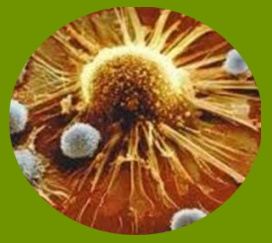
Cryphonectria parasitica tendrils on chestnut tree bark (Photo: Ministry of Agriculture and Regional Development Archive, Ministry of Agriculture and Regional Development, Bugwood.org)



6/22/2014

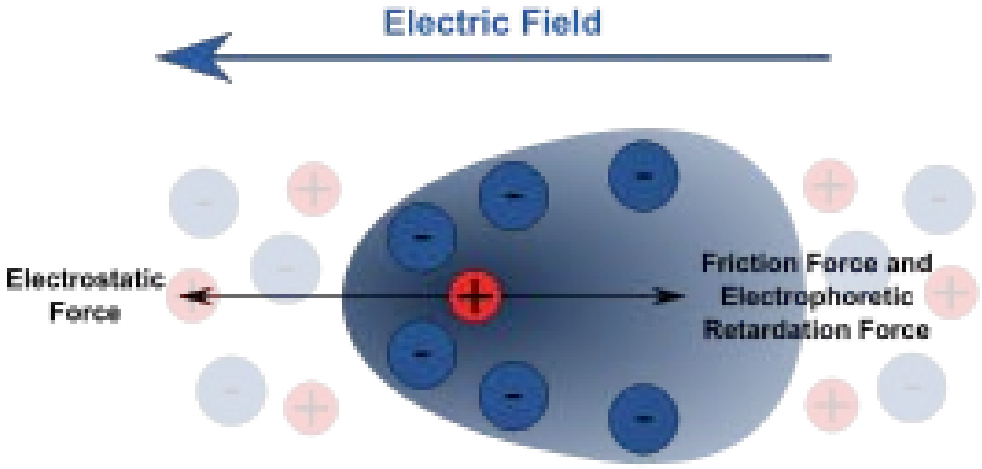
Gel Electrophoresis and Analysis

B3 Summer Science Camp at Olympic High School

Dr. Jennifer Weller

Lab Method: Gel electrophoresis

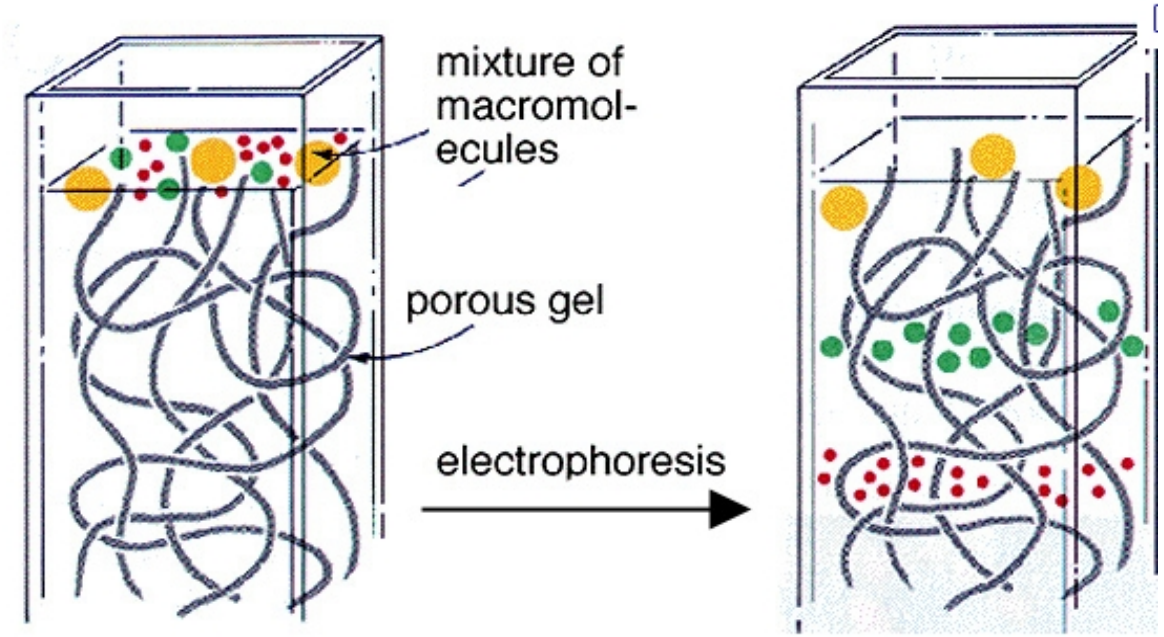
- Electrophoresis: separating molecules in a charged field. Charged molecules will migrate towards the opposite charged electrode under a voltage potential.
- The rate at which molecules move is affected by the ions in solution and the total amount of charge on the molecule over its total size and shape.



http://en.wikipedia.org/wiki/Electrophoresis

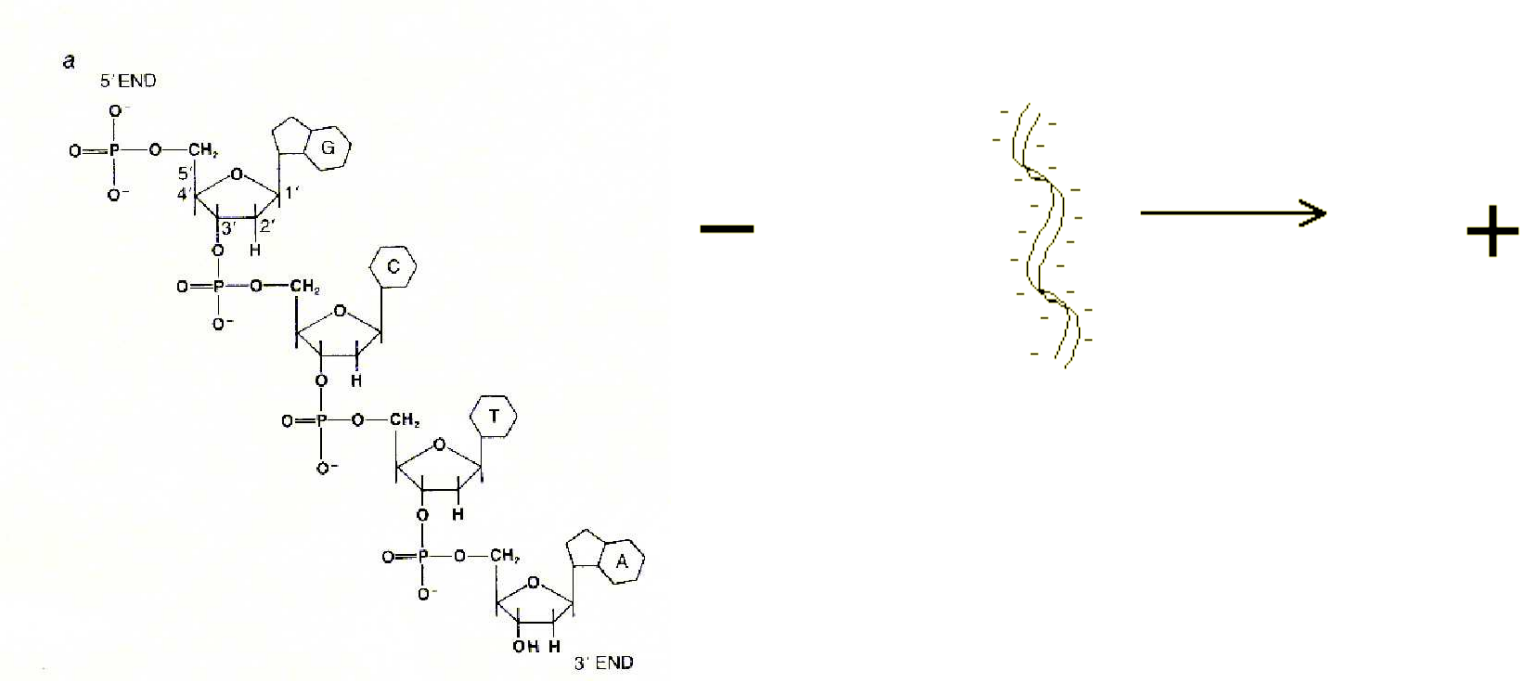
Lab Method: Gel electrophoresis

- A gel matrix produces a sieve-like environment. It is mostly liquid but does give some inert support – there is an average pore size of interlaced strands.
  - The matrix retards diffusion, reduces convection, etc.
  - The pores impose some size/shape selection



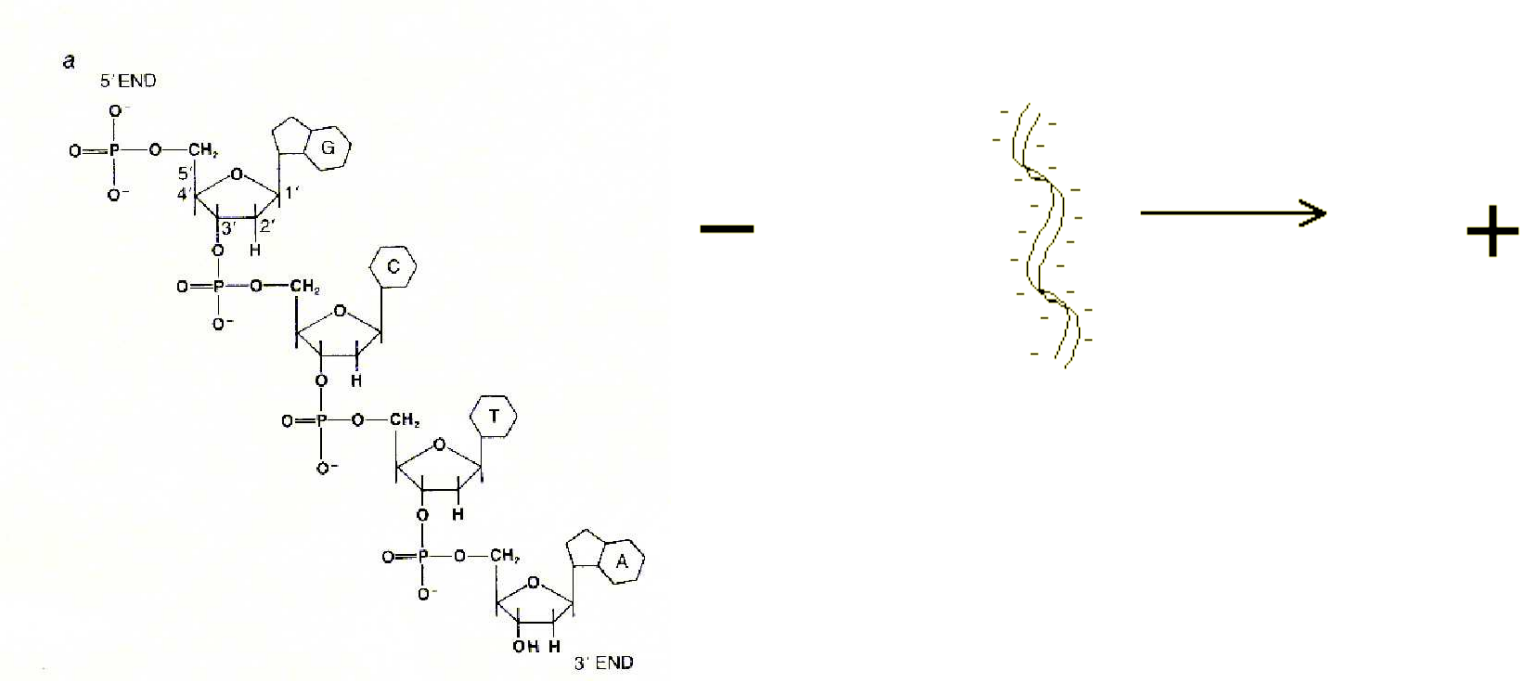
Lab Material: DNA in Gel electrophoresis

- DNA has the same ‘shape’ and a constant charge to mass ratio (one negative charge for every nucleotide, on the phosphate) – why would we use gel electrophoresis in this case?



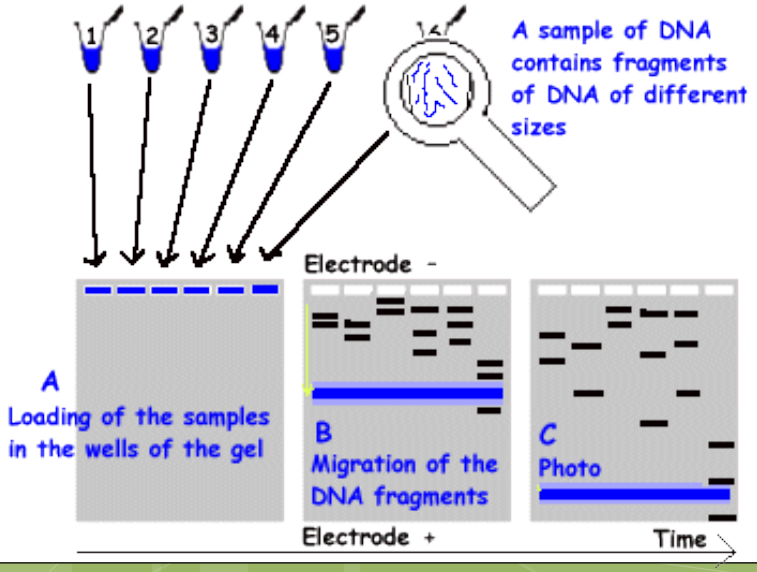
Lab Material: DNA in Gel electrophoresis

- DNA has the same ‘shape’ and a constant charge to mass ratio (one negative charge for every nucleotide, on the phosphate) – why would we use gel electrophoresis in this case?

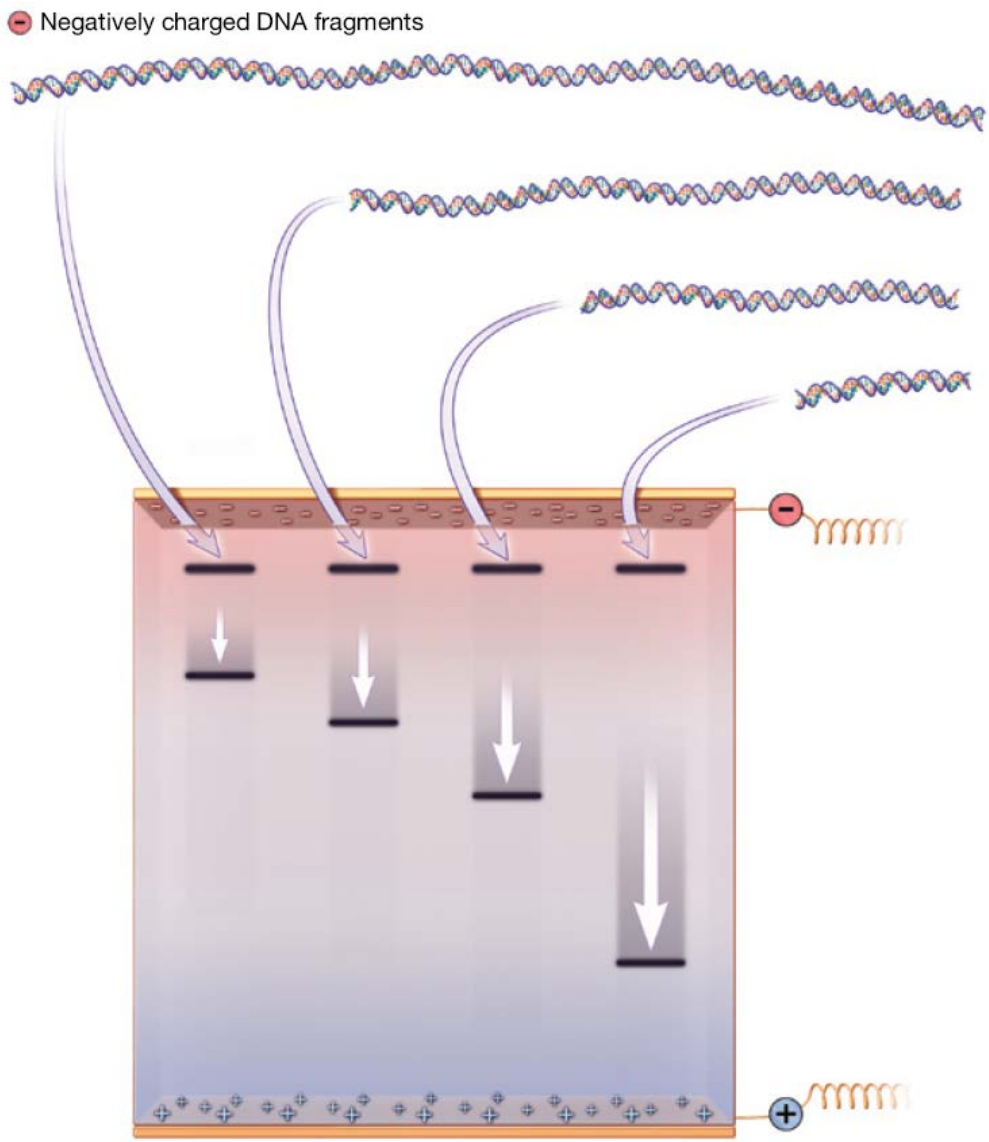


Lab Material: DNA in Gel electrophoresis

- If the length of DNA molecules is different then the gel causes the separation of longer and shorter fragments.
- Why would the length be different?
  - Use restriction enzymes to cut the DNA into defined fragments
  - Use PCR to copy out DNA fragments of defined length
  - The intact DNA has a defined length (plasmids, viruses)

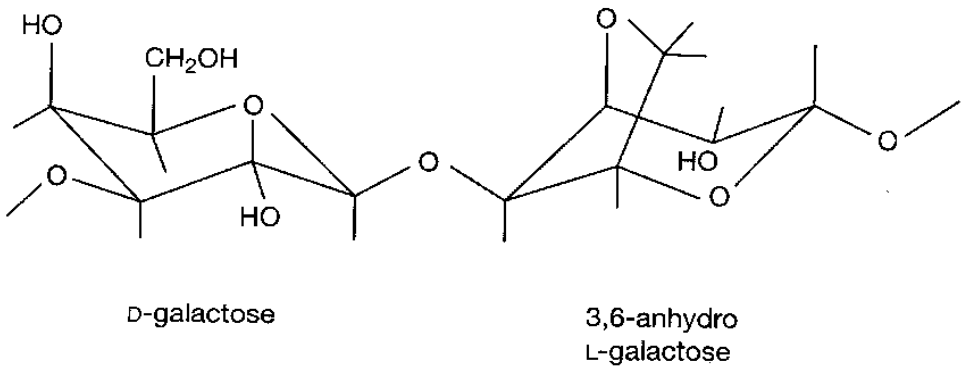


Shorter fragments work their way through the gel faster than longer fragments


Negatively charged DNA fragments

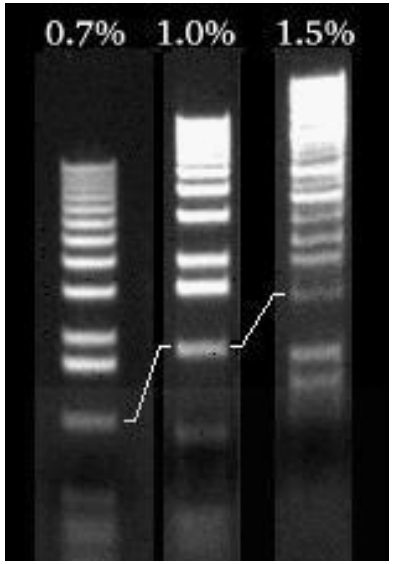
Chemistry: An agarose gel is made of long polysaccharides extracted from seaweed.

- The polysaccharide is used at 0.5-3% concentration
  - This is a weight:volume calculation: 1% means 1gm of agarose in 100ml of buffer (we usually use Tris-Borate-EDTA buffer).
  - Higher conc. → more tightly interwoven strands → smaller pore size so it is harder for long fragments to migrate in the gel
- The range of separation is quite large (100-50,000 bp) but the resolution (how well bands that are close together in size can be separated) is rather low.



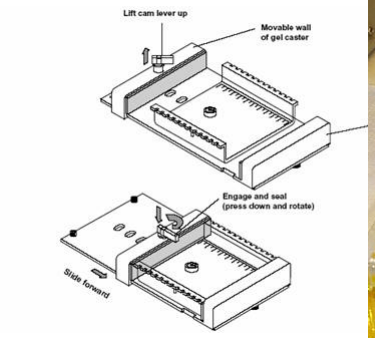
D-galactose

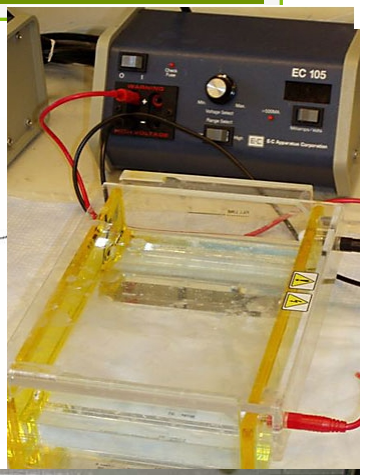
3,6-anhydro
L-galactose

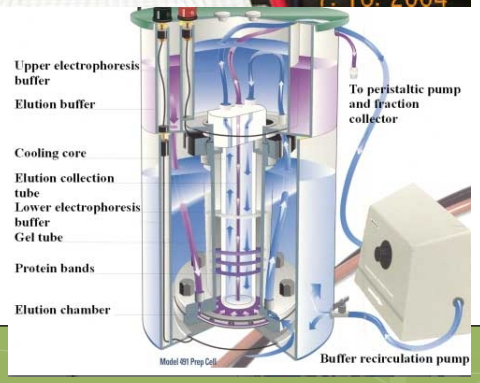


Gel Electrophoresis instrument components

- Required: a power supply, connectors= leads, electrophoresis chamber with buffer reservoirs (tank), gel cassette (casting tray with dams).
  - Power supply maintains constant voltage or power
  - Leads connect electrodes on the electrophoresis chamber (tank) to the power supply
  - The buffer reservoirs help maintain the environment (buffering to keep the molecules intact and salts for electrical conductivity)
  - The gel cassette contains the casting tray in which the gel material was formed
    - A comb is used to form sample wells during the gelling stage, but is removed prior to electrophoresis.
- Optional
  - A recirculating pump may be used to mix buffer components back together during a long run
  - A chiller or fan may be used if the resistance is high and the material is likely to heat up.

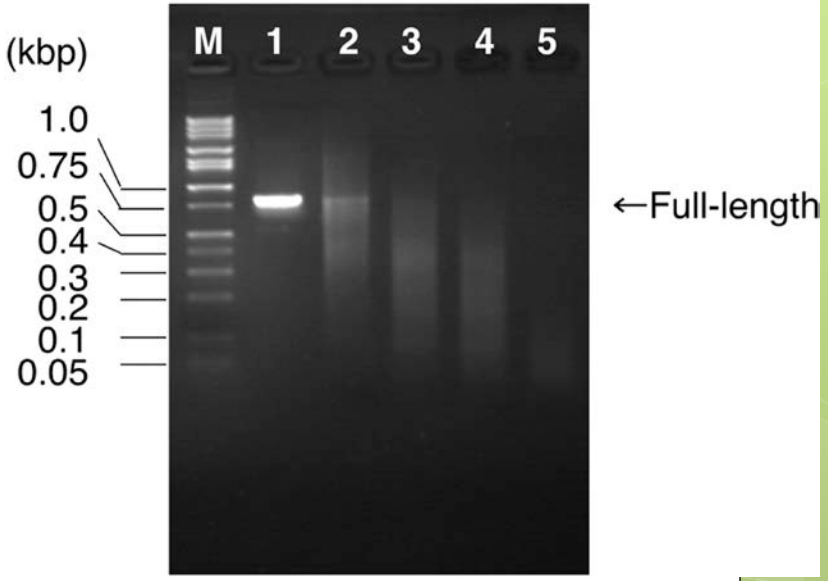


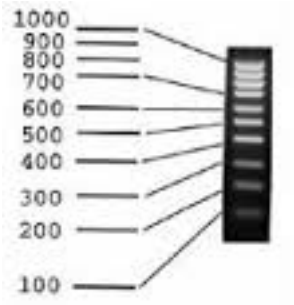


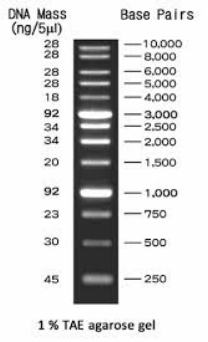


Gel Electrophoresis sample details

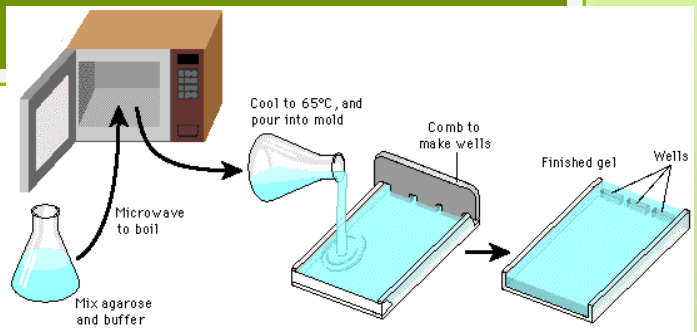
- The sample must have a gel loading buffer (GLB) added to it
  - The sample is dissolved in TE buffer – it will float in your electrophoresis buffer
  - Loading buffer has some glycerol in it (neutral) and some colored compounds
  - It is usually ‘4X’ or ‘6X’.
    - In the first case use 1 part loading buffer to 3 parts sample (so the total is 4 parts)
- The gel must have at least one lane of a DNA ladder on it – this is usually put in the edge lanes of the gel.
  - This has DNA fragments whose lengths we already know – this gives us a ruler for judging our samples.
  - This also gives us a positive control that the gel ran properly.
  - There are MANY commercial ladders available – some only give the length of each band and some give the mass per band as well.

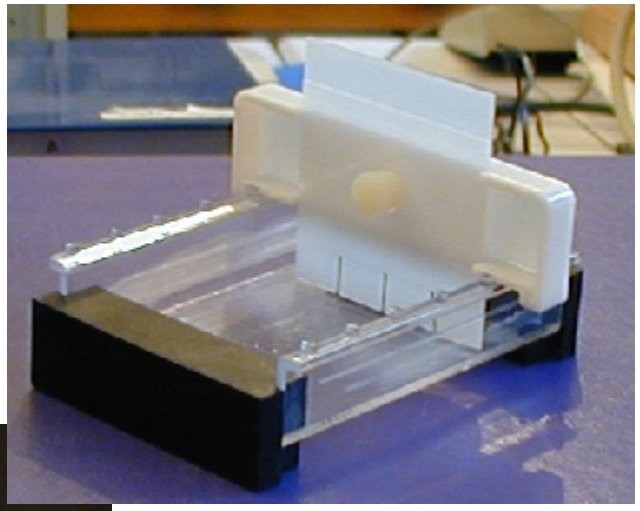


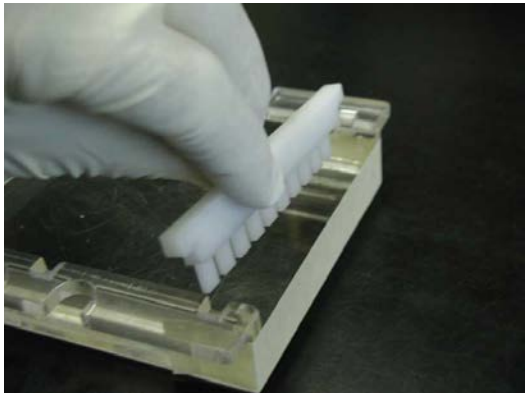


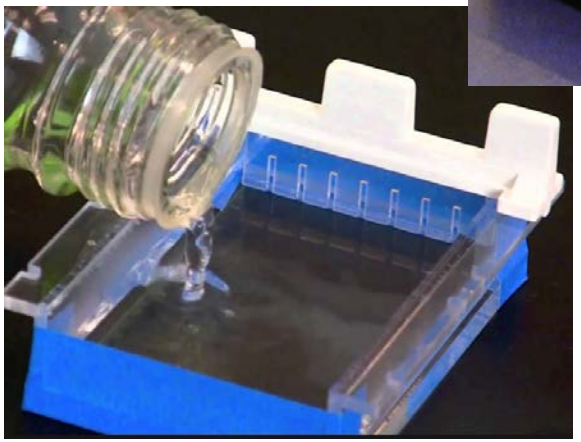


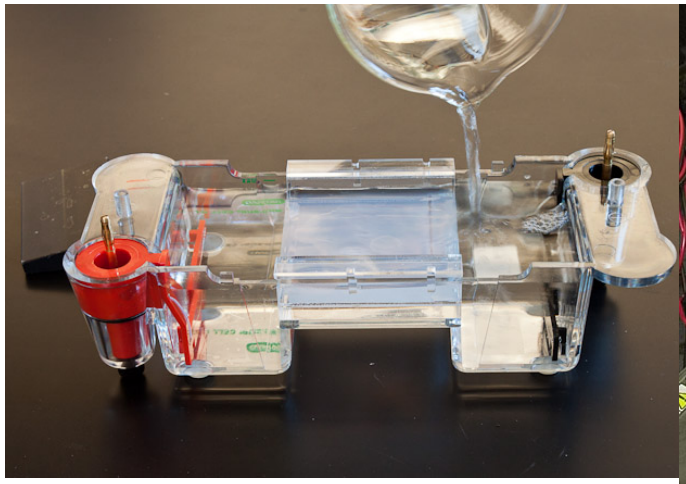

UV Stratalinker 1800


Cool to 65°C, and pour into mold
Comb to make wells
Finished gel
Wells
Microwave to boil
Mix agarose and buffer

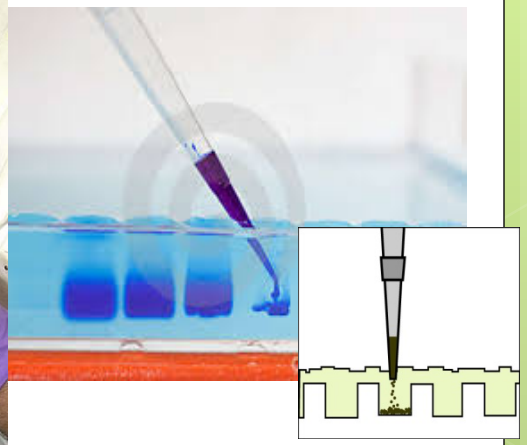


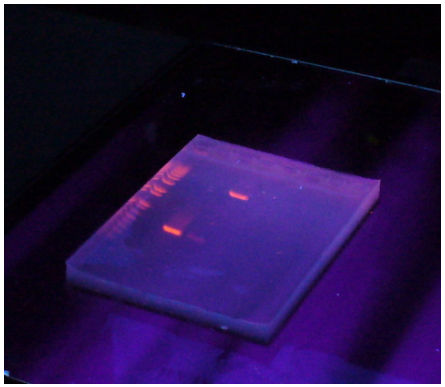


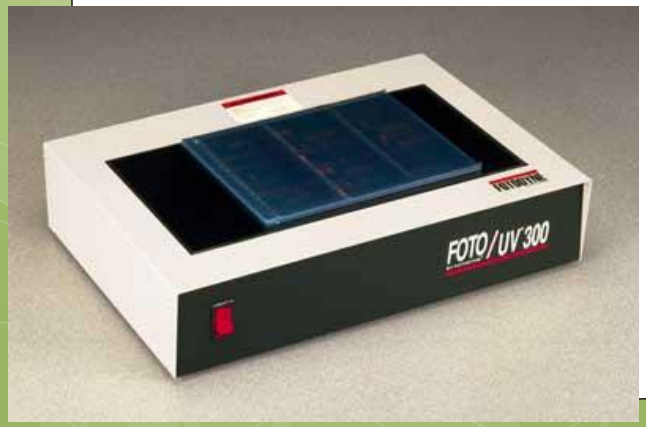


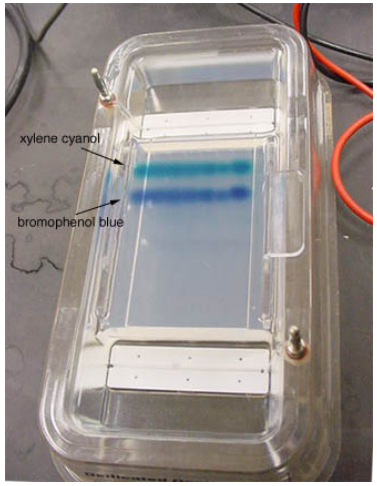


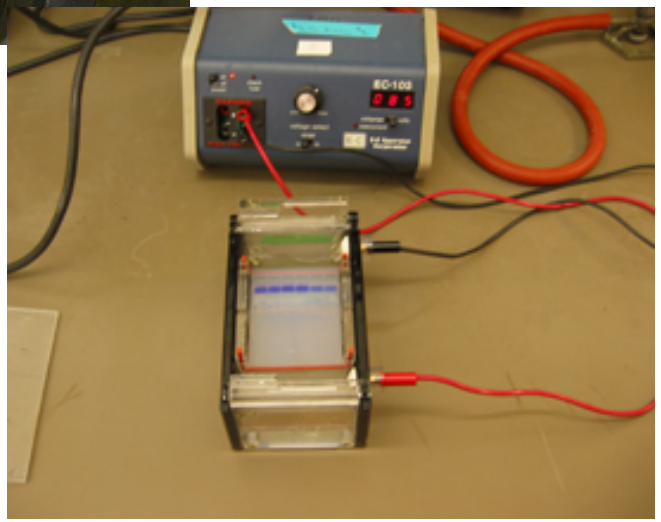

RADIOACTIVE






FOTO/UV 300

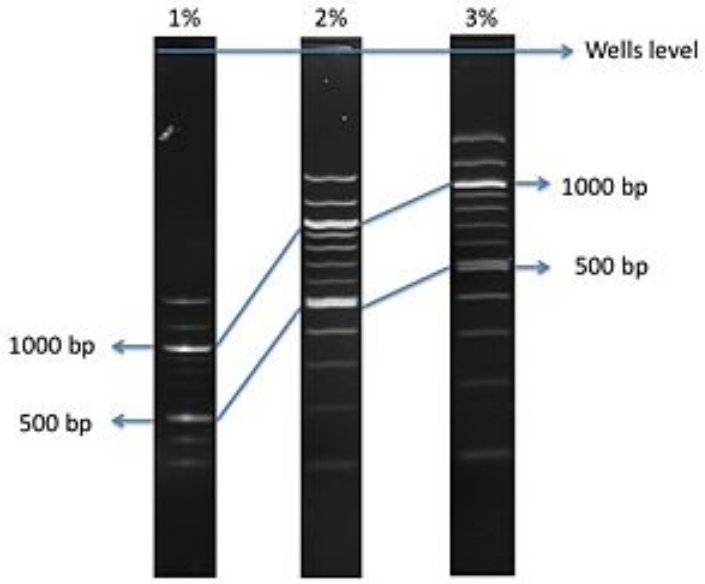

xylene cyanol
bromophenol blue



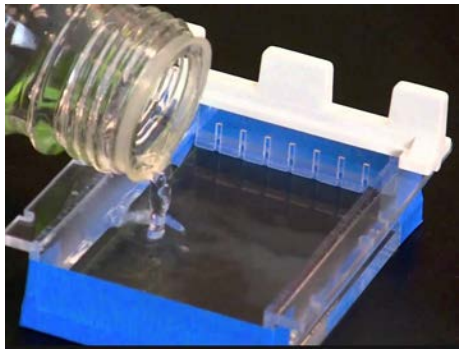
Picking the agarose concentration: gel material from different sources vary slightly in separating ability – the supplier should document how to make up the right concentration for your experiment.

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

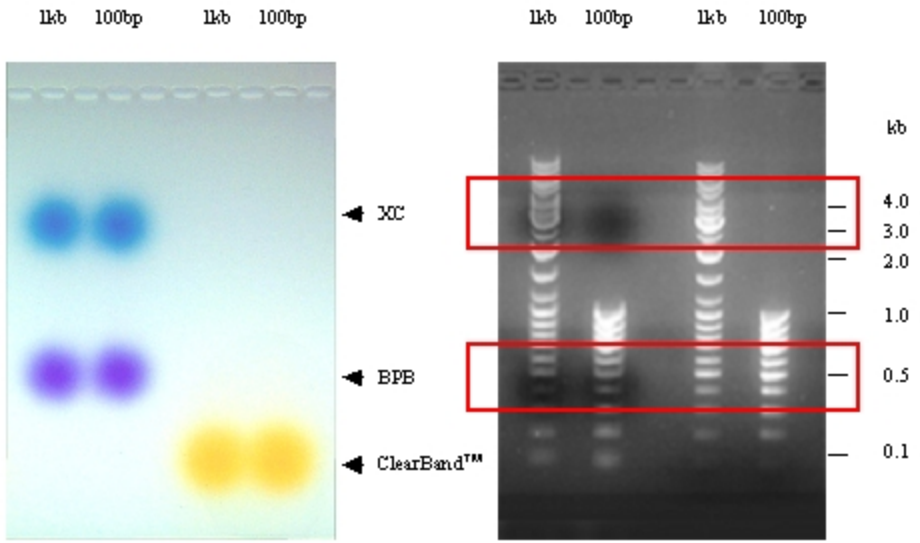


The gel and sample are clear- how can you keep track of the speed of electrophoresis?



- Visible dyes are used to judge approximate travelling distance of the sample in the gel.
  - Bromophenol blue (BPB) and xylene cyanol (XC) are the most common tracking dyes
    - Note: They have a net negative charge and so they migrate in the same direction as DNA in the lectric field.

1.5% agarose in 1X TBE buffer with 1:10,000 of 10mg/ml ethidium bromide.



Dye ‘sizes’: BPB ~300bp, XC ~4kbp)

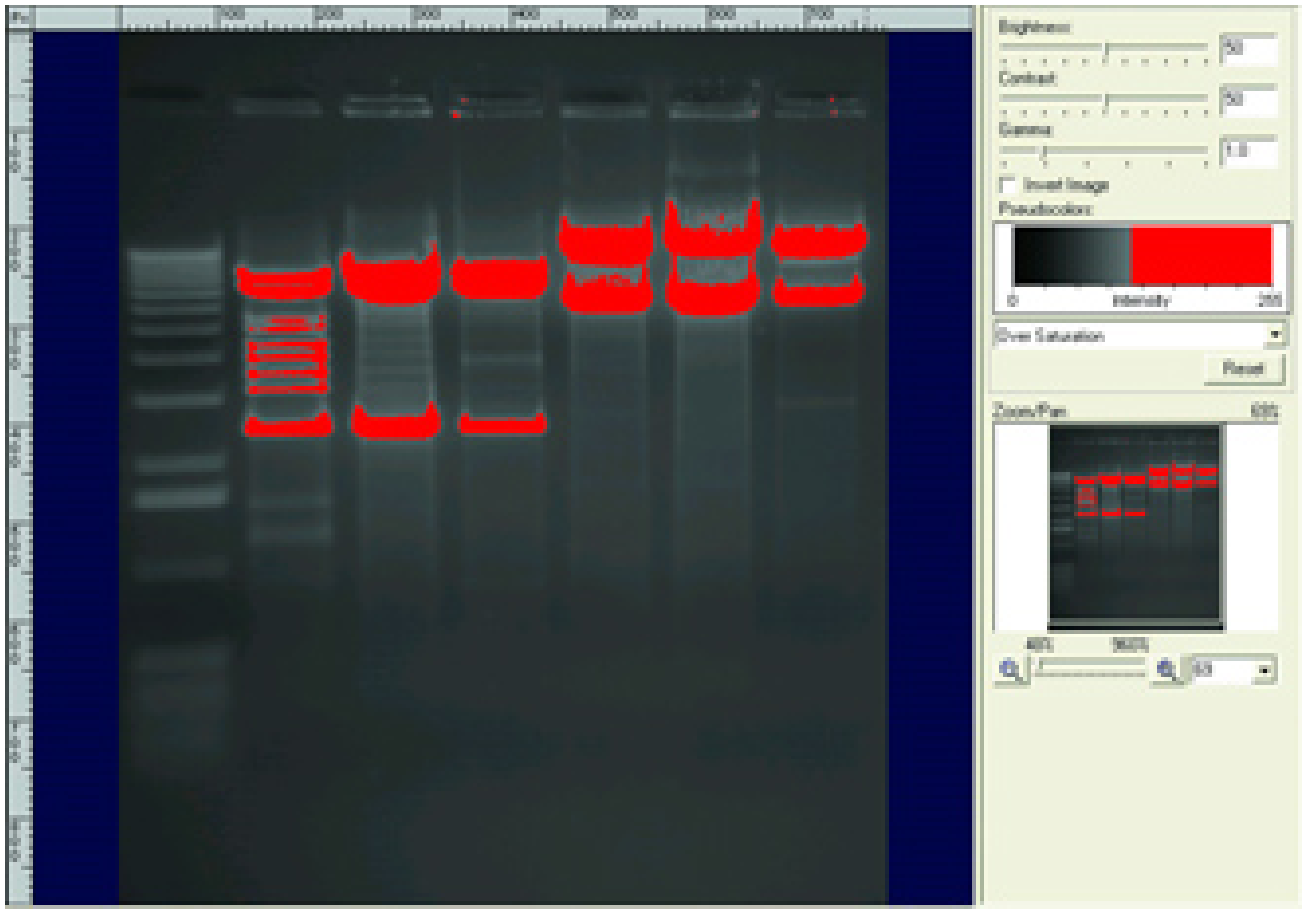
Preparation notes for Agarose gels.

- Best band resolution comes with thin combs (<1mm) and wide teeth.
  - Clean everything! With deionized water.
  - Use a very thin buffer layer covering the gel.
- Use 0.5X or 1X TBE for best resolution of DNA
- Let agarose powder hydrate in the buffer for ~2min before heating it in a microwave – add powder to buffer slowly, with swirling, to prevent clumping.
- Heat in the microwave on high at 20 sec intervals, swirling between.
  - Wear goggles
  - Wear heat-protective gloves
  - Point mouth of flask away from yourself and anyone else when you remove it from the microwave and when you swirl it around.
- Cool to 50-60C before pouring
  - You can run cool water over outside of flask while swirling, for about 1 minute
  - You can put it in 60C water bath for 15 minutes.
  - Try to avoid bubbles.
  - If you are using ethidium bromide add it after the solution has cooled just *prior to pouring*.
- Let gel set 30 minutes before putting it in the tank and loading the sample.

Web sites showing principles and animation of gel electrophoresis

- Gel Electrophoresis Virtual Lab
- DNA manipulation - Gel Electropheresis www.dnai.org

Gel Data Analysis



Gel Analysis – what we can learn

- Looking at a gel can tell us (quantitatively) if we
  - got a product that stains like nucleic acid
  - ***If*** we included a size standard we can set up a sizing curve to estimate the length of our product.
  - ***If*** we also have quantities for the amount of each band in the DNA ladder we can use the intensity of the bands to estimate the amount of DNA in our product. All of this requires an image and software that can analyze that image according to the parameters we have described.

Gel Analysis with software

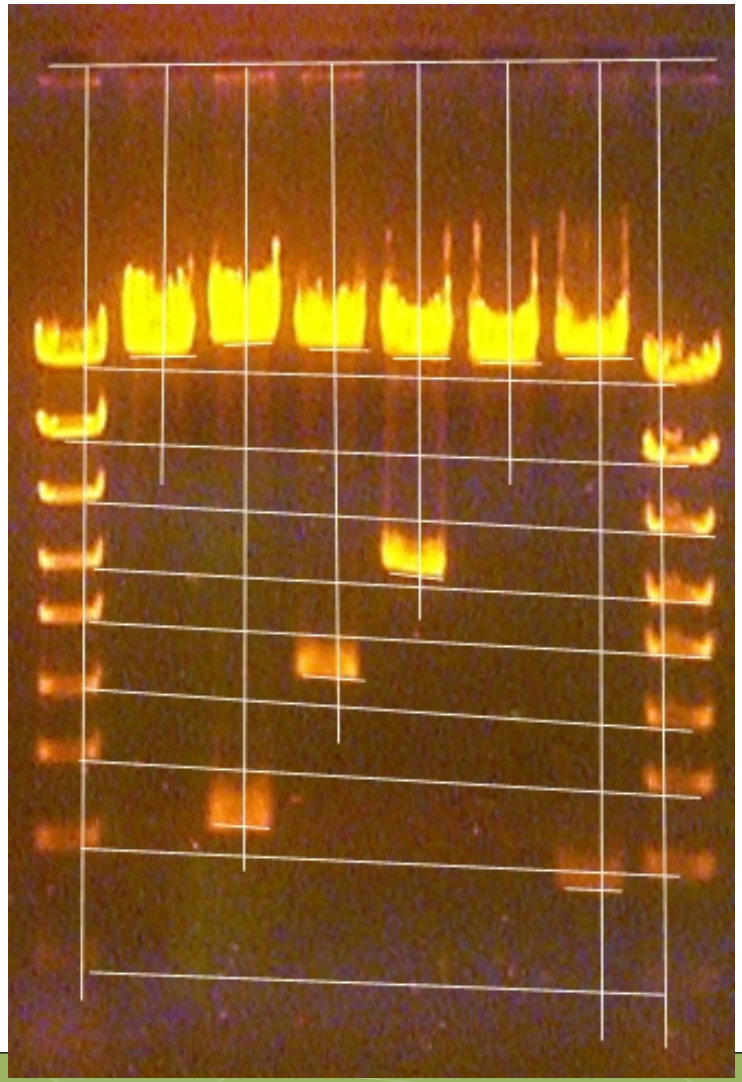
- Looking at a gel can tell us (quantitatively) if we got a product that stains like nucleic acid, and if we included a size standard we can set up a sizing curve to interpolate the length of our product. If we also have quantities for the amount of each band in the DNA ladder we can use the intensity of the bands to estimate the amount of DNA in our product. All of this requires an image and software that can analyze that image according to the parameters we have described.

Software programs use size standard length and mass to provide approximations for your unknown bands.

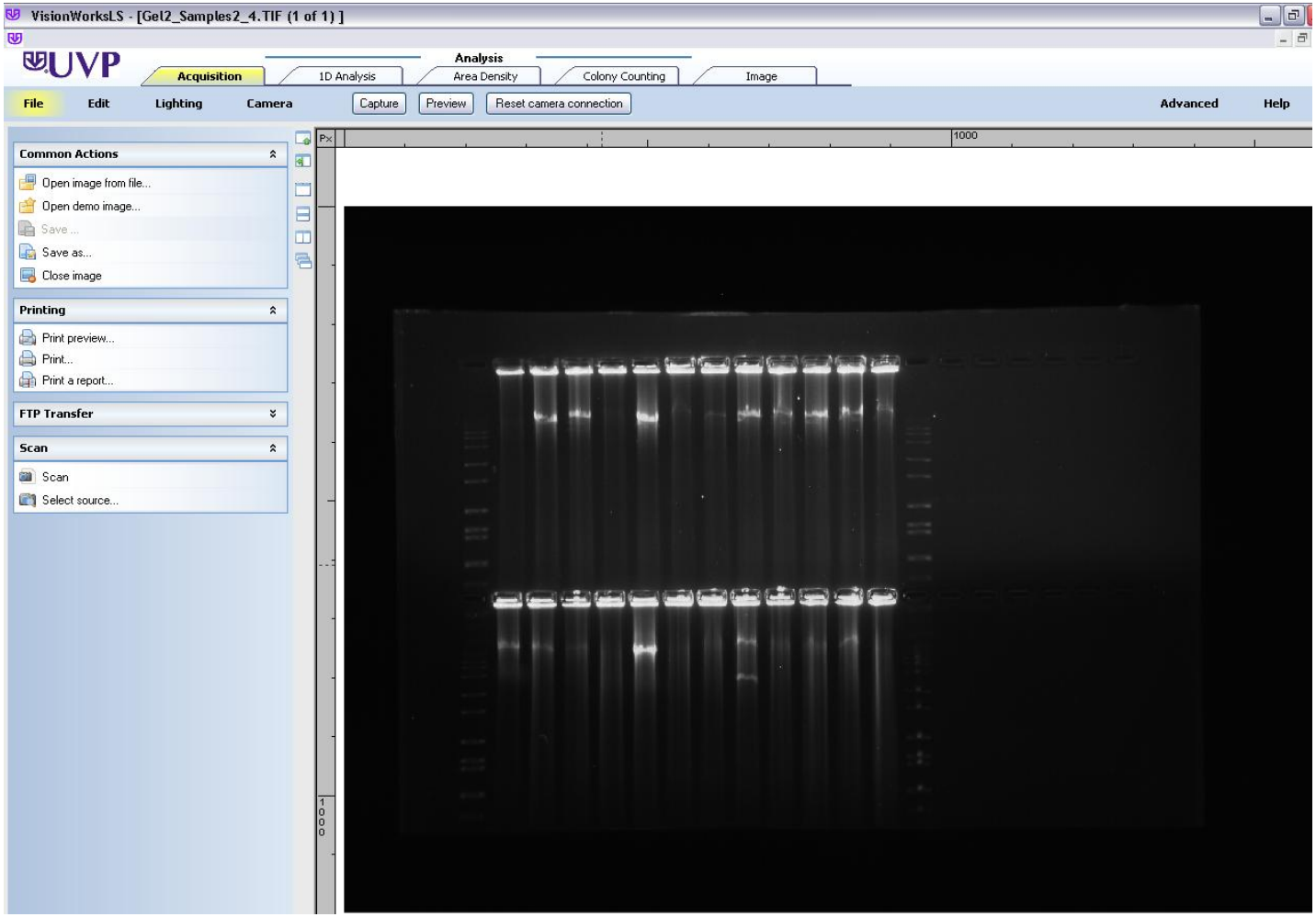
The software can correct for defects in the gel such as bright spots, the ‘smile’ migration shown here.

(note that the smile problem shows why you should include size standards on both edges if an accurate size estimate is important to you).

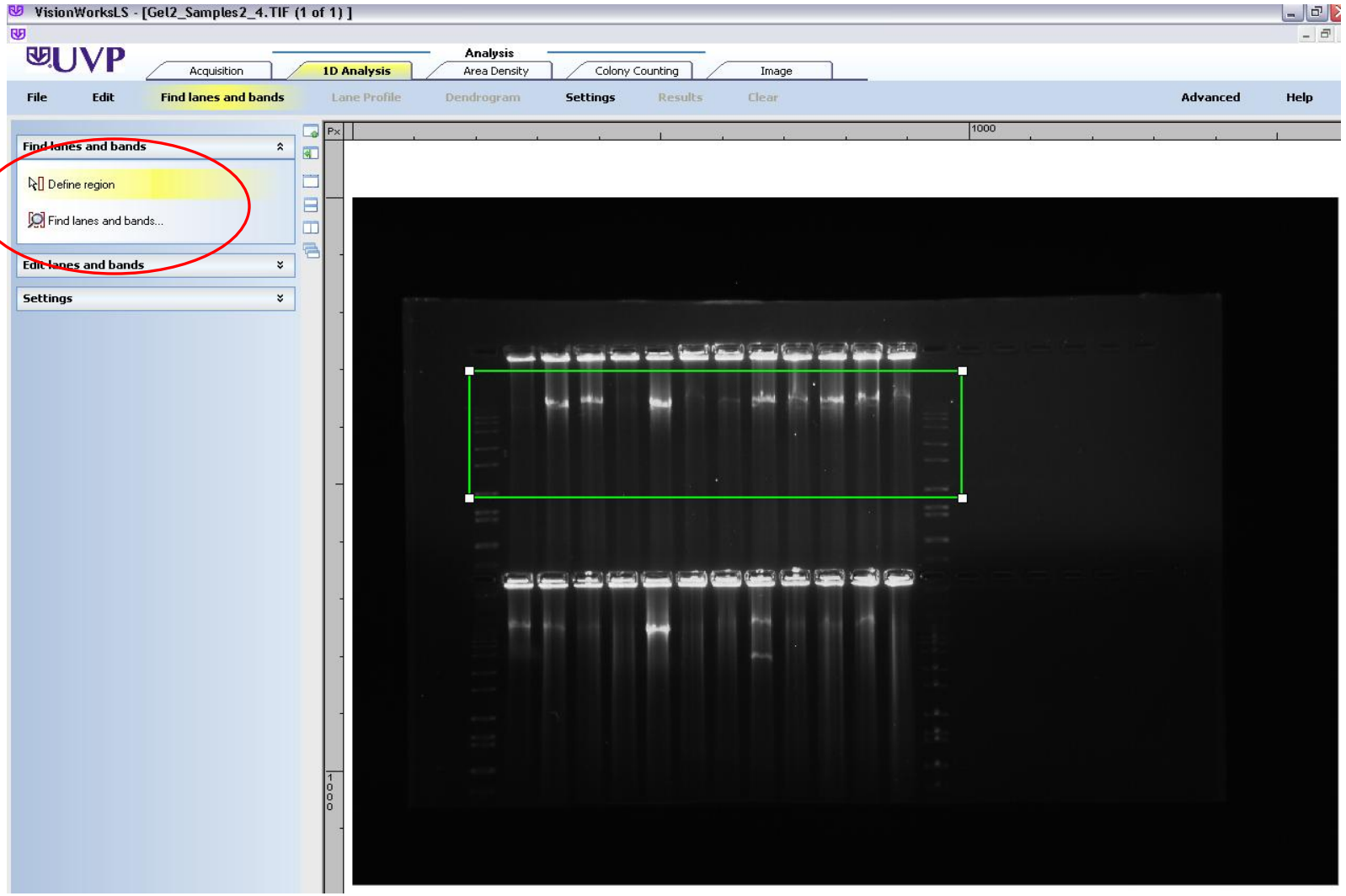
Does the color matter?
No, software uses grey-scale tif images (but make sure it can handle the encoding and information resolution, 8-bit, 16-bit etc).



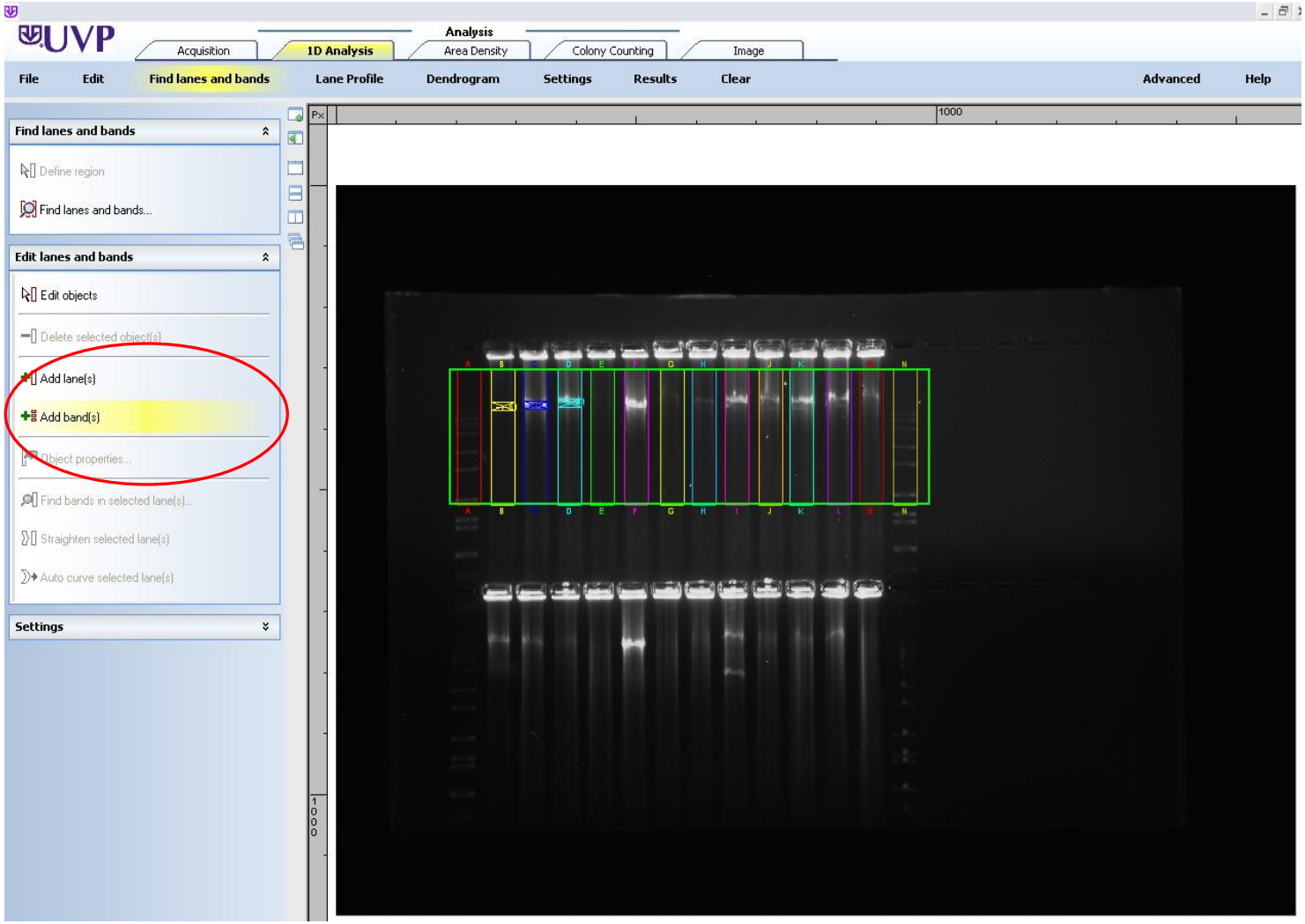
VisionWorksLS tutorial



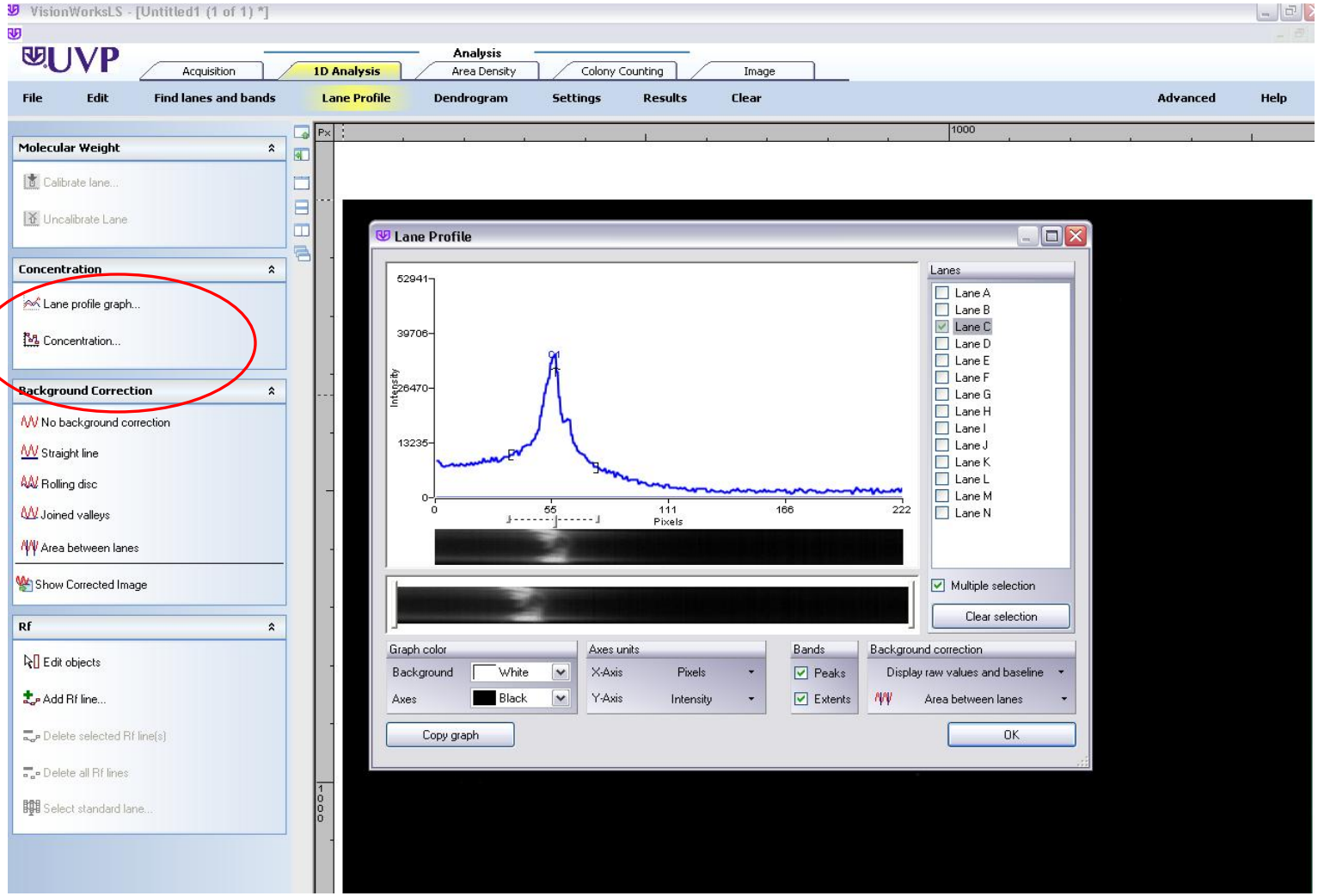
VisionWorksLS tutorial



VisionWorksLS tutorial



VisionWorksLS tutorial



VisionWorksLS tutorial

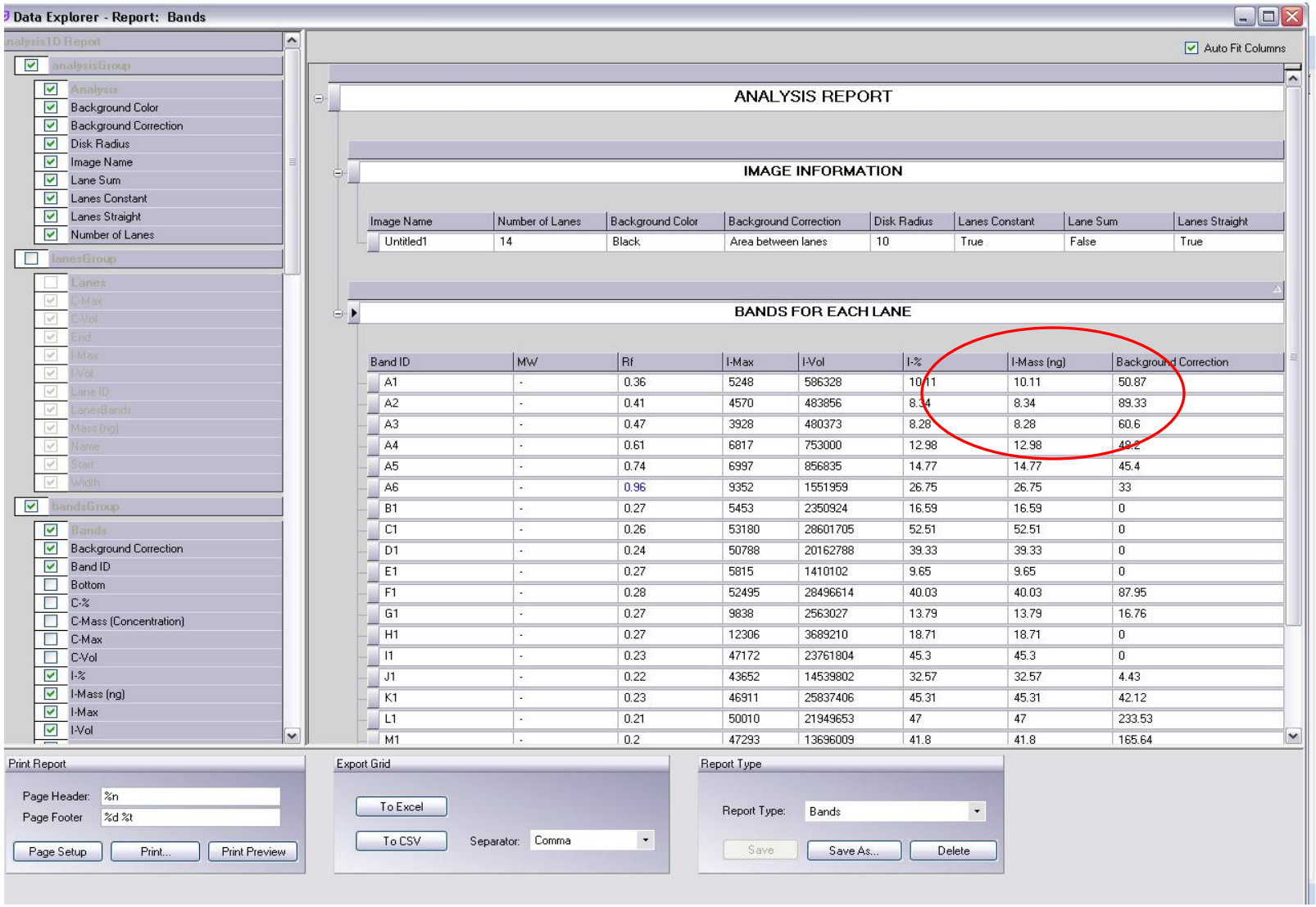


Image Name	Number of Lanes	Background Color	Background Correction	Disk Radius	Lanes Constant	Lane Sum	Lanes Straight
Untitled1	14	Black	Area between lanes	10	True	False	True

Band ID	MW	Rf	I-Max	I-Vol	I-%	I-Mass (ng)	Background Correction
A1	-	0.36	5248	586328	10.11	10.11	50.87
A2	-	0.41	4570	483856	8.34	8.34	89.33
A3	-	0.47	3928	480373	8.28	8.28	60.6
A4	-	0.61	6817	753000	12.98	12.98	48.2
A5	-	0.74	6997	856835	14.77	14.77	45.4
A6	-	0.96	9352	1551959	26.75	26.75	33
B1	-	0.27	5453	2350924	16.59	16.59	0
C1	-	0.26	53180	28601705	52.51	52.51	0
D1	-	0.24	50788	20162788	39.33	39.33	0
E1	-	0.27	5815	1410102	9.65	9.65	0
F1	-	0.28	52495	28496614	40.03	40.03	87.95
G1	-	0.27	9838	2563027	13.79	13.79	16.76
H1	-	0.27	12306	3689210	18.71	18.71	0
I1	-	0.23	47172	23761804	45.3	45.3	0
J1	-	0.22	43652	14539802	32.57	32.57	4.43
K1	-	0.23	46911	25837406	45.31	45.31	42.12
L1	-	0.21	50010	21949653	47	47	233.53
M1	-	0.2	47293	13696009	41.8	41.8	165.64