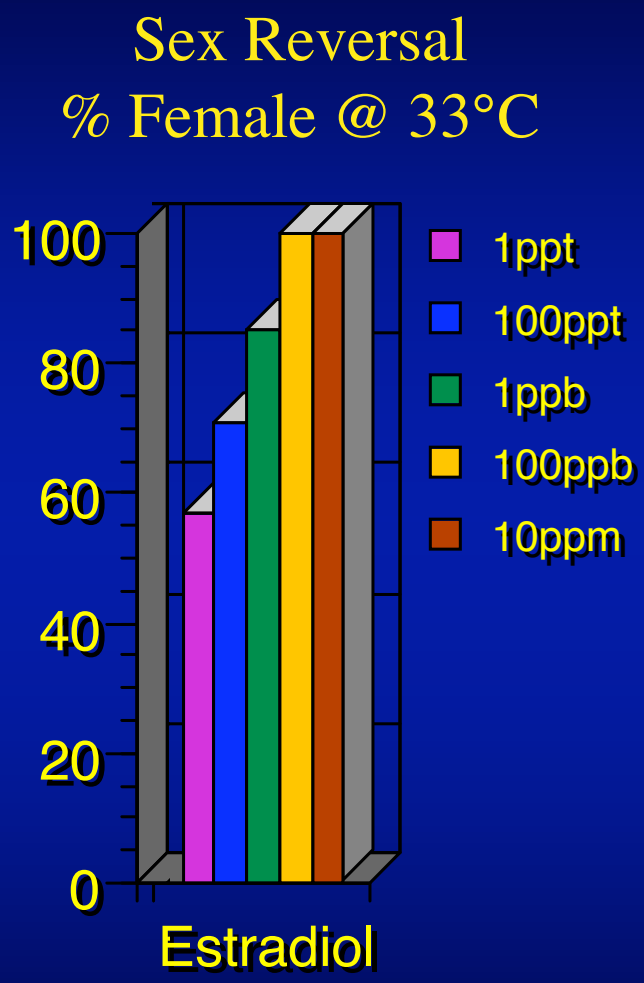
Techniques in Reproductive Biology

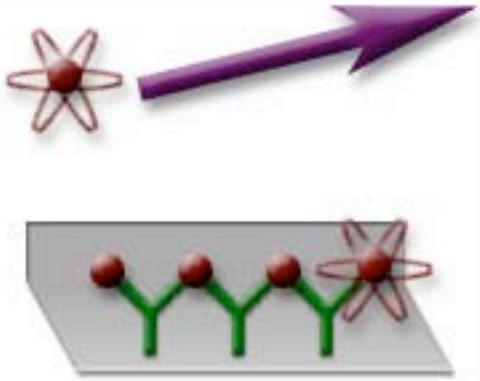
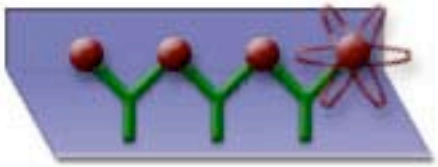
Bioassay

- Use of a known biological response
- Develop a dose response curve
- Access unknowns
- Still extensively used



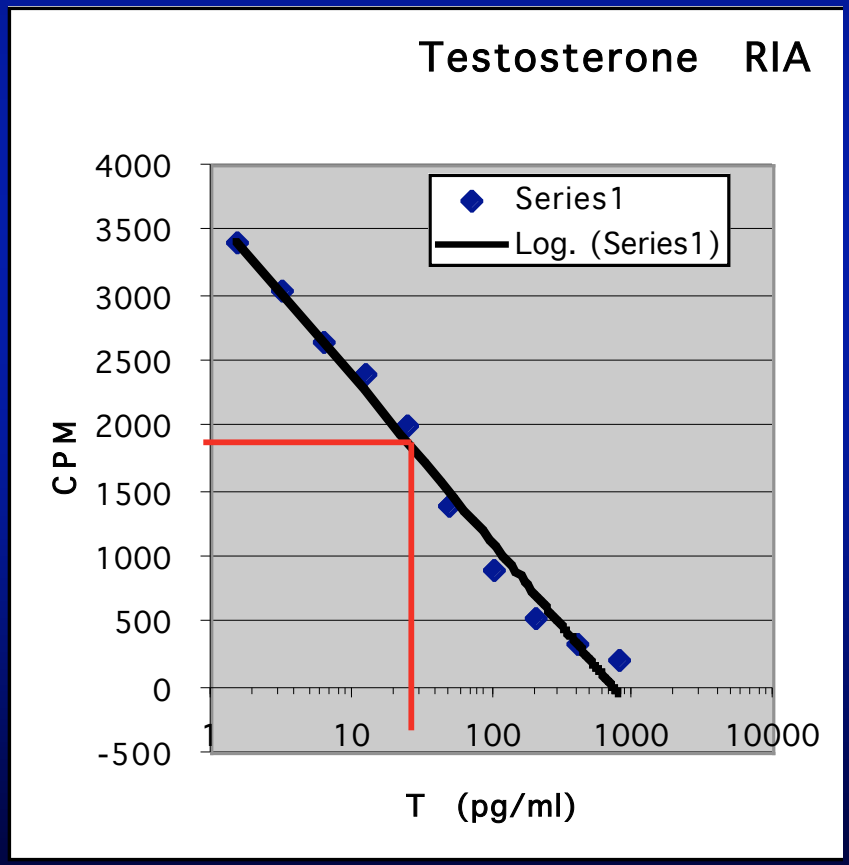
Radioimmunoassay

- Used to measure quantitatively hormone concentrations in blood, receptors in tissue, etc.
- A competitive binding assay using
  - Radio = radioactive label
  - Immuno = specific antibody
  - Assay = quantitative approach

REAGENTS: Ab specific for hormone (coating the filter) Unknown sample with hormone Allow time to react Wash away unbound substances	**POSITIVE SAMPLE** high level of hormone	**NEGATIVE SAMPLE** low level of hormone
REAGENTS: ^{125}I-labeled hormone Allow time to react Wash away unbound radiolabeled hormone		
PROCEDURE: measure radioactivity in a gamma counter RESULT: amount of radioactivity is inversely proportional to the concentration of hormone in the sample.		

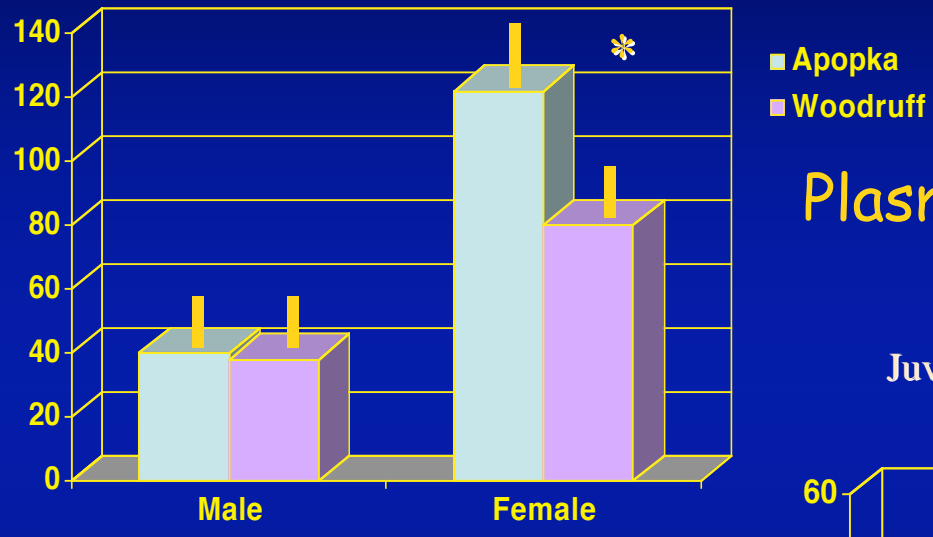
				CPM 1	CPM 2	MEAN CPM
				CPM 1	CPM2	
TC				14595	15187	14891
NSB				87	96	92
B0		0		2919	2898	2909
STD	1	1.5625		2862	2868	2865
STD	2	3.125		2750	2719	2735
STD	3	6.25		2614	2672	2643
STD	4	12.5		2352	2434	2393
STD	5	25		2014	1980	1997
STD	6	50		1414	1388	1401
STD	7	100		935	869	902
STD	8	200		535	511	523
STD	9	400		308	339	324
STD	10	800		209	206	208

- 1900 cpm = 28 pg/tube
- If:
  - 100 µl/tube
- Then
  - Concentration in ml
  - 10 X 28 pg/tube
  - 280 pg/ml



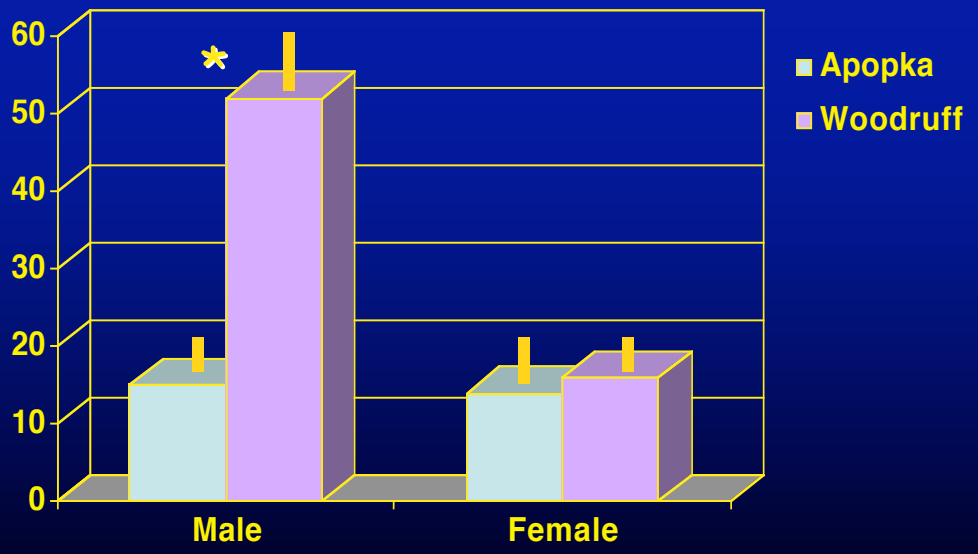
Plasma Estradiol (pg/ml)

Juvenile Alligators - 9 mo old



Plasma Testosterone (pg/ml)

Juvenile Alligators - 9 mo old



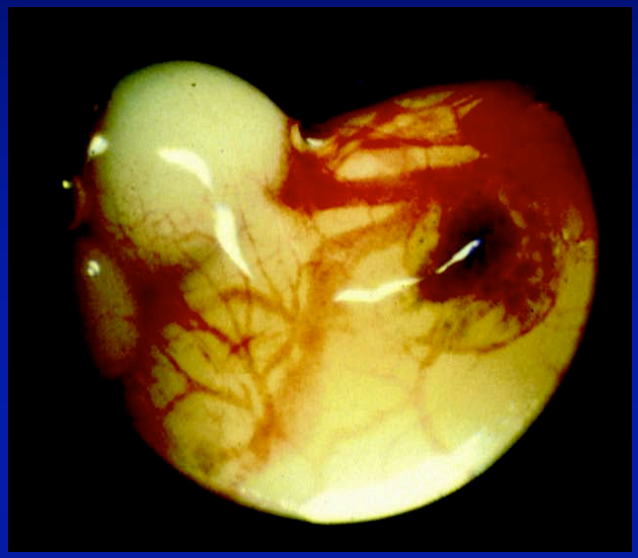
Guillette et al. (1994) Environ. Health Perspec. 102:680-688

Surgery

- Earliest technique
  - 1) "Extirpation"
    - Remove tissue
  - 2) See what happens
  - 3) Replacement
  - Still used extensively
  - Interpretation can be difficult

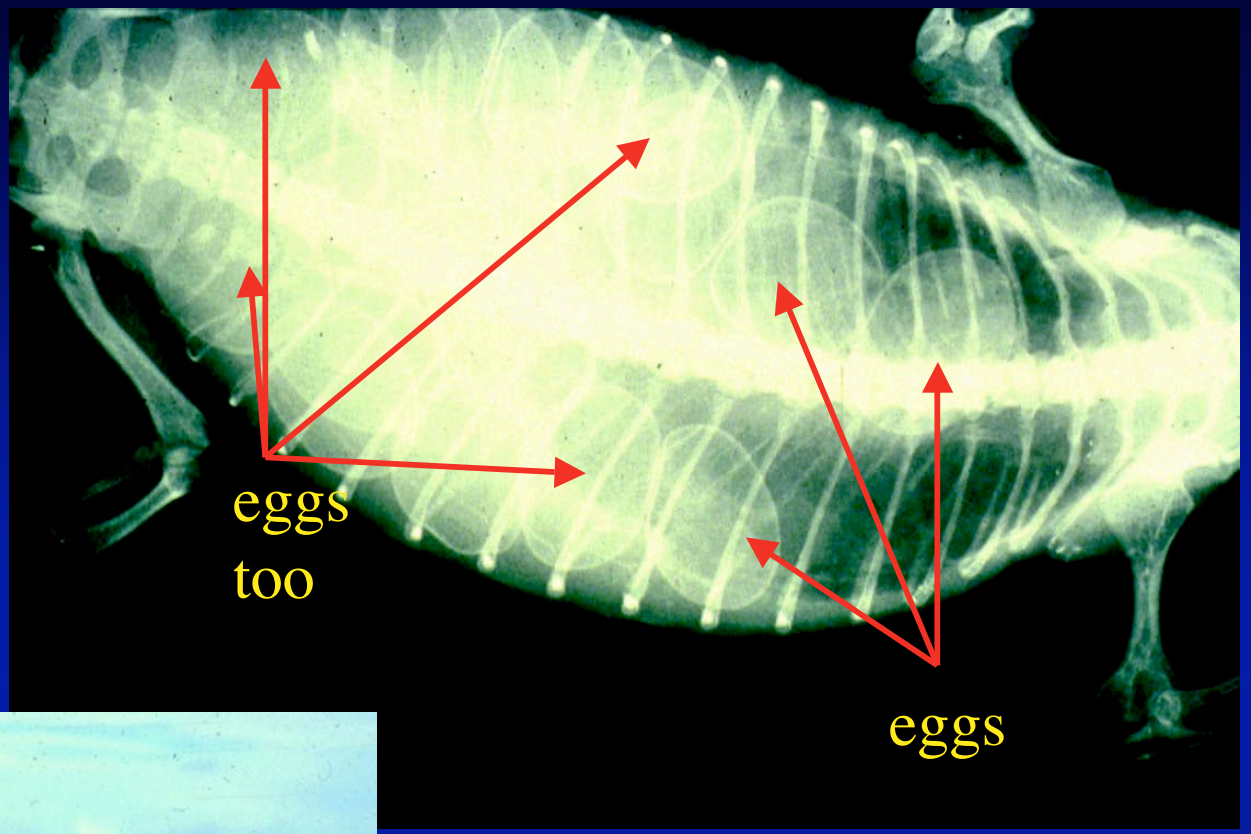
X-Ray & Other Imaging

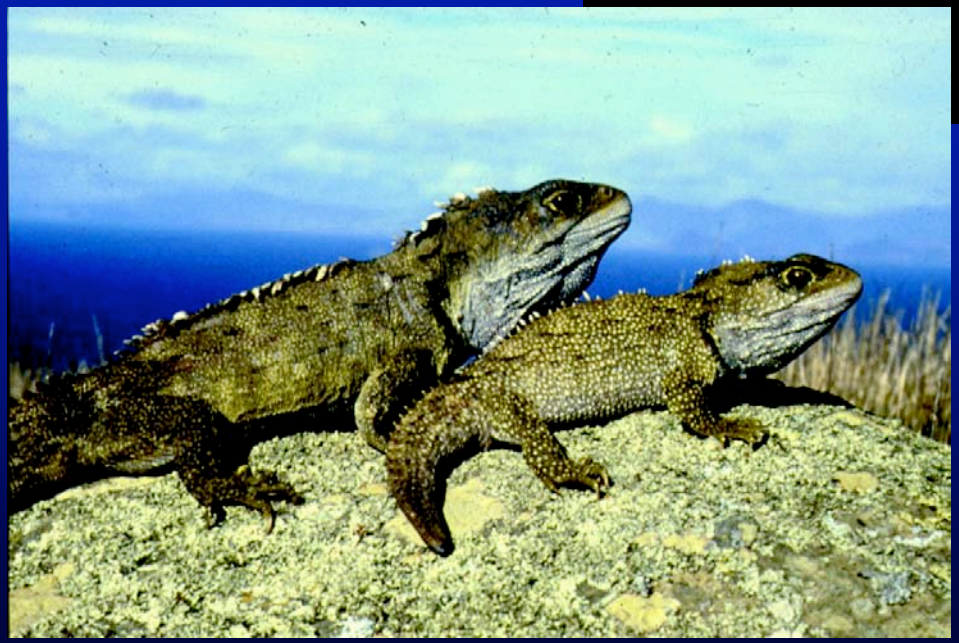
- Modern imaging technology extensive
  - X-ray; MRI; CAT scan
- Used for non- to minimally invasive 'view'
- Can provide extensive information



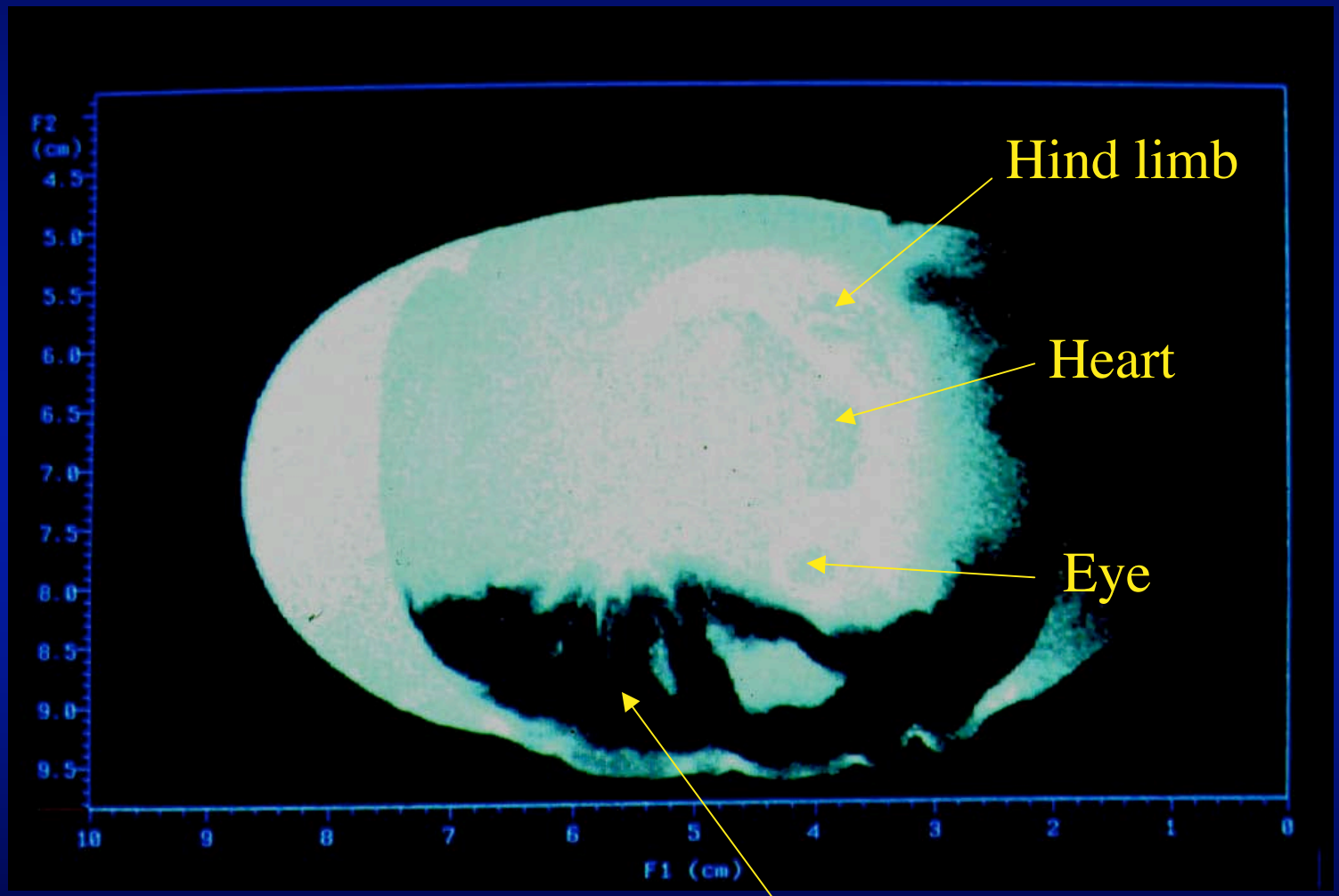
Laparoscopy of Tuatara ovary

X-ray of gravid tuatara



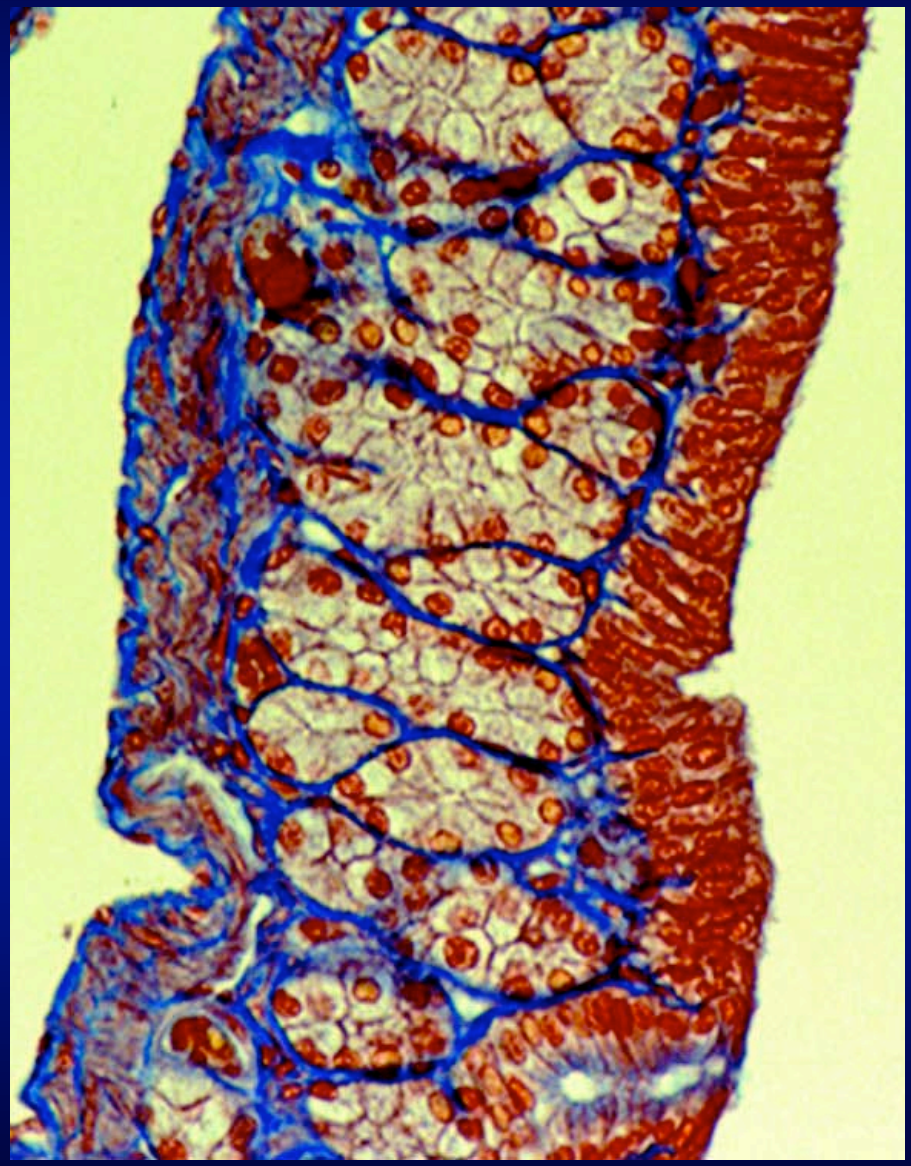


MRI of an
Alligator egg



Histology

- Sections of tissues cut at micrometer thickness or smaller
- Provides view of tissue at cellular or subcellular level
- Initial process of many other processes



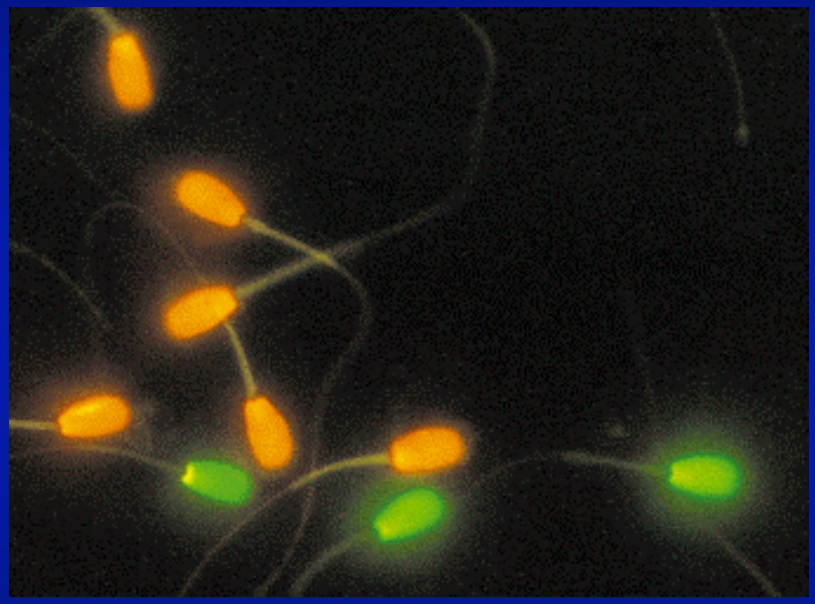
SEM/TEM

- Electron microscopy allows sub cellular view of cells
- Powerful tools for anatomy and physiology
- SEM: tissue dried - coated with gold
- TEM: tissue fixed - cut at < 1 μm

SEM of uterine surface with
Egg shell fibers extruding

Histochemistry

- Used to examine specific enzymatic reactions of cells
  - Can be used to identify pathological tissues
  - Or live and dead tissue

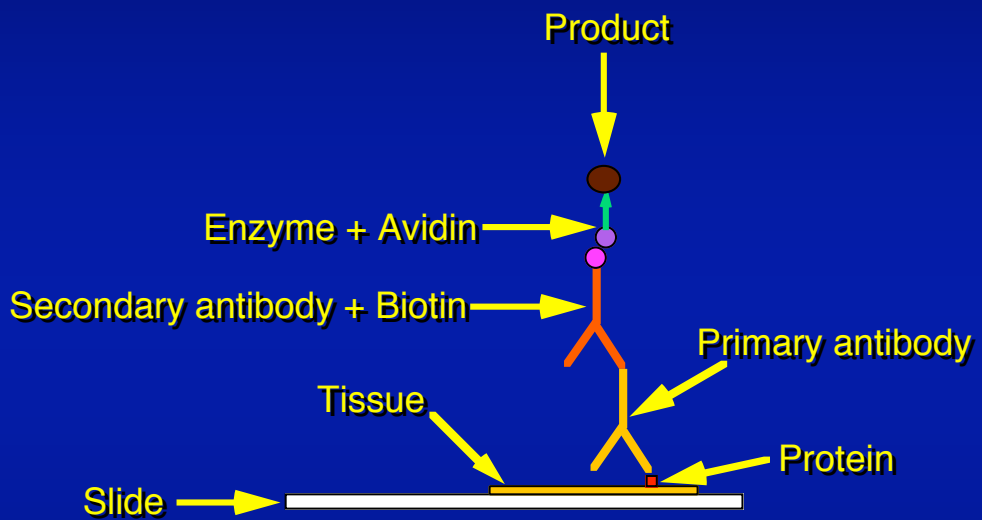


- Bull sperm
  - live - green
  - dead - red

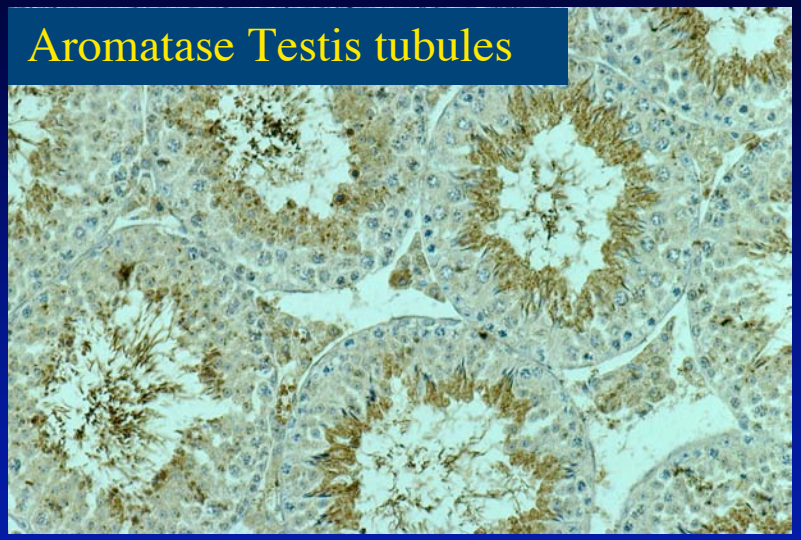
Immunocytochemistry

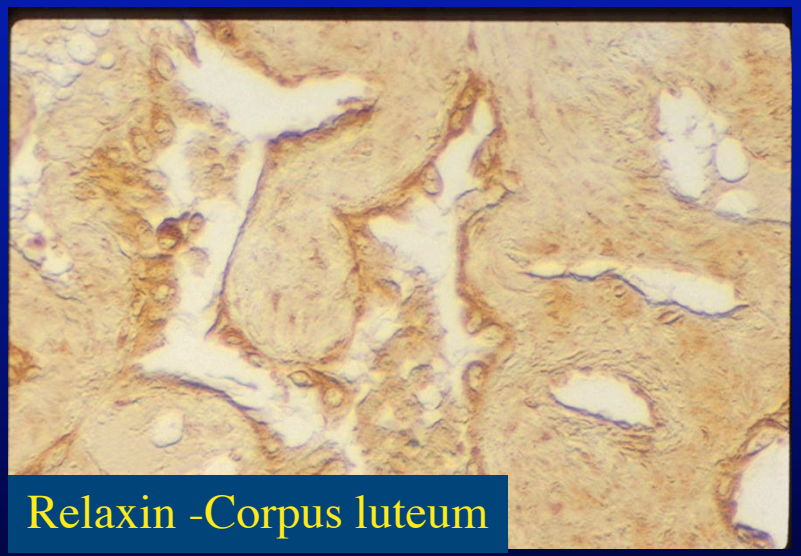
- Used to identify location of protein in a cell/tissue
- Use of a specific antibody and targeted enzyme reaction

Immunocytochemistry

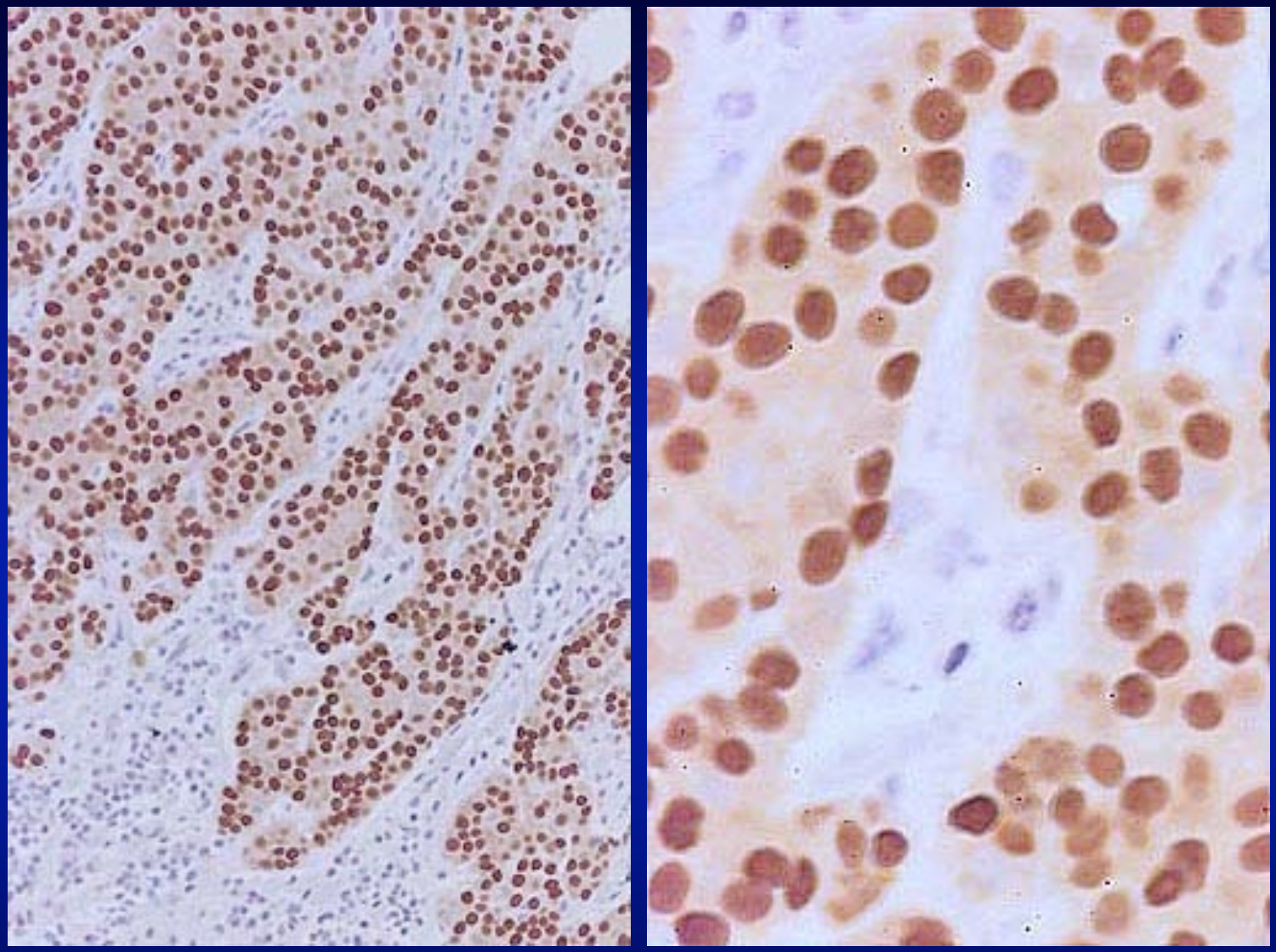


Aromatase Testis tubules





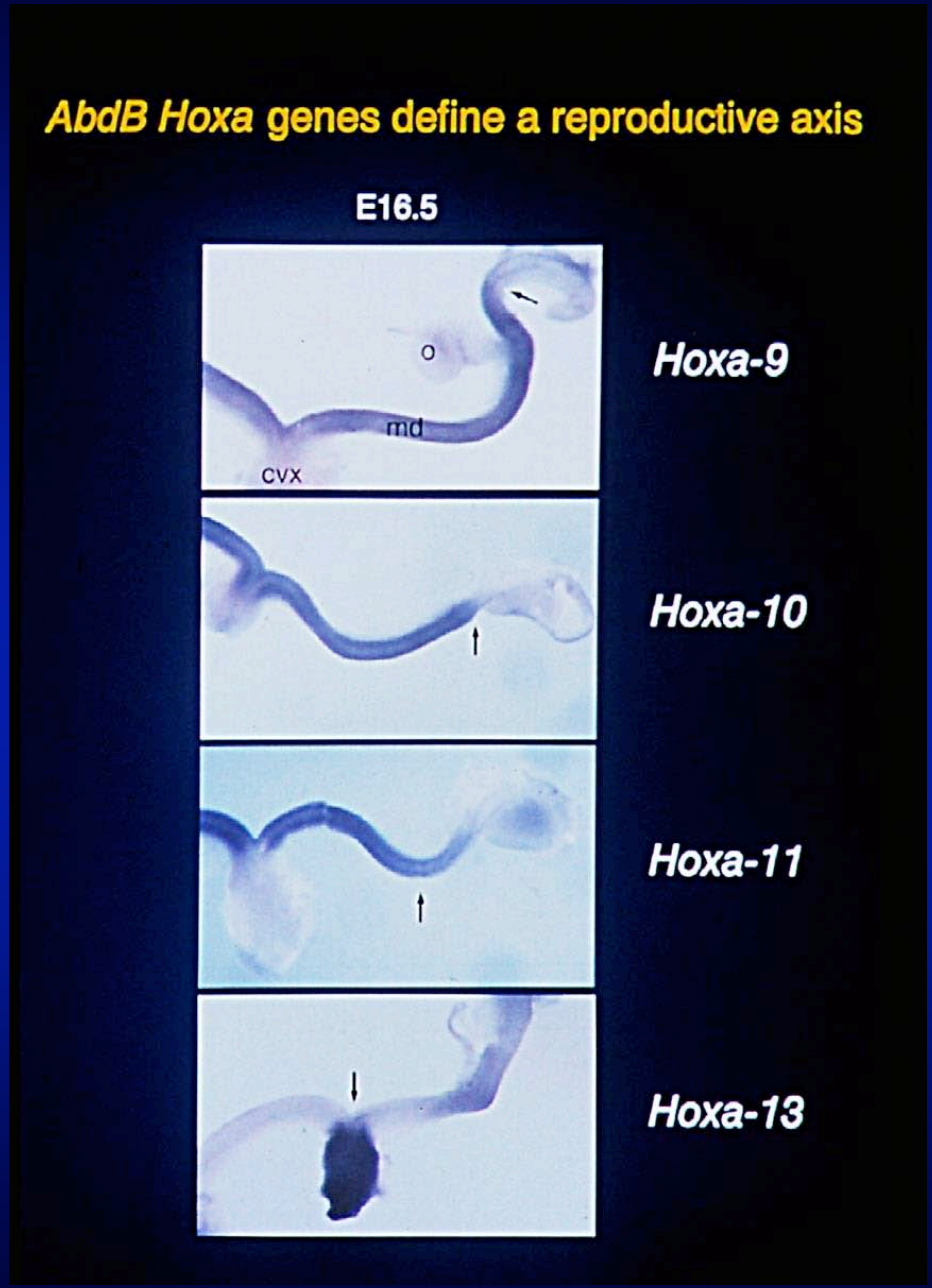
Relaxin -Corpus luteum



ICC for ER in Uterine tissue

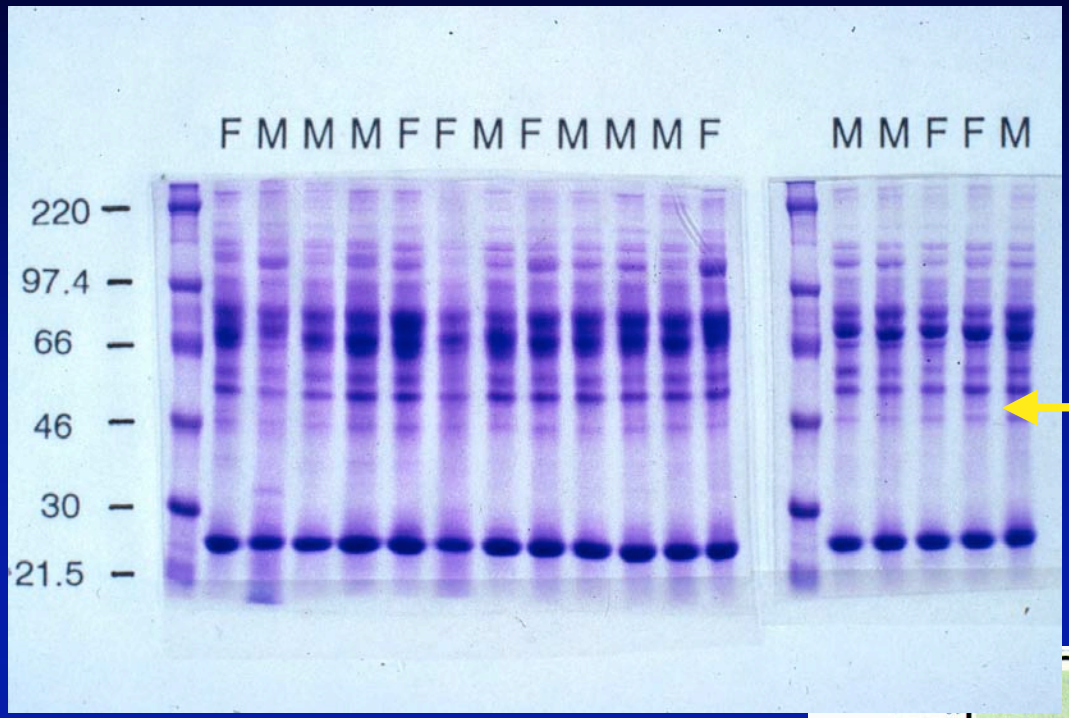
In situ Hybridization

- Used to localize specific mRNA in tissue or cell
- Targeted probe complementary to the mRNA



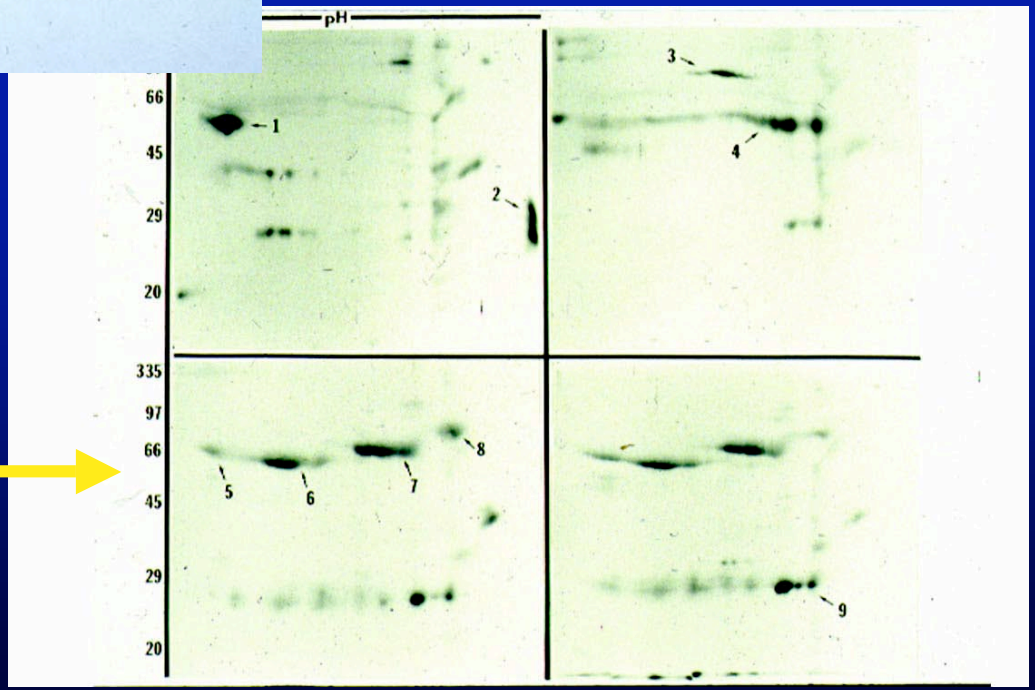
Electrophoresis

- Use molecular weight &/or charge to separate chemicals
  - proteins or RNA or DNA commonly isolated using electrophoresis
  - 1 or 2 dimensions



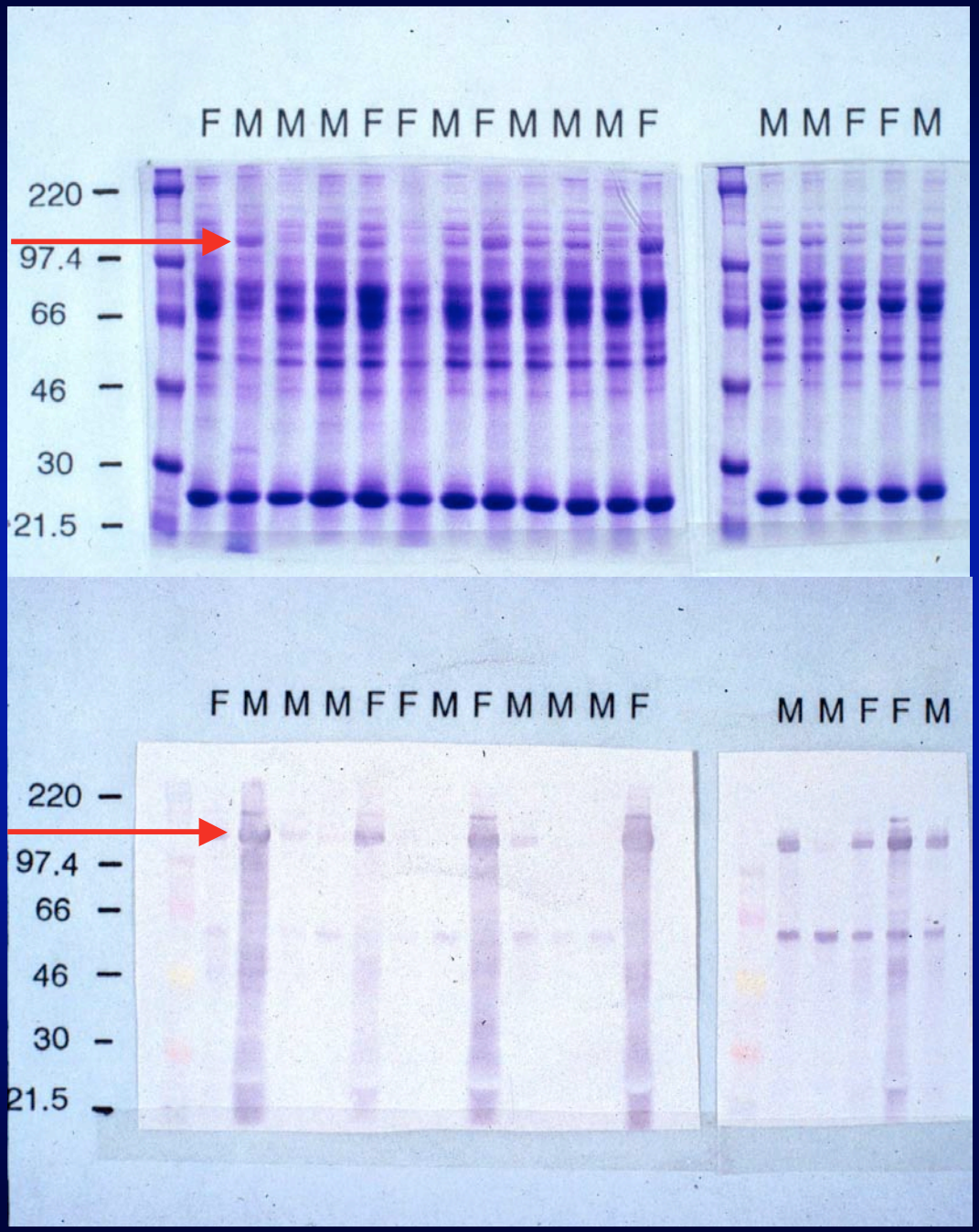
1D-SDS PAGE for
Serum proteins in fish

2D-SDS PAGE for
Oviduct proteins in gator



Western blots

- Protein from electrophoretic gel transferred to membrane
- 'Stained' with a specific antibody
  - ICC on gel blot
- Identifies a specific band associated with a specific protein

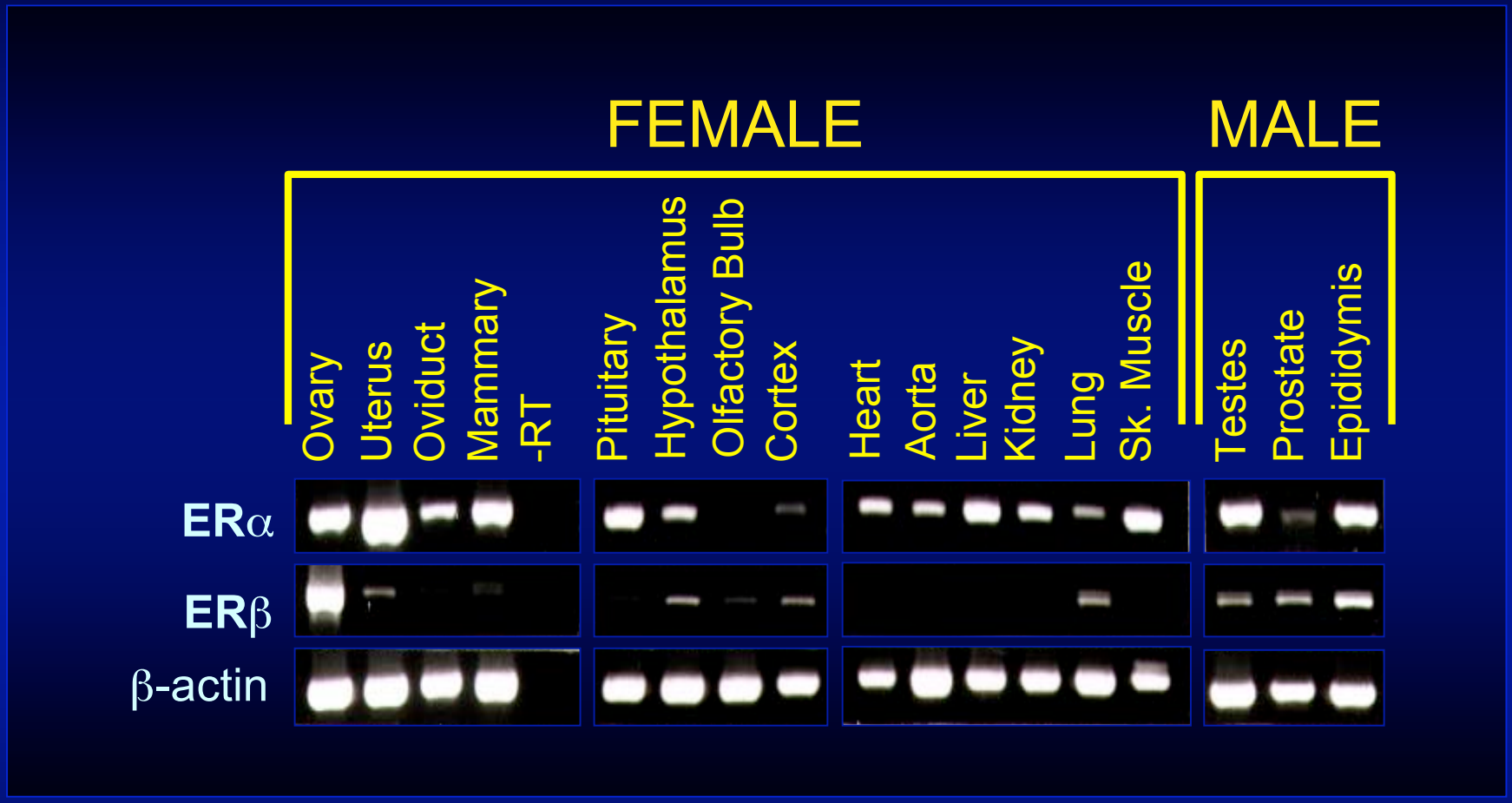


1D PAGE for Vtg

Western for Vtg

RNA/Northern Blot

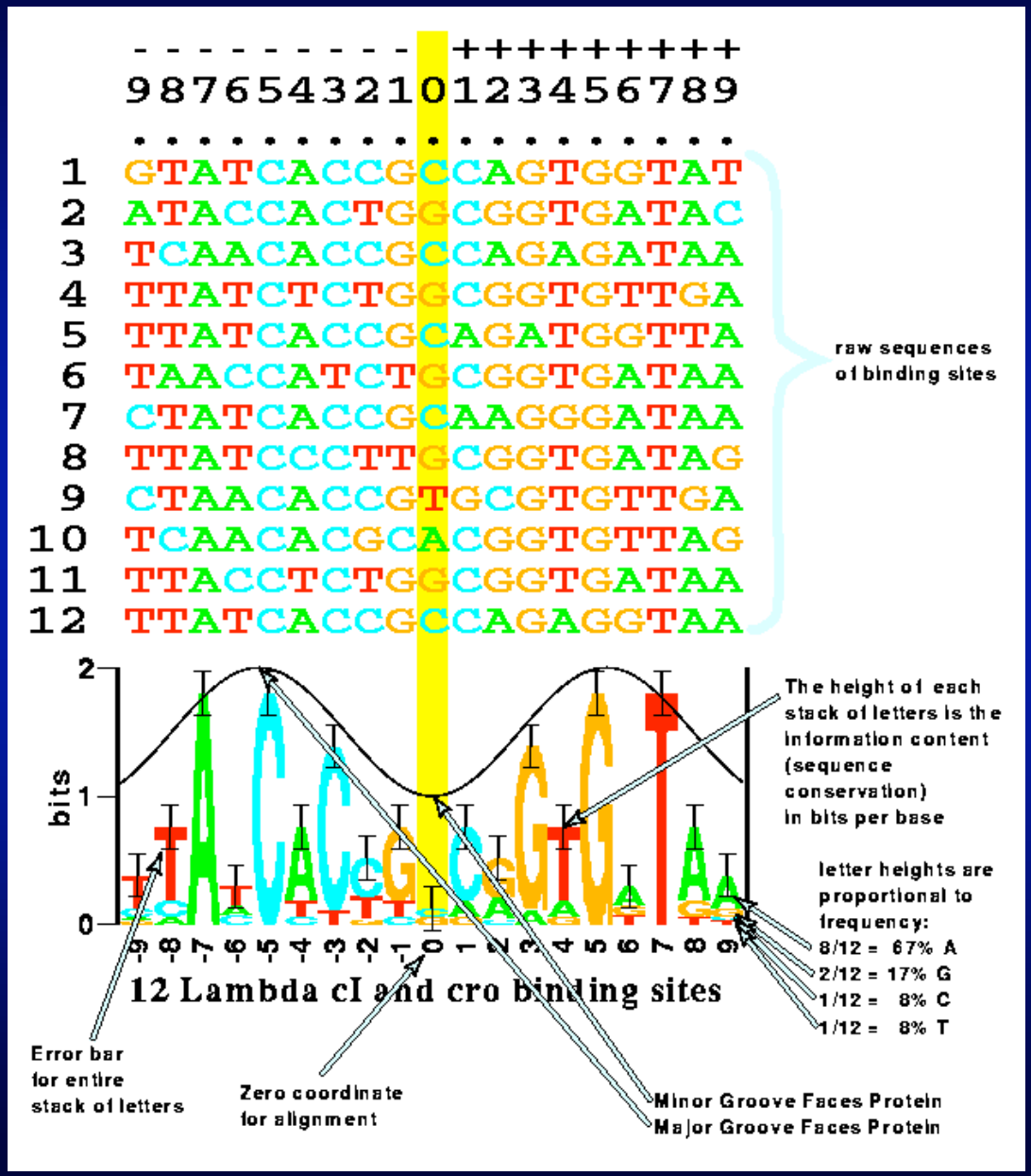
- Electrophoresis of RNA
- Transfer to membrane and probe with complementary radioactive cDNA
- Expose X-ray film/plate

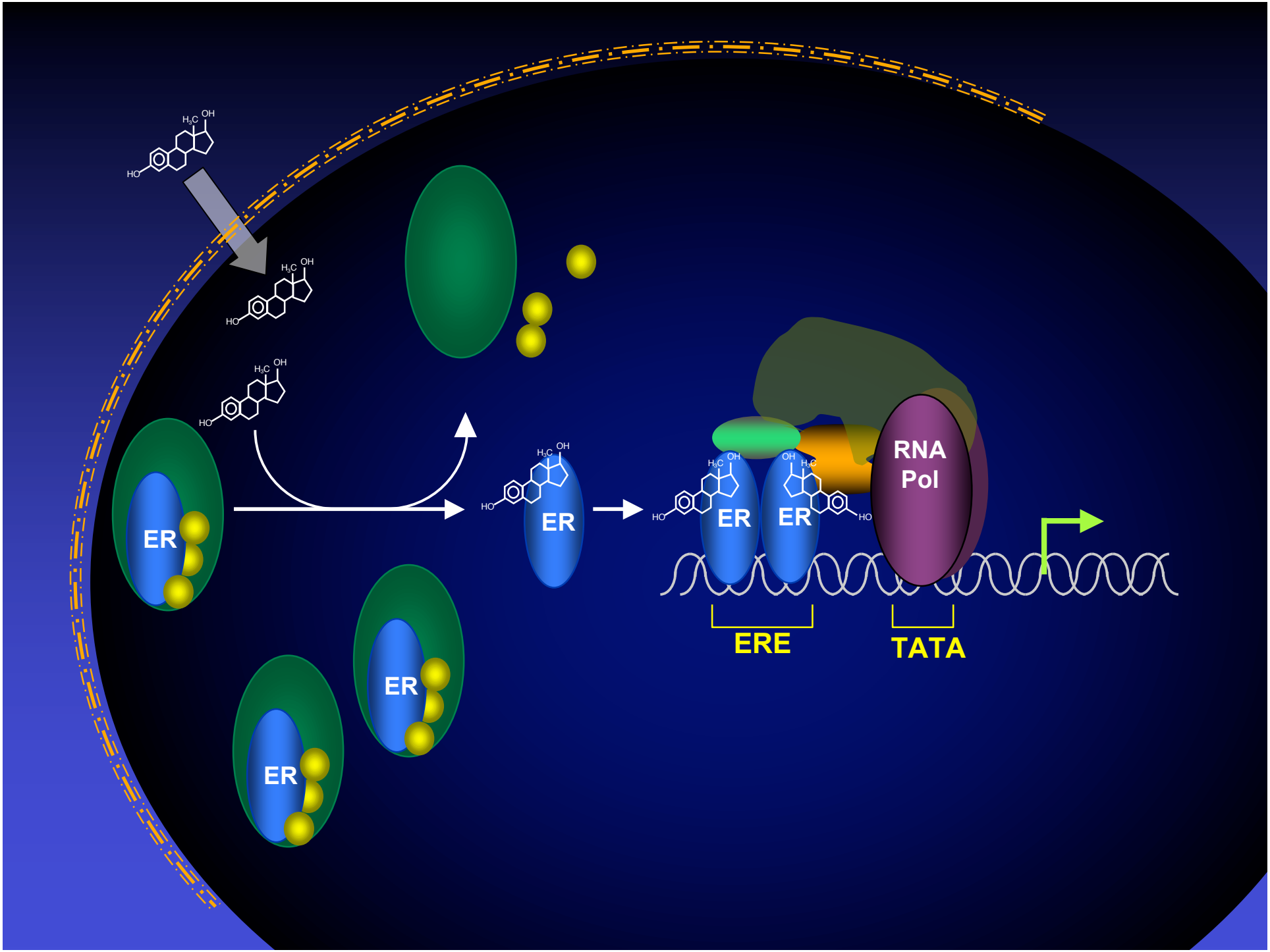


JF Couse and KS Korach (1999) *Endocrine Reviews*. 20:358-417.
JF Couse et al. (1997) *Endocrinology*. 138:4613-4621.

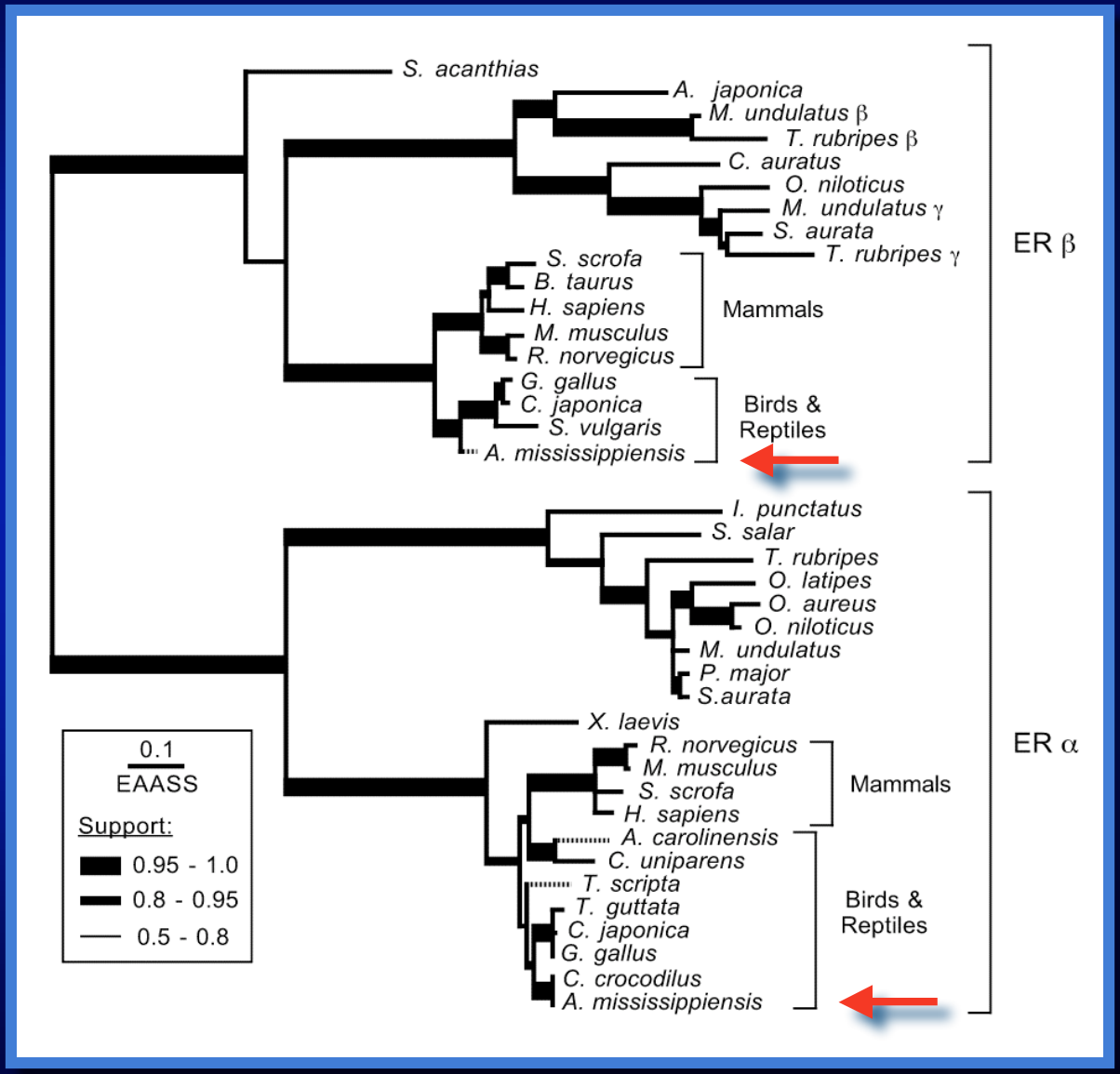
Couse, 1999

DNA Sequencing




ER
ER
ER
ER
ER
ER
RNA
Pol
ERE
TATA

Phylogeny of ER

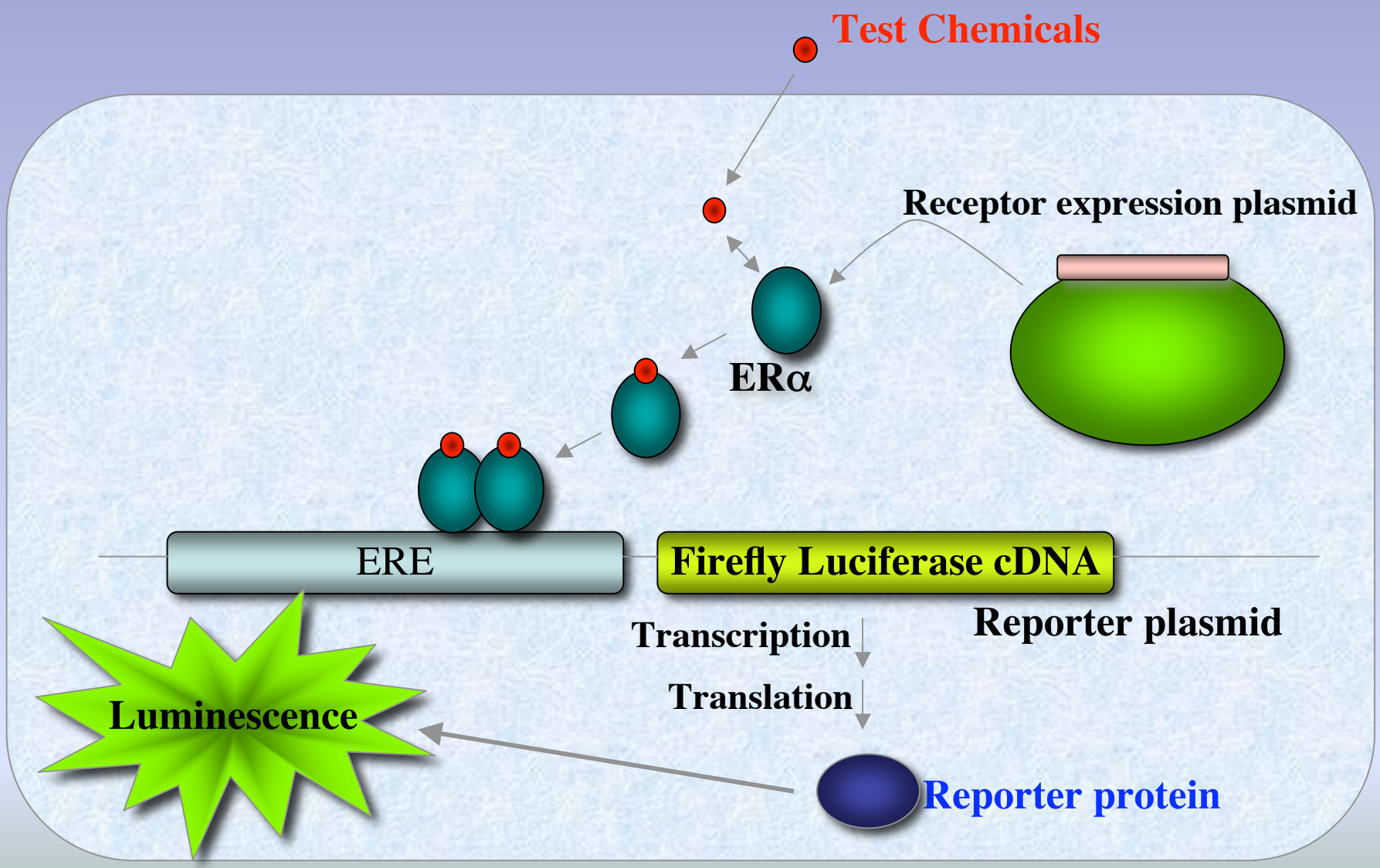


Katsu, et al., Gen. Comp. Endo. (2003)

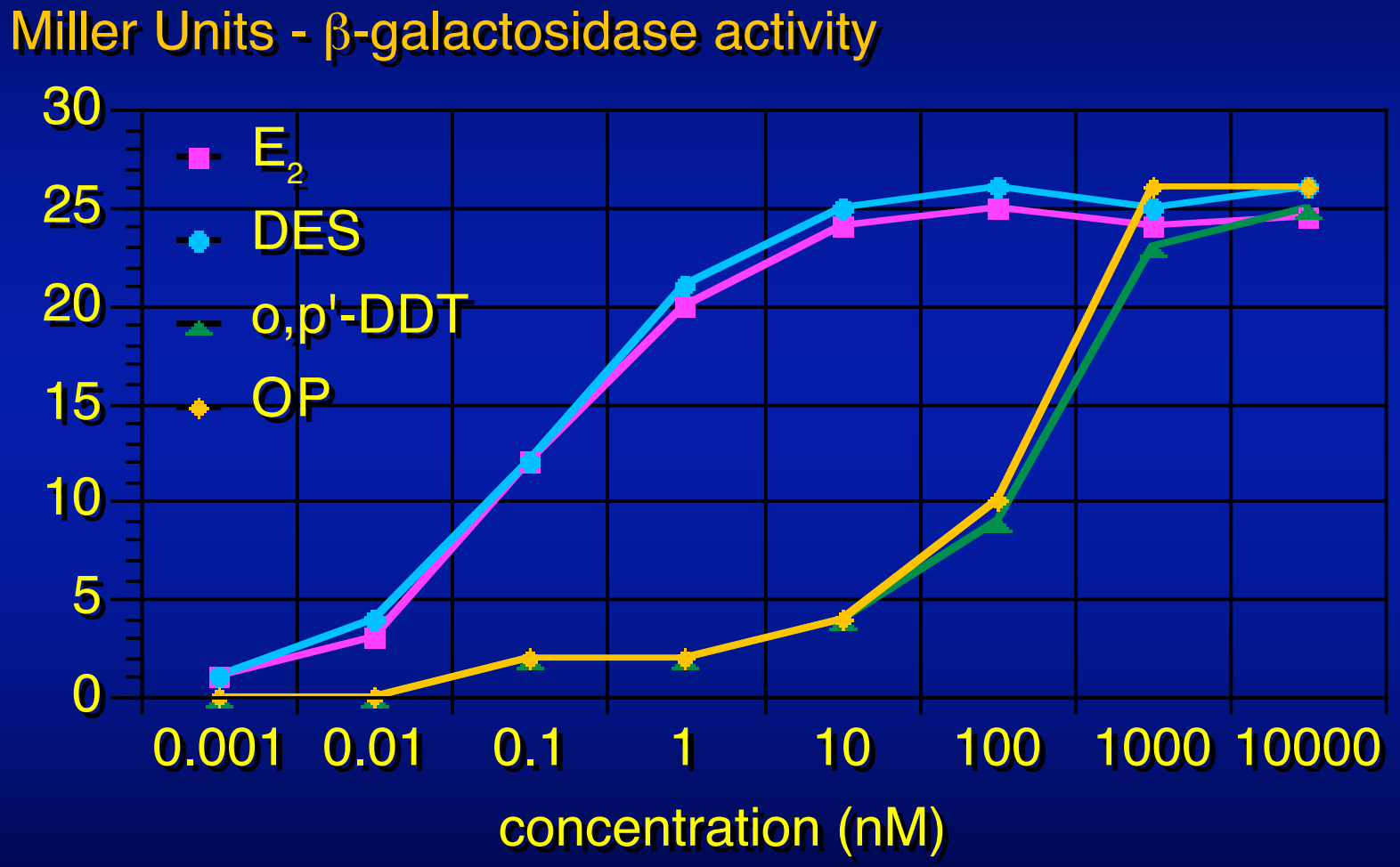
Transfected Cell Line

- Develop a test 'organism'
- Insert a receptor-gene construct if interest for testing
  - Human ER or AR with reporter gene

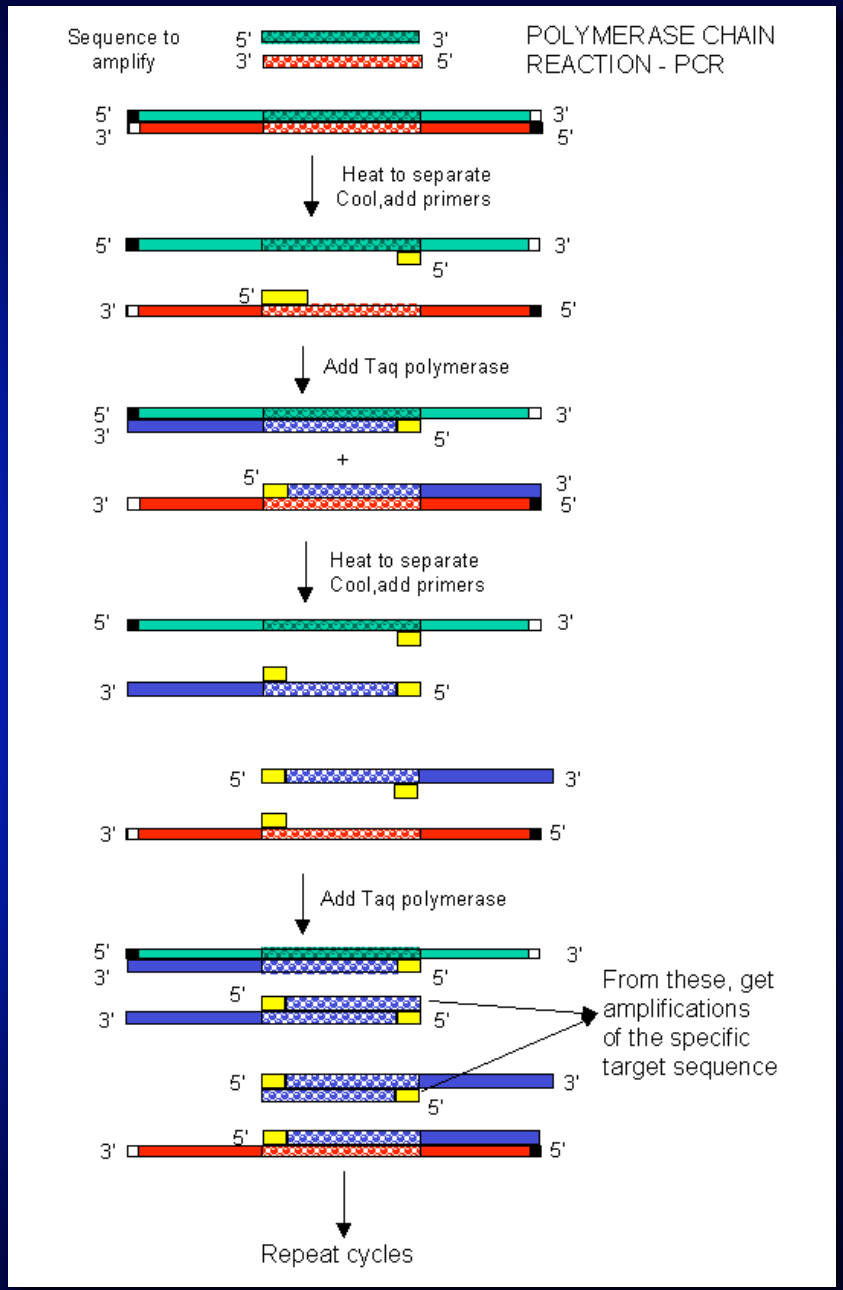
Reporter Gene Assay using ERE-Luc



Yeast Estrogen Screen (YES) Assay



Arnold et al., 1996, Environ. Health Perspec.



PCR

- Use of natural DNA/RNA mechanisms