Molecular Cytogenetics

Molecular Cytogenetics of Fishes

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CYTOGENETICS

Is the study of the structure and properties of chromosomes, chromosomal behaviour during mitosis and meiosis, chromosomal influence on the phenotype and the factors that cause chromosomal changes (Hare and Singh, 1979).

Fish exhibit the greatest diversity of all vertebrates, making this group extremely attractive for the study of a number of evolutionary questions

Major advances in chromosomal studies have been achieved in the current 'molecular cytogenetic era' based on detecting DNA sequences on chromosomes, parts of chromosomes or even whole genomic DNA by fluorescence in situ hybridization or in situ hibridization

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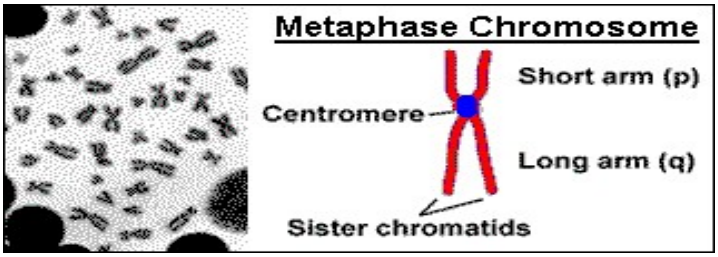
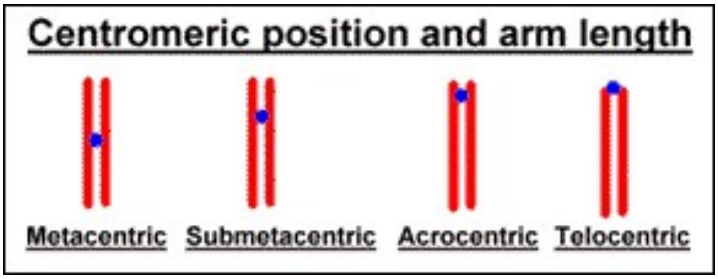
Nomenclature of chromosomes





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BANDING OF CHROMOSOMES

- G - Banding
- Q - Banding
- C - Banding
- R - Banding
- T - Banding
- NOR - Banding
- High Resolution Banding
- Restriction Endonuclease Banding

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Fluorescence in situ Hybridization (FISH)

- FISH - a process which vividly paints chromosomes or portions of chromosomes with fluorescent molecules
- Opening picture - Human M-phase spread using DAPI stain

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Fluorescence in situ Hybridization (FISH)

- Identifies chromosomal abnormalities
- Aids in gene mapping, toxicological studies, analysis of chromosome structural aberrations, and ploidy determination

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Fluorescence in situ Hybridization (FISH)

- Used to identify the presence and location of a region of DNA or RNA within morphologically preserved chromosome preparations, fixed cells or tissue sections

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Fluorescence in situ Hybridization (FISH)

- This means you can view a segment or entire chromosome with your own eyes
- Was often used during M phase but is now used on I phase chromosomes as well

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Fluorescence in situ Hybridization (FISH)

- Advantage: less labor-intensive method for confirming the presence of a DNA segment within an entire genome than other conventional methods like Southern blotting

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FISH Procedure

- Denature the chromosomes
- Denature the probe
- Hybridization
- Fluorescence staining
- Examine slides or store in the dark

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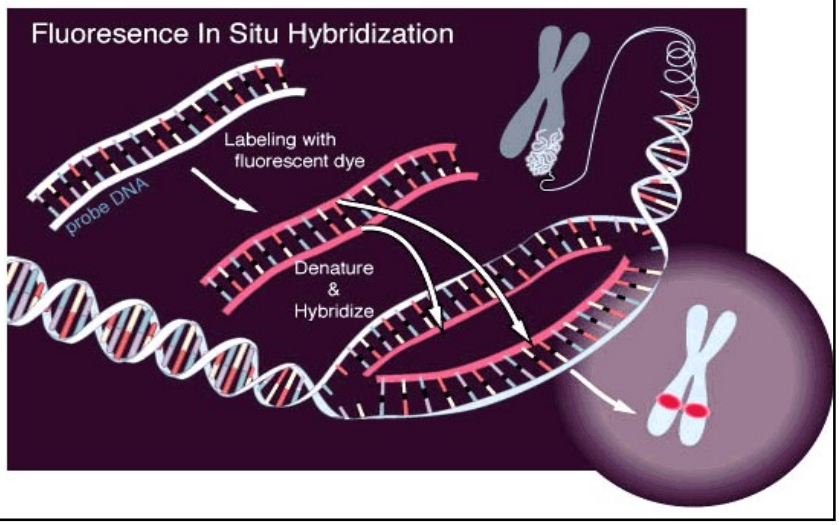
FISH Procedure



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FISH Uses

- Detection of high concentrations of base pairs
- Eg: Mouse metaphase preparation stained with DAPI (a non-specific DNA binding dye with high affinity for A-T bonds)

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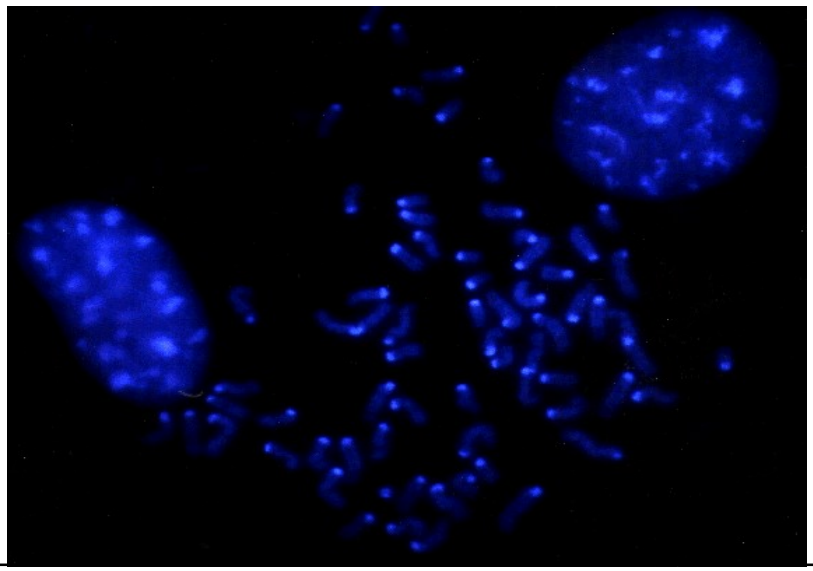
FISH Uses



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FISH Uses

- Centromere regions stained brighter - means they are rich in A-T bonds
- Also used in germ cell or prenatal diagnosis of conditions such as aneuploidies

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FISH and Telomeres

- Telomeric probes define the terminal boundaries of chromosomes (5’ and 3’ ends)
- Used in research of chromosomal rearrangements and deletions related to cell aging or other genetic abnormalities

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FISH and Telomeres

- Special telomeric probes specific to individual chromosomes have been designed
- Probe is based on the TTAGGG repeat present on all human telomeres

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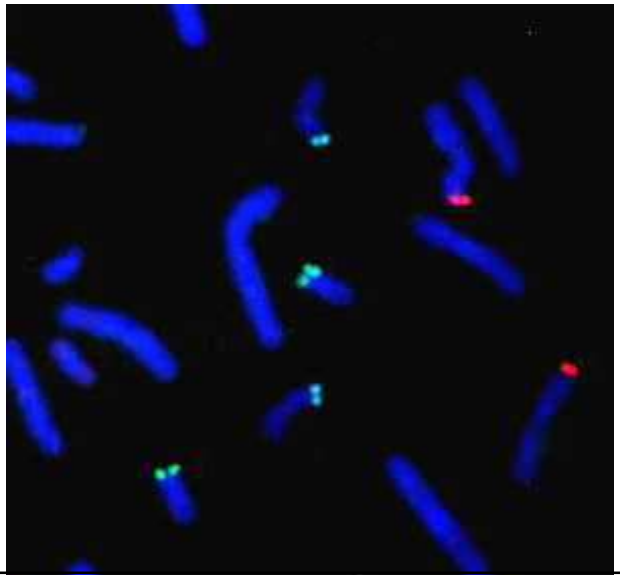
FISH and Telomeres



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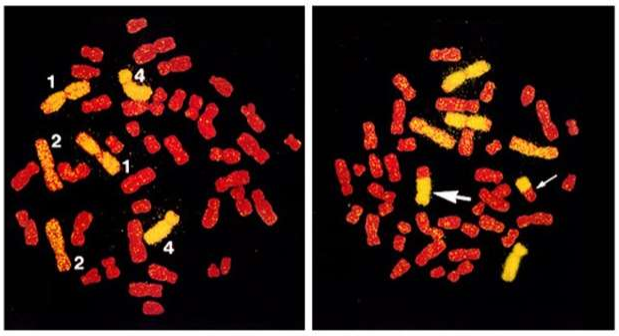
FISH – Mutasi in zebrafish



normal abnormal (translocation)

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In situ Hybridization

- ✓ISH is a molecular biologic technique that follows the principles of assays, with the added requirement of maintaining a monucleic acid hybridizationrphologic context.
- ✓The components of an ISH assay can be mapped to preanalytic, analytic, and postanalytic variables that must be understood and considered in laboratory quality control.
- ✓As with any assay, an adequate sample representative of the lesion being investigated is of paramount importance. In many clinical settings, ISH is used in a similar way as IHC, as an adjunctive test following routine histologic evaluation. This has the advantage of allowing histology to guide selection of the most representative sample. However, the limitation is that routine tissue processing is far from standardized.

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Probes

- **<u>Probe</u> is a nucleic acid that**
  - **can be labeled with a marker which allows identification and quantitation**
  - **will hybridize to another nucleic acid on the basis of base complementarity**
- **Types of labels**
  - **Radioactive (^{32}P, ^{35}S, ^{14}C, ^{3}H)**
  - **Fluorescent**
    - **FISH: fluorescent in situ hybridization**
      - **chromosomes**
  - **Biotinylated (avidin-streptavidin)**

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Hybridization

- **Nucleic acid <u>hybridization</u> is the formation of a duplex between two complementary sequences**
- **Intermolecular hybridization: between two polynucleotide chains which have complementary bases**
  - **DNA-DNA**
  - **DNA-RNA**
  - **RNA-RNA**
- **<u>Annealing</u> is another term used to describe the hybridization of two complementary molecules**

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Denaturation - Renaturation

Double-stranded DNA

Single-stranded DNA

Initial Base pairing

Renatured DNA

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Solid Support Hybridization

- **Solid support hybridization: DNA or RNA is immobilized on an inert support so that self-annealing is prevented**
- **Bound sequences are available for hybridization with an added nucleic acid (probe).**
- **Filter hybridization is the most common application:**
  - **Southern Blots**
  - **Dot/Slot Blots**
  - **Northern Blots**
- **In-silica hybridization (glass slides)**
  - **in situ hybridization (tissue)**
  - **Chromosomal (FISH)**
  - **Microarrays**

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Southern Blots

- **Southern blotting is a procedure for transferring denatured DNA from an agarose gel to a solid support filter where it can be hybridized with a complementary nucleic acid probe**
- **The DNA is separated by size so that specific fragments can be identified**
- **Procedure:**
  - **Restriction digest to make different sized fragments**
  - **Agarose gel electrophoresis to separate by size**
  - **Since only single strands bind to the filter, the DNA must be denatured.**
  - **Denaturation to permit binding to the filter (NaOH)**
  - **Transfer to filter paper (capillary flow)**
  - **Hybridization to probe**
  - **Visualization of probe**

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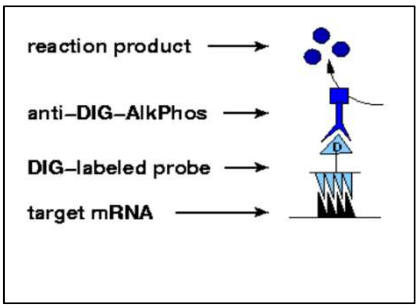
Methods

Procedure ISH

Aromatase probe size is 526 kb



Probe 1:1000 for 16h
Anti-Dig AP 1:2000
Hybridization temperature 65°C

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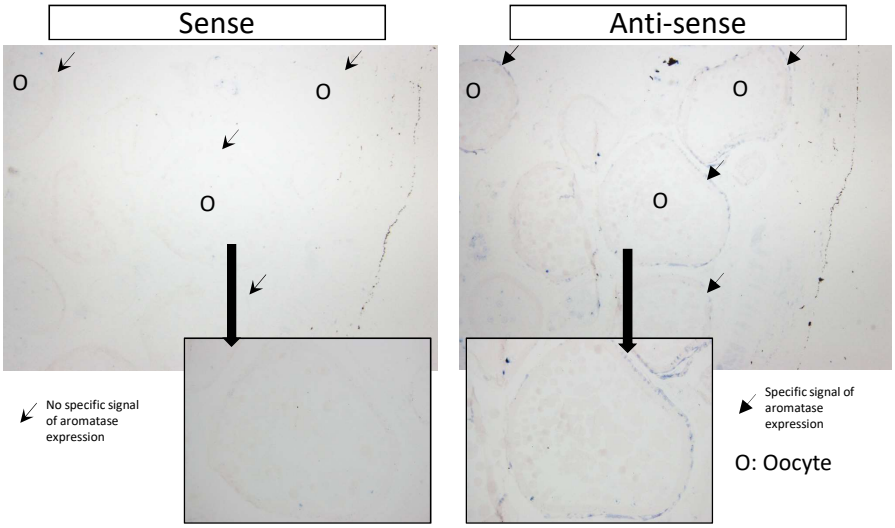
Cyp19a1 (ovarian-type aromatase) expression of the adult ovary



Aromatase expression was not detected in the slide with sense probe, in other hand it was specific observed in the slide with anti-sense probe

Aromatase expression was observed only in mature oocyte of adult ovary

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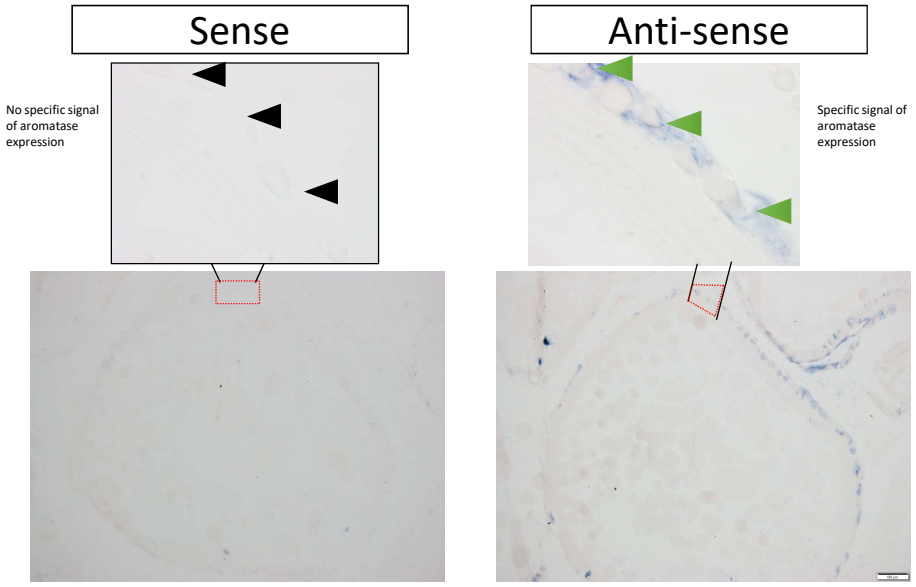
Cyp19a1 (ovarian-type aromatase) expression in the follicle



Probes is specific for aromatase expression in pejerrey

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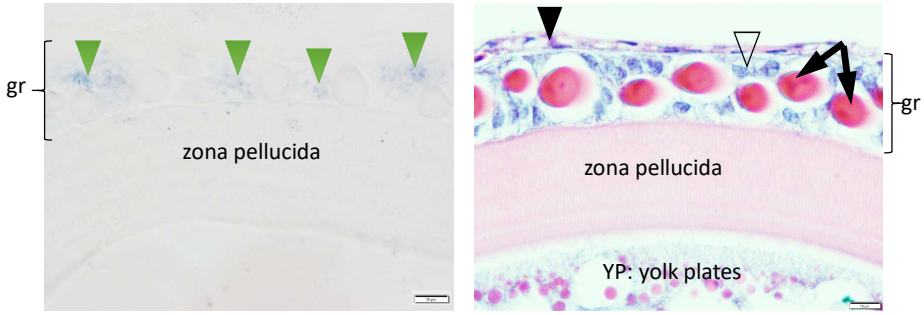
Aromatase expressed in which cell of oocyte?



Theca indicates a pyriform-like cell gr: the granulosa cell layer

the nucleus of prismatic follicular cells Aromatase expression

Aromatase expression was observed in granulosa cell of mature oocyte

How is expression in larvae during gonadal sex differentiation?

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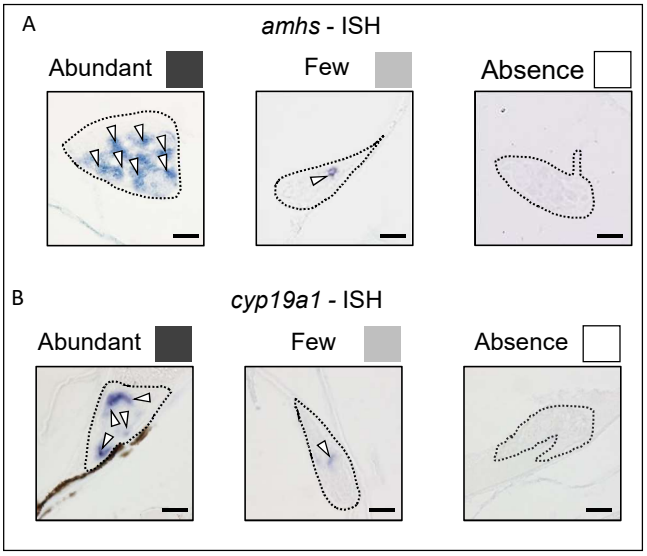
Amh and aromatase expressed in larvae



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