Image Processing in Bioinformatics

part of “Fortgeschrittene Methoden in der Bioinformatik”

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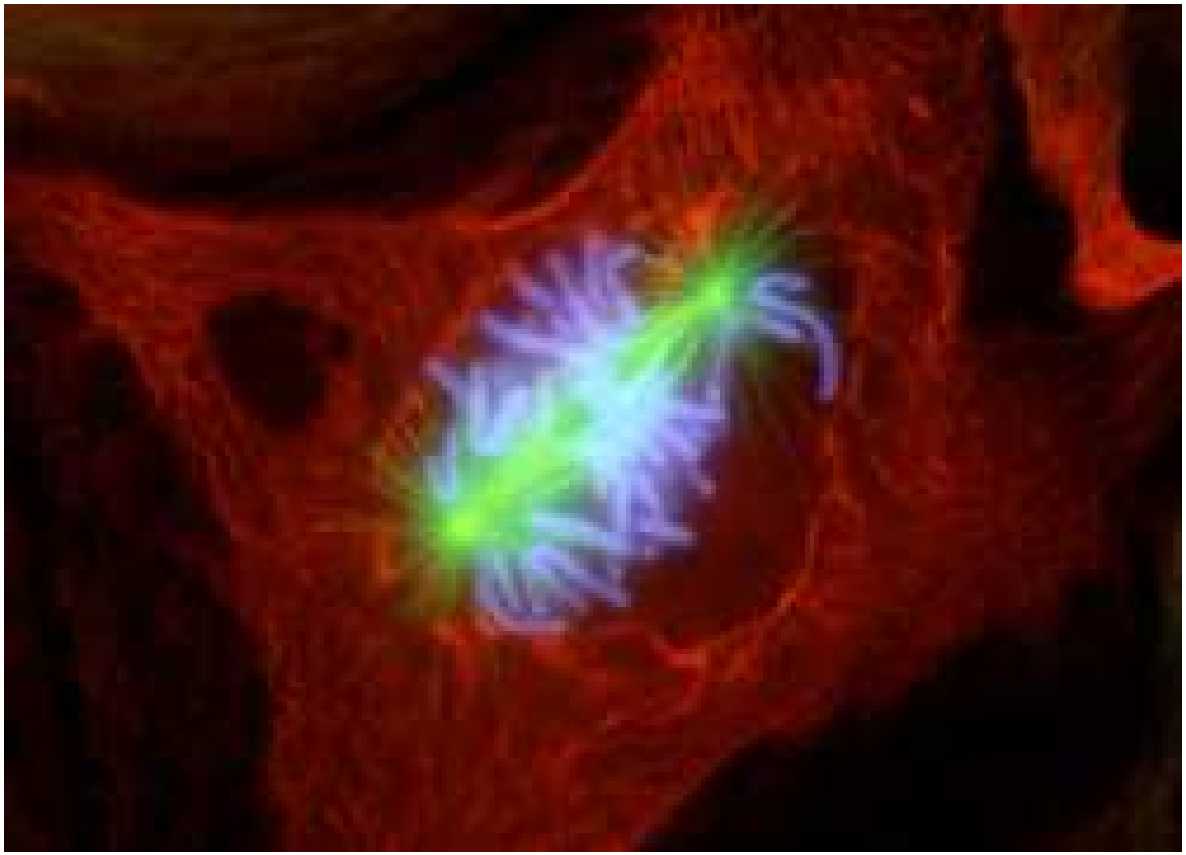
Outline

Image Data in Bioinformatics

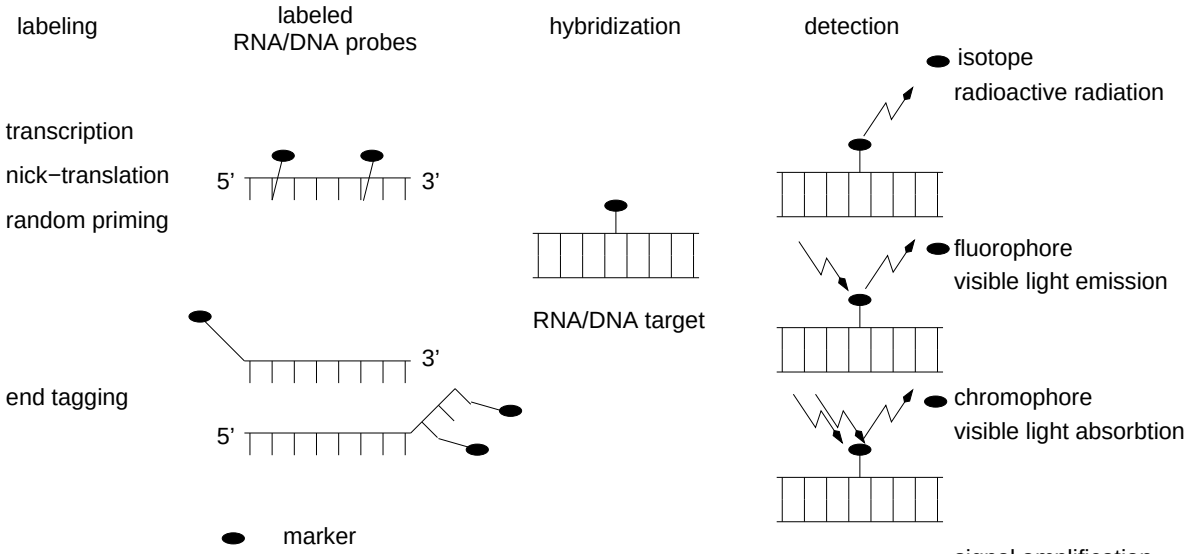
- *in situ* hybridization (ISH)
  - localization of DNA/RNA targets
  - DNA/RNA probes
  - radio-, flurescence- or antibody-labeling
  - for light- and electrone microscopy
  - multicolor ISH
  - tissue slices, whole-amount objects
  - live-cell imaging
- immunohistochemistry (IHC)
  - localization of protein targets
  - anti-target antibodies as lables
  - detection systems as for ISH
  - Multi-Epitope-Ligand-Cartography/Toponomics
  - tissue slices, whole-amount objects

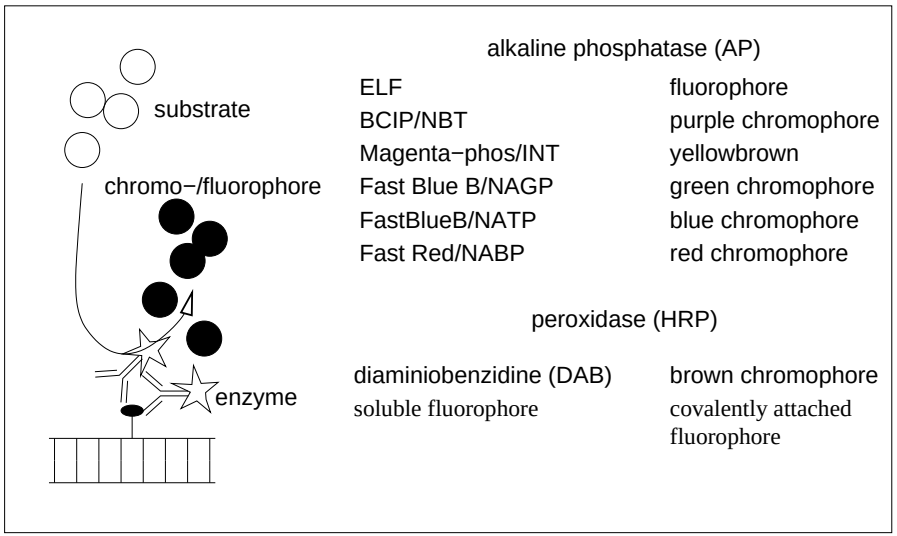
Example

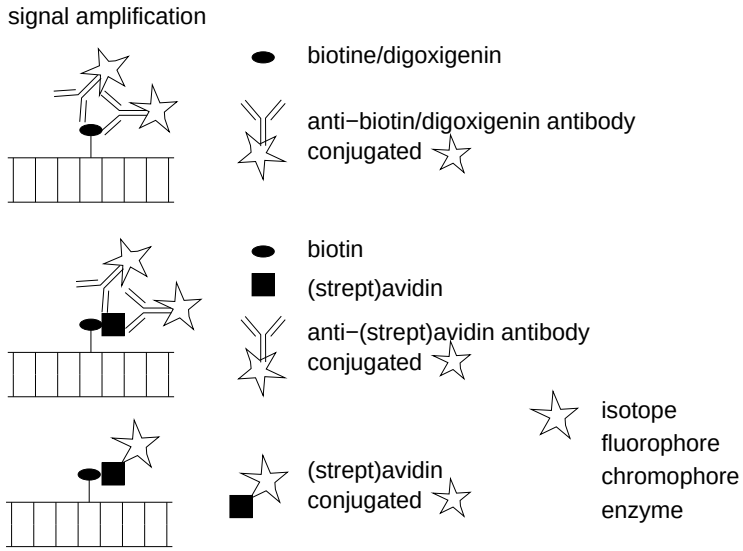


http://www.youtube.com/watch?v=P7m3WfzgZdI

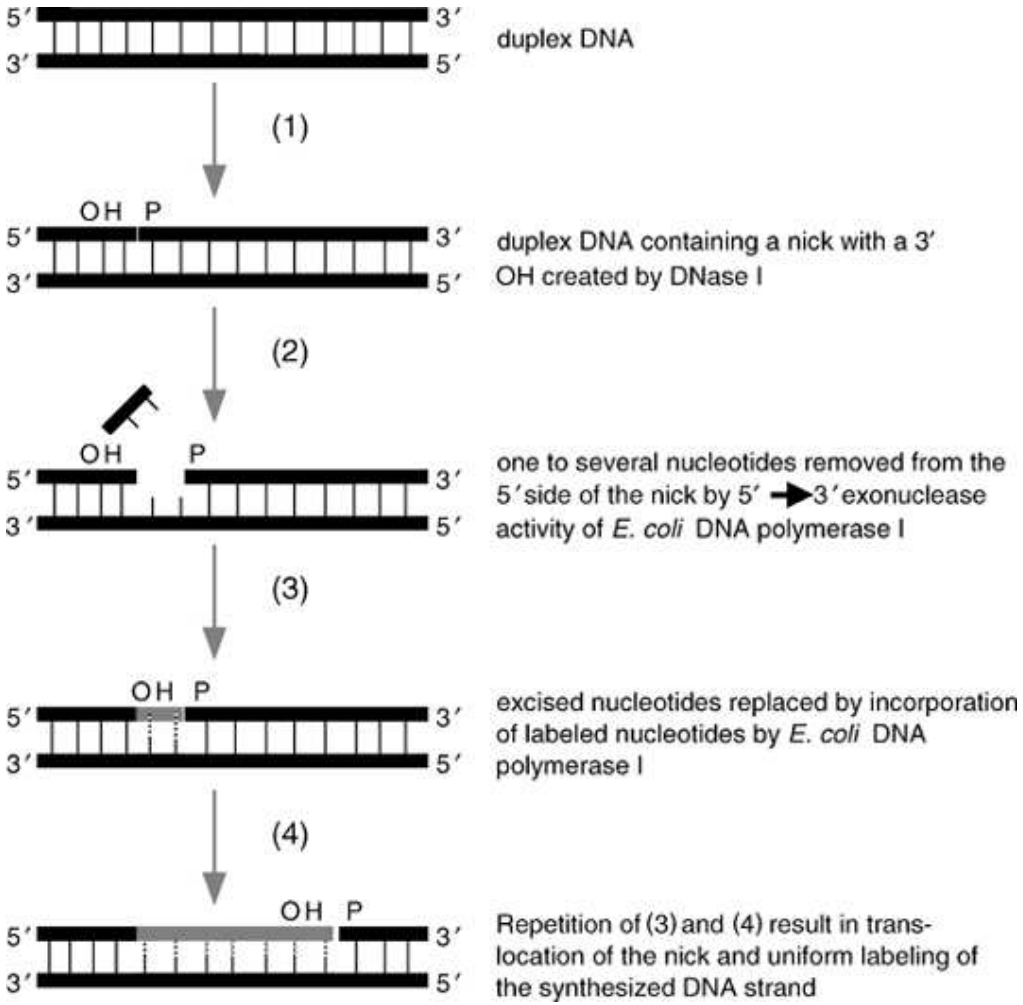
Concept of *in situ* hybridization



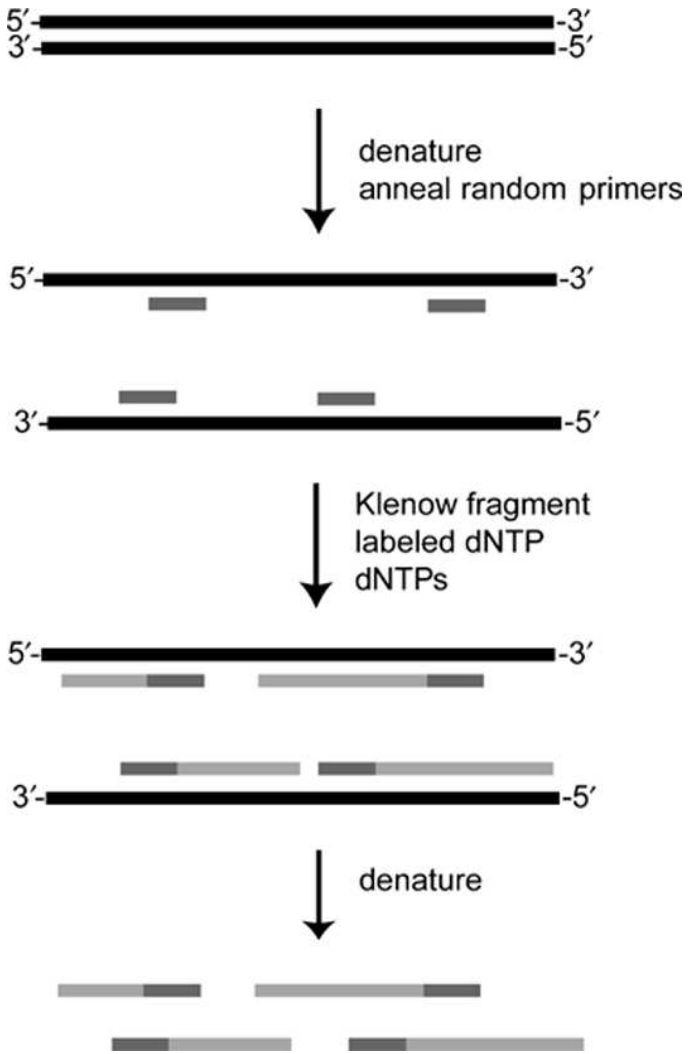




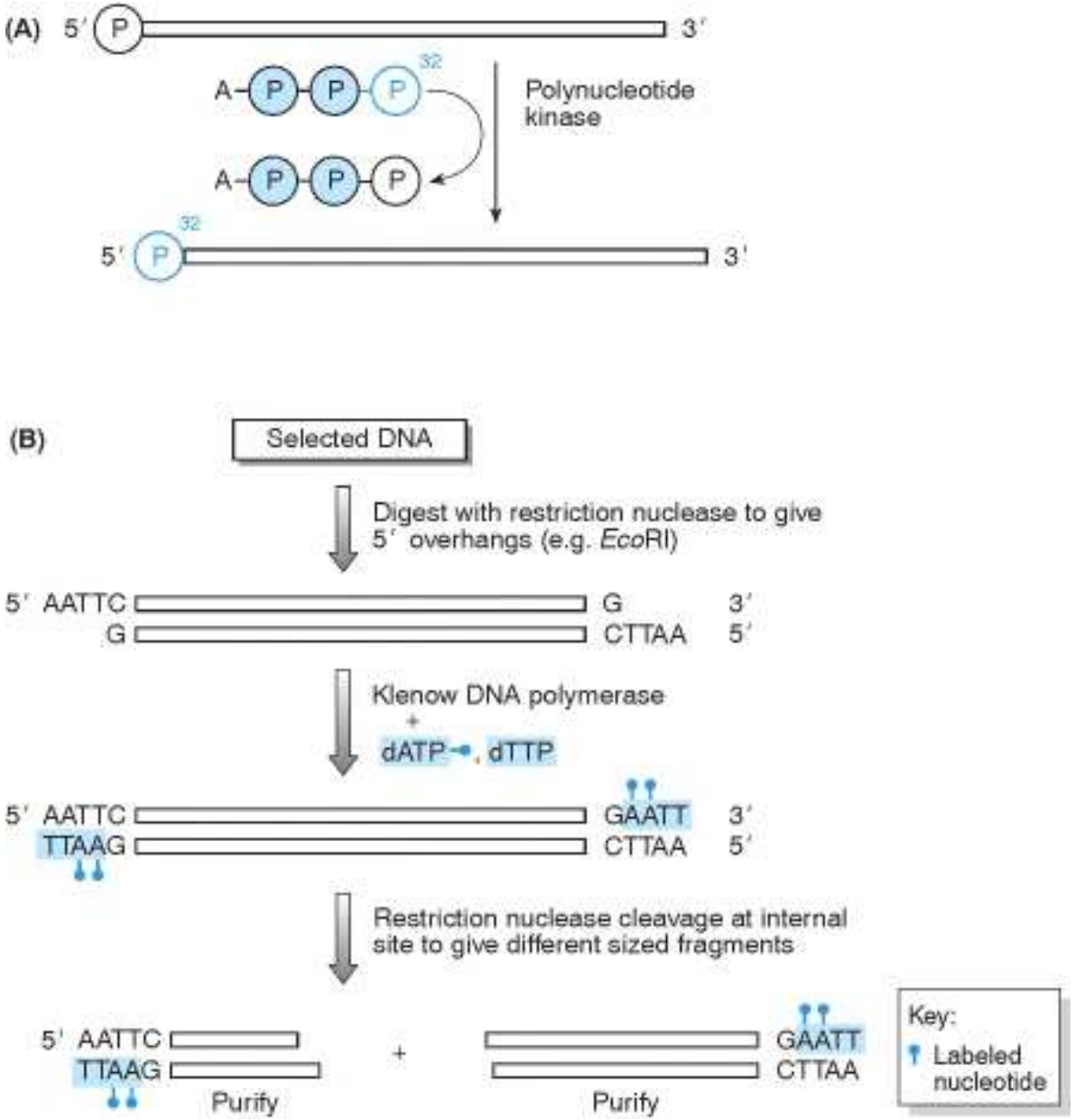
Labeling: Nick Translation



Labeling: Random Priming



Labeling: 5'-end Labeling



Labeling: Radioactive Labeling

- ^{35}S – 87.2 days
- ^{32}P – 14.3 days
- ^{3}H – 12.43 years

Labeling: Radioactive Labeling

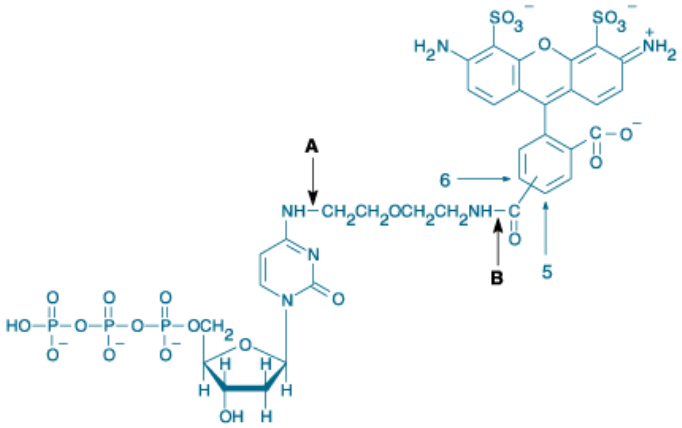
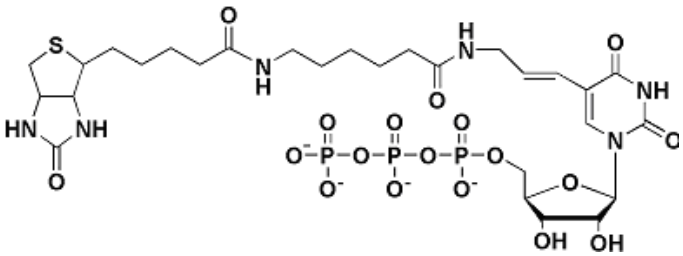
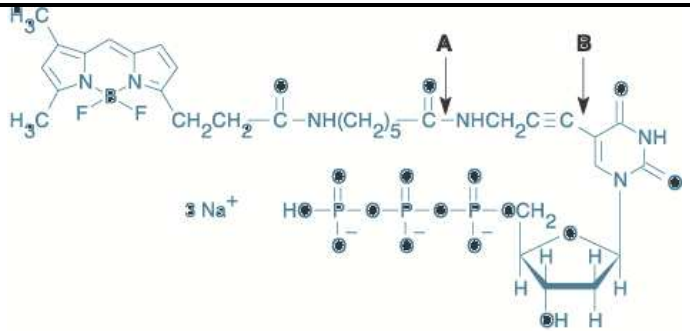
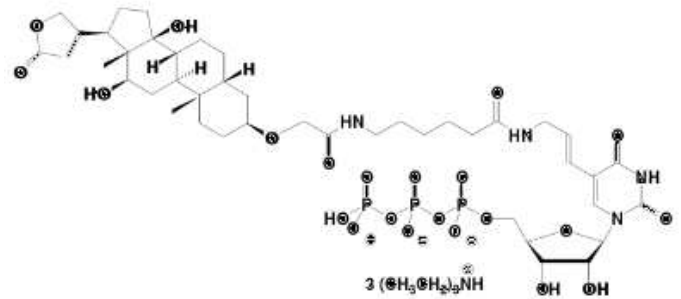
disadvantages

- safety problems
- isotopes vary in exposure time and stability only
- instability
- time-consuming

advantages

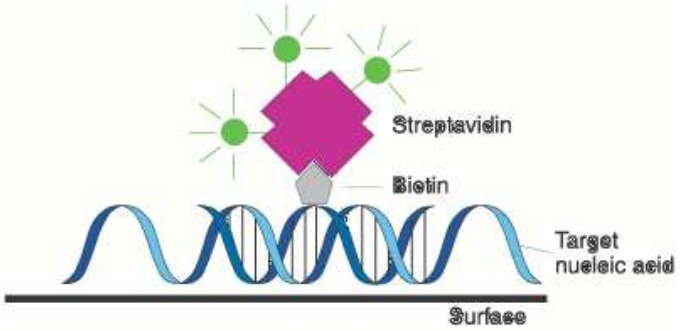
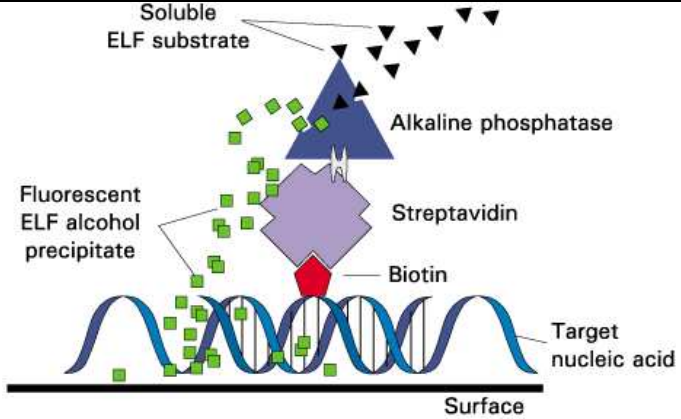
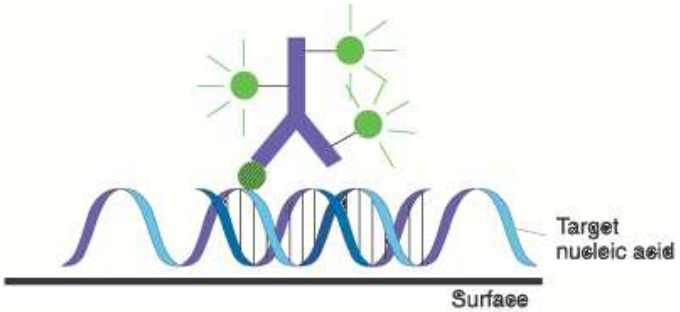
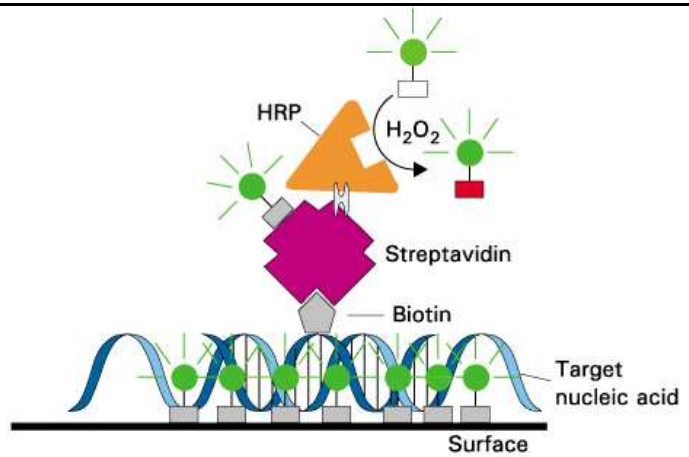
- high sensitivity, signal amplification
- lower probe concentration (10-50 fold)
- chemically identical probes
- easy access to the target

Labeled Nucleotides

Fluorophore	Marker
	
Alexa Fluor dCTP	Biotin UTP
	
BODIPLY dUTP	Digoxigenin UTP

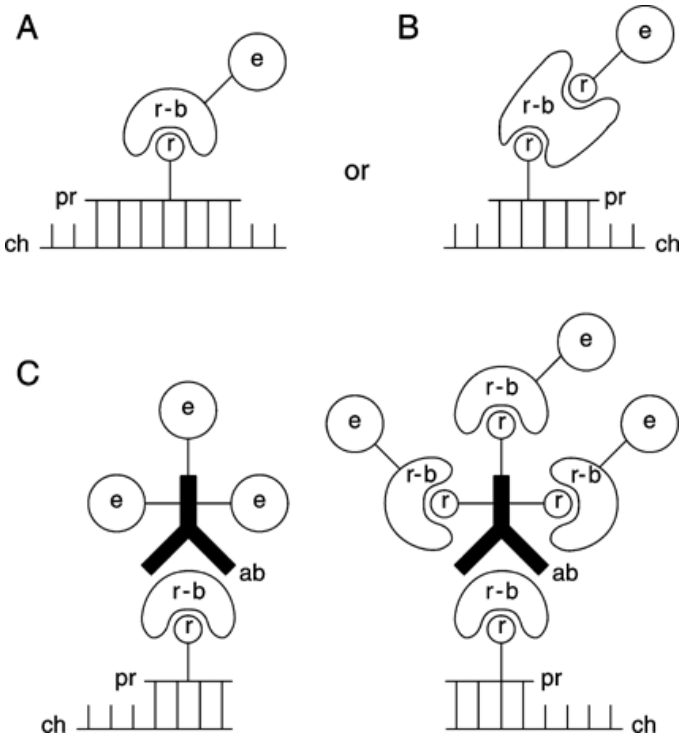
Spacer arms allow base paring and make the marker accessible to detection.

Detection Systems: Signal Amplification

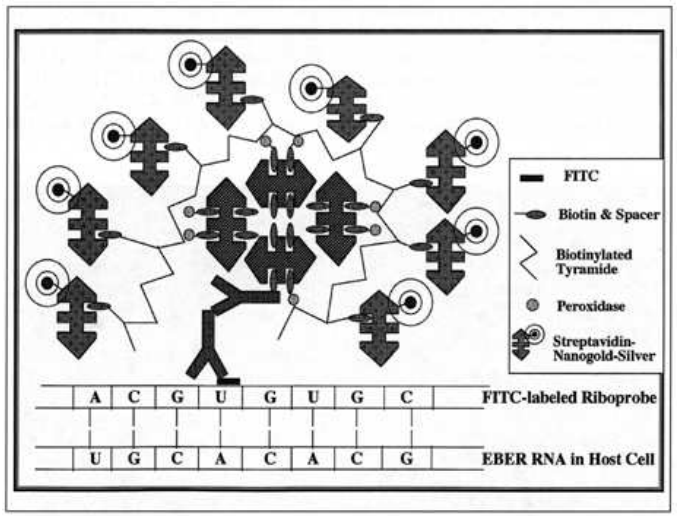
several dye moities per marker	enzymatic amplifiaction
	
	

Detection Systems: Strong Signal Amplification

light microscopy



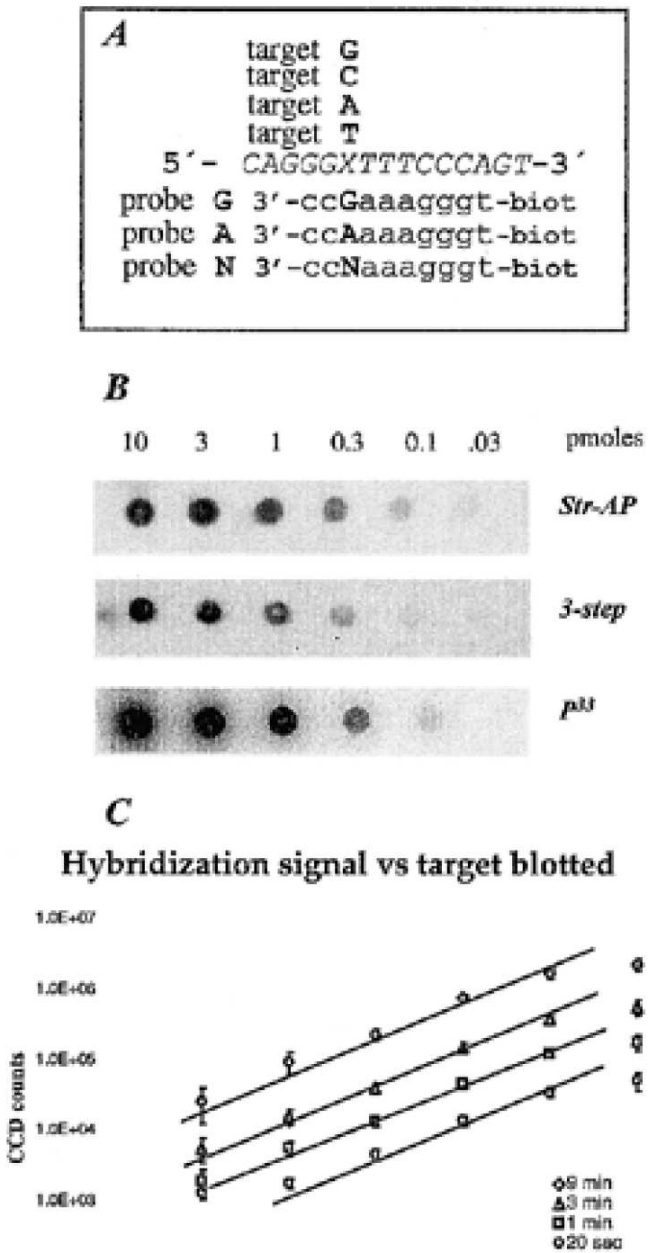
electron microscopy



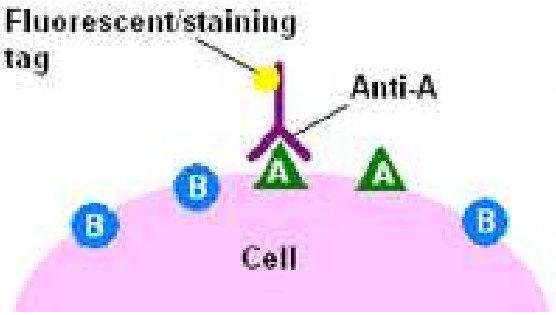
Why is this interesting to bioinformaticians?

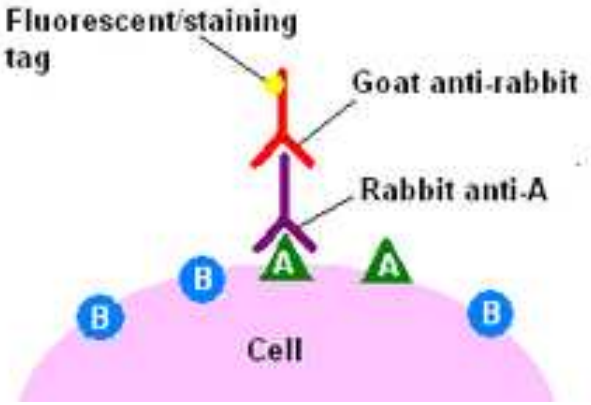
- biotin labeling: endogenous biotin can cause false positives
- digoxigenin labeling: only in *Digitalis sp.*, otherwise less false positives
- unspecific binding events? (avidin versus streptavidin)
- Can low amounts of targets be detected?
- Do labeling and amplification steps allow quantitative analysis?
- Is the amount of target in a linear relationship to the staining intensity?

Amount of Target RNA/DNA Versus Staining Intensity

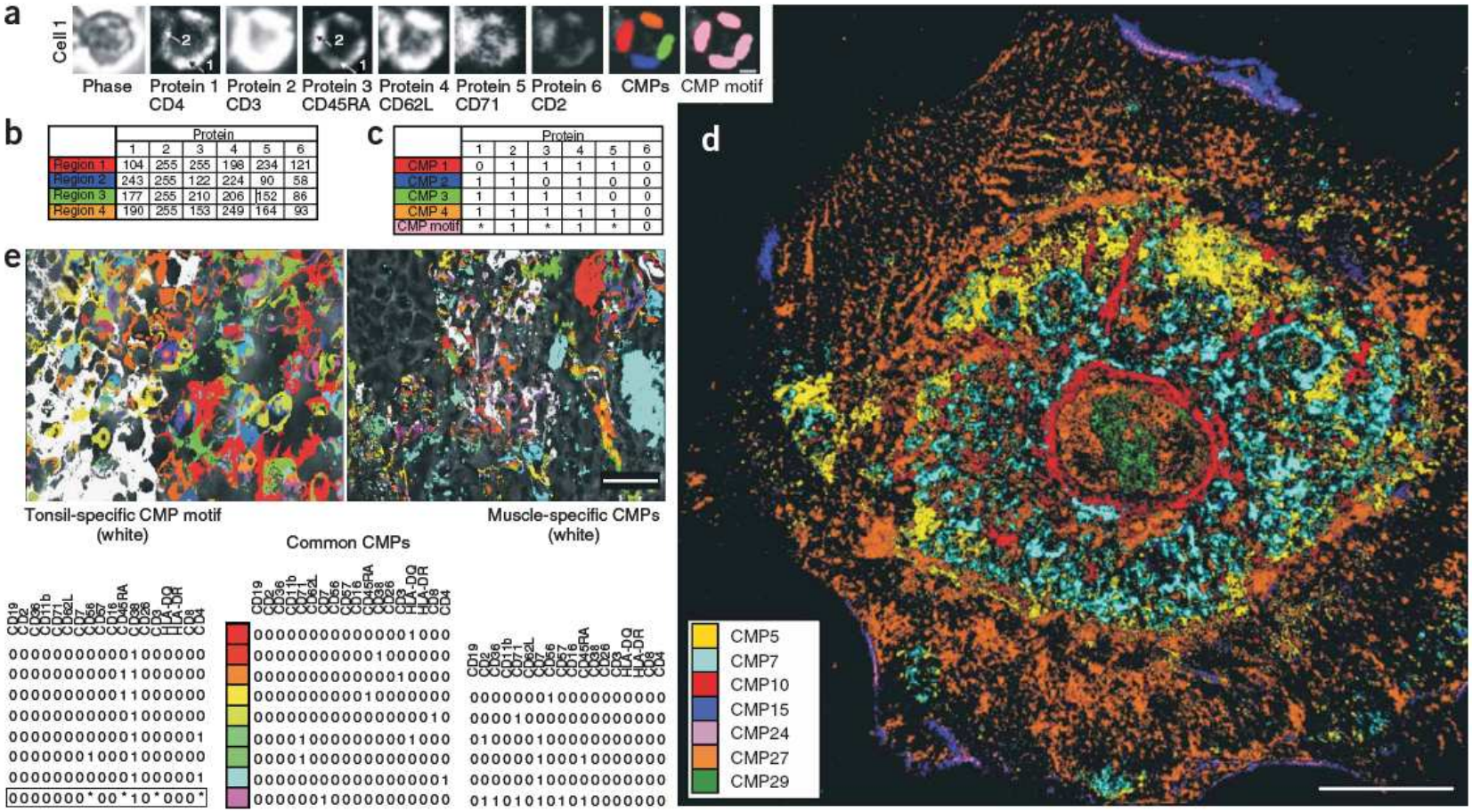


Direct and Indirect Immunohistochemistry





MELK and Toponomics



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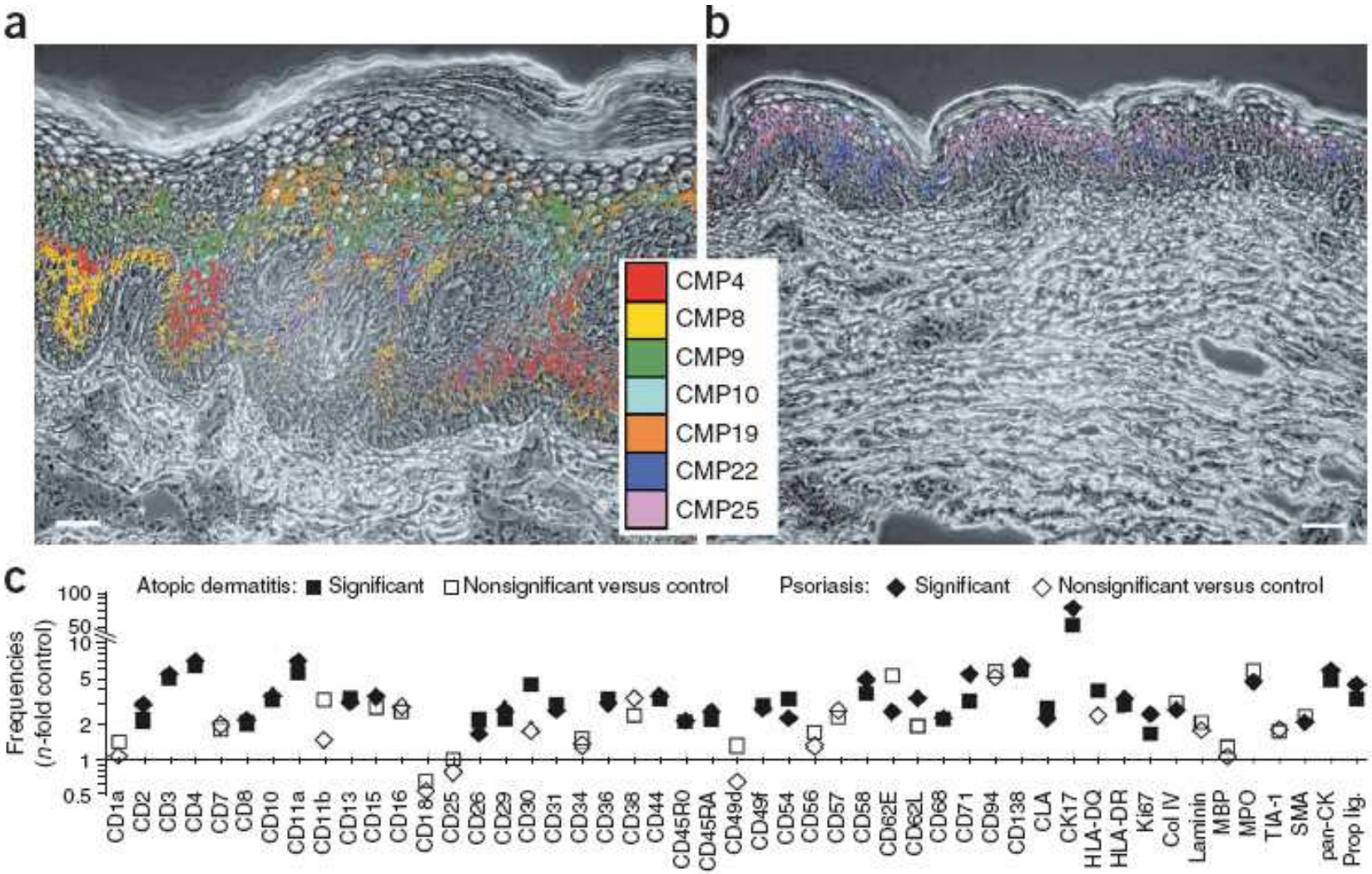
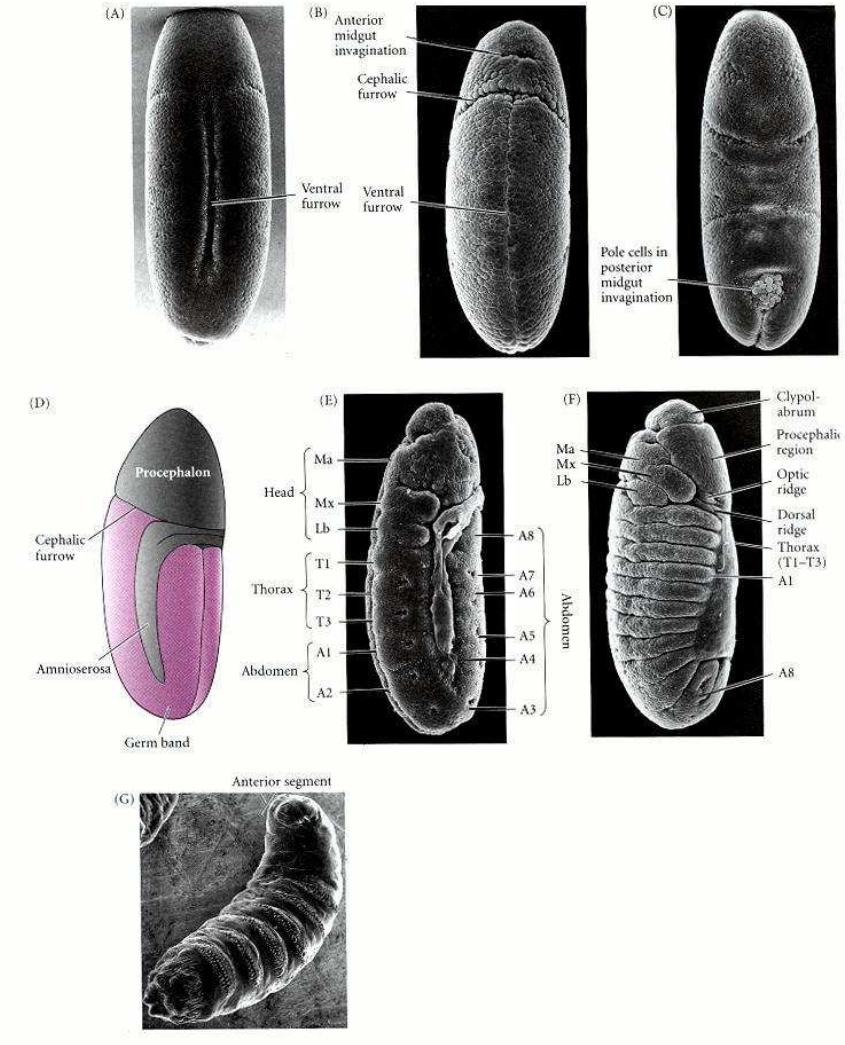
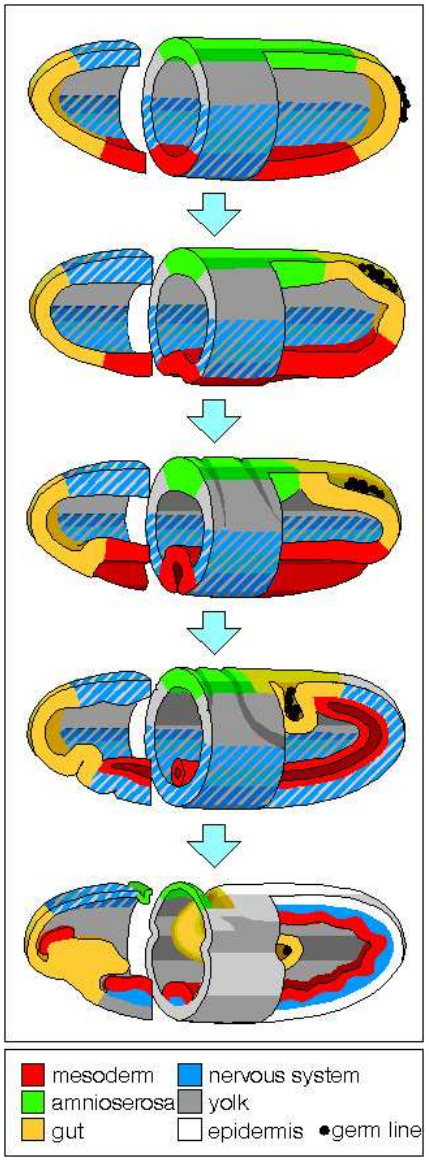


Databases

- BDGP - FlyExpress (fly)
- ZFIN (zebrafish)
- GEISHA (chicken)
- EMAGE (mouse)

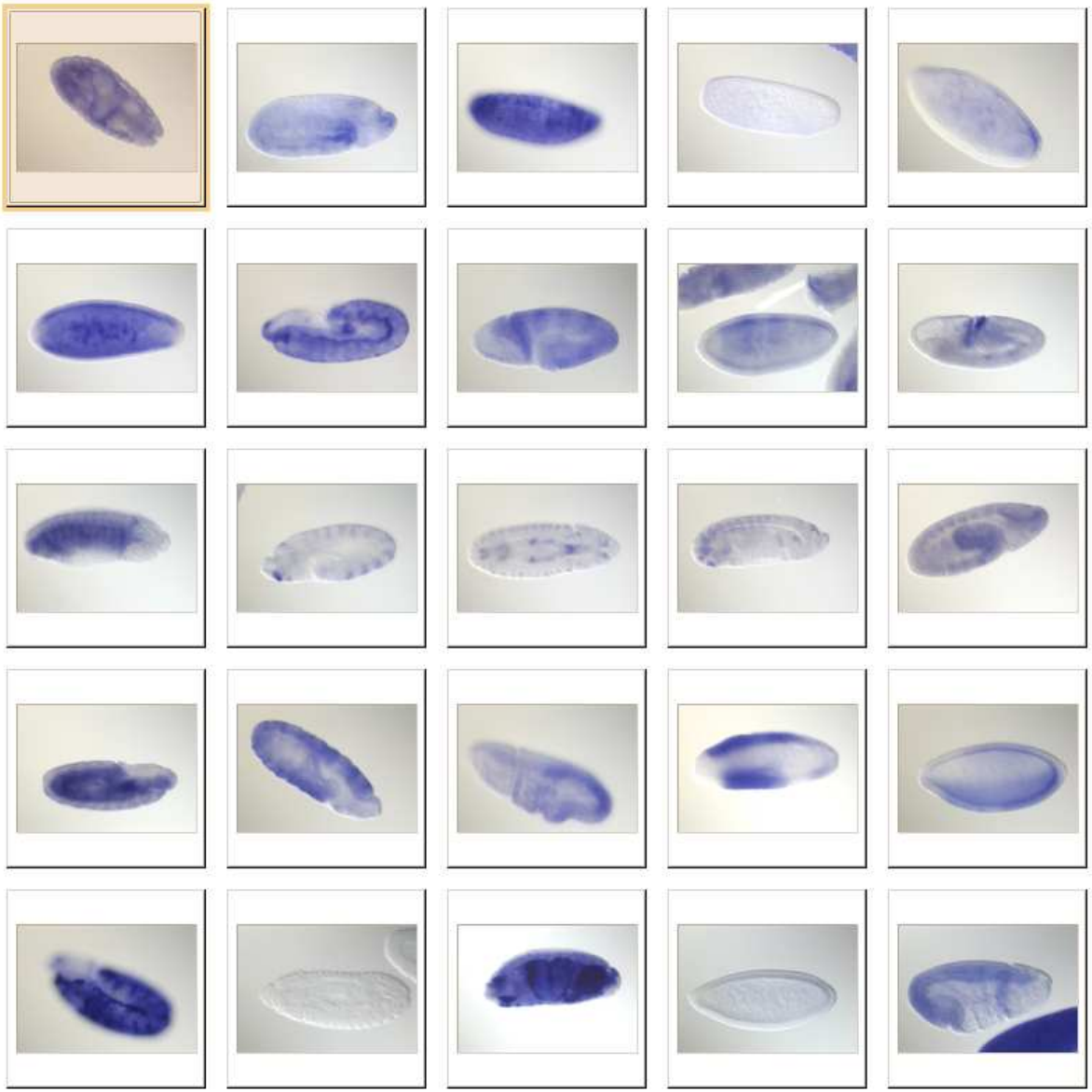
Drosophila Development





Youtube:
http://www.youtube.com/watch?v=Lb6TJzTLg_E

BDGP Raw Data



From the *in situ* image to the comparison of spatial expression patters

pay attention to:

- images from the same developmental stage and
- same view
- alignment of anterior – anterior, ventral – ventral, etc.
- inferrence of target localization/concetration from staining
- quantitative/qualitative measure of similarity/correlation

From the *in situ* image to the comparison of spatial expression patters

processing steps:

- cut out embryo
- determine view, orientation
- standardize embryos
- extract staining
- compare distribution of stain from embryo 1 and embryo 2

Be Careful with Biological Conclutions

- mRNA distribution does not necessarely reflect protein distribution
- mRNAs *in situ* patters are found to overlap
  - does not necessarely mean interaction of the gene products (A interactis with B)
  - does not necessarely indicate co-regulation (A and B are regulated by a set of common TFs)
  - does not necessarely indicate a positive regulatory interaction (A enhances B or B enhances A)