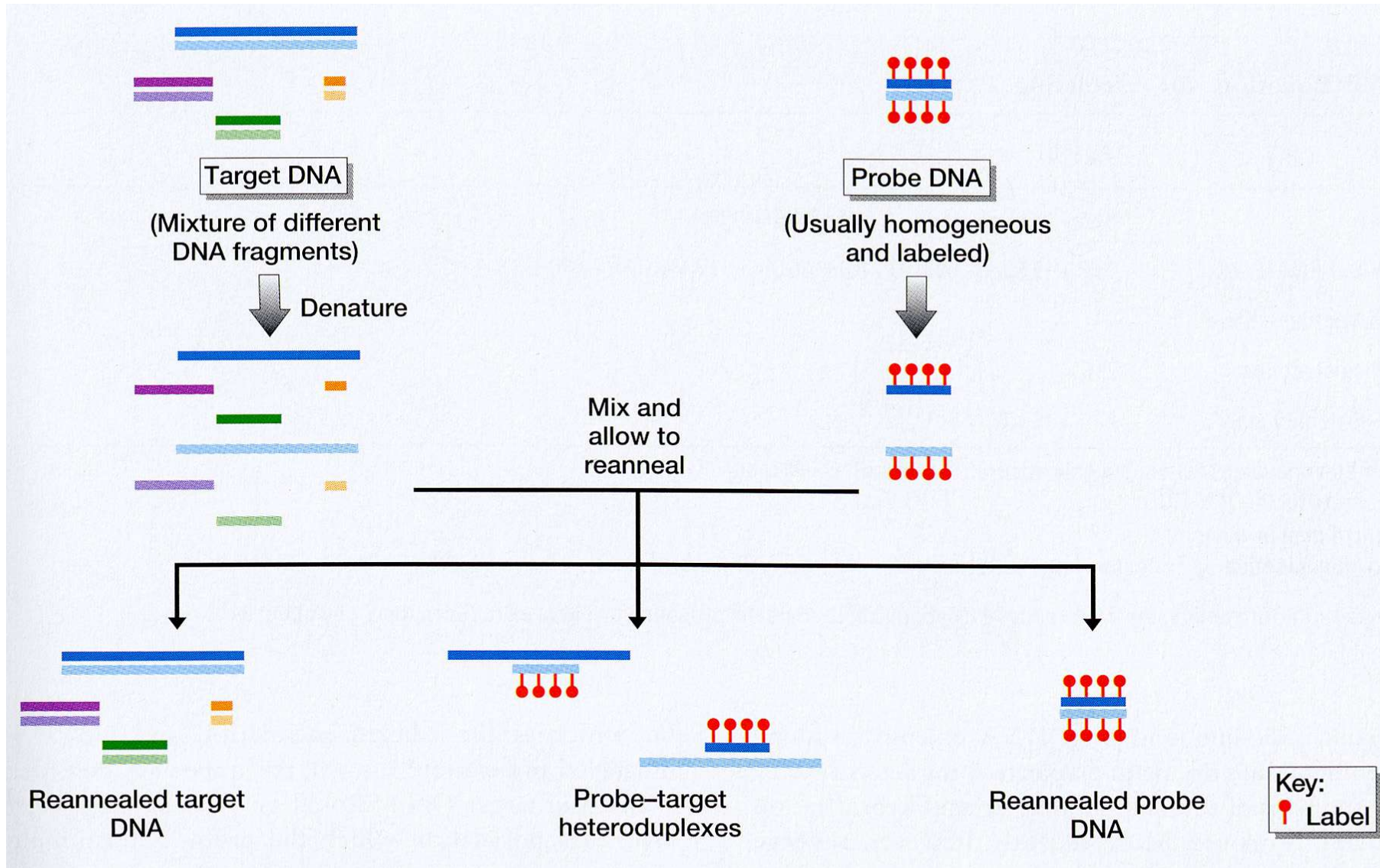


Genetik für Bioinformatiker

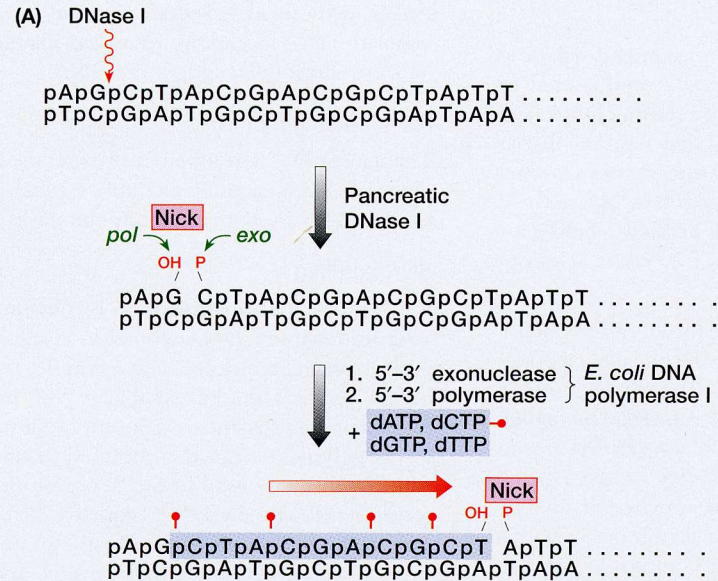
Vorlesung 18.05.04

(Reinhard Ullmann, MPI Berlin)

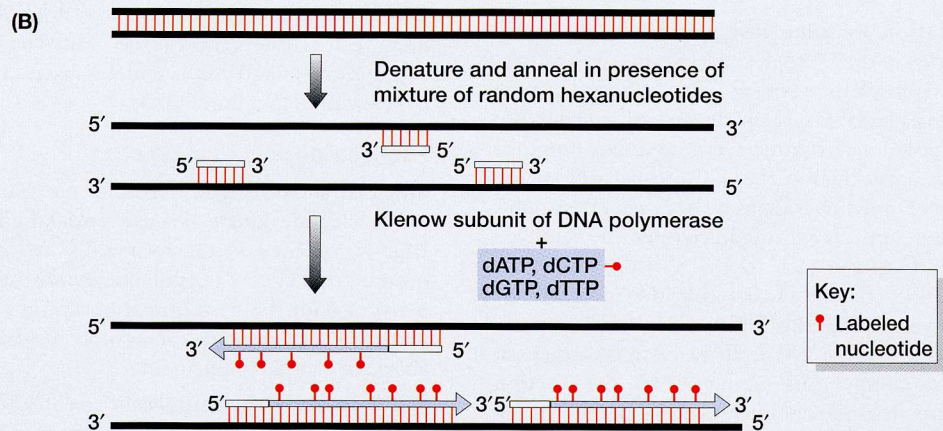
Hybridisieren



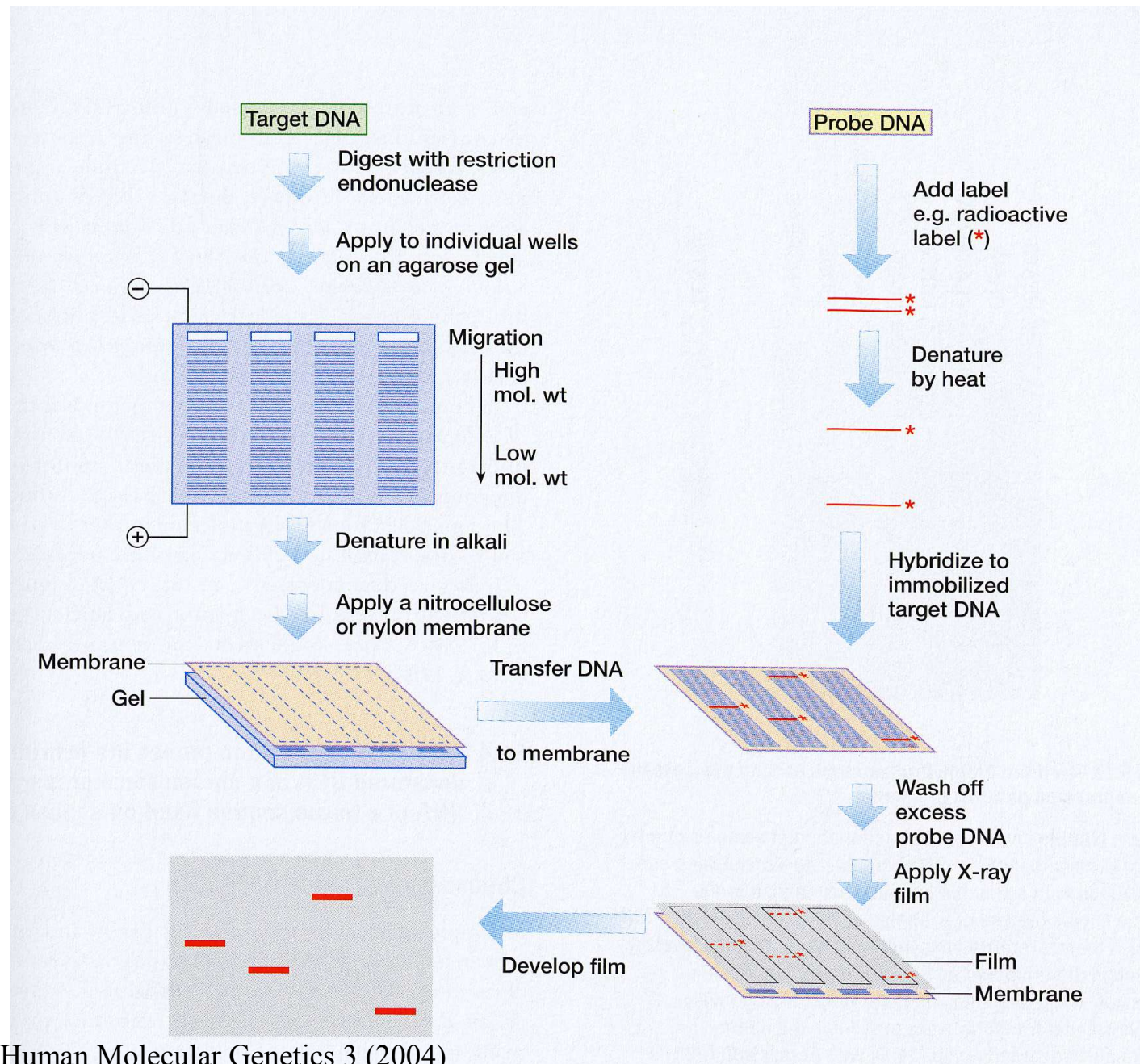
Nick Translation Assay



Random Priming



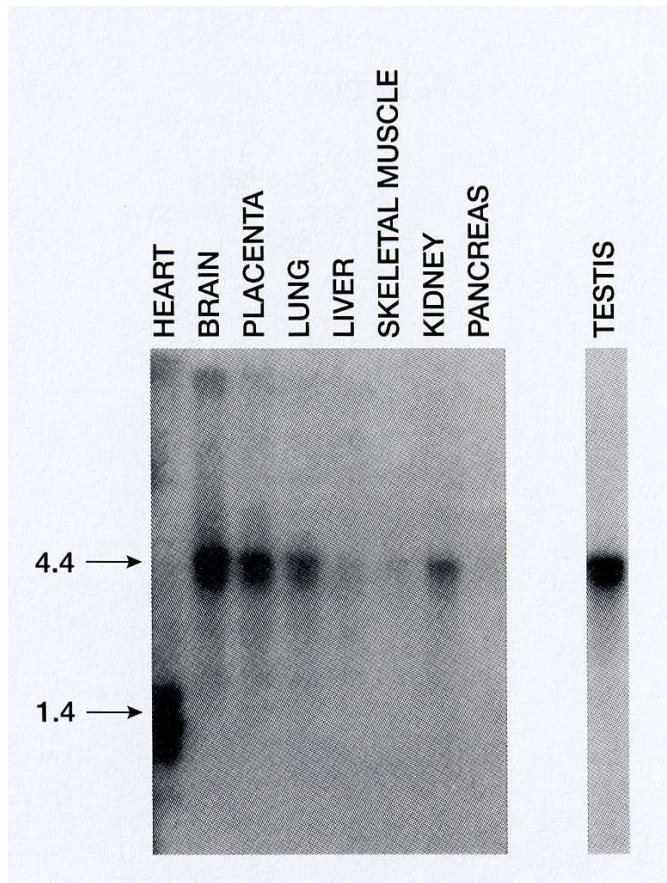
Southern blot



Stringenz

- + Fragmentlänge**
- + Basenzusammensetzung (GC-Gehalt)**
- + Ionenstärke (z.B Natriumionen)**
- + Formamid (senkt Schmelzpunkt der DNA; T_m)**
- + Temperatur**

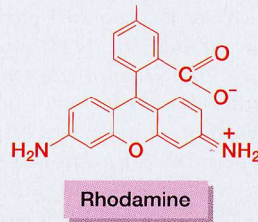
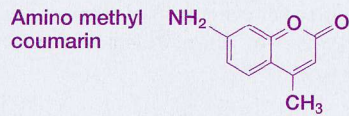
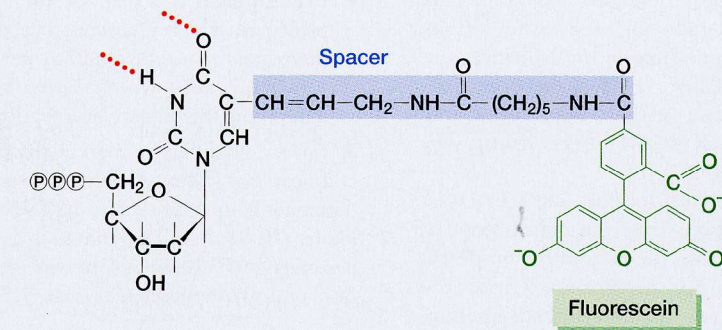
Northern Blot



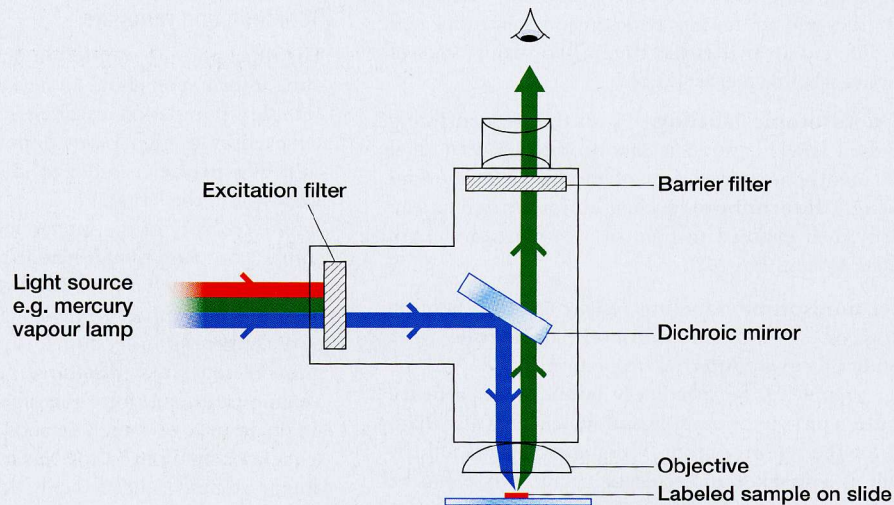
in situ Hybridisierung



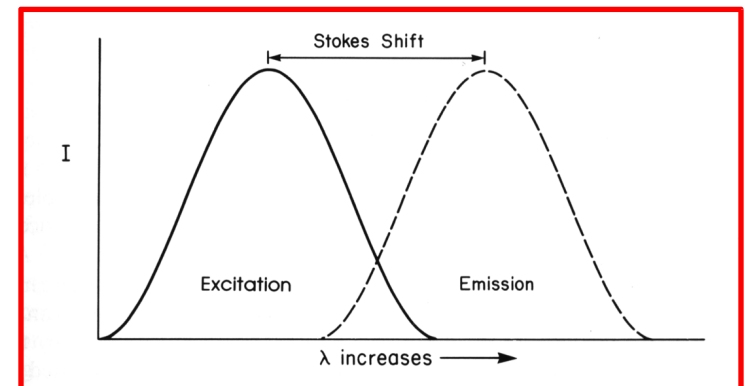
(A)

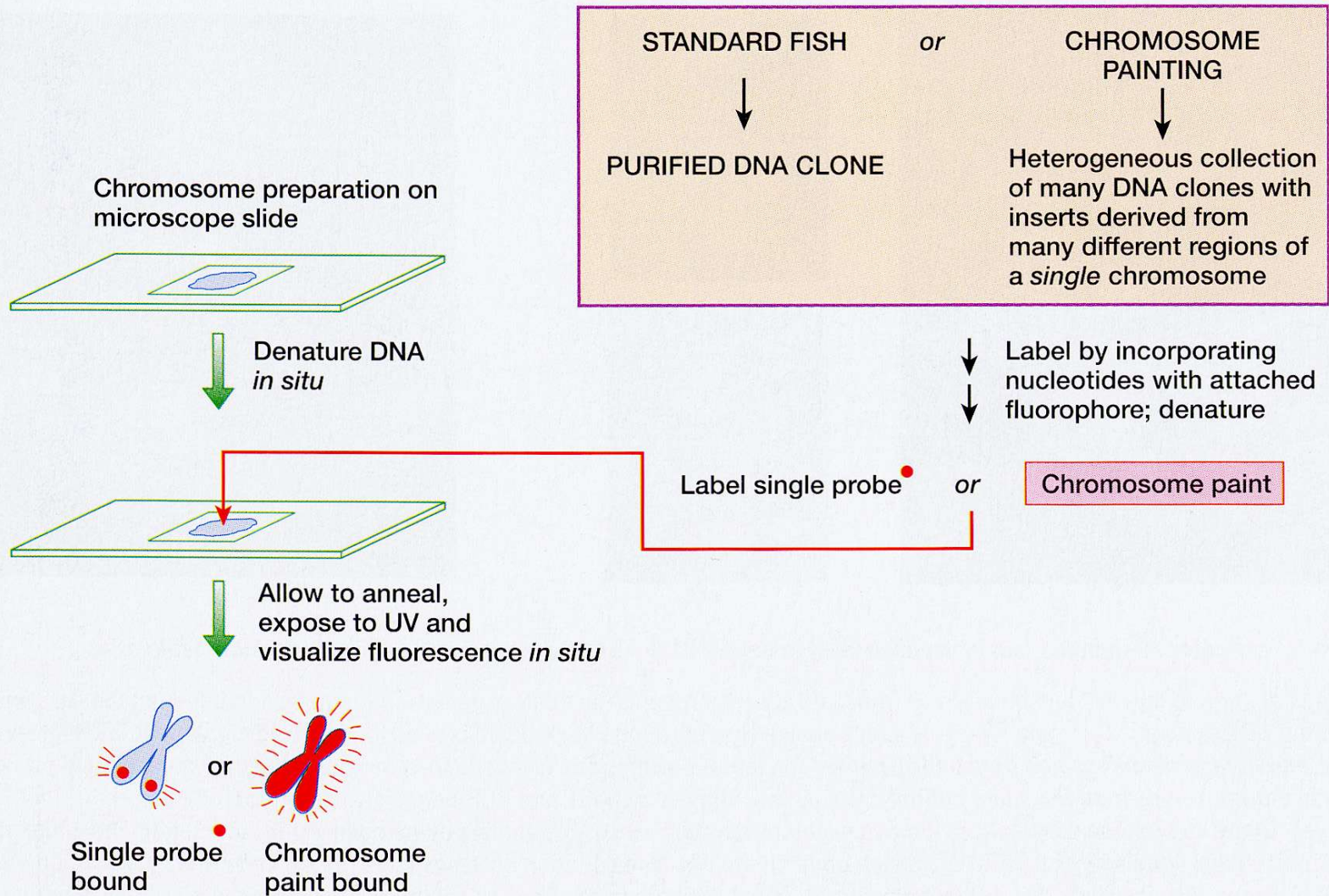


(B)



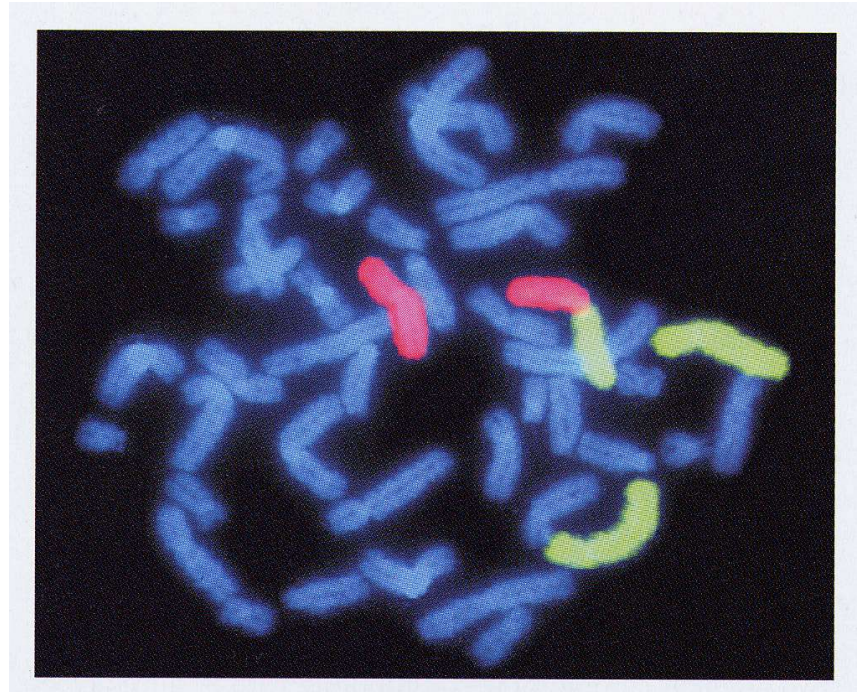
Prinzip der Fluoreszenz





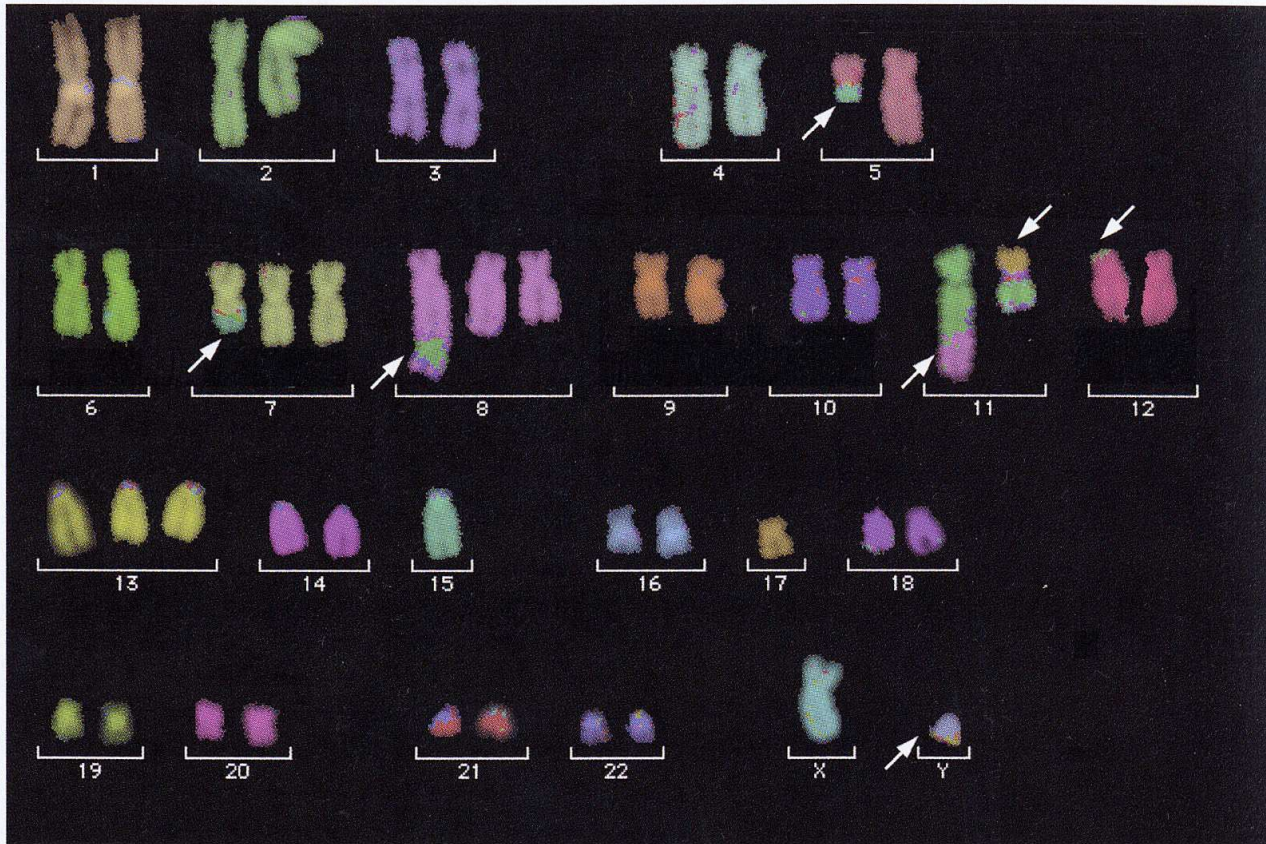


Fluoreszenz in situ Hybridisierung
(FISH) mit Bacterial artificial
chromosome clone (BAC)



FISH mit whole chromosome probes

Multicolor-FISH

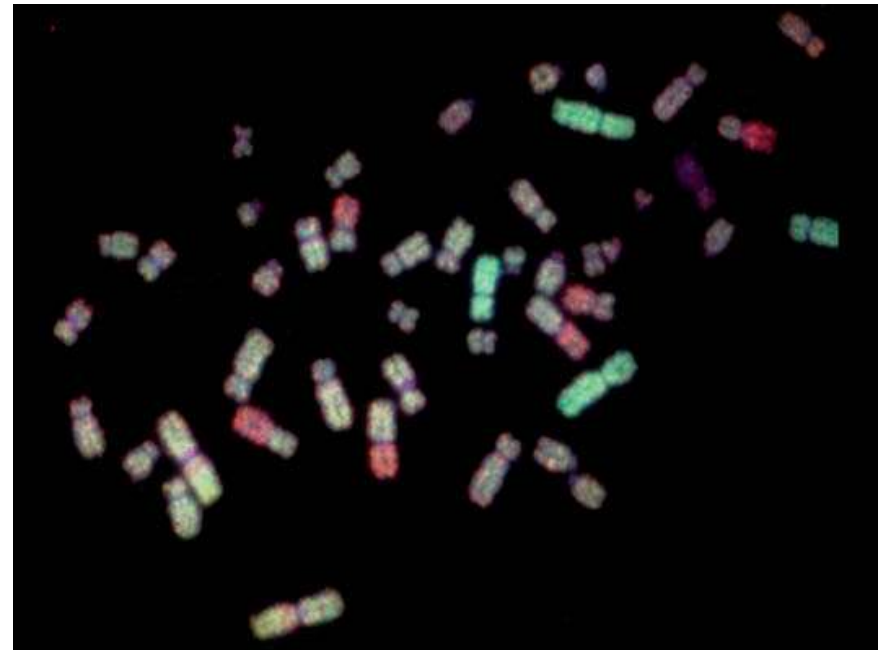
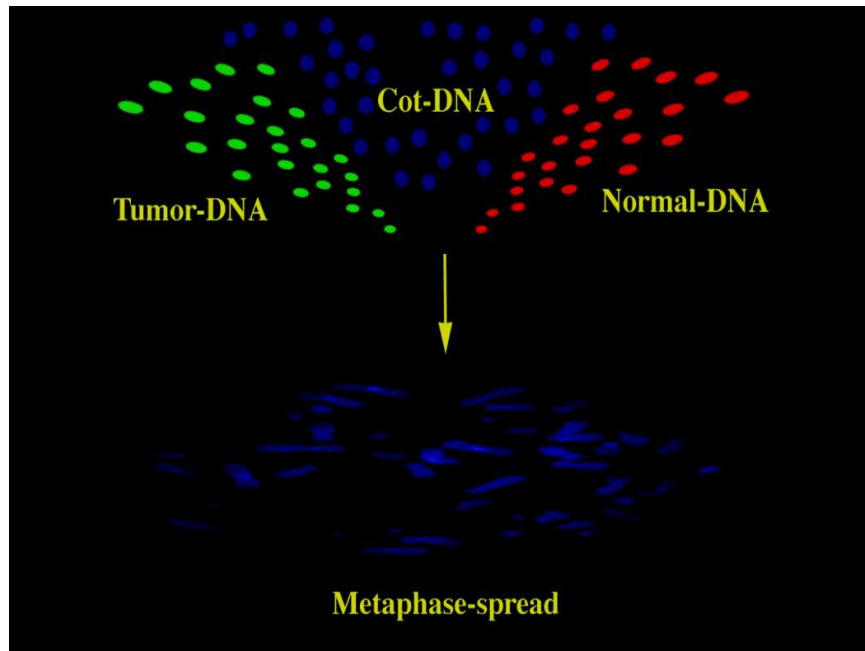


Markierungsschema

#	DEAC Ex 426nm Em 480nm	FITC Ex 502nm Em 530nm	Spectrum Orange™ Ex 558nm Em 588nm	Texas Red® Ex 595nm Em 615nm	Cy™5 Ex 640nm Em 670nm
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
X					
Y					

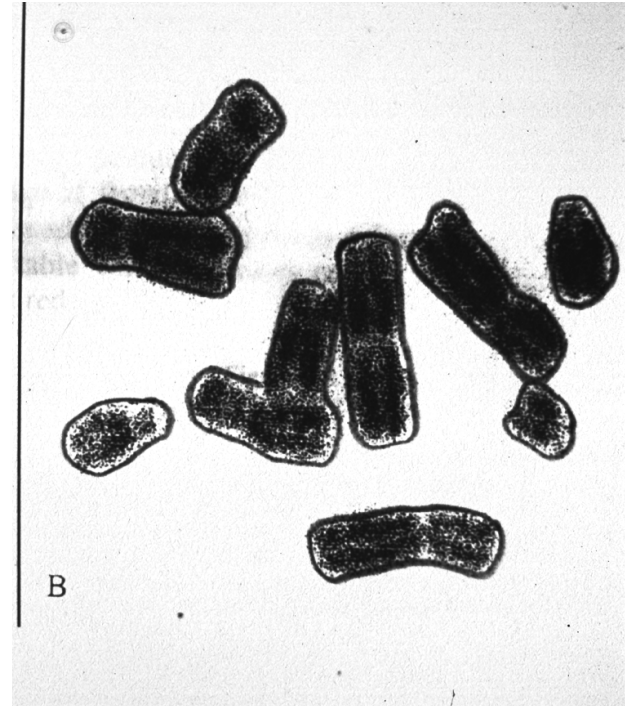
Ex = Excitation Spectra, Em = Emission Spectra

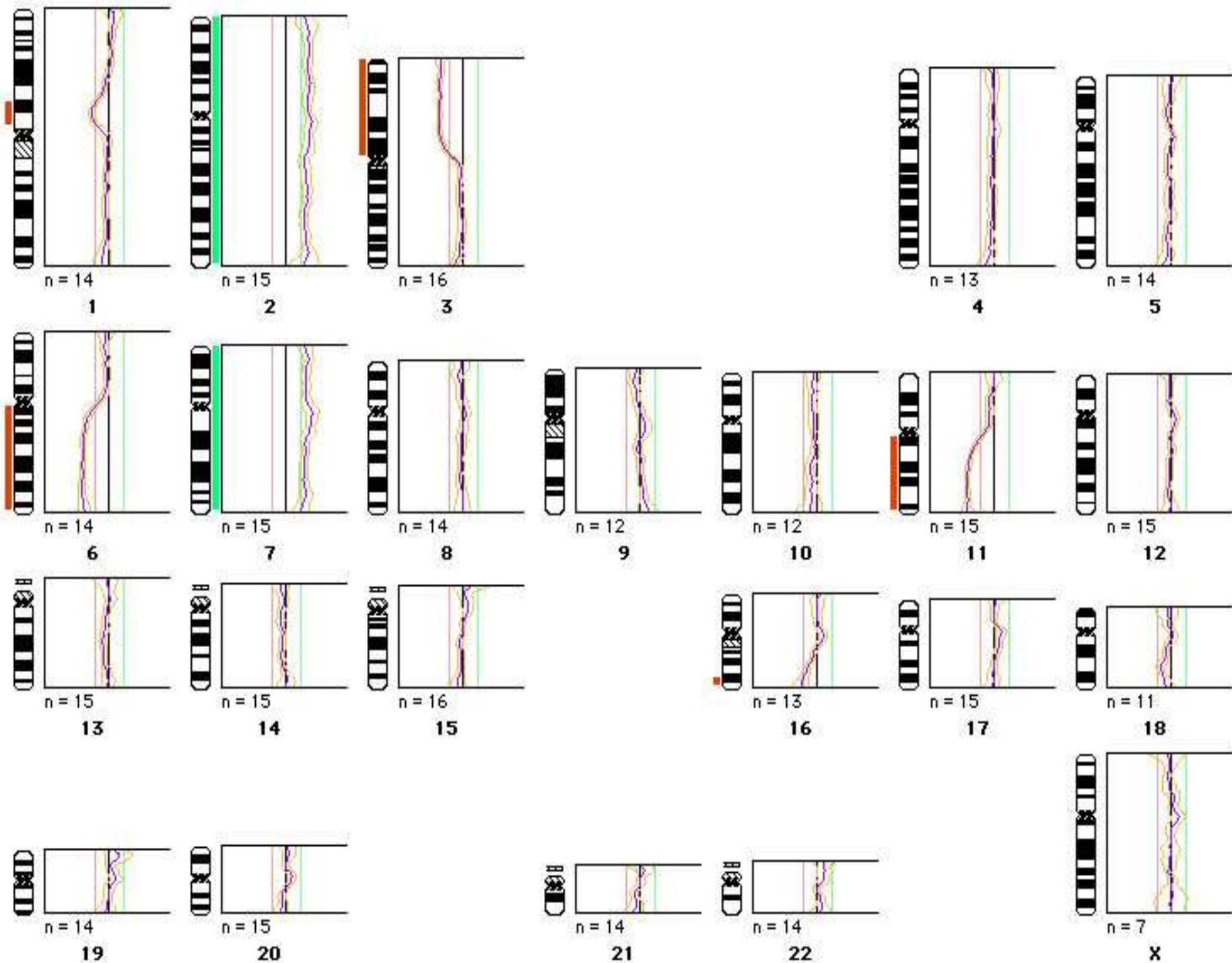
Comparative Genomische Hybridisierung (CGH)



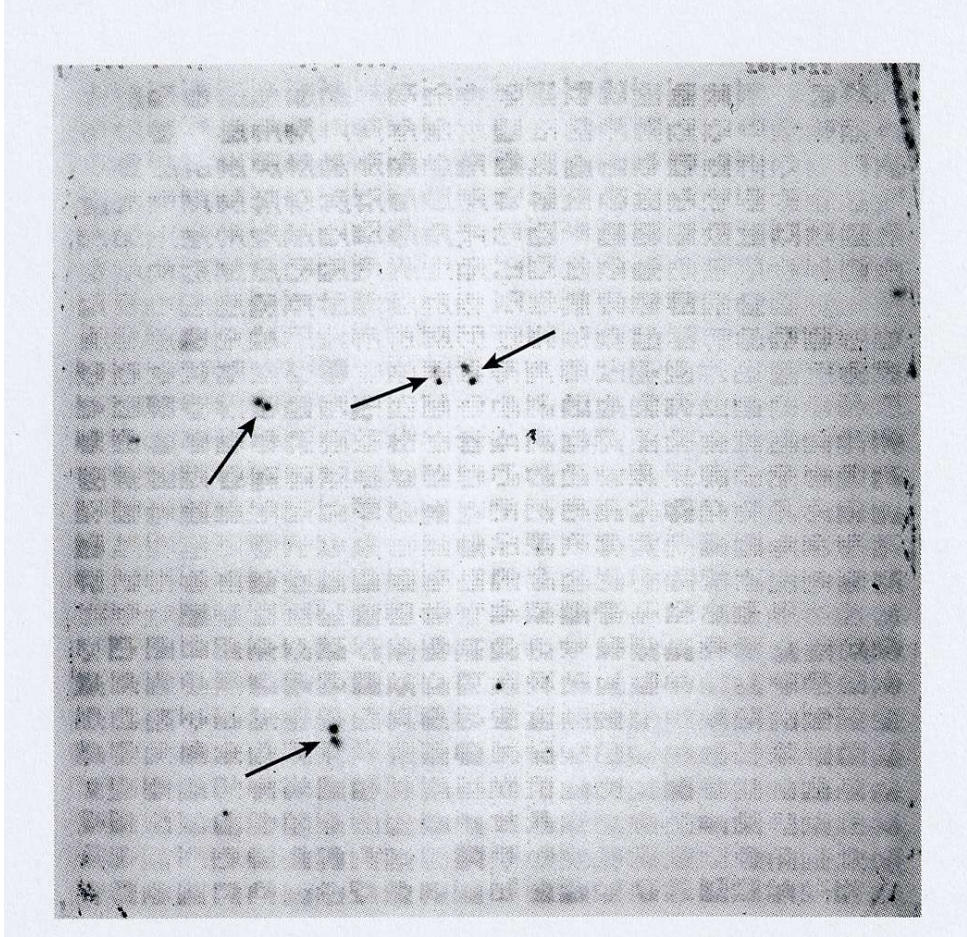
Auswertung

- Segmentierung
- Normalisierung
- Sortieren
- Ratio





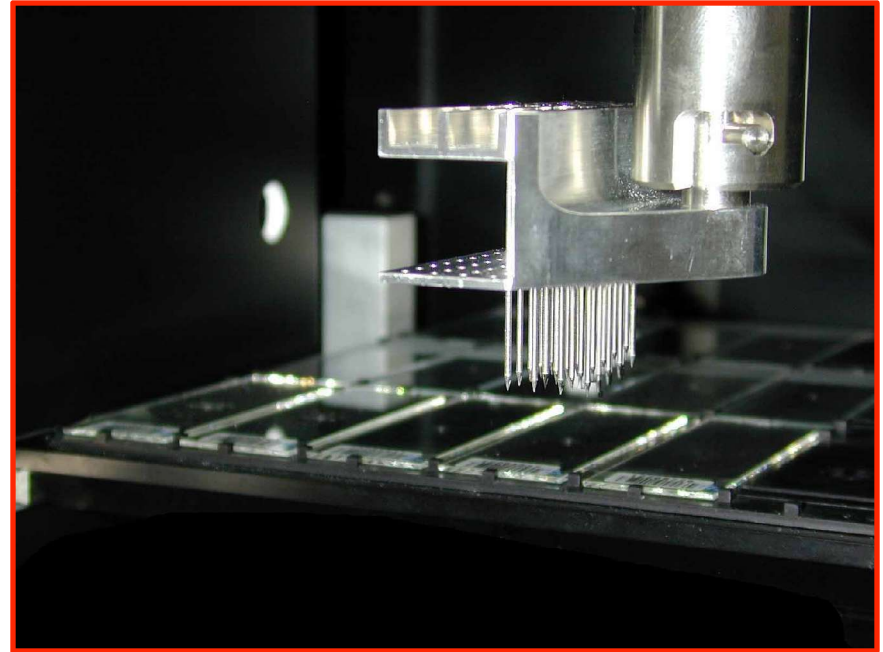
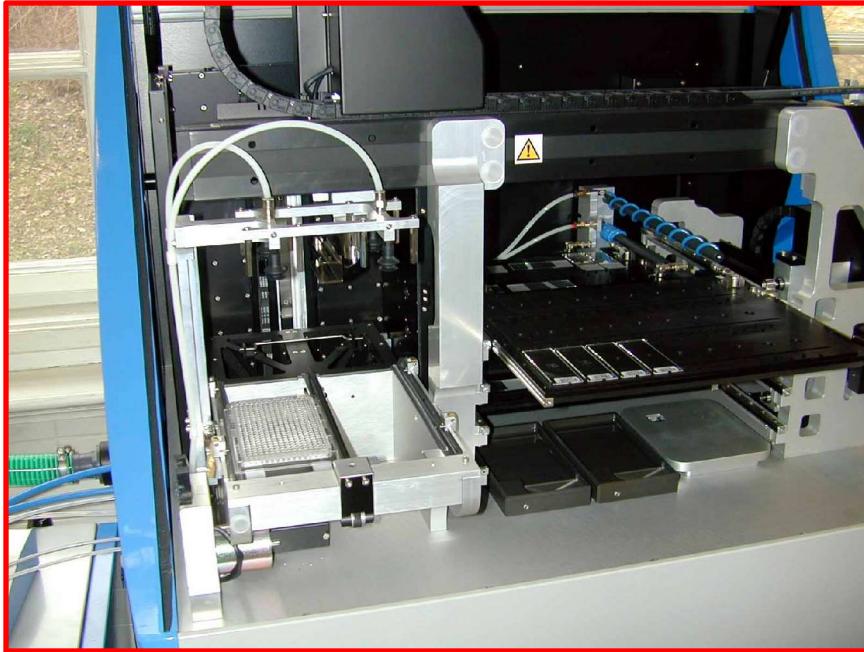
Gridded clone hybridization filters



**Umgekehrt zu Northern
und Southern Blot:**

**Sonden sind gespottet und die zu
untersuchende DNA ist in der
Hybridisierungslösung!**

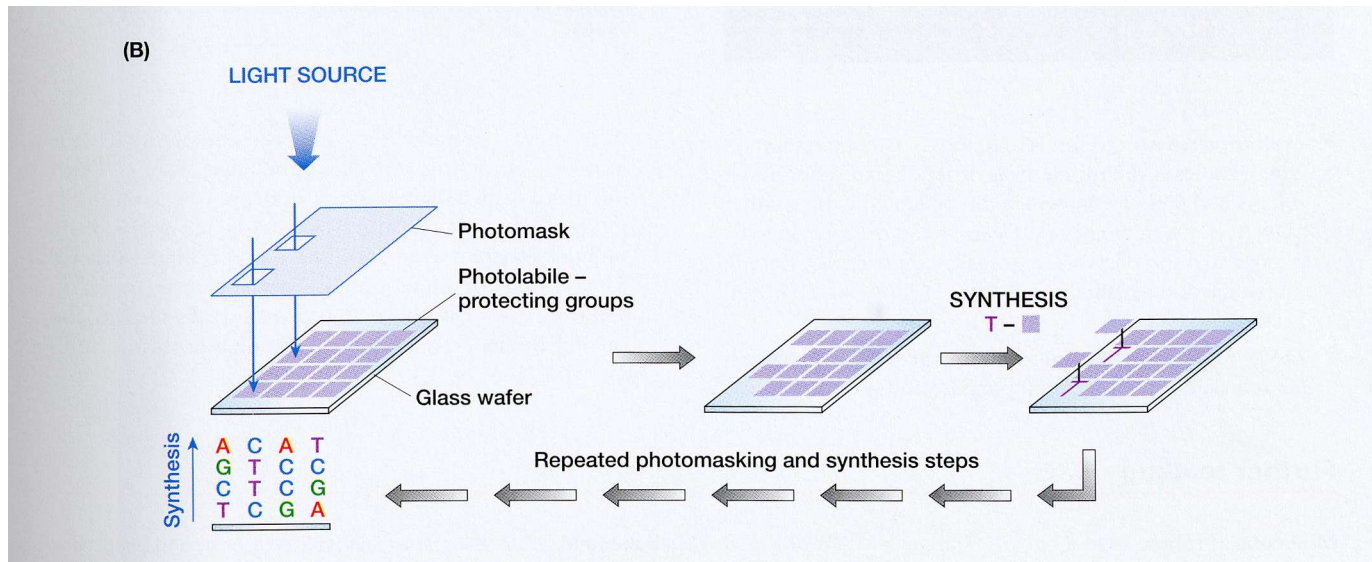
Arrayer für die Produktion von DNA arrays

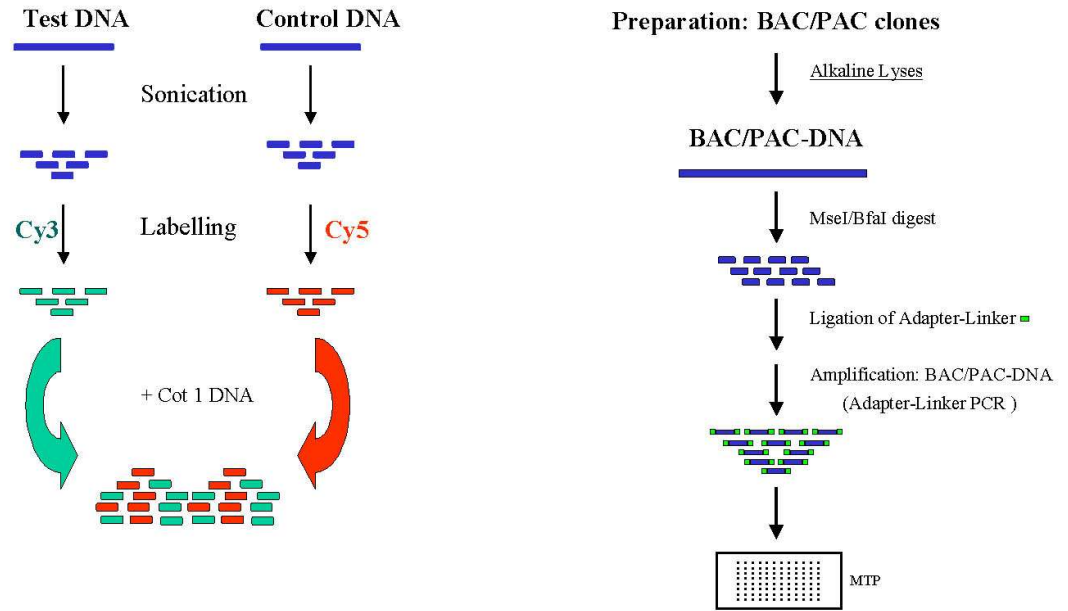


Zwei Prinzipien

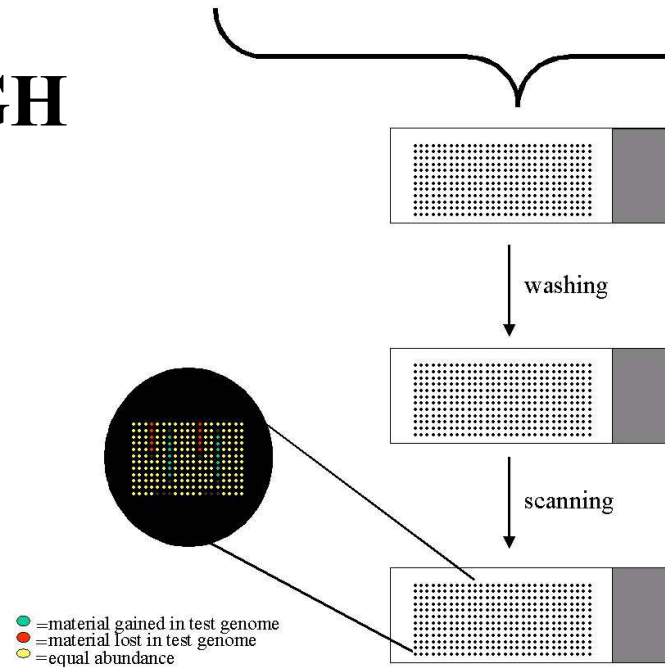
- + Kontaktpotten: Nadel hat Kontakt zum Objektträger
- + Ink-jetting: wie bei Farbdruckern

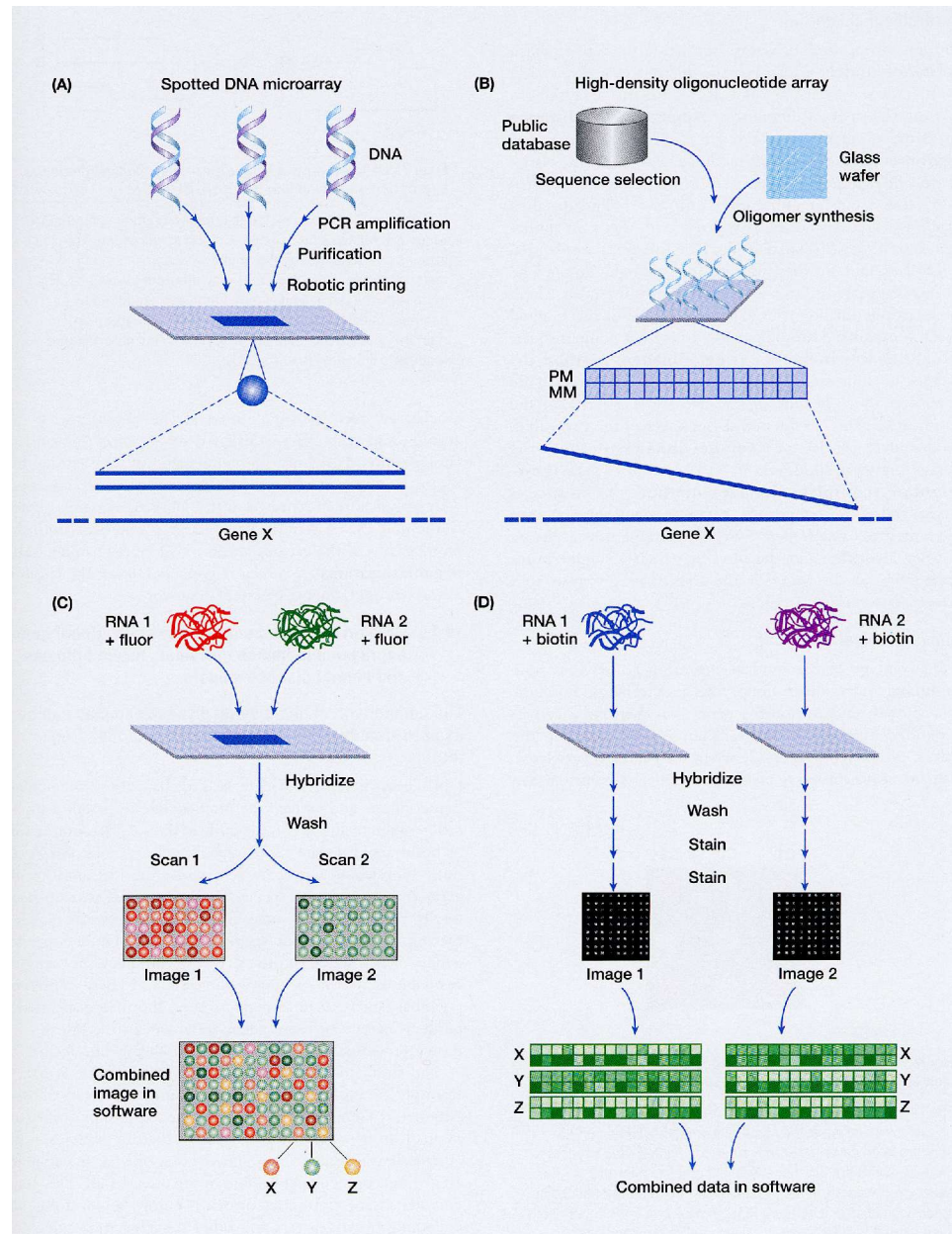
Oligonucleotide Array



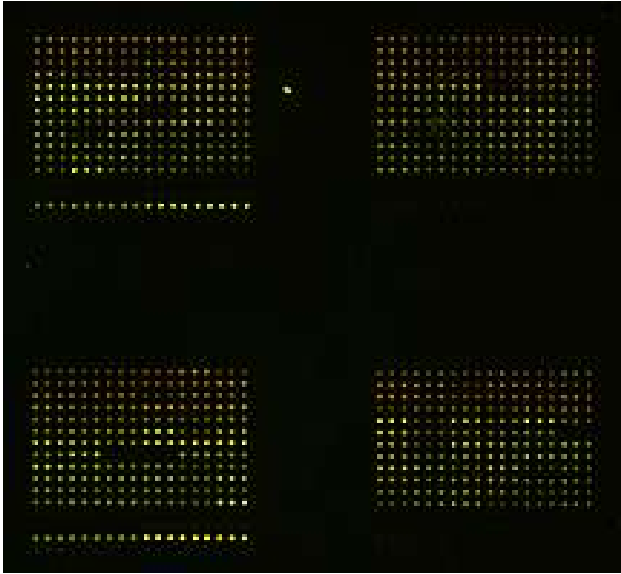


Prinzip der ArrayCGH

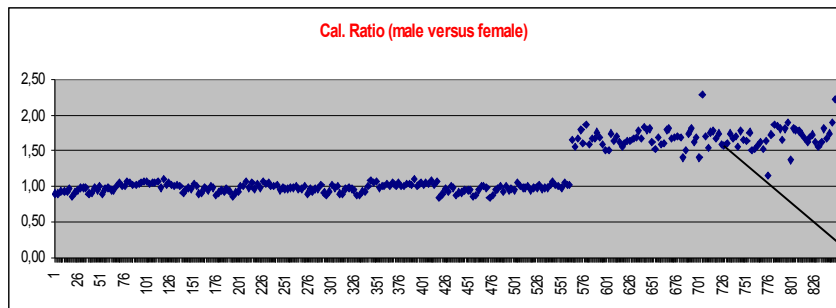




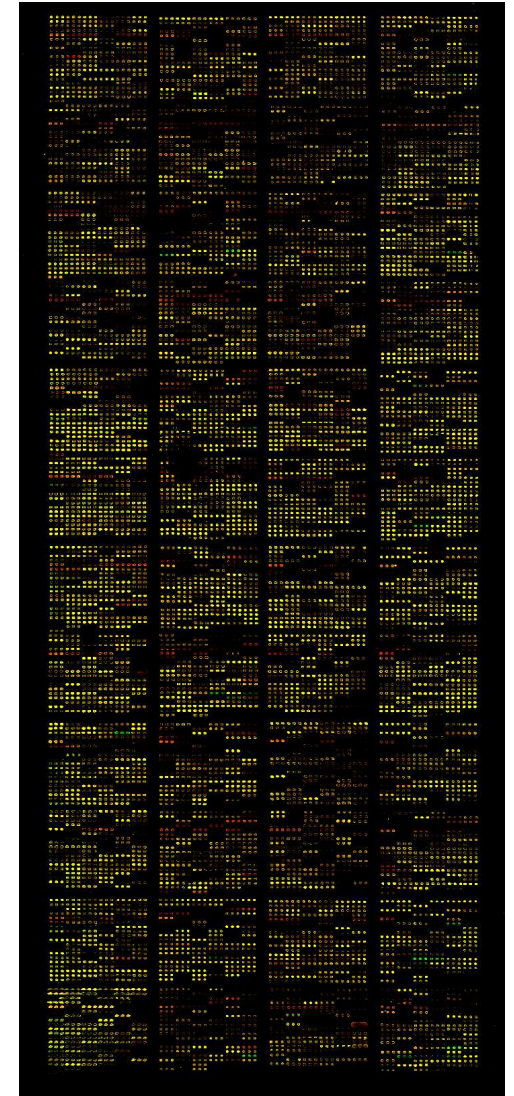
ArrayCGH

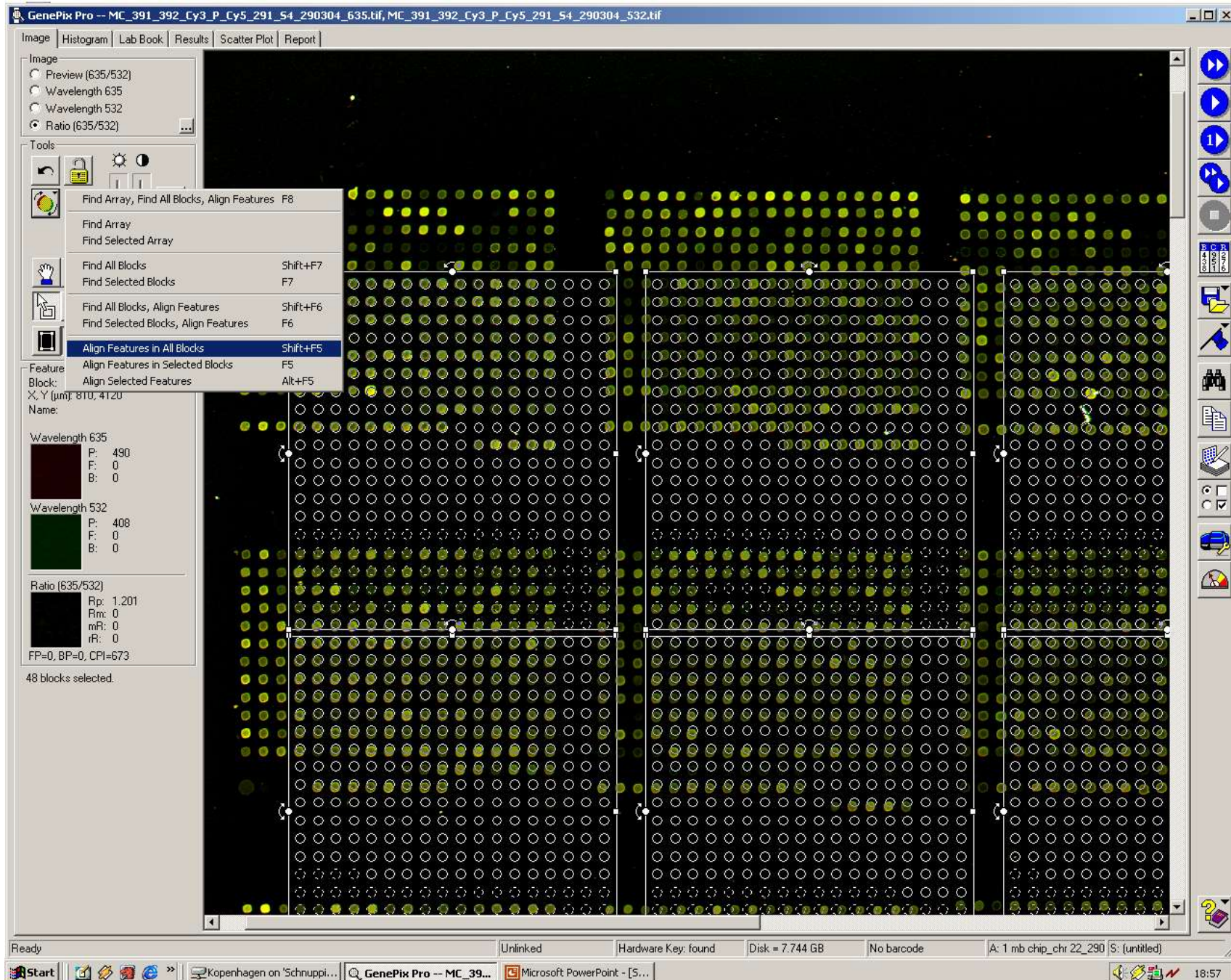


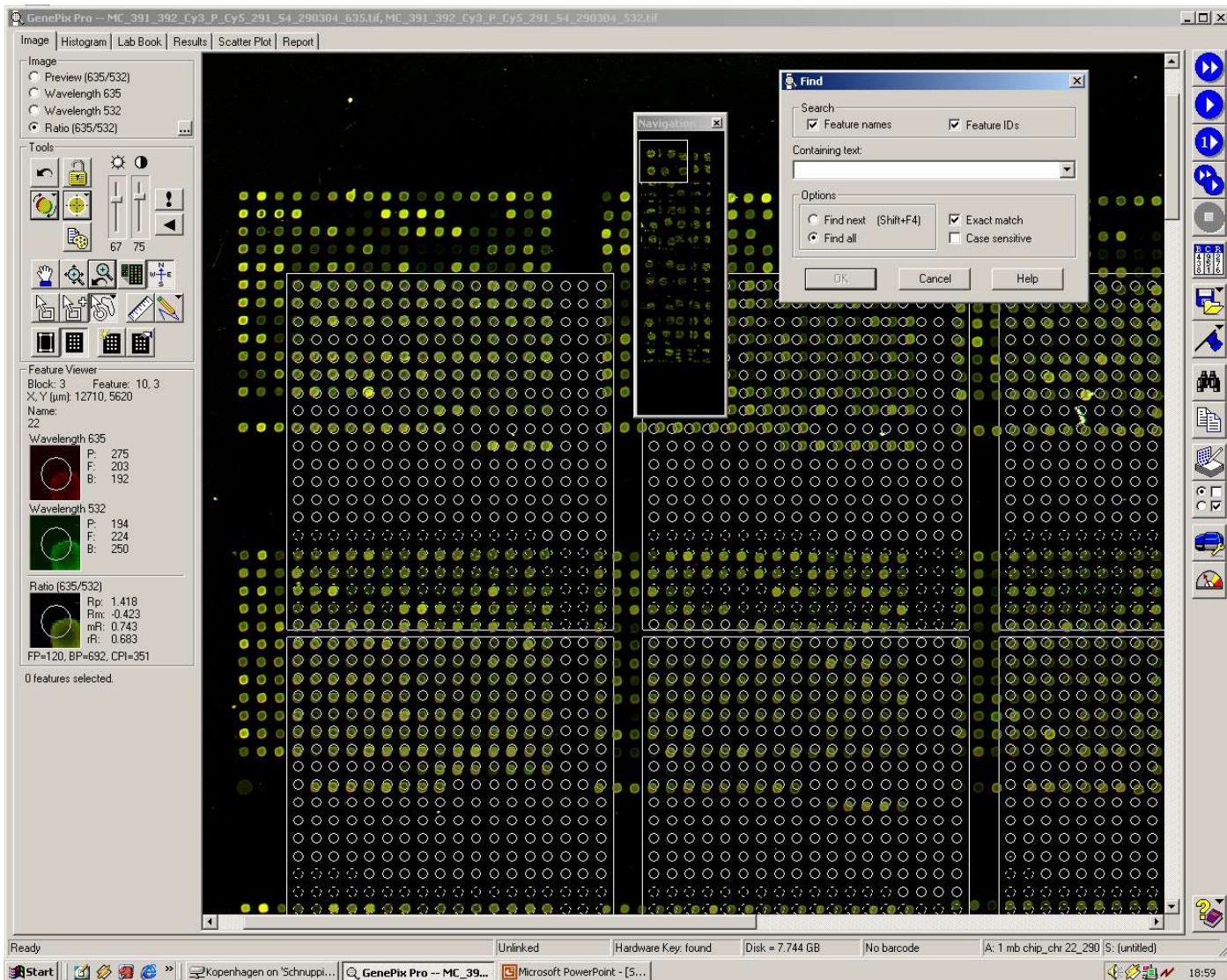
Männlich/Weiblich Hybridisierung

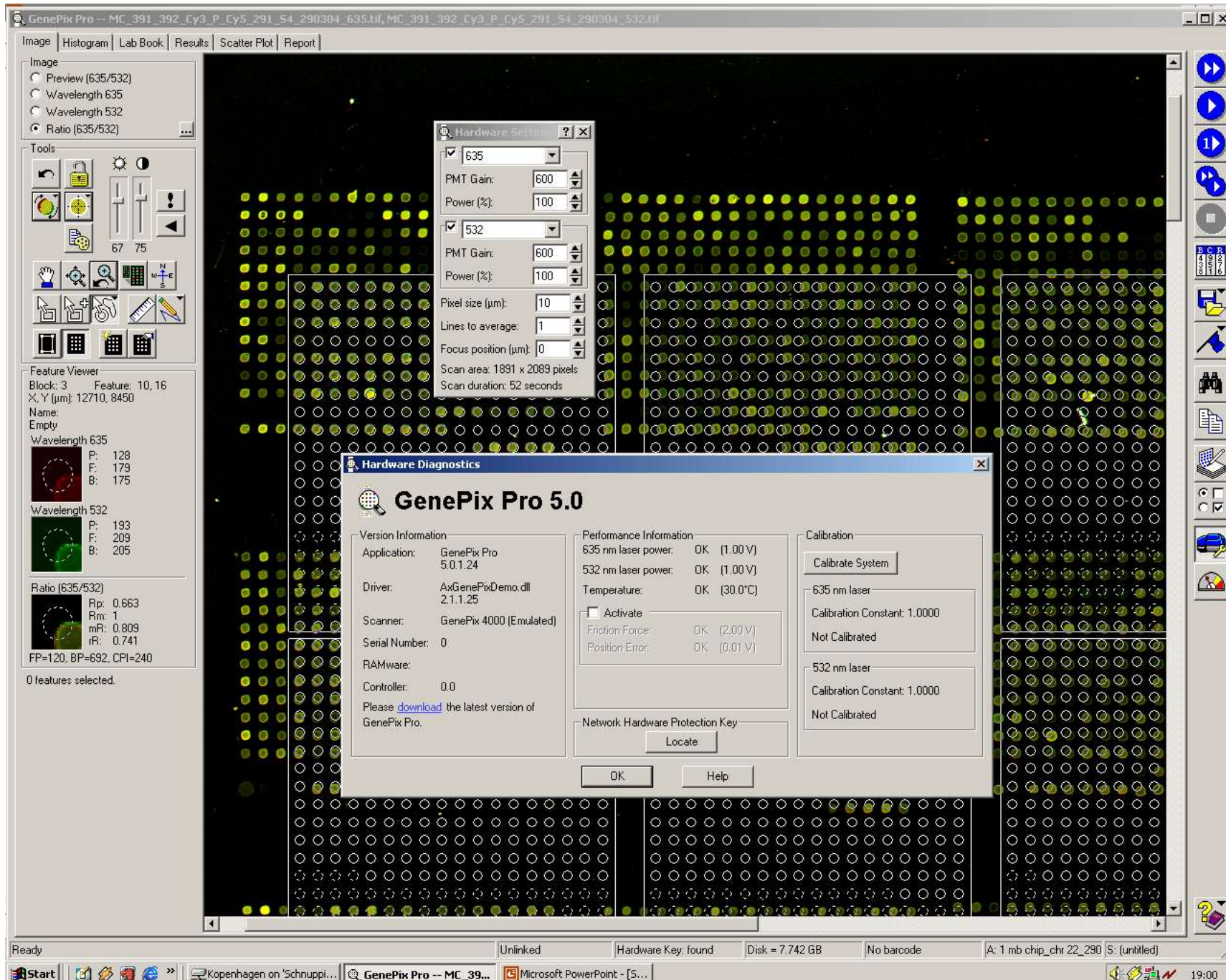


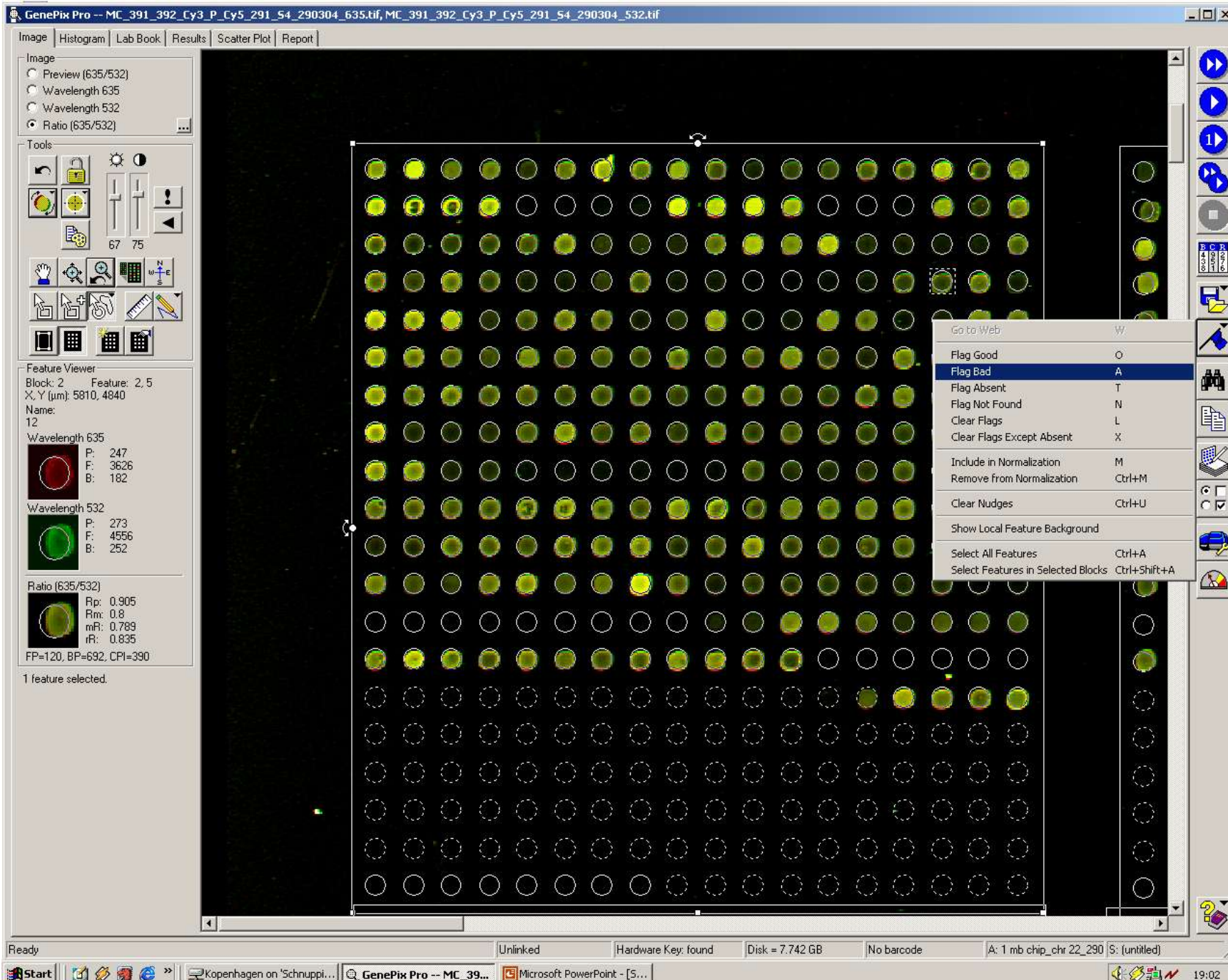
Chr.X



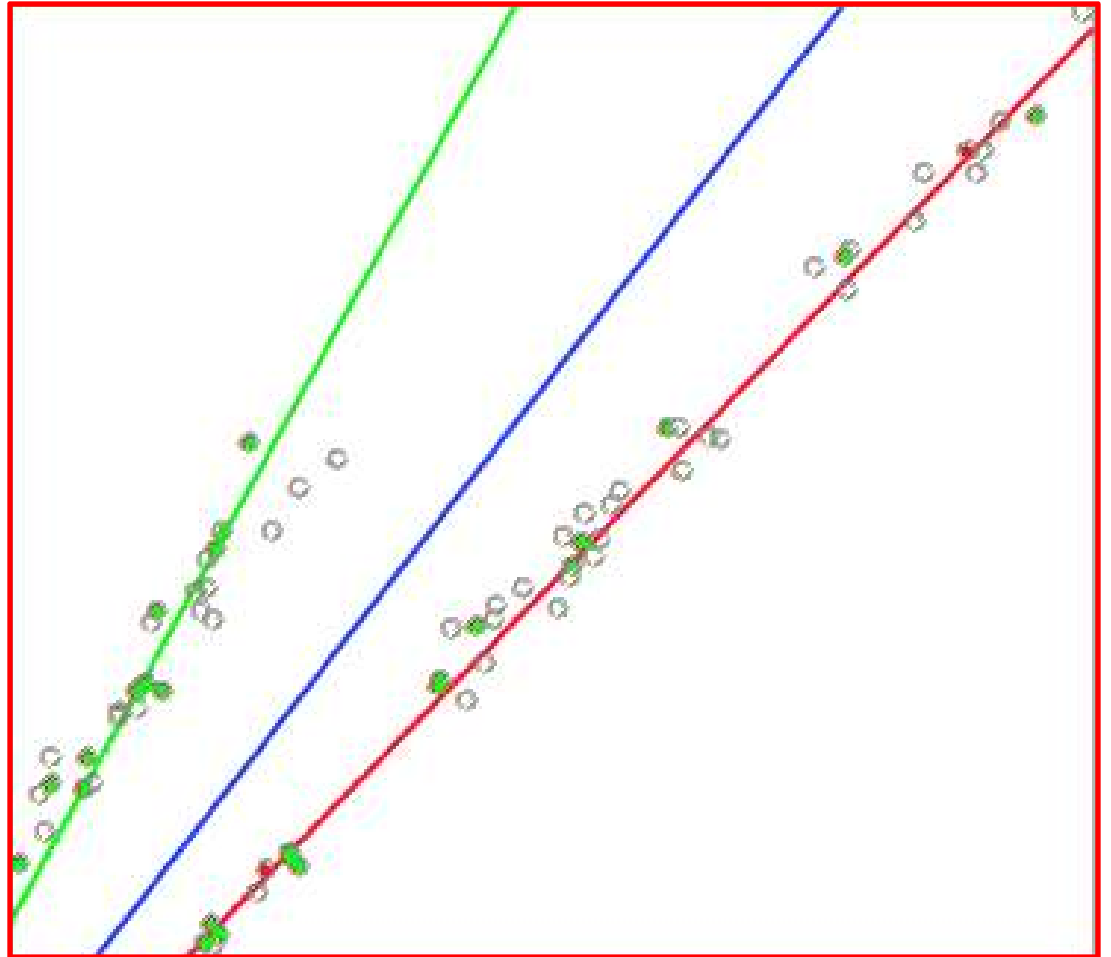
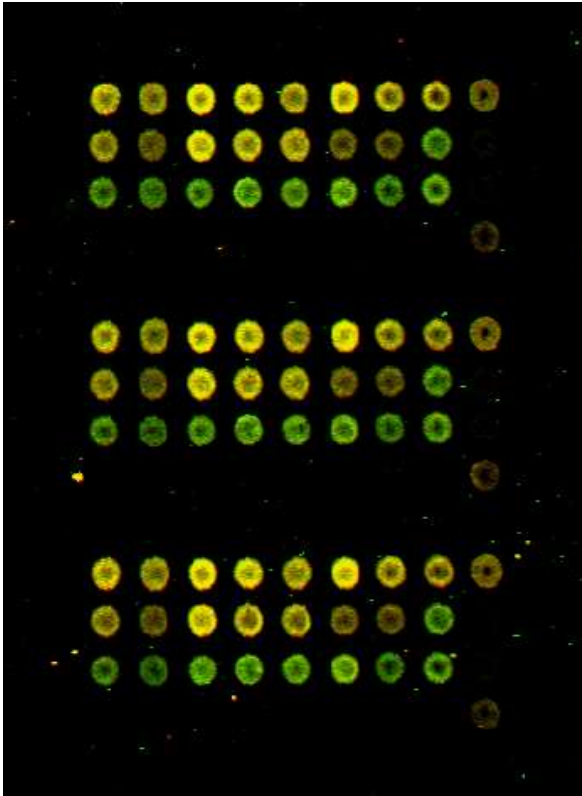


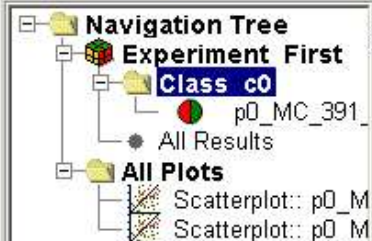




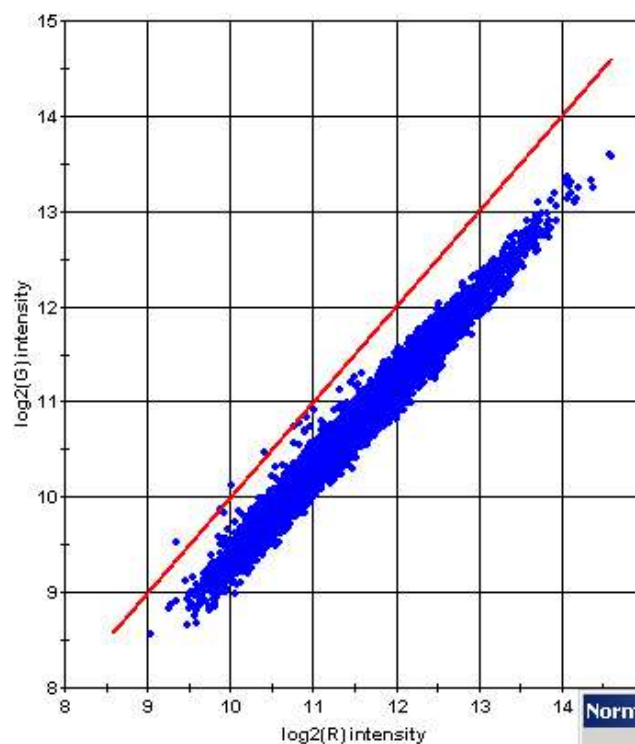


Problem der Normalisierung



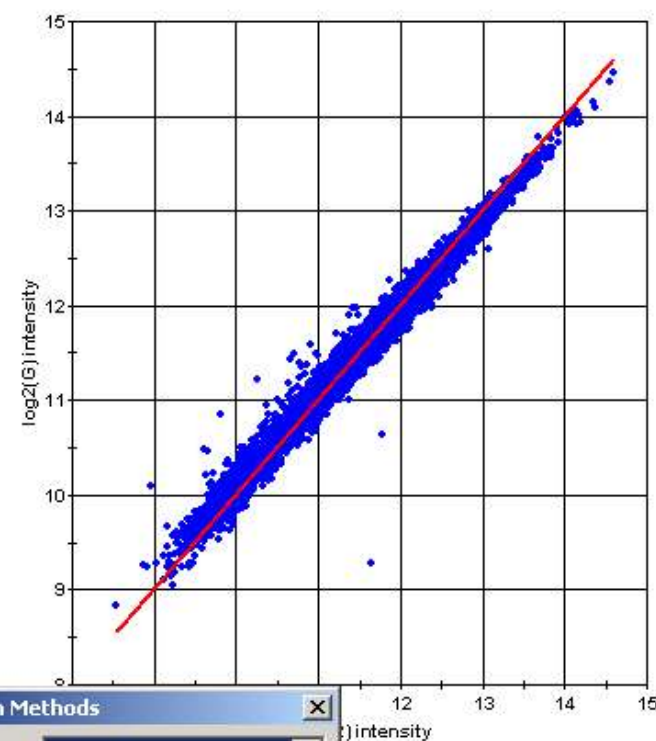


Scatterplot: p0_MC_391_392_Cy3_P_Cy5_291_S4...

Scatterplot $\log_2(R)$ vs. $\log_2(G)$ 

Slide Scatterplot: p0_MC_391_392_Cy3_P_Cy5_291_S4...

Scatterplot: p0_MC_391_392_Cy3_P_Cy5_291_S4...

Scatterplot $\log_2(R)$ vs. $\log_2(G)$ 

Slide Scatterplot: p0_MC_391_392_Cy3_P_Cy5_291_S4...

Normalisation Methods

Choose a method:

GlobMean

GlobMean

GlobMedian

GlobMeanSubgrid

GlobMedianSubgrid

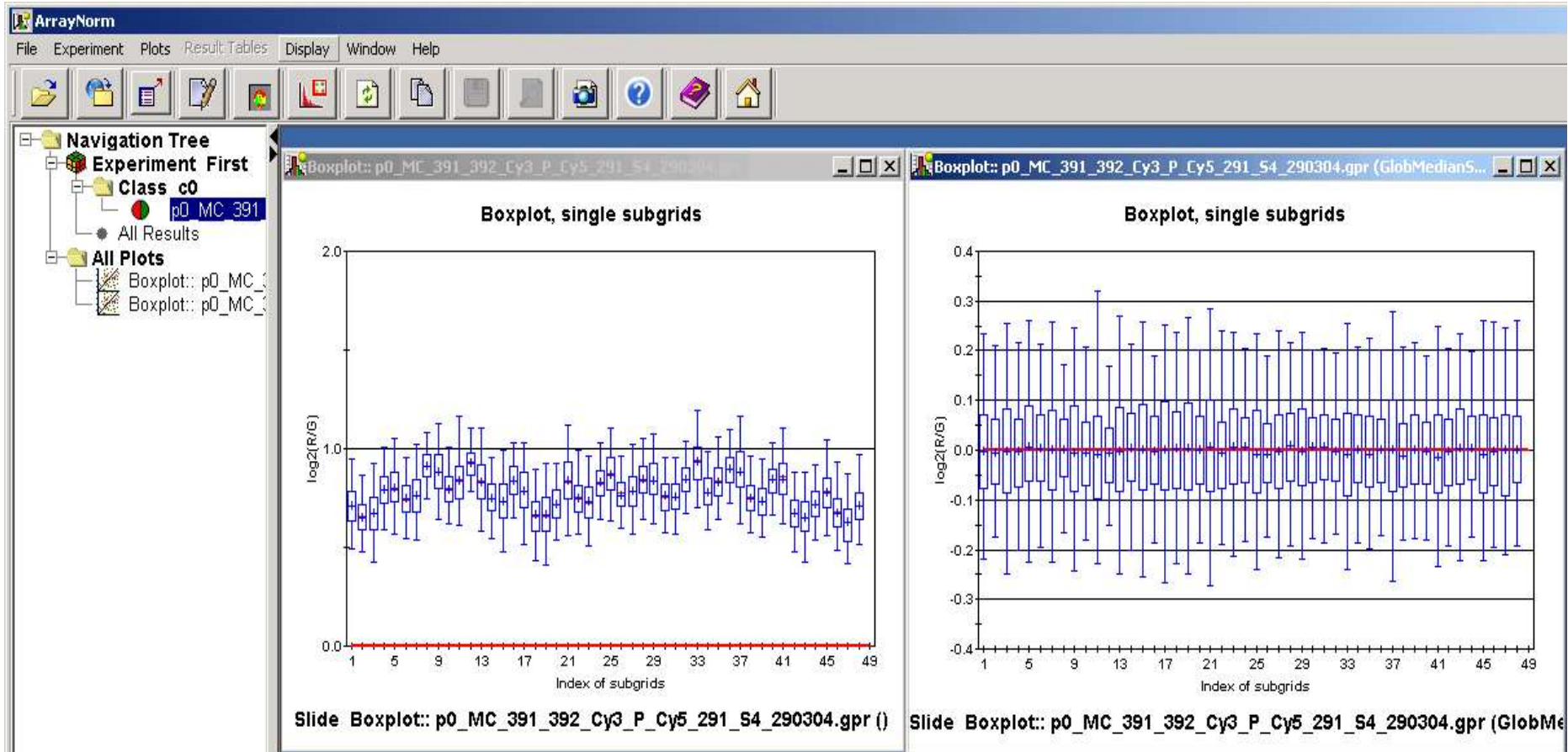
LowessRegression

LowessRegressionSubgrid

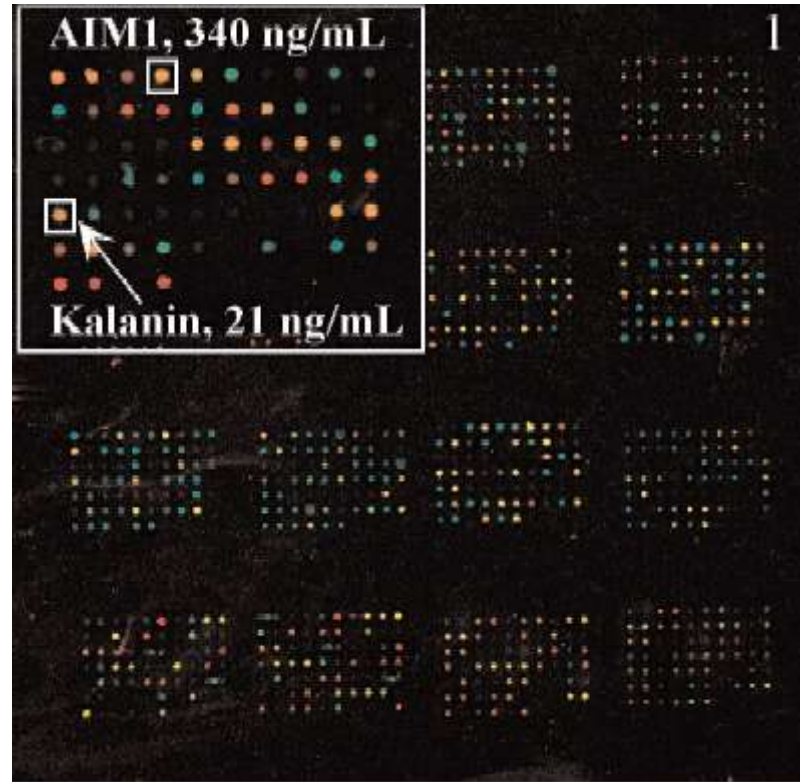
UseDyeswapPairs

UseControlSpots

Ok

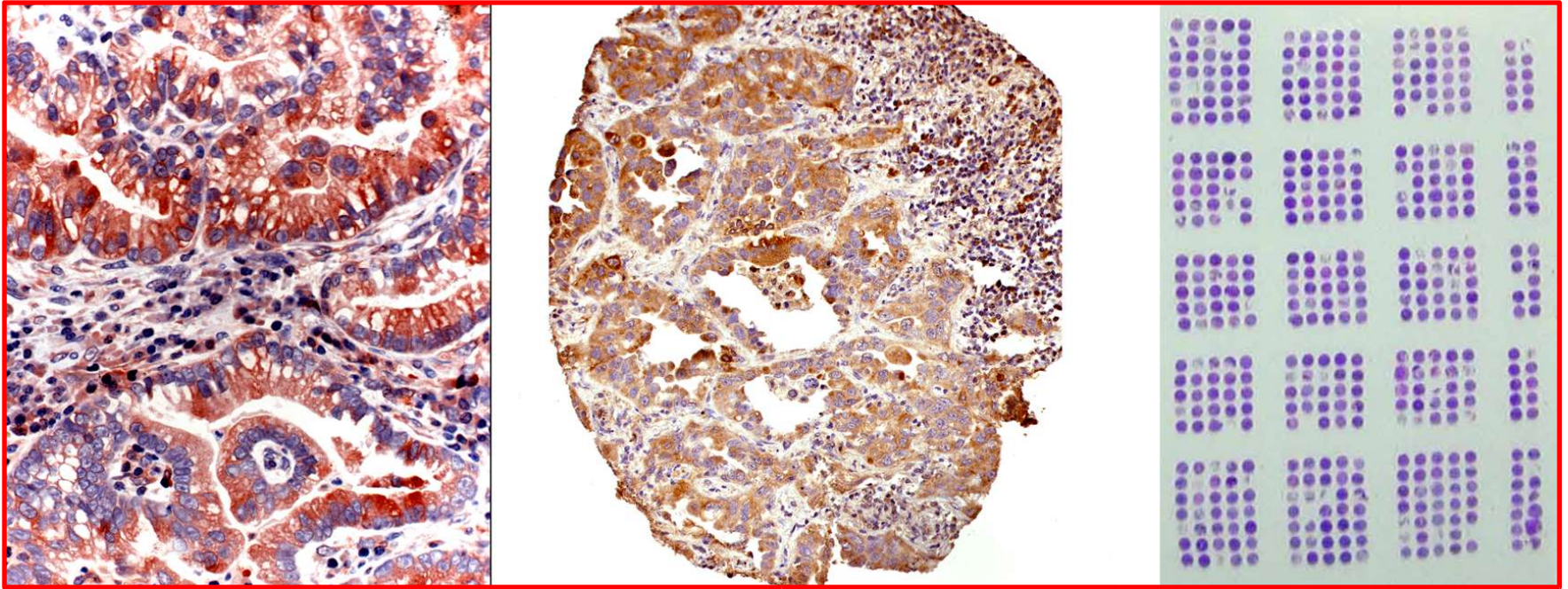


Proteinarrays



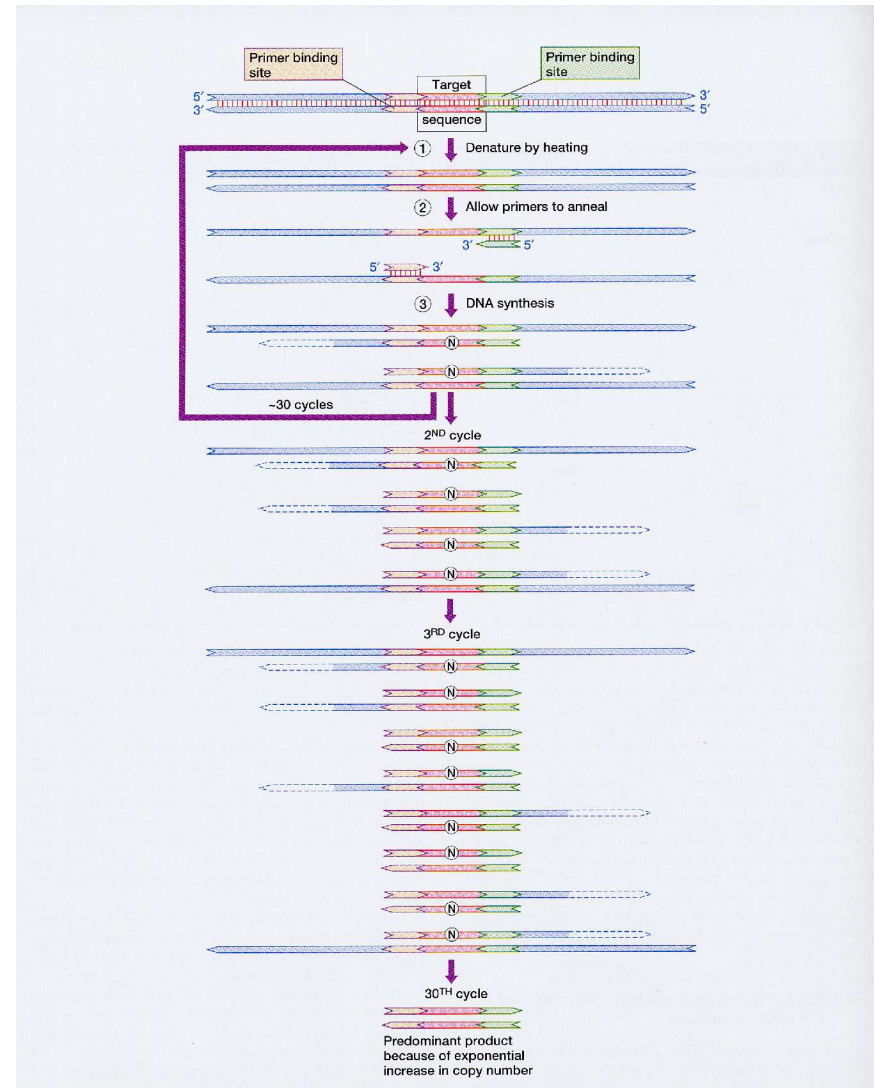
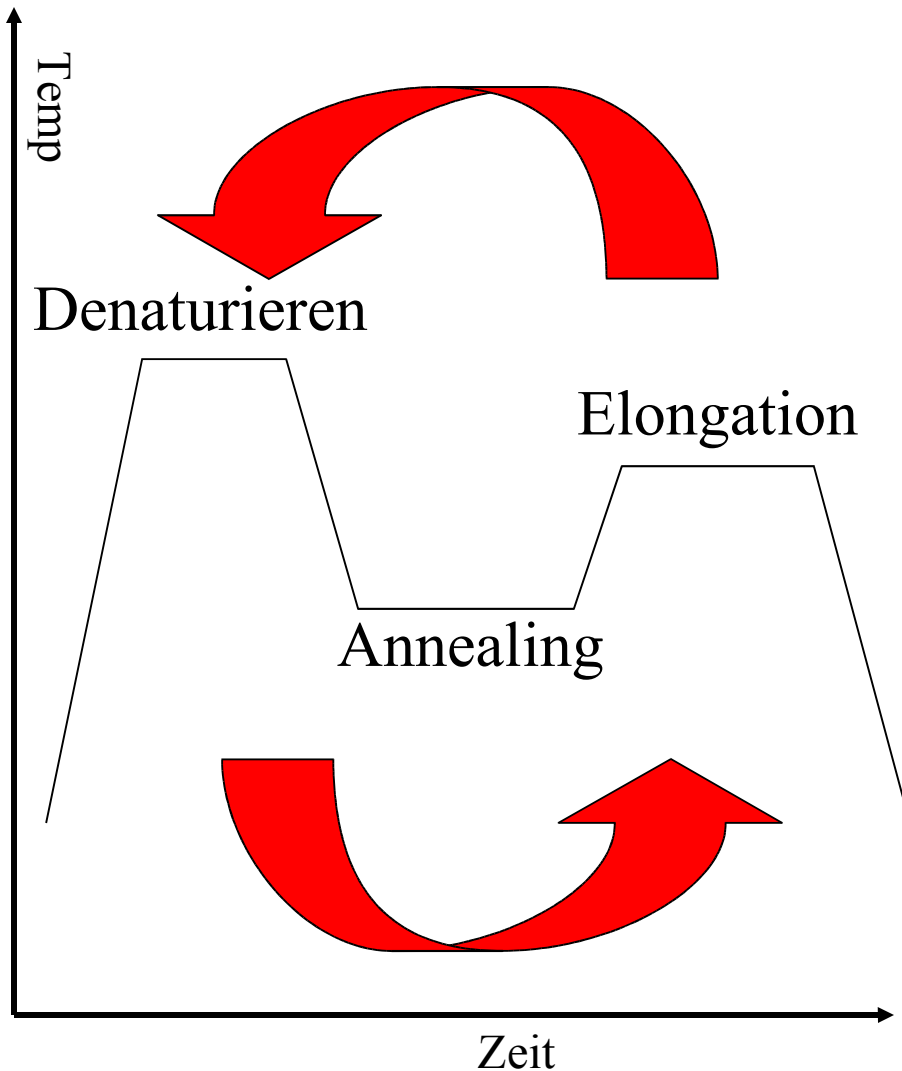
Haab BB et al., Genome Biology 2001

Tissuemicroarrays



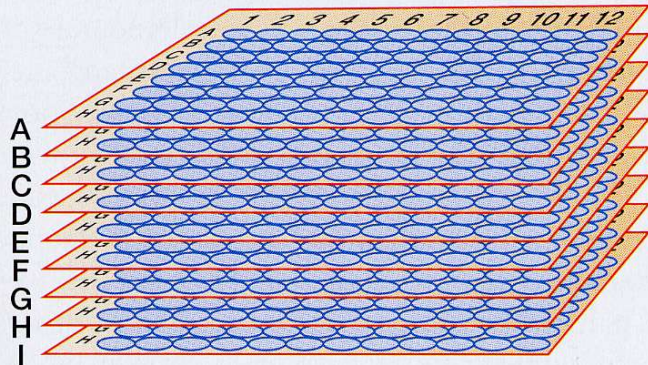
Gewebe wird aus einem Spenderblock gestanzt und in einen Empfängerblock eingebracht: geeignet für Untersuchungen von DNA, RNA und Protein

Polymerase Chain Reaction (PCR)



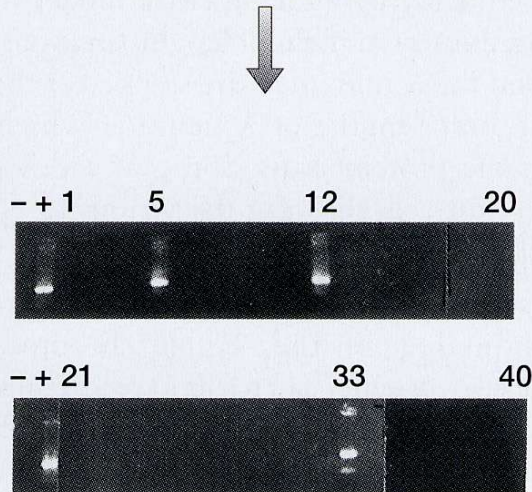
(A) Primary screening

PCR screening strategy for the ICI-YAC library. The 35 000 clones are individually grown in 360 microtiter dishes. Cultures from nine dishes (864 YACs) are combined and used to make one master pool DNA sample for screening



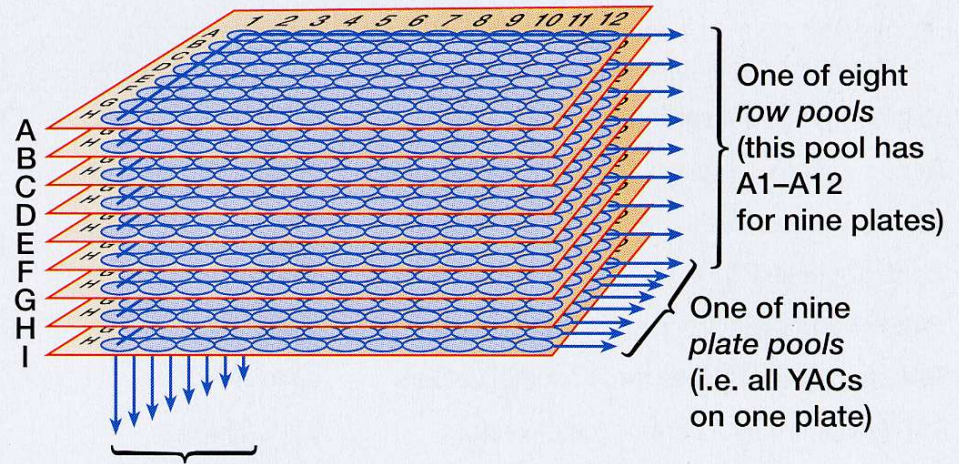
Mixture of 864 YAC DNAs = 1 *master pool*

DNA sample analyzed by PCR



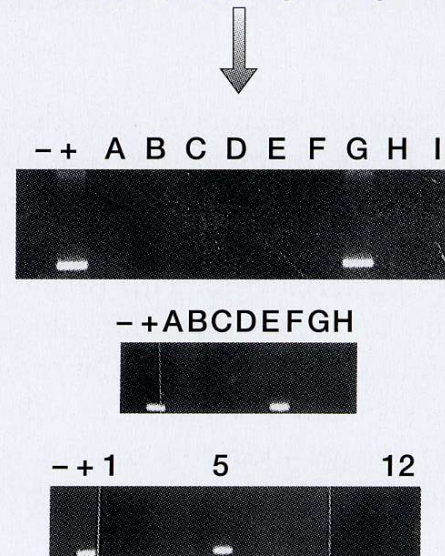
(B) Secondary screening

Three-dimensional screening is achieved by analysis of DNAs prepared for plate, row and column pools



One of 12 *column pools*
(this pool has A1-H1 for nine plates)

DNA samples analyzed by PCR



RNA Interference

