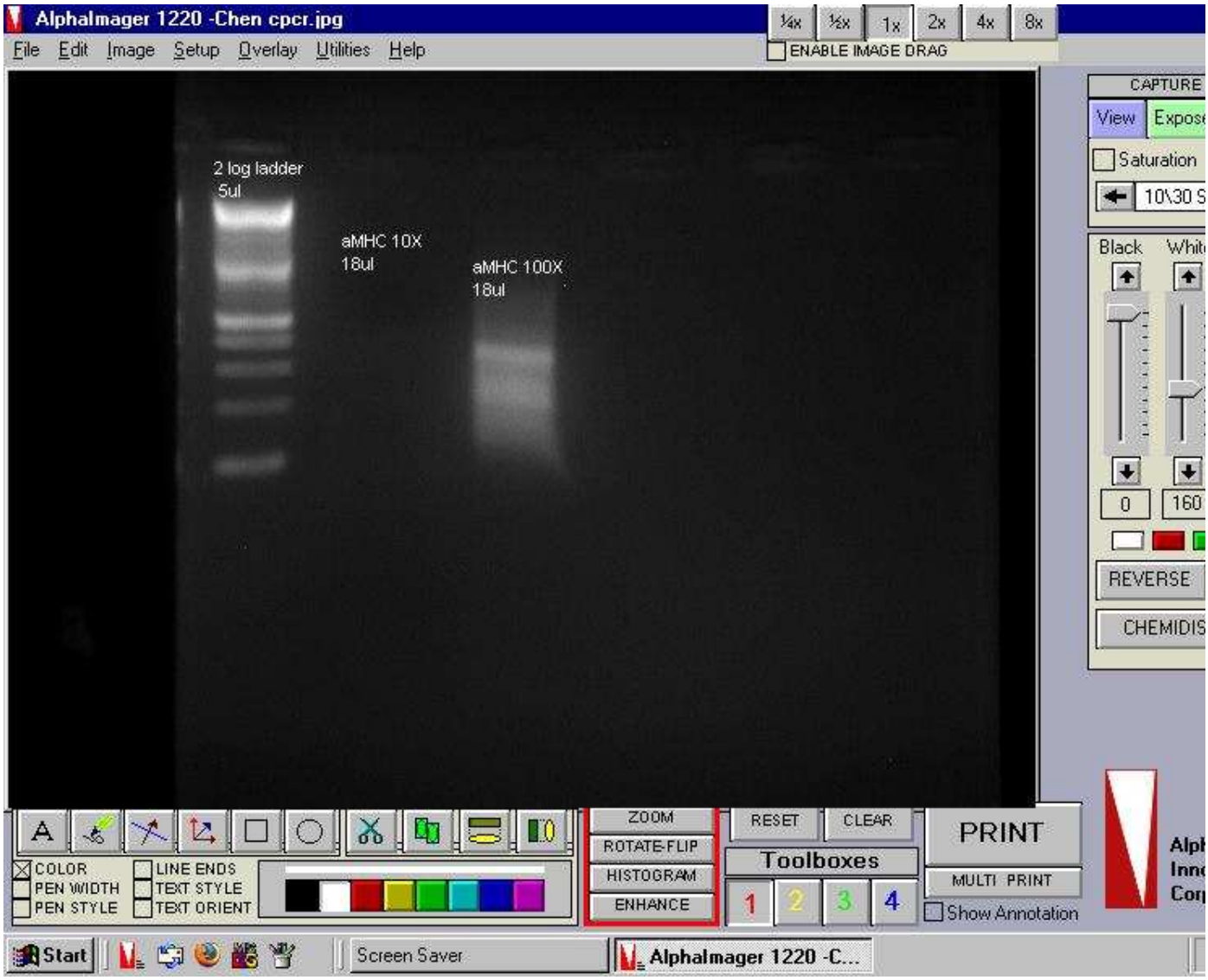
- thaw cells on ice; cool tubes
- add 3.4ul beta-mercaptoethanol to cells
- incubate on ice for 10 mins
- divide into tubes:
    - 6 tubes: 20ul (DNA from minipreps done with Klenow #1-6)
    - 2 tubes: 40ul (old ligations done with Pfu; 50/100ng)
- add 1ul DNA to each; gently swirl
- incubate on ice for 30 mins
- incubate at 42C for 45 secs (used PCR machine)
- put on ice for 2 mins
- add:
    - 400ul SOC to old ligations tubes
    - 200ul SOC to minipreps
- incubate at 37C for 1 hour with shaking
- plated 200ul for all of them-->plates are in at 37C incubator

Tried to redo aMHC PCR (wasn't sure what Josh's protocol was on Friday)

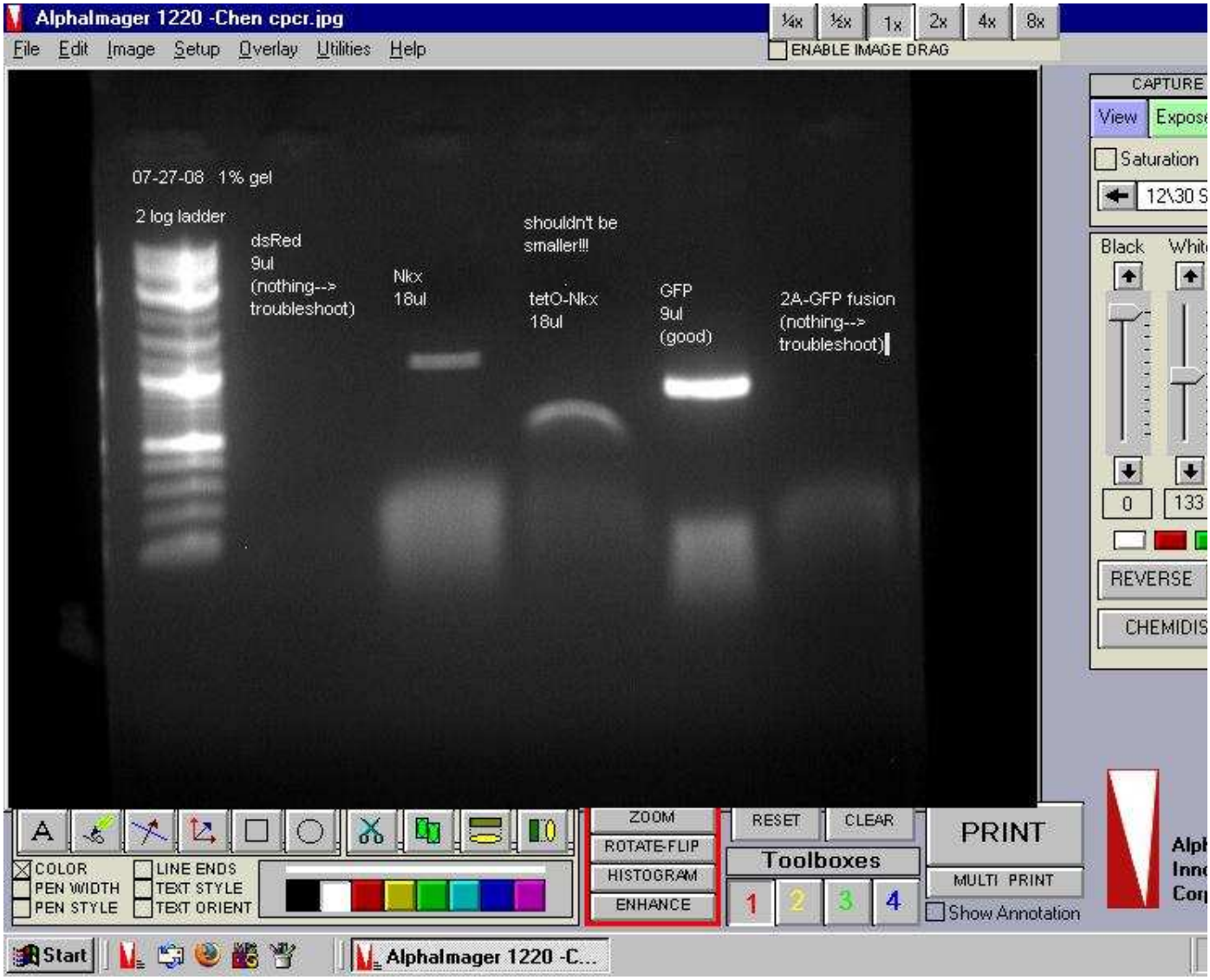
alphaMHC
32ul H2O?
5ul KOD buffer
5ul dNTPs
3ul MgSO4?
1ul KOD Pol
1.5ul 5'oligo (aMHC-5')
1.5ul 3' oligo (aMHC-NeoR-3')
1ul 10X/100X DNA

Temp program: 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 10 secs -->repeat 39X; 70C for 2 mins

Ran it out on a 2% gel --> 100X looks good (some banding above?); there was no band for the 10X



ran out dsRed from Friday and redid the tetO-Nkx and 2A-GFP on a 1% gel --> the dsRed didn't work; tetO-Nkx showed a smaller band than Nkx (I'm still not convinced that the Nkx worked); 2A-GFP didn't work



GATA4 and Nkx2.5 have high GC content in some areas and I think that's why our PCRs haven't worked so far. I tried redoing the PCR's using the protocol Josh put up in his notebook.

These need to be run out on a gel and checked

07/25/08

Couldn't run out the MHC because the machine was stuck at 95C (saw it a little late), but I tried to restart it, this time the machine got stuck at 60. We probably shouldn't use the bigger tube PCR machine since it got stuck on Sunday (07/27) as well. The other machine was busy, and the KOD Pol was probably ruined by now.

Tried 2A-GFP fusion

2A-GFP
29ul H2O?
5ul KOD buffer
5ul dNTPs
3ul MgSO4?
1ul KOD Pol

3ul 5'oligo (2A-GFP-5')
3ul 3' oligo (GFP-3')
0.5ul 2A(13 cycles)
0.5ul GFP (13 cycles)

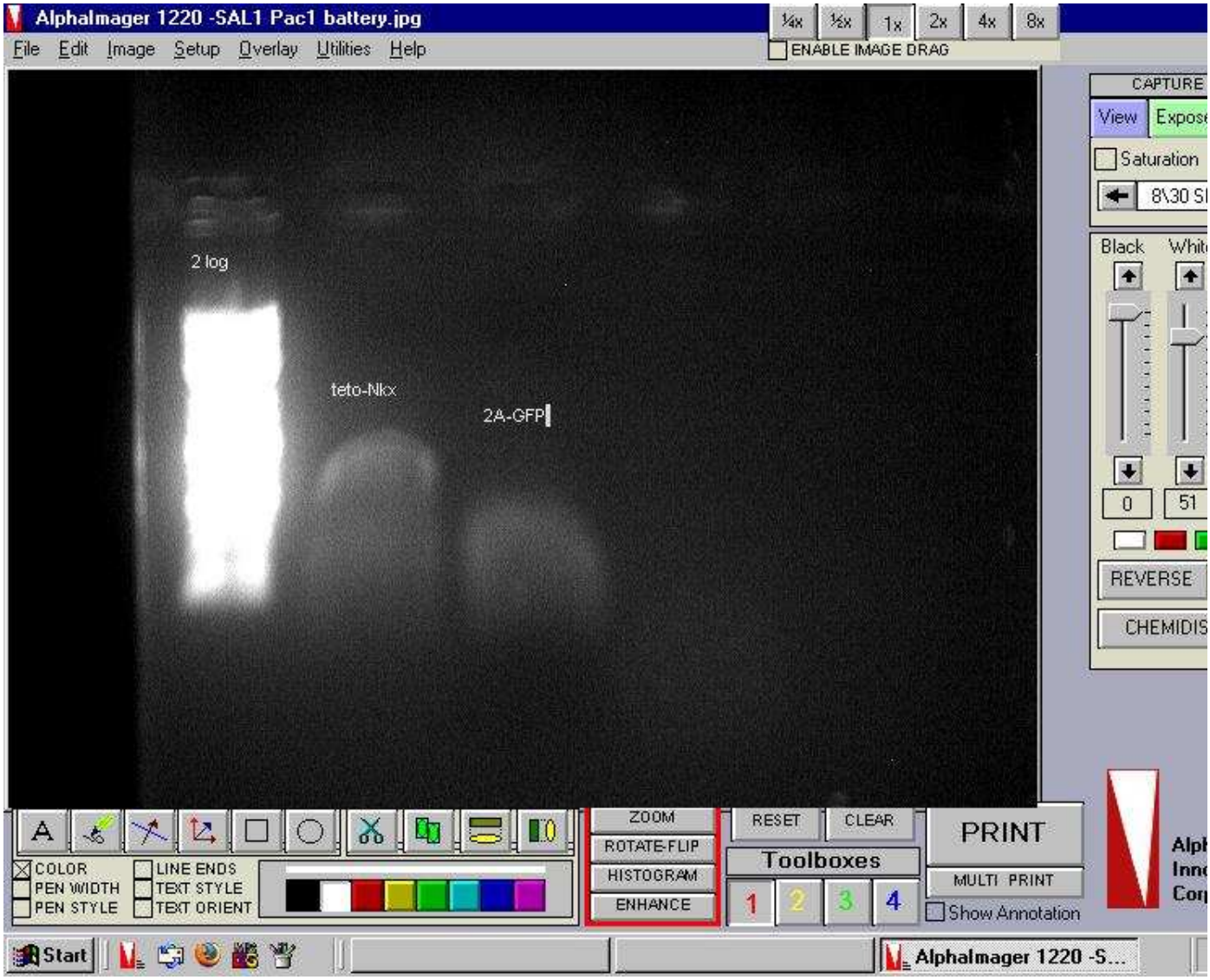
Temp program: 55C for 5 and 60C for 25 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 15 secs -->repeat 4X; 70C for 2 mins 95C for 2 mins; 95C for 20 secs, 60Cfor 10 secs, 70C for 15 secs -->repeat 24X; 70C for 2 mins

Tried to PCR dsRed at higher annealing temperature

dsRed
32ul H2O?
5ul KOD buffer
5ul dNTPs
3ul MgSO4?
1ul KOD Pol
1.5ul 5'oligo (2A-dsRed-5')
1.5ul 3' oligo (dsRed-3')
1ul plvtrKRAB-red (10ng)

Temp program: 95C for 2 mins; 95C for 20 secs, 61Cfor 10 secs, 70C for 15 secs -->repeat 9/24X; 70C for 2 mins

Ran out tetO-Nkx and 2A-GFP on a 1% gel --> it came out kind of blurry...look at 07/27 for a repeat of this



07/24/08

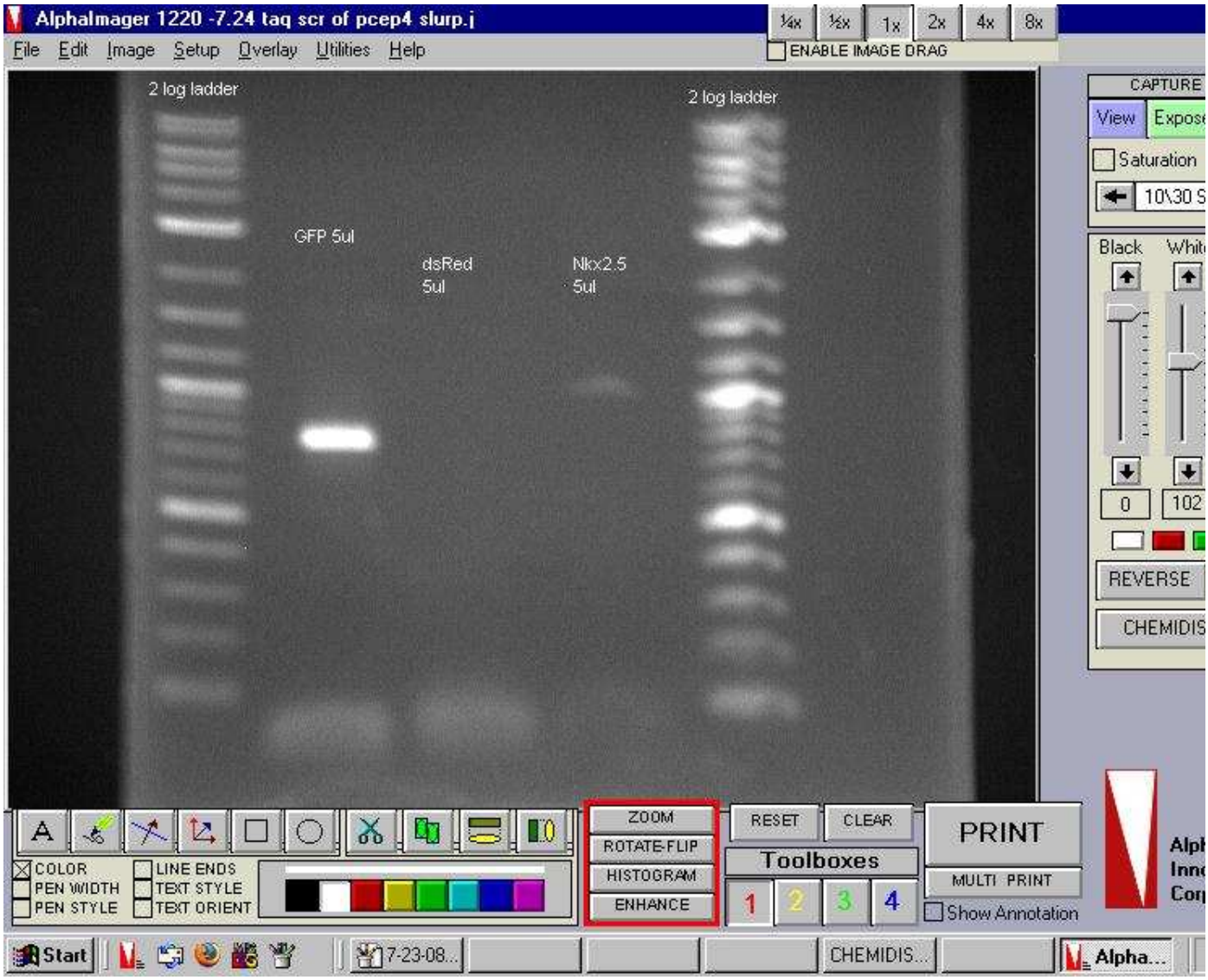
Nkx 2.5	dsRed	GATA4	tetO	2A for Nkx	GATA4(tetO)
5ul KOD buffer	5ul KOD buffer	5ul KOD buffer	5ul KOD buffer	5ul KOD buffer	5ul KOD buffer
5ul dNTPs	5ul dNTPs	5ul dNTPs	5ul dNTPs	5ul dNTPs	5ul dNTPs
3ul MgSO4?	3ul MgSO4?	3ul MgSO4?	3ul MgSO4?	3ul MgSO4?	3ul MgSO4?
1ul KOD Polymerase	1ul KOD Polymerase	1ul KOD Polymerase	1ul KOD Polymerase	1ul KOD Polymerase	1ul KOD Polymerase
32ul H2O?	32ul H2O?	32ul H2O?	32ul H2O?	32ul H2O?	32ul H2O?
1ul Nkx2.5 (9-6-07)	1ul plvtrKRABred (10ng)	1ul GATA4 (8-19-07)	1ul ptripz (iGEM box #1)	1ul 2A (iGEM box #1)	1ul GATA4 (8-19-07)
1.5ul 3'oligo	1.5ul 3'oligo	1.5ul 3'oligo	1.5ul 3'oligo	1.5ul 3'oligo	1.5ul 3'oligo
1.5ul 5'oligo	1.5ul 5'oligo	1.5ul 5'oligo	1.5ul 5'oligo	1.5ul 5'oligo	1.5ul 5'oligo

***I should've written down which oligos, and I'll start doing that from now on, but Joshua was there and confirmed the ones that were used

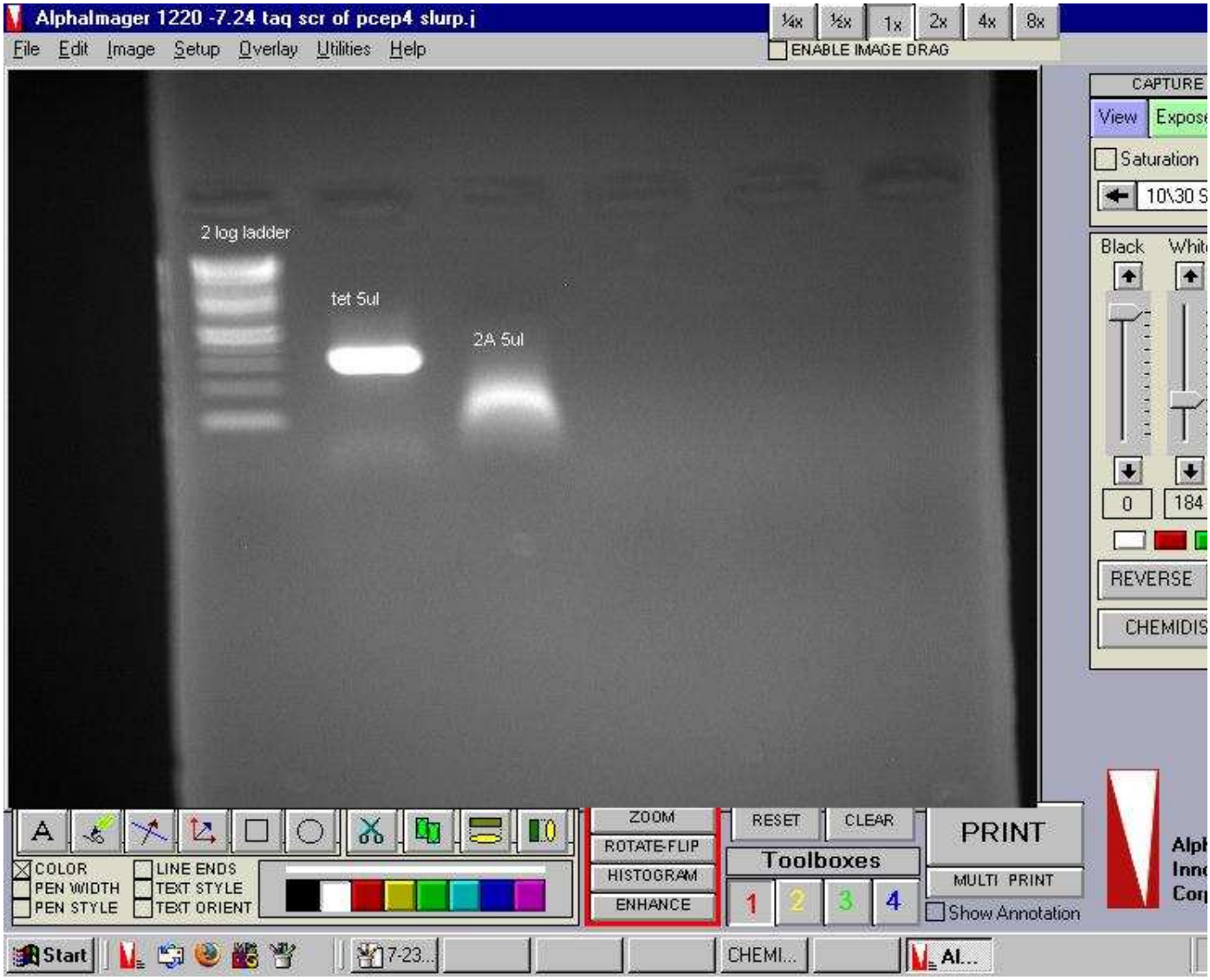
Temperature programs:

1. Nkx2.5 + dsRed: 95C for 2 mins; 95C for 20 secs, 56Cfor 10 secs, 70C for 15 secs -->repeat 9/24X; 70C for 2 mins
2. GATA4 + GATA4(tetO): 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 20 secs -->repeat 9/24X; 70C for 2 mins
3. tetO + 2A for Nkx: 95C for 2 mins; 95C for 20 secs, 55Cfor 10 secs, 70C for 10 secs -->repeat 9/24X; 70C for 2 mins

Ran out GFP(Josh's) + dsRed + Nkx2.5 on a 1% gel --> dsRed and Nkx2.5 didn't work

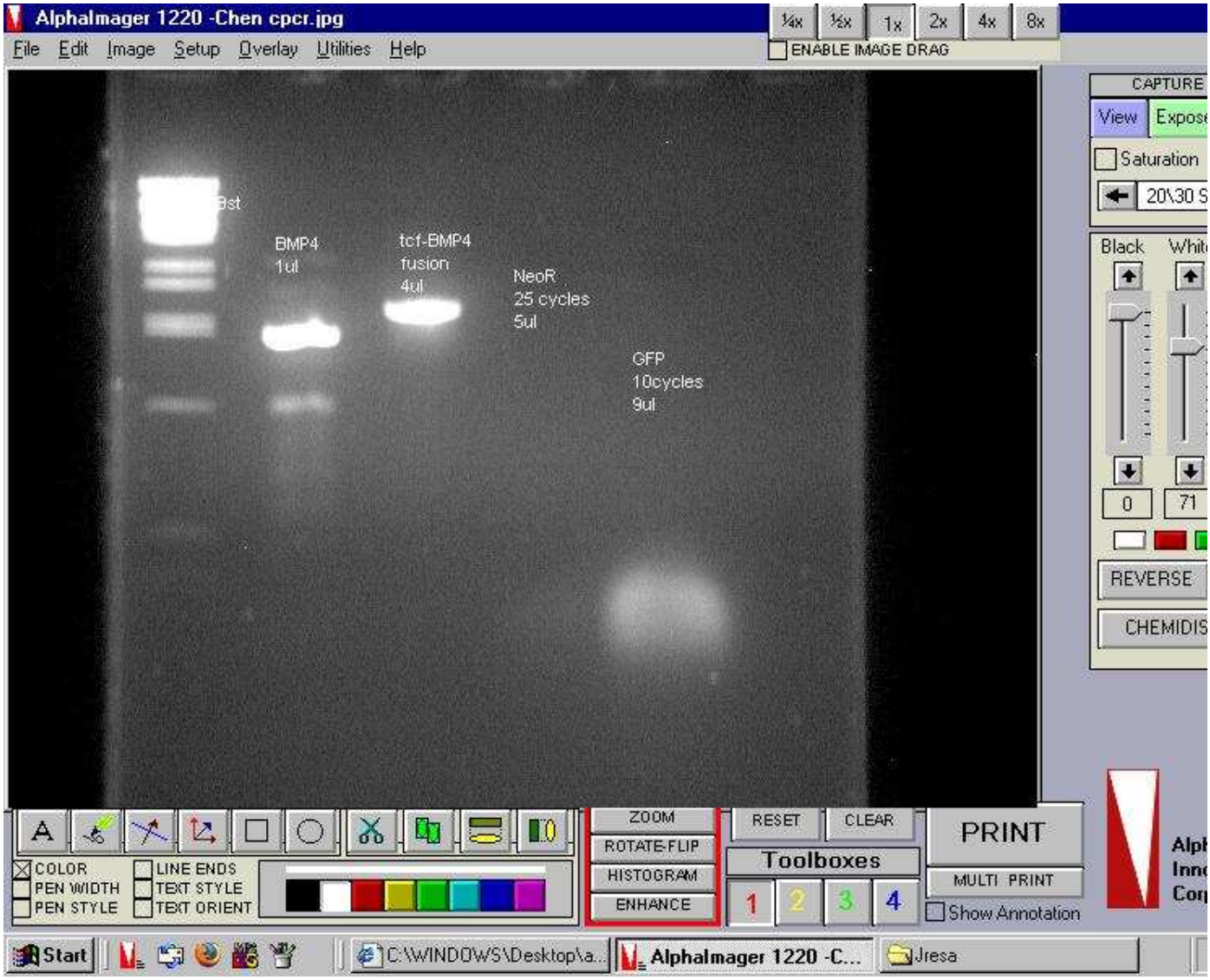


Ran out tetO + 2A for Nkx on a 2% gel --> both looked good



07/22/08

Ran out tcf-bmp4 fusion on a 1% to double check that the gel isolation was good since it was overloaded -->looks good! On the same gel, I ran out NeoR? (25 cycles) and GFP (10cycles) and it doesn't look like either worked



Retried GFP isolation from pWPI

32ul H2O?
5ul KOD buffer
5ul dNTP's
1ul KOD Polymerase
3ul MgSO4?
1.5ul 5' oligo
1.5ul 3' oligo
1ul pWPI (10ng/ul)

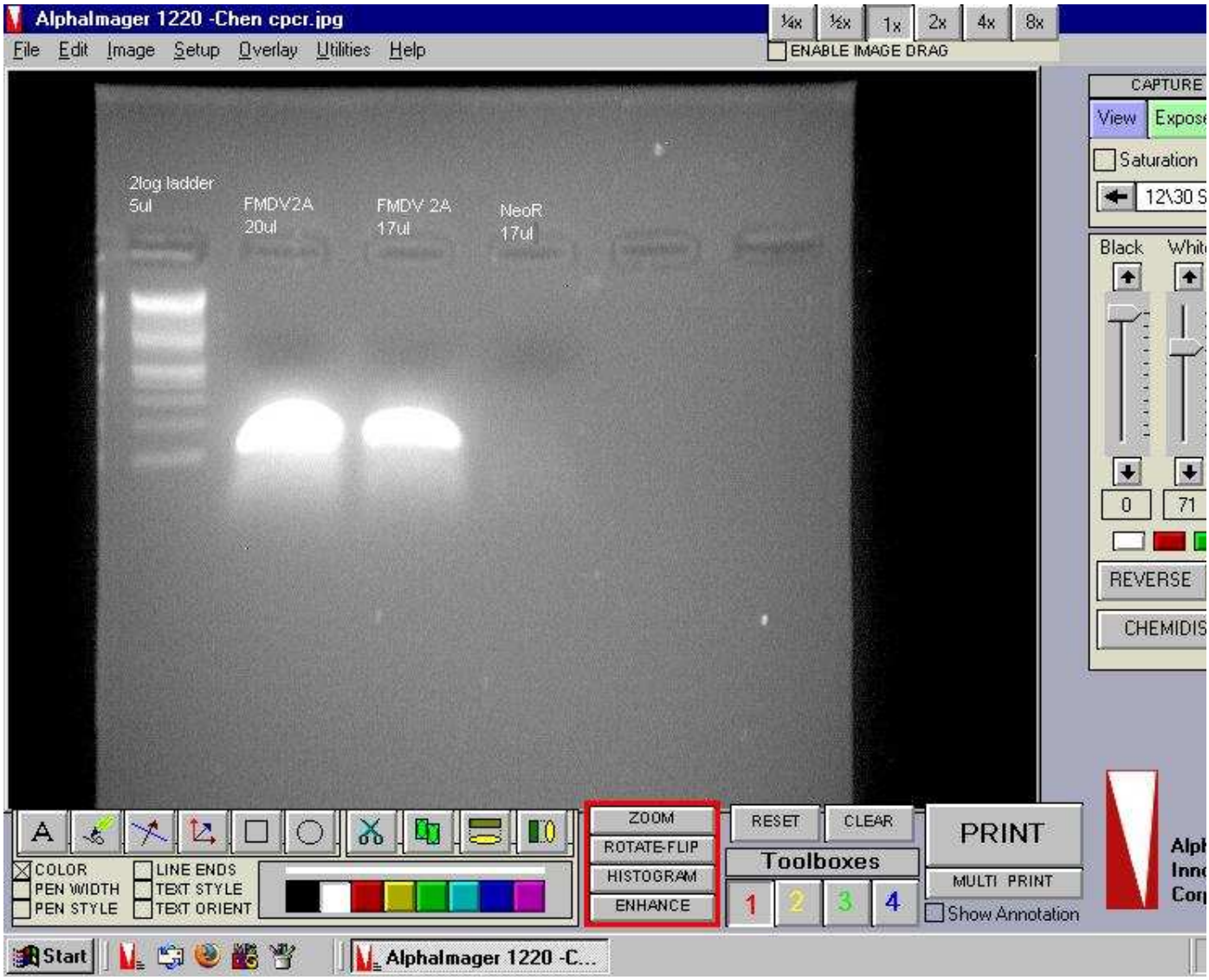
Program: 95C for 2 mins 95C for 20 secs, 55Cfor 10 secs, 70C for 15 secs -->repeat 9/24X 70C for 2 mins

I took out the 10 cycles tube, but left the 25 cycles one in the PCR machine

NeoR? - since the isolation didn't work again, I started talking to Andy about it and apparently it's hard to sometimes isolate a piece that's high in GC content on some stretches. So I downloaded this program, and we figured out that it IS in fact high in GC content. Apparently there's a separate protocol for that --> ask Jen

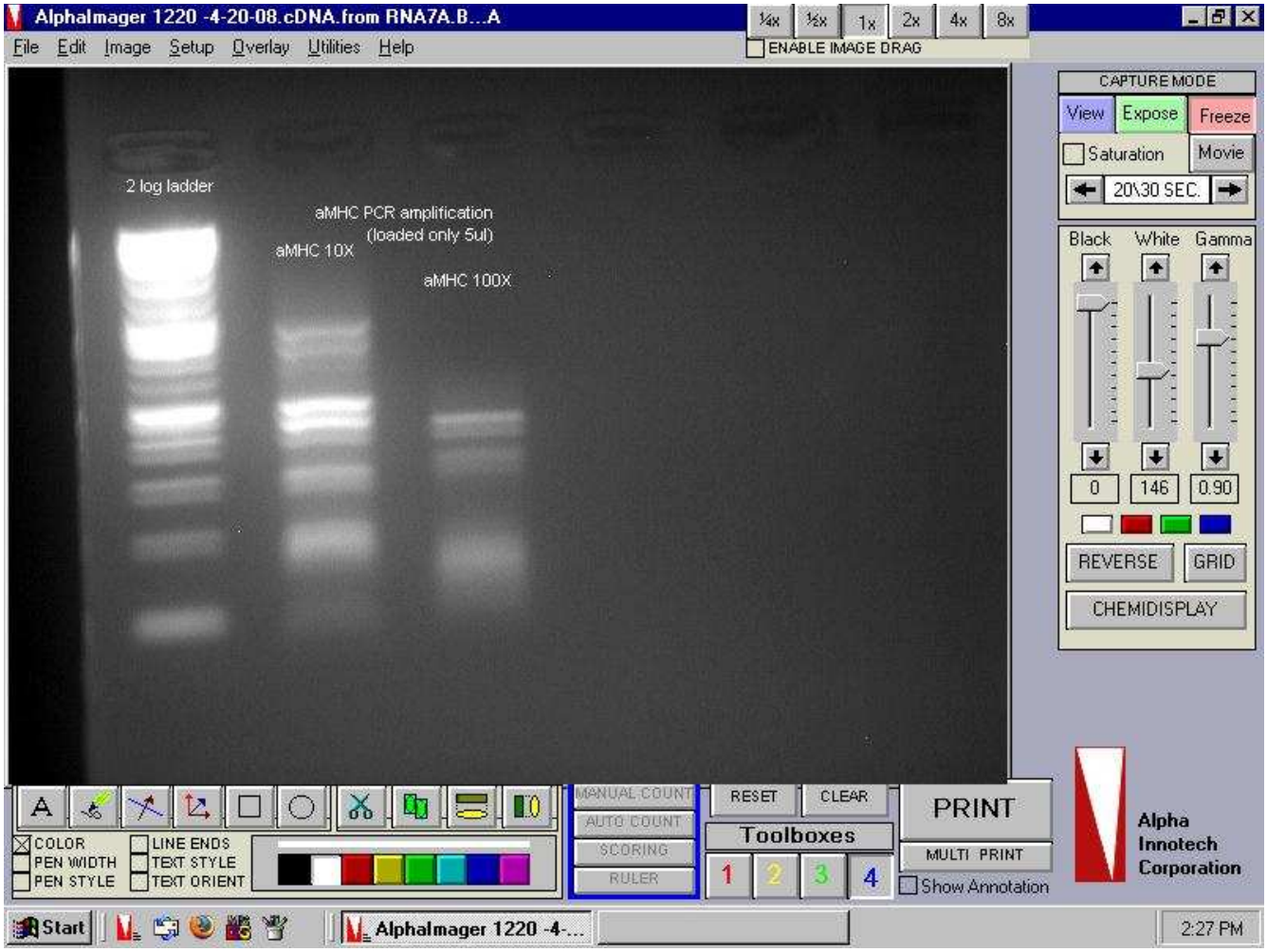
Ran out and isolated FMDV2A? (same gel also has NeoR? , more DNA loaded just to make sure there's nothing). It was a little

overloaded so we might want to double check. I also used the cleaning protocol to isolate it from the gel



07/20/08

Ran out on a 2% gel (2log ladder, 5ul 10X, 5ul, 100X) for aMHC PCR with 45 cycles



Redid alphaMHC PCR, this time with 40 cycles

aMHC
32.5ul H2O?
5ul KOD buffer
5ul dNTP's
1ul KOD Polymerase
2.5ul MgSO4?
1.5ul 5' oligo
1.5ul 3' oligo
1ul 10X/100X DNA

Program: 95C for 2 mins 95C for 20 secs, 55Cfor 10 secs, 70C for 10 secs -->repeat 39X 70C for 2 mins (left in PCR machine)

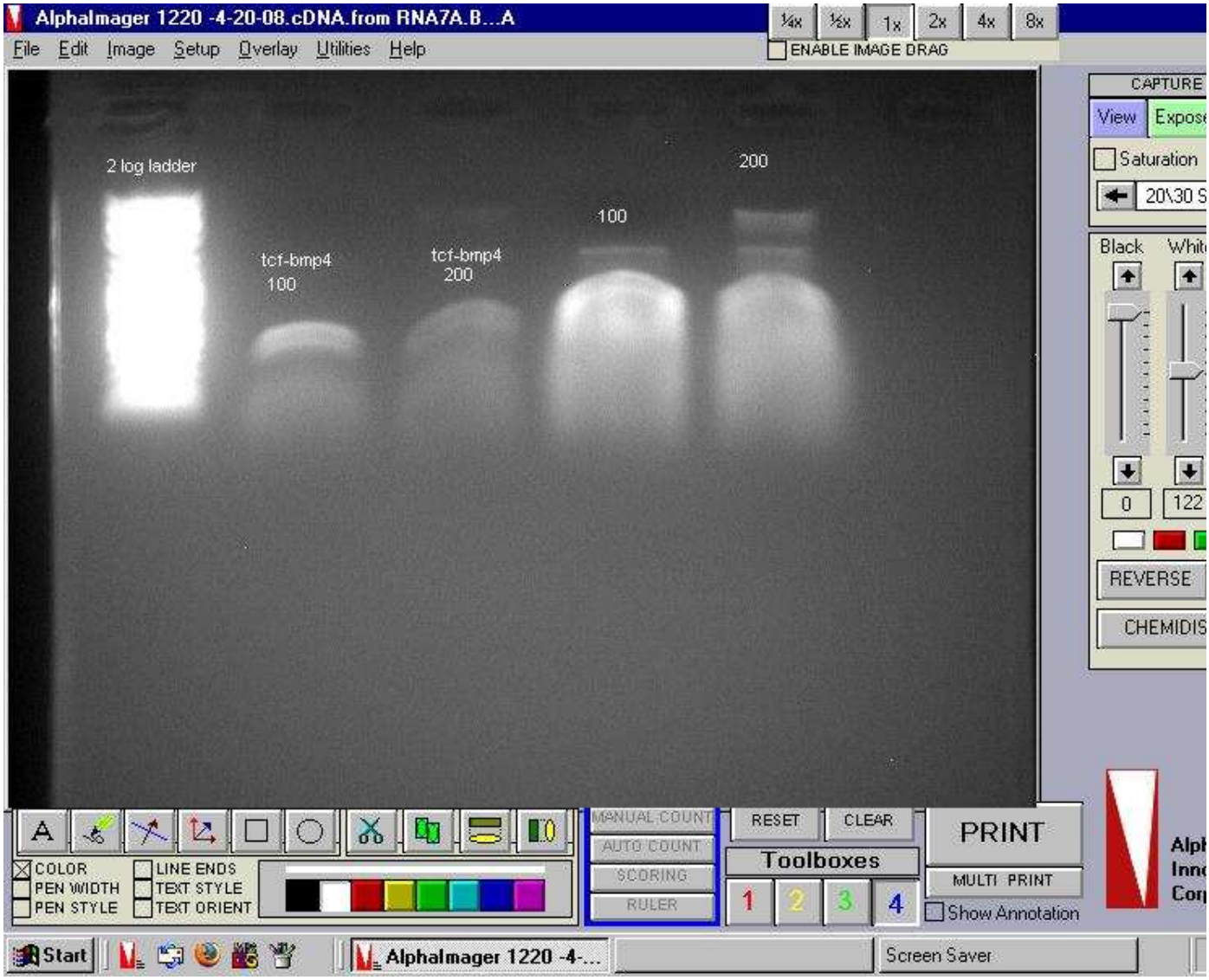
Attempted TCF-BMP4 fusion

29ul H2O?
5ul KOD buffer
3ul MgSO4?
5ul dNTP's

1ul KOD Polymerase
3ul 3' oligo
3ul 5' oligo
0.5ul TCF assembly (7-16)
0.5ul BMP4 assembly (10 cycles)

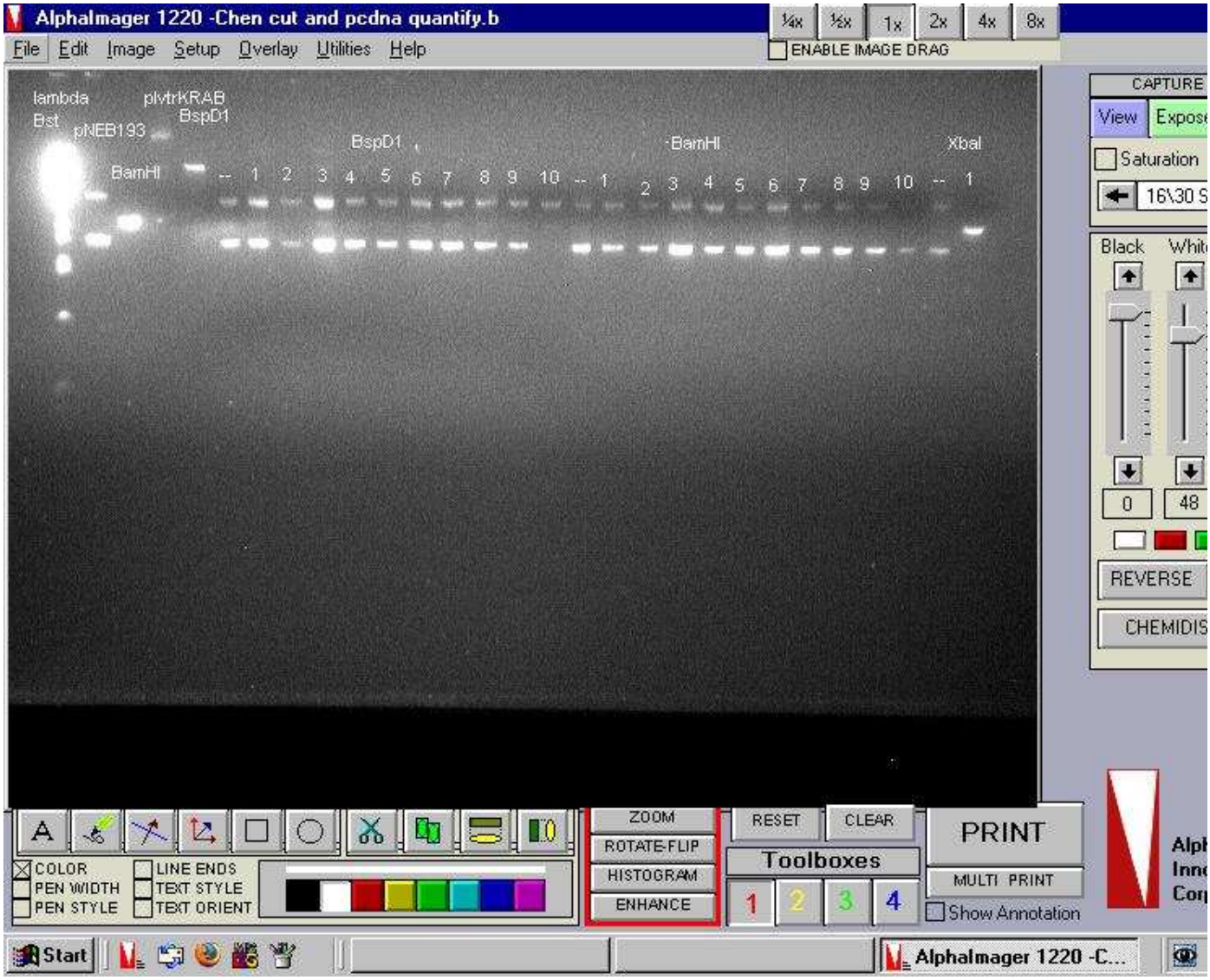
Program: 95C for 2 mins 95C for 20 secs, 55Cfor 10 secs, 70C for 30 secs -->repeat 19X 70C for 2 mins

Checked on 1% gel



07/19/08

Ran out the digests on a 0.8 % gel



pNEB193 modification didn't work --> need to find a new plasmid.

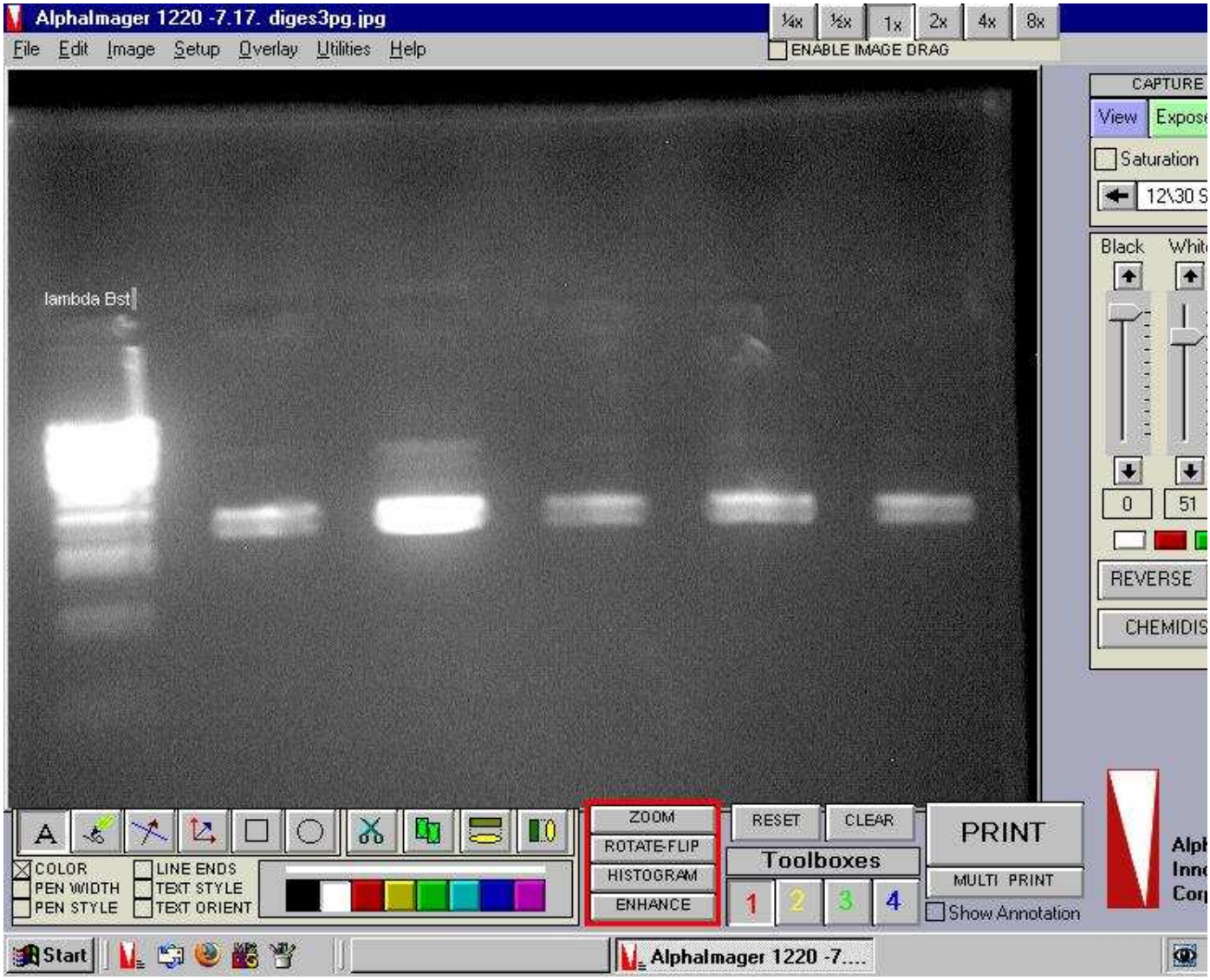
Redid alphaMHC PCR, this time with 45 cycles

aMHC
32.5ul H2O?
5ul KOD buffer
5ul dNTP's
1ul KOD Polymerase
2.5ul MgSO4?
1.5ul 5' oligo
1.5ul 3' oligo
1ul 10X/100X DNA

Program: 95C for 2 mins 95C for 20 secs, 55Cfor 10 secs, 70C for 10 secs -->repeat 44X 70C for 2 mins

07/18/08

Quantified pNEB193 DNA from minis. Loaded #1,3,5,7,9



Restriction enzyme digests: Reaction Setup

10 tubes	**10 tubes**	**+ control XbaI?**	**- control BamHI?**	**- control BspD1?**	**- control (XbaI?)**	**pNEB193 BamHI?**	**plvtrKRAB BspD1?**
2ul NEB 3	2ul NEB 4	2ul NEB 2	2ul NEB 3	2ul NEB 4	2ul NEB 2	2ul NEB3	2ul NEB4
2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA	2ul BSA
1ul BamHI?	1ul BspD1?	1ul XbaI?	no enzyme	no enzyme	no enzyme	1ul BamHI?	1ul BspD1?
1ul DNA	1ul DNA	1ul DNA	1ul DNA	1ul DNA	1ul DNA	0.5ul pNEB193	0.5ul plvtrKRAB
14ul H2O?	14ul H2O?	14ul H2O?	15ul H2O?	15ul H2O?	15ul H2O?	14.5ul H2O?	14.5ul H2O?

--> incubate at 37C for 1 hour

Used GENECLEAN Turbo Kit to isolate DNA from agarose gel

- added 400ul Turbo Salt Solution; mix well (melted at 55C and made sure it was homogenous)
- transfered 600ul to column+catch tube;
- centrifuge for 5 secs; empty catch tube (repeated for what was left)
- add 500ul Turbo Wash; centrifuge; empty catch tube
- centrifuge again (5 mins) and empty catch tube
- add 30ul Turbo Elution Solution and incubate for 5 mins at room temperature

- move to another tube, centrifuge for 1 min
- -->all 3 tubes are now in the iGEM box

Ran out 7-18-08 alphaMHC that Josh had PCRed with 50 cycles picture again, it looks like there may have been too many cycles since there are bands above the needed size

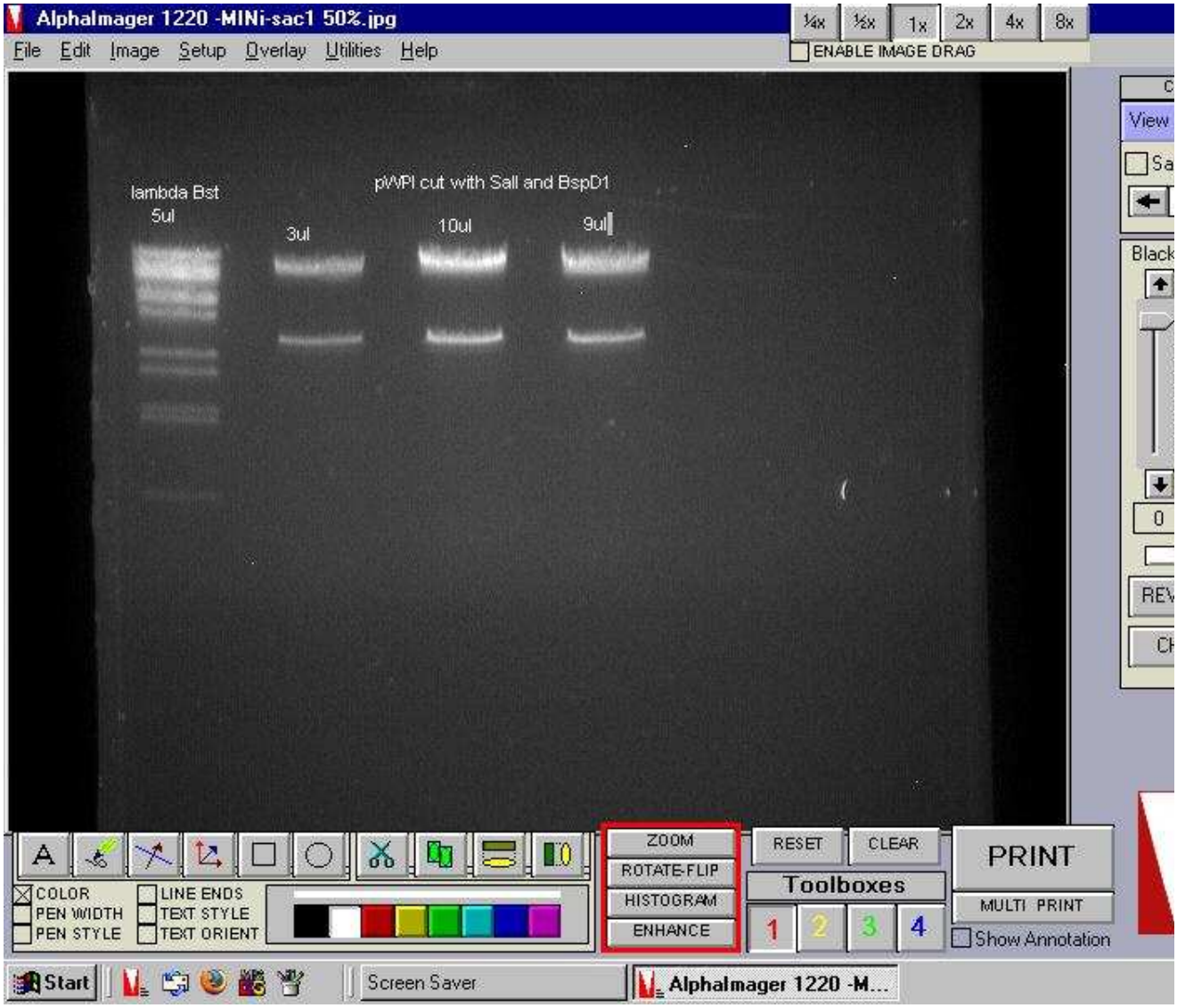
07-18_alphaMHC_after_PCR.jpg

07/16/08

PNEB193 plasmid – retry cutting with BamHI? ? , filling in sticky ends, religating --> should now get a unique ClaI? ? site

1. Restriction enzyme digest with BamHI? ? * 2ul NEB 3 * 2ul BSA * 1ul BamHI? ? * 1ul pNEB193 plasmid * 14ul H2O? ? * --> at 37C for one hour --> now have 500ng plasmid in 20ul
2. BamHI? ? can't be heat inactivated; GENECLEAN Turbo Kit * added 100ul Turbo Salt Solution; mix well * transfer all of it (120ul) to column+catch tube * centrifuge for 5 secs; empty catch tube * add 500ul Turbo Wash; centrifuge; empty catch tube * centrifuge again (5 mins) and empty catch tube * add 30ul Turbo Elution Solution and incubate for 5 mins at room temperature * move to another tube, centrifuge for 1 min * -->now have 500ng plasmid in 30ul
3. Used Klenow to fill in sticky ends * 15ul plasmid DNA * 2ul NEB2 buffer * 1ul Klenow * 2ul dNTP mix * -->at 25C for 15 minutes -->now have 233ng in 20ul * to stop Klenow activity: add 0.4ul of 0.5M EDTA (final concentration= 10mM); at 75C for 20 mins
4. Ammonium acetate precipitation (to clean DNA from EDTA) * add 1ul glycogen * 7ul ammonium acetate * 70ul 100% ethanol * mix and leave on ice for 5 mins. * centrifuge for 15 mins at 4C (in fridge) * decant everything except the pellet * add 100ul 70% ethanol </

- 8-24_pWPI_cut_wSalI+aMHC.jpg:



I	Attachment	Action	Size	Date	Who	Comment
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	07-18_pNEB193_quantification.jpg	manage	102.9 K	01 Aug 2008 - 19:06	SiminaTicau	
	07-22_Bmp4-tcf__NeoR__GFP_diagnostic_30mins_later.jpg	manage	88.9 K	03 Aug 2008 - 14:58	SiminaTicau	
	07-22_FMDV2A_isolation__NeoR_double_check.jpg	manage	81.4 K	03 Aug	SiminaTicau	

				2008 - 14:58	
	07-24_GFP__dsRed__Nkx_2.5.jpg	manage	82.2 K	03 Aug 2008 - 14:59	SiminaTicau
	07-24_tet__2A.jpg	manage	71.7 K	03 Aug 2008 - 15:00	SiminaTicau
	07-25_tet-Nkx__2A-GFP.jpg	manage	86.5 K	03 Aug 2008 - 15:07	SiminaTicau
	07-27_aMHC.jpg	manage	67.2 K	03 Aug 2008 - 15:08	SiminaTicau
	07-27_dsRed__tetO-Nkx__2A-GFP.jpg	manage	74.6 K	03 Aug 2008 - 15:09	SiminaTicau
	07-28_gata__gata_(tet)_(plg)_and_aMHC.jpg	manage	75.1 K	03 Aug 2008 - 15:09	SiminaTicau
	6-26_A20_assembly_with_07_oligos.jpg	manage	22.9 K	01 Jul 2008 - 22:44	SiminaTicau
	6-28_A20_assembly.jpg	manage	17.4 K	04 Jul 2008 - 17:38	SiminaTicau
	6-28_A20_gel_isolation.jpg	manage	18.1 K	04 Jul 2008 - 17:39	SiminaTicau

7-01_quantification_of_minis.jpg	manage	21.7 K	04 Jul 2008 - 18:48	SiminaTicau
7-04_pNEB193_cut_with_BspDI_(2).jpg	manage	35.6 K	05 Jul 2008 - 14:54	SiminaTicau
7-05_pNEB193_cut_BamHI_and_ClaI_-_Copy.jpg	manage	30.0 K	05 Jul 2008 - 18:17	SiminaTicau
7-19_pNEB193_BamHIBspD1_digests.jpg	manage	97.0 K	01 Aug 2008 - 19:07	SiminaTicau
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7-20_tcf-bmp4_fusion.jpg	manage	77.7 K	01 Aug 2008 - 19:08	SiminaTicau
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				15:17	
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7-31_dsRed.jpg	manage	84.6 K	03 Aug 2008 - 14:52	SiminaTicau
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8-02_aMHC-NeoR_fusion.jpg	manage	70.7 K	03 Aug 2008 - 15:48	SiminaTicau
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8-03_PCR_machine_test.jpg	manage	72.0 K	03 Aug 2008 - 16:06	SiminaTicau
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8-03_redid_ClaI_digests-_new_NEB4.jpg	manage	72.6 K	03 Aug 2008	SiminaTicau

				- 16:07	
	8-04_dsRed_PCR_using_PanTaq.jpg	manage	76.4 K	07 Aug 2008 - 19:42	SiminaTicau
	8-05_2A-GFP_fusion_failed.jpg	manage	84.9 K	07 Aug 2008 - 19:42	SiminaTicau
	8-06_dsRed_and_rtTA_PCR.jpg	manage	69.7 K	07 Aug 2008 - 19:43	SiminaTicau
	8-06_tet-Nkx_and_2A-GFP_fusions.jpg	manage	85.1 K	07 Aug 2008 - 19:43	SiminaTicau
	8-07_2A_for_Nkx_construct.jpg	manage	76.5 K	20 Aug 2008 - 20:50	SiminaTicau
	8-07_2A_for_Nkx_construct_(later).jpg	manage	76.6 K	20 Aug 2008 - 20:51	SiminaTicau
	8-07_GATA_and_2A-dsRed_fusion.jpg	manage	77.0 K	20 Aug 2008 - 21:04	SiminaTicau
	8-07_aMHC_construct.jpg	manage	83.5 K	20 Aug 2008 - 20:51	SiminaTicau
	8-08_2A-dsRed_gel_isolation.jpg	manage	115.9 K	20 Aug 2008 - 20:58	SiminaTicau

8-08_tet-Nkx_amplification.jpg	manage	76.4 K	20 Aug 2008 - 20:58	SiminaTicau
8-10_aMHC_construct_PCR_fusion.jpg	manage	77.2 K	20 Aug 2008 - 21:03	SiminaTicau
8-10_aMHC_construct_WORKED.jpg	manage	70.5 K	20 Aug 2008 - 21:03	SiminaTicau
8-10_aMHC_construct_and_2A-dsRed_fusion.jpg	manage	76.4 K	20 Aug 2008 - 21:01	SiminaTicau
8-11_2A-dsRed_fusion.jpg	manage	79.1 K	20 Aug 2008 - 21:19	SiminaTicau
8-12_BamHI_dugest_-_blurry.jpg	manage	82.0 K	20 Aug 2008 - 21:19	SiminaTicau
8-12_BspD1_and_ClaI_digests.jpg	manage	112.5 K	20 Aug 2008 - 21:20	SiminaTicau
8-13_cmv_pcr.jpg	manage	72.3 K	20 Aug 2008 - 21:25	SiminaTicau
8-13_pbluescript_digest.jpg	manage	96.7 K	20 Aug 2008 - 21:26	SiminaTicau
8-13_tet-Nkx_construct.jpg	manage	92.3 K	20 Aug 2008 -	SiminaTicau

				21:27	
8-16_GATA4_PCR_failed.jpg	manage	68.8 K	20 Aug 2008 - 21:30	SiminaTicau
8-16_GATA4_minis_quantification.jpg	manage	76.2 K	20 Aug 2008 - 21:29	SiminaTicau
8-17_A20,_TCF,_aMHC_colony_PCR.jpg	manage	75.9 K	23 Aug 2008 - 15:08	SiminaTicau
8-17_GATA4_PCR_succes.jpg	manage	68.0 K	23 Aug 2008 - 15:09	SiminaTicau
8-17_rtTA_colony_PCR.jpg	manage	85.6 K	23 Aug 2008 - 15:10	SiminaTicau
8-18_2A_gata_5_and_3_(for_nkx_construct).jpg	manage	68.6 K	23 Aug 2008 - 15:10	SiminaTicau
8-18_tcf,_cmv,_a20_minis_digests_=).jpg	manage	73.0 K	23 Aug 2008 - 15:11	SiminaTicau
8-18_tet-Nkx-2A_and_gata_for_tet_and_gata-2a-dsred.jpg	manage	73.5 K	23 Aug 2008 - 15:12	SiminaTicau
8-19_Joshs_tcf,_cmv,_a20_digests.jpg	manage	80.6 K	23 Aug 2008 - 15:13	SiminaTicau
8-21_pWPI_w_SalI_+_A20,_TCF,_pbluescript_maxis.jpg	manage	84.2 K	25 Aug 2008	SiminaTicau

				- 13:45	
	8-23_aMHC_quant_1-2ng.jpg	manage	99.0 K	25 Aug 2008 - 13:58	SiminaTicau
	8-23_pWPI_cut_gel_isolation.jpg	manage	89.1 K	25 Aug 2008 - 14:02	SiminaTicau
	8-23_pWPI_cut_wSalI+aMHC_quant.jpg	manage	76.0 K	25 Aug 2008 - 14:05	SiminaTicau
	8-23_tet-Nkx-2A,_tet-Nkx,_2A-dsRed_all_for_pblue_and_quant._of_pWPI.jpg	manage	78.0 K	25 Aug 2008 - 14:05	SiminaTicau
	8-24_pWPI_cut_wSalI+aMHC.jpg	manage	77.6 K	25 Aug 2008 - 14:15	SiminaTicau