Clinical Consequences of Subtelomeric Chromosomal Rearrangements

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Objectives

- Understand basic chromosome structure and function
- Describe methods to detect abnormalities at chromosomal ends
- Discuss clinical effects of these abnormalities

Chromosome Structure

DNA Packing Ratio [starting DNA length/final length]:

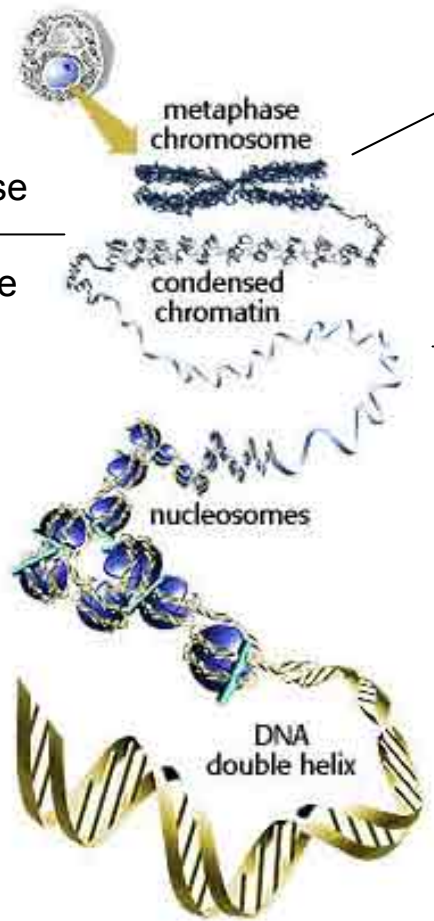
~10,000 metaphase

~1000 interphase

40 - ‘beads on a string’

6 - nucleosome

1 – DNA helix



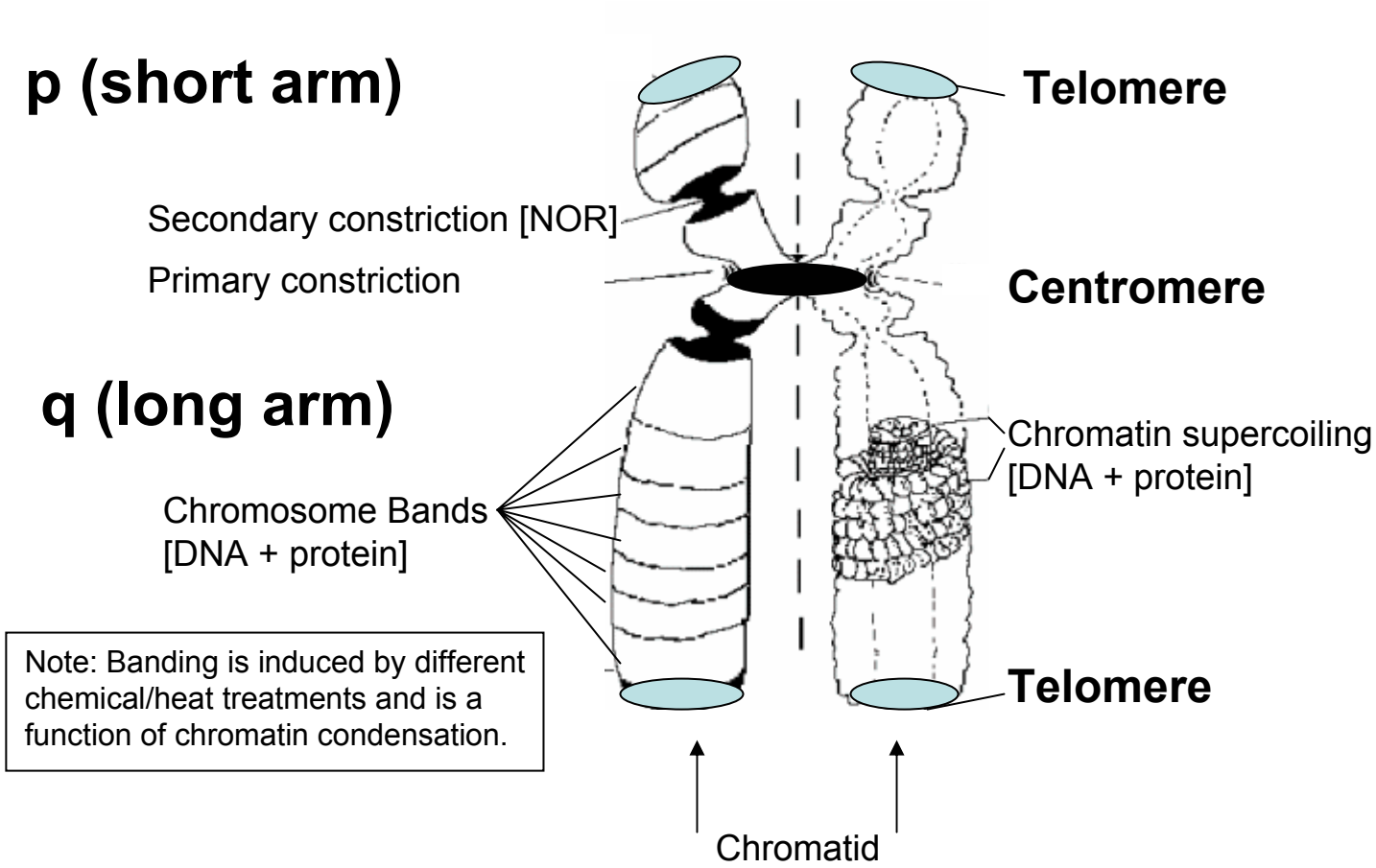
Chromatin packs tightly into metaphase chromosomes; 46 human chromosomes per somatic cell (23 from each parent)

Additional levels of chromatin compaction (supercoiling)

DNA wrapped around histone protein octamers (chromatin)

This year is 50th anniversary of reporting of structure of DNA (Watson, Crick, Franklin); 4 nucleotides – A pairs with T and G with C to form double helix

Metaphase Chromosome Anatomy

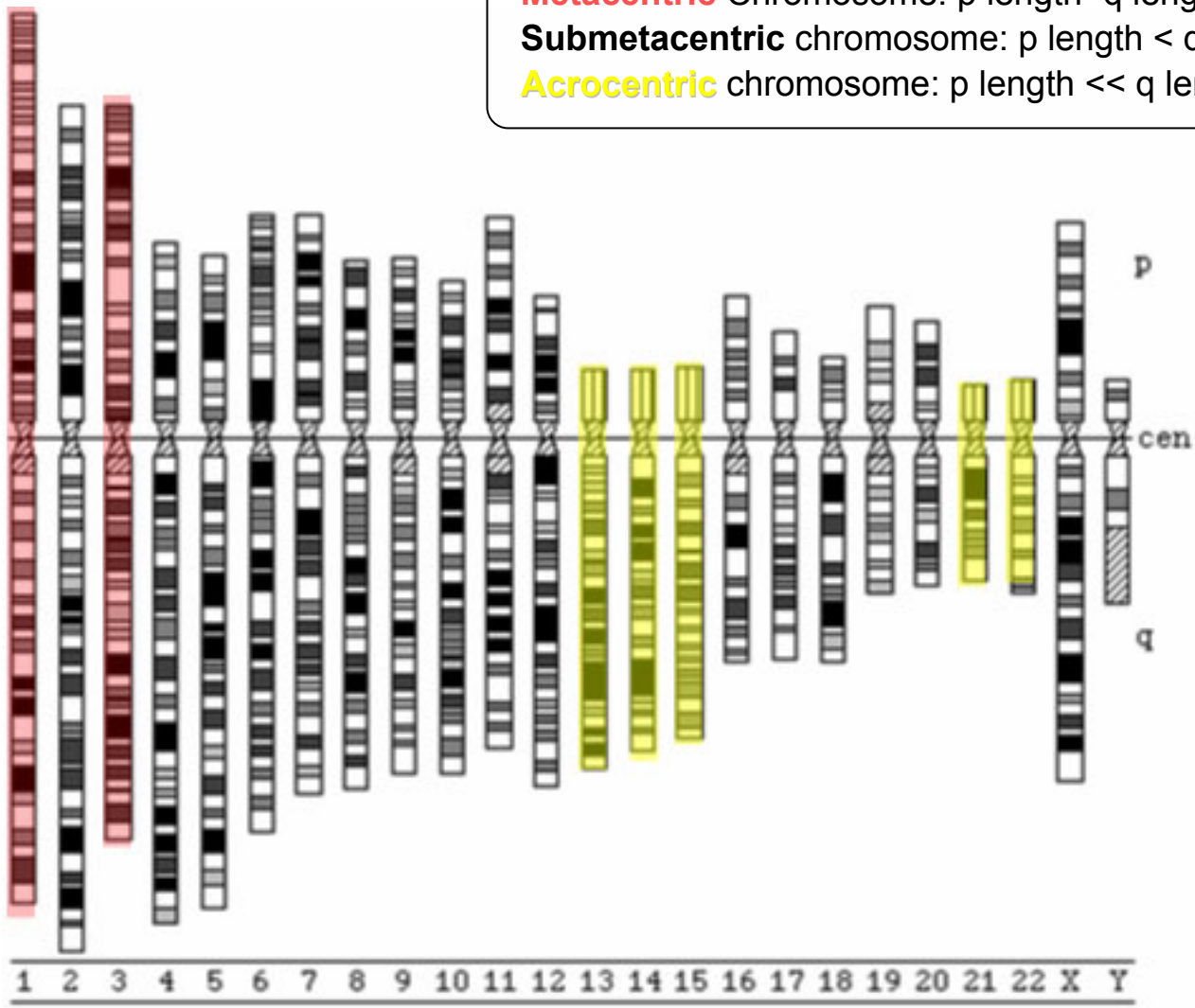


Centromeres

- Specialized protein-DNA structure in human chromosomes
- Chromosome-specific with some shared homologies (*CDE-I, CDE-II, CDE-III*) between chromosomes
- Reponsible for accurate segregration of chromosomes in somatic cells and germ cells
- Position determines whether chromosome is metacentric, submetacentric or acrocentric

Human Chromosomes: Relative Size

Metacentric Chromosome: p length=q length
Submetacentric chromosome: p length < q length
Acrocentric chromosome: p length << q length



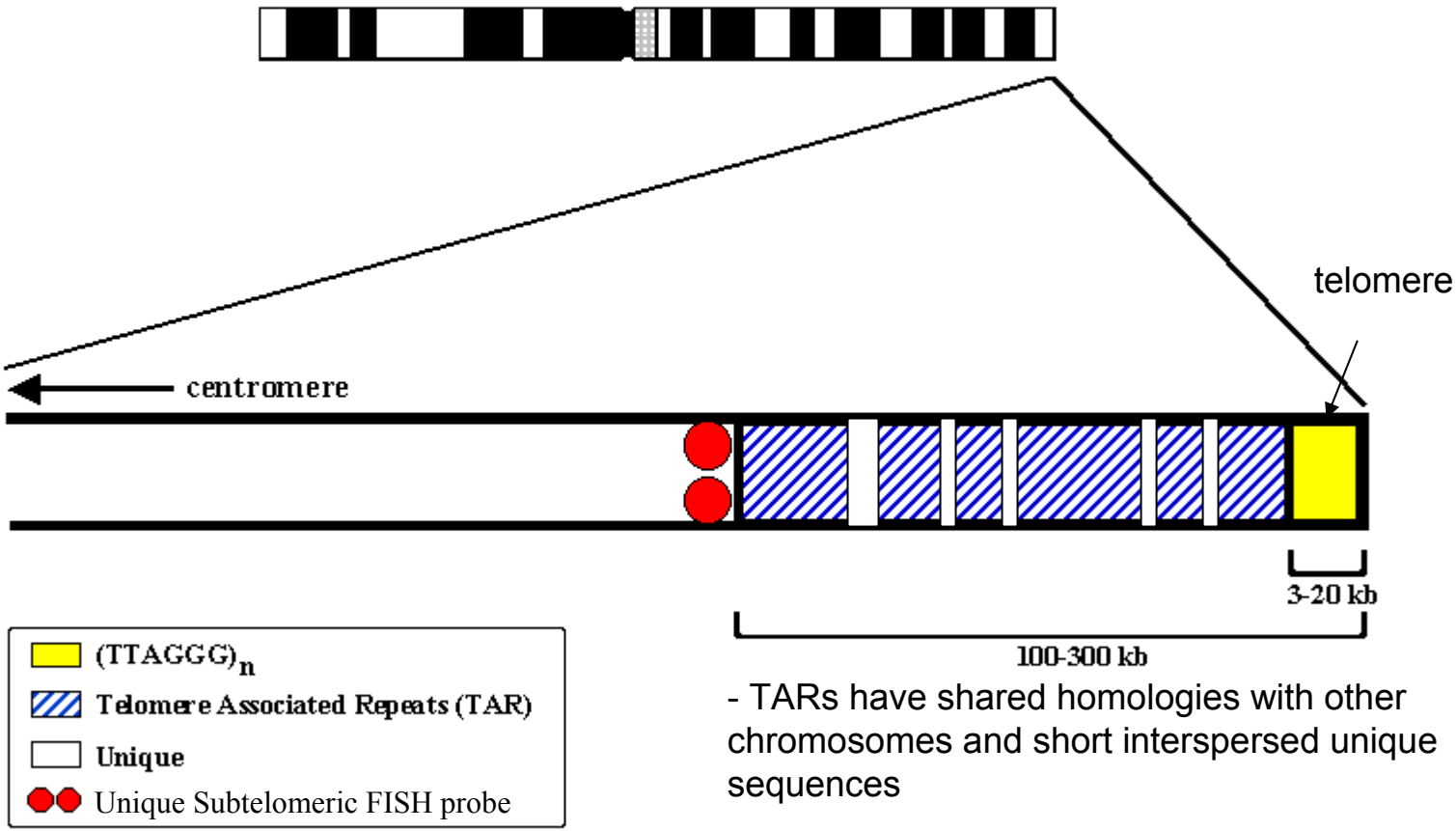
Chromosome Length ($\times 10^6$ bp) 260 → 45

Total length per haploid genome: 3×10^9 bp

Telomeres

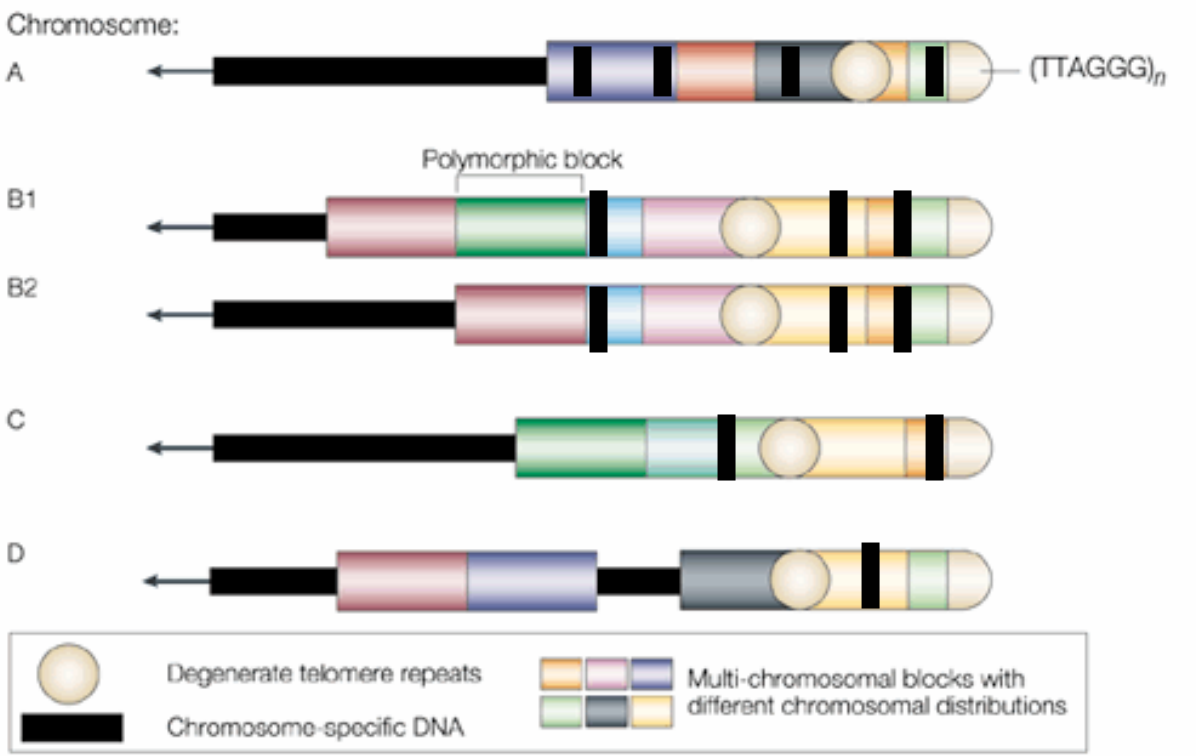
- Specialized protein-DNA structures containing long stretches of $(TTAGGG)_n$ repeats (3-20kb) at the ends of all chromosomes
- Shield chromosomes from degradation and end-to-end fusions with other chromosomes
- Loss of telomere repeats is associated with aging (senescence in somatic cells)
- In contrast, germ line and cancer cells have an enzyme (telomerase) to restore loss and maintain telomere length

Structure of Chromosome Ends



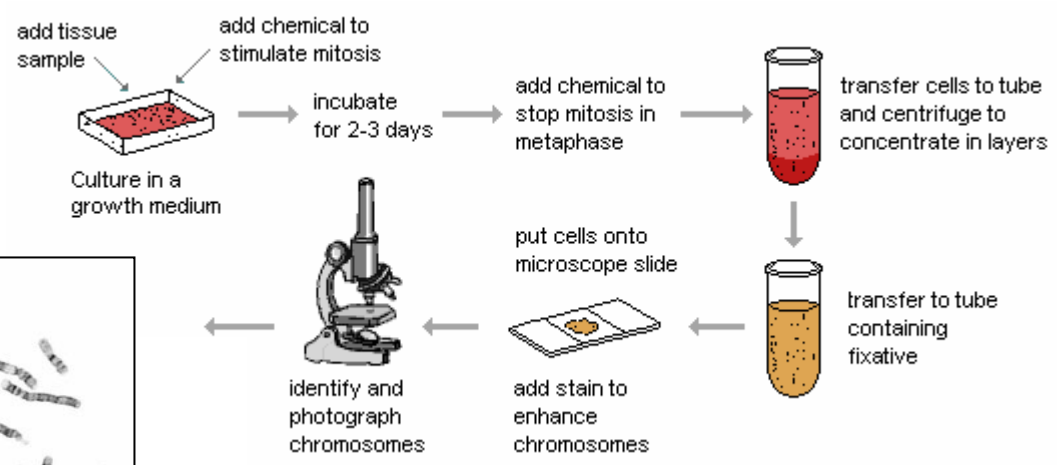
- TARs have shared homologies with other chromosomes and short interspersed unique sequences

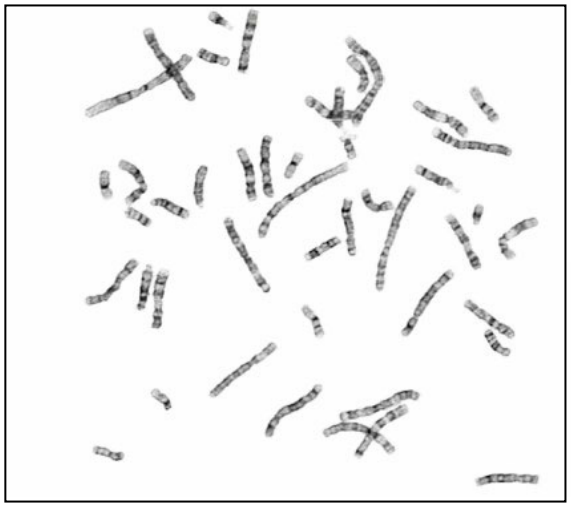
Schematic of TAR Structure



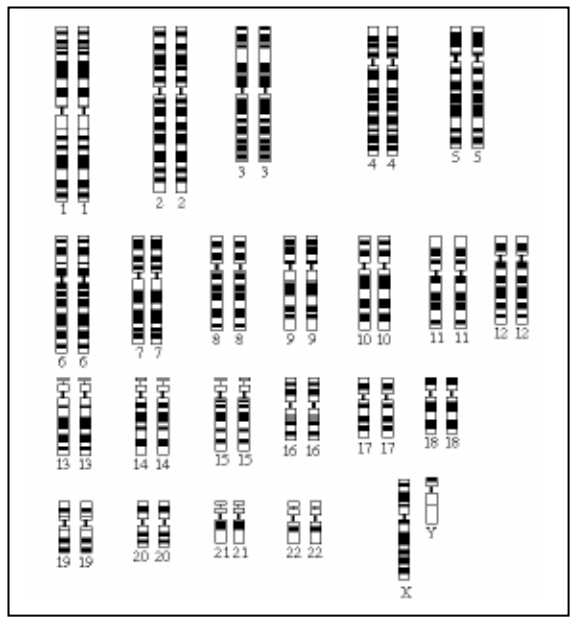
Modified from Nature Review Genetics 3:91, 2002; TAR = telomere associated repeats.

Chromosome Preparation





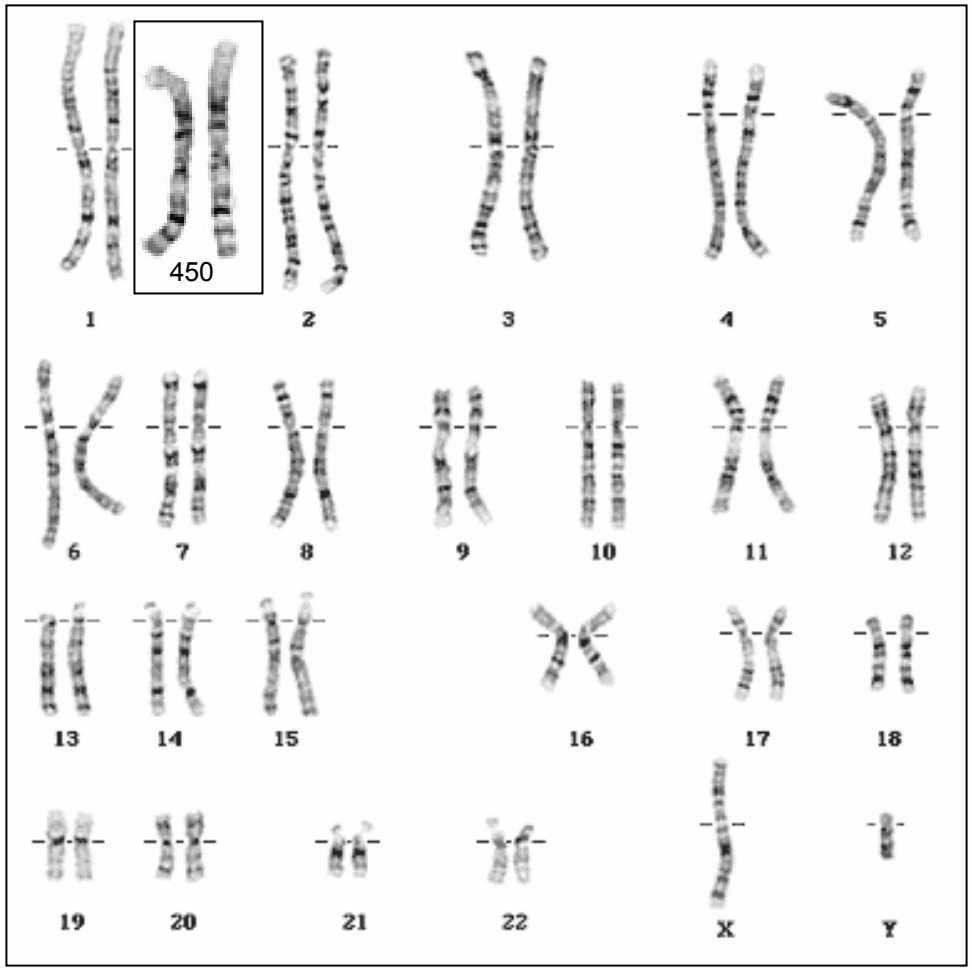
Metaphase with GTG-banding



Karyotype

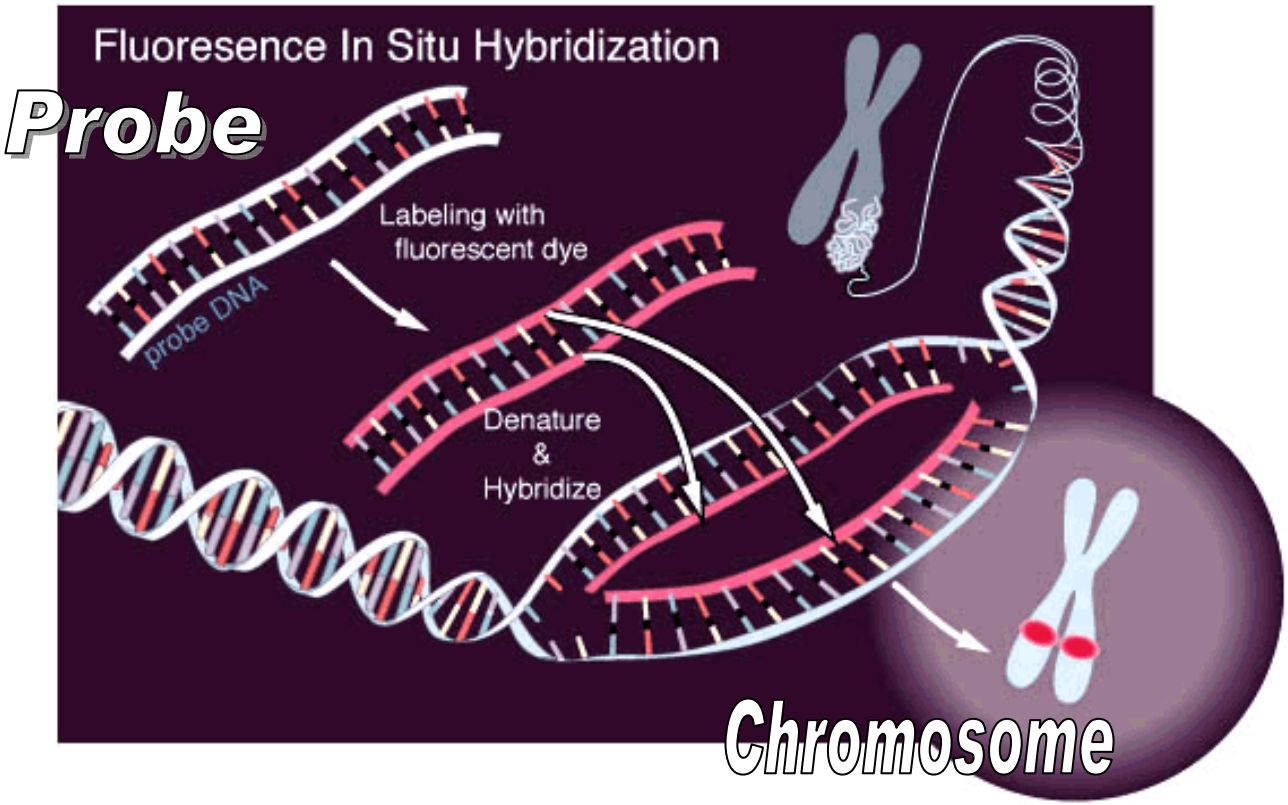
Light G-bands are gene rich, have a lower density of repetitive sequences as compared to dark G-bands. Chromosome ends are generally light G-bands.

Normal Male Karyotype (>550 band resolution)



Each chromosomal band has about 5 million base pairs of DNA (~50 genes); exchanges between terminal ends difficult to detect since light staining.

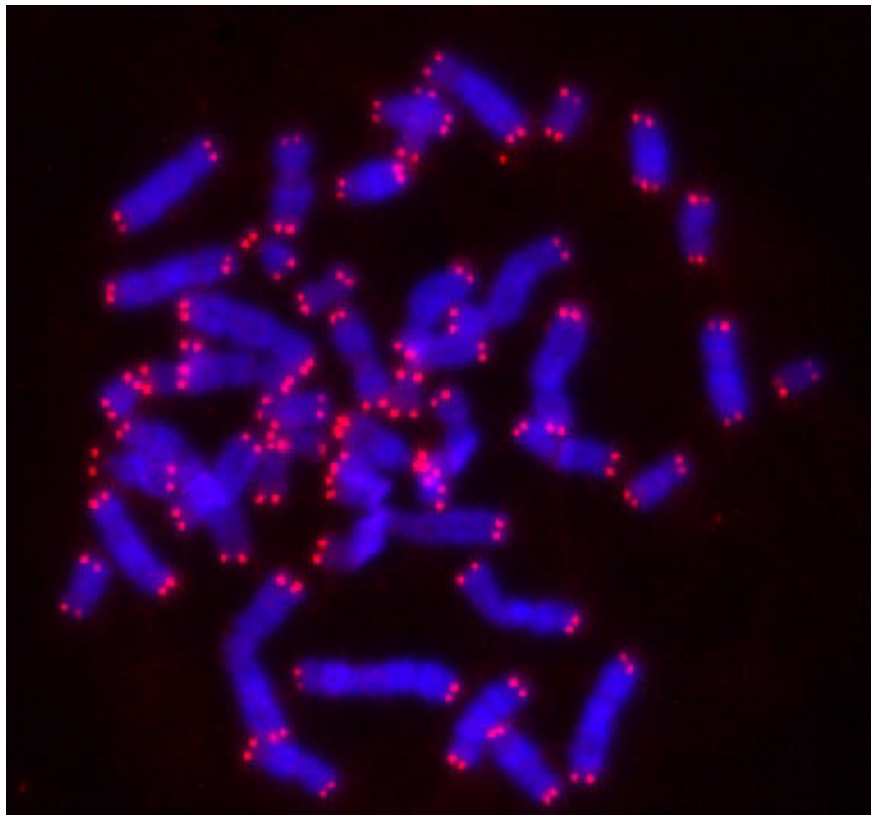
F.I.S.H.: A Molecular Cytogenetic Test



Complementary nucleic acid and chromosomal target DNA bind noncovalently; binding detected by fluorescence.

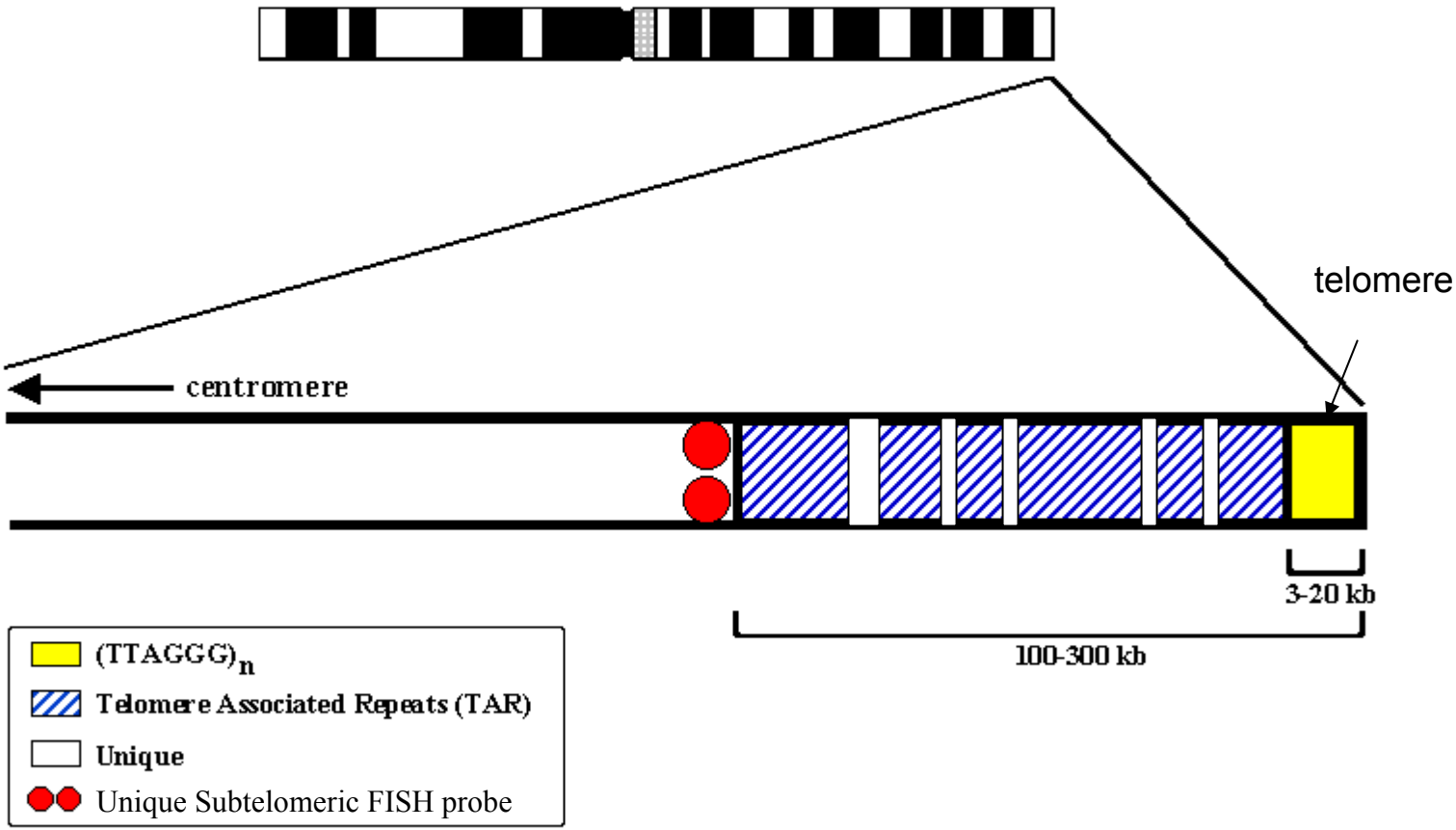
This test is used in the clinical cytogenetics laboratory to diagnose chromosome abnormalities.

Hybridization of Telomere Repeat Sequence Probe $(TTAGGG)_n$ to Human Metaphase Cell

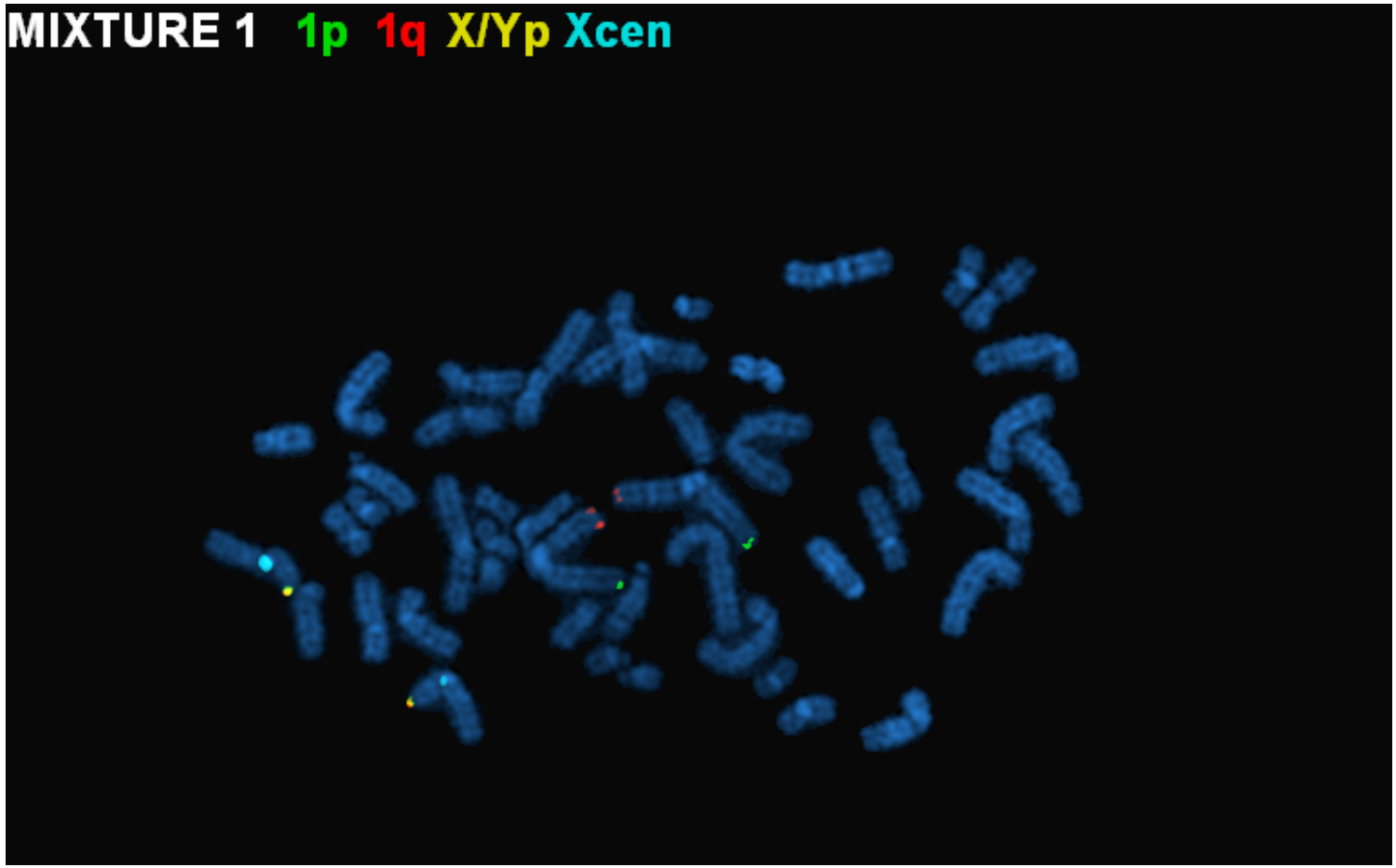


Telomeres are present on both ends of all chromosomes.

Structure of Chromosome Ends

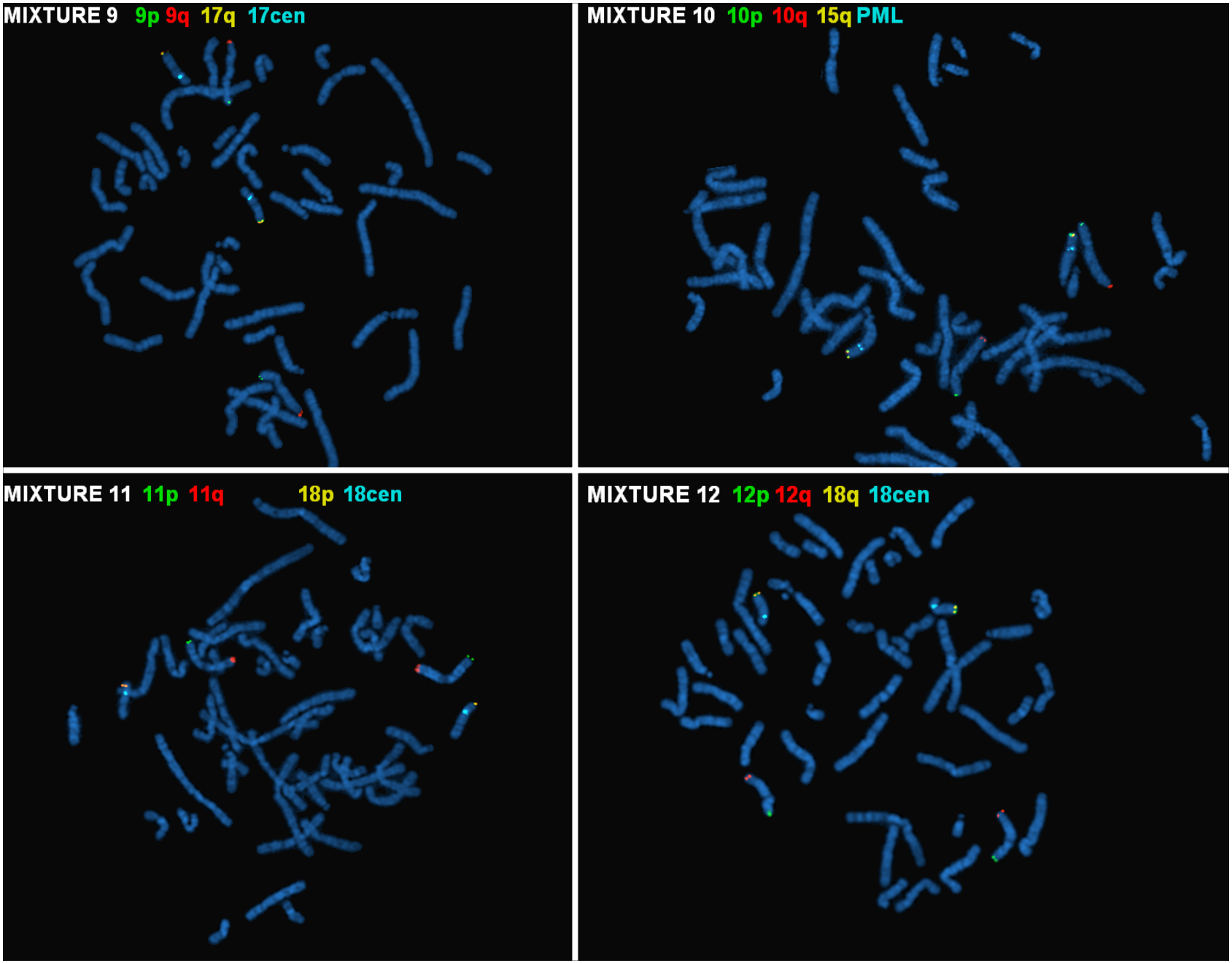


Subtelomeric FISH for Chromosome 1 and the Short Arms of Chromosomes X and Y

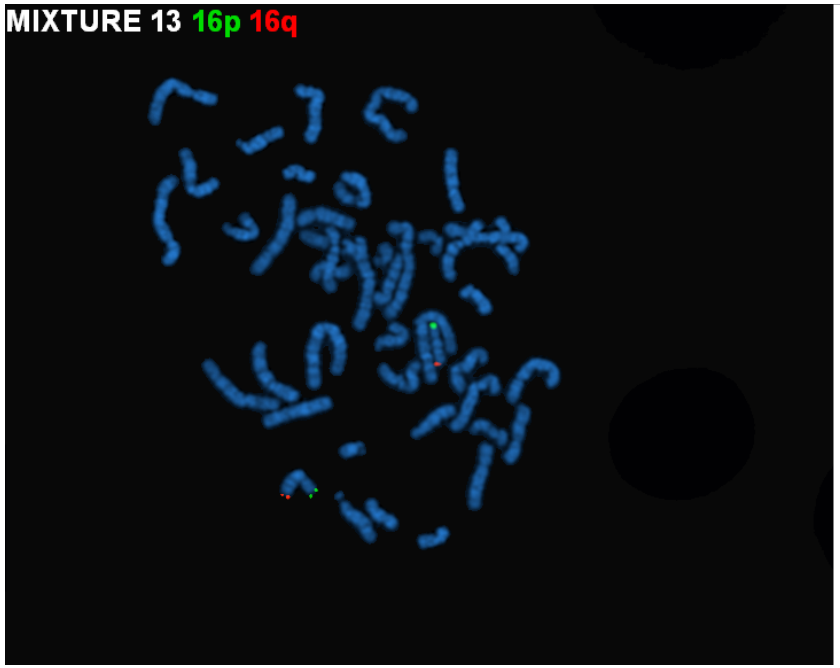


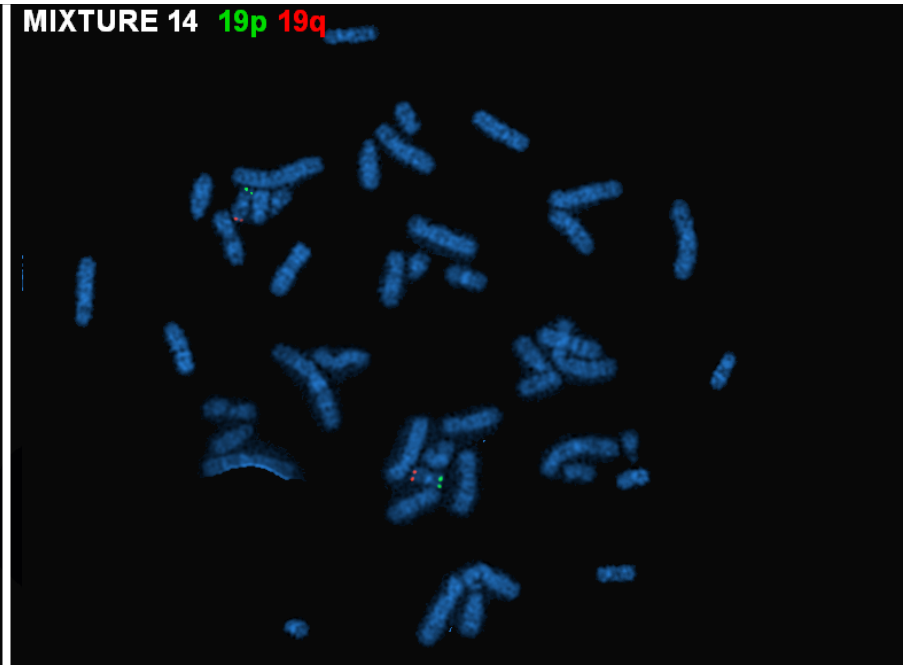
Normal hybridization; Xp and Yp share homology for this probe; X centromere probe identifies the X chromosome.

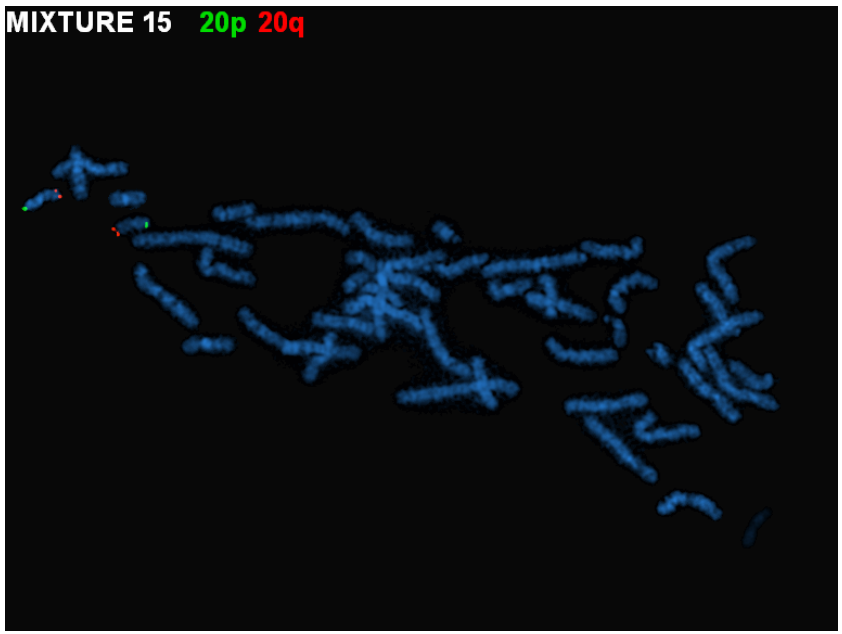
Different Probe Mixtures on Normal Cells



Different Probe Mixtures on Normal Cells

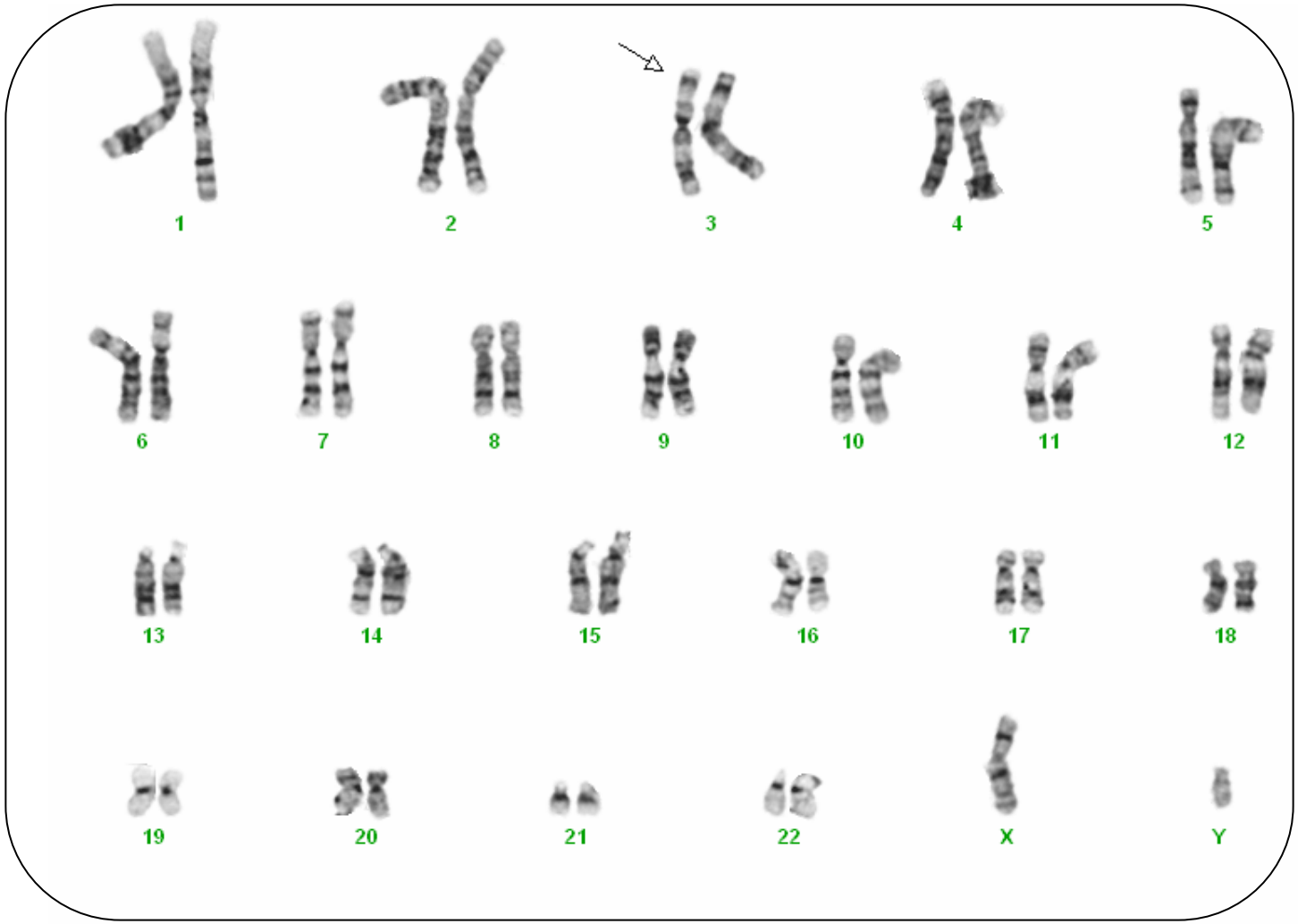






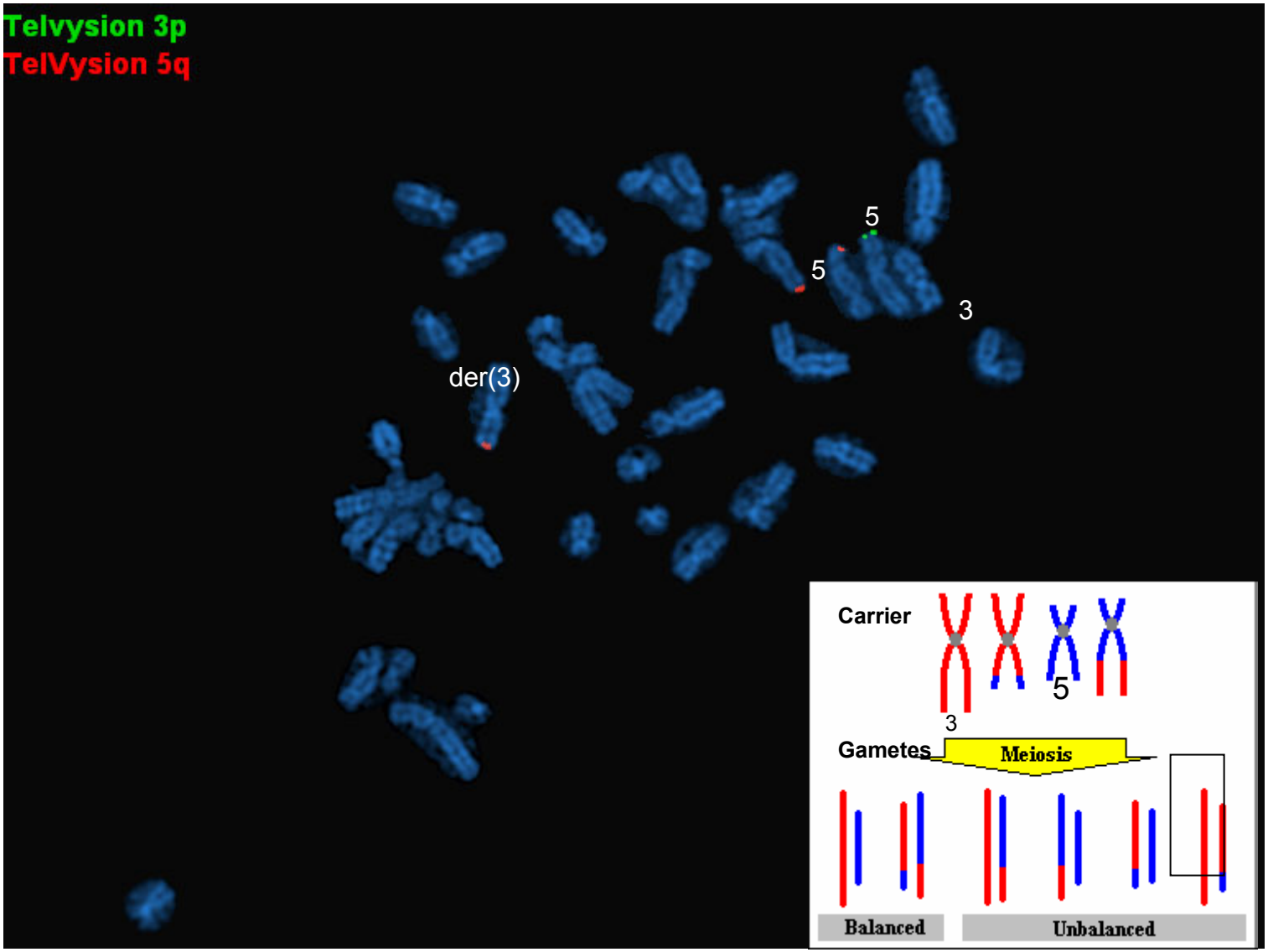
Surveillance of all telomeres (n=42) currently requires a minimum of 15 different hybridizations.

Detection of an Abnormal Chromosome 3p by GTG-Banding



46,XY,der(3) t(3;?)(p25;?) [CVS tissue]

Characterization of a Chromosome 3p Subtelomeric Abnormality



46,XY,der(3) t(3;5)(p25;q35.3)mat.ish der(3)(D3S4559-,GS3508+) [CVS tissue]

Characteristics of Commercial Subtelomeric FISH Probes

- 60,000 – 170,000 bp in length (average is ~110,000 bp)
- Comprised of unique sequence and suppressed repetitive DNA
- Recombinant or cloned DNA
- Not all are chromosome specific, several have cross hybridization to other chromosomes*
- Some detect polymorphisms**

* Probe for 3q cross-hybridizes with 6p; 4p with 17p; 8q with 11p; 10 p with 12p; 11p with 16p/17p/20p; 16q with 4q/9q/10p/16p/18p; 17p with 11p. ** Probes for 2q, 7p, Xp detect polymorphisms)

Subtelomeric FISH studies have been reported on:

- Couples with a history of multiple pregnancy losses (MPL)
- Individuals with idiopathic mental retardation (IMR)
  - common causes of MR have been ruled out including high resolution chromosome analysis
- Control normal individuals to validate probes; individual case reports with cytogenetically detectable abnormalities

What were the findings?

- Couples with MPL: No demonstrated increase in subtelomeric translocations.
  - Finding suggests that subtelomeric rearrangements are compatible with survival to term
- Normal Individuals: Polymorphisms were detected with some subtelomeric probes.
  - Demonstrates that family studies are necessary to distinguish a true chromosomal imbalance from a familial variant
  - New probes have been developed for some polymorphic probes

What were the findings? (cont'd)

- Children with IMR
  - Mild MR:
    - 0.5% have subtelomeric abnormalities
      - *Knight and Flint, 2000 [1/182]*
      - *many smaller studies with no detected cases*
  - Moderate to severe MR with physical anomalies:
    - ~4-5% have subtelomeric abnormalities (range of 0-9%)
      - *(Knight et al, 1999 [21/284]*
      - *Knight and Flint, 2000 [1/182]*
      - *Joyce et al, 2001[0/200]*
      - *Rossi et al, 2001[13/200]*
      - *many smaller studies*

Why are the results so variable in this group?

Potential Explanations for the Variability in Detection of Subtelomeric Rearrangements in IMR

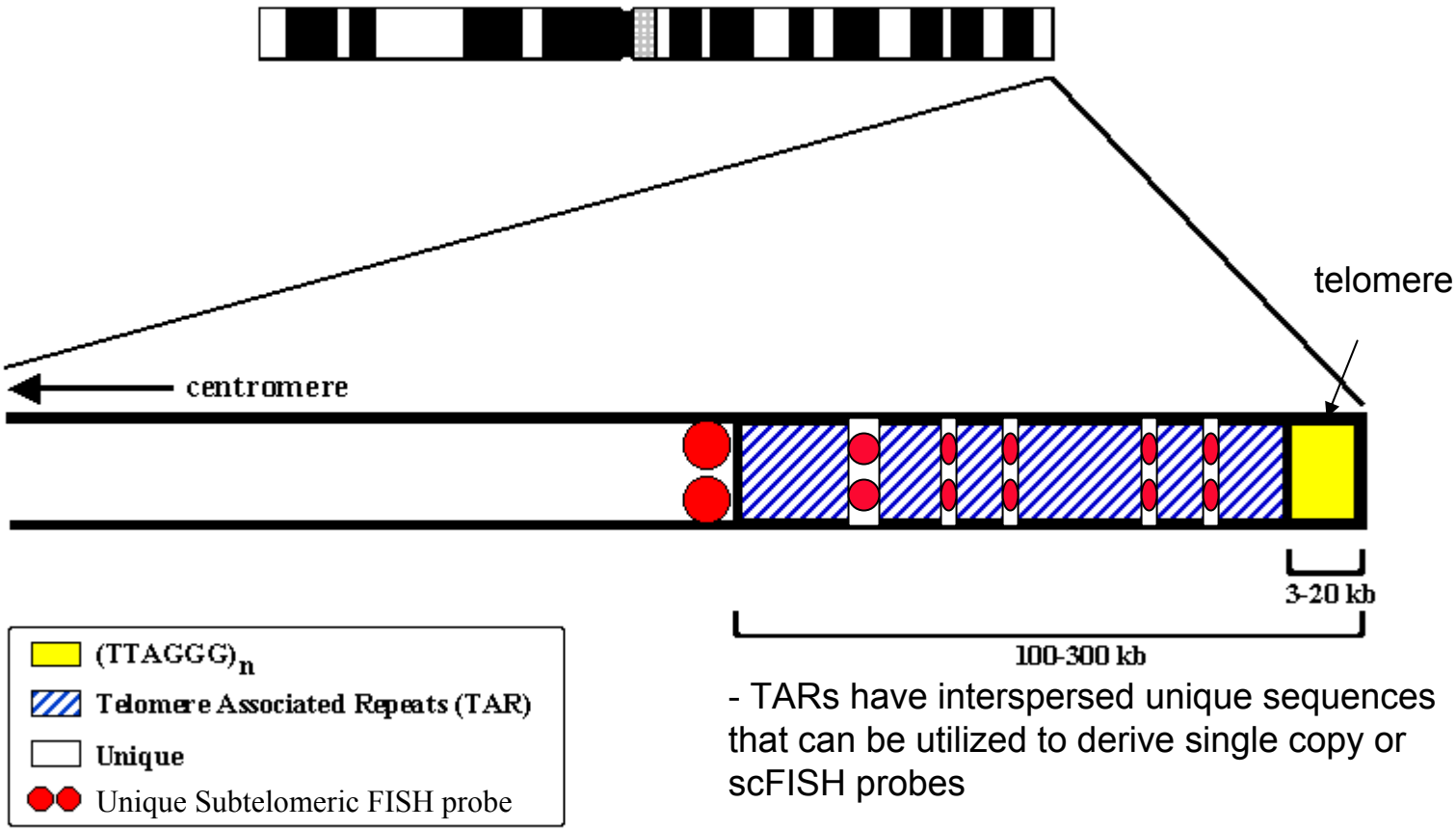
- ***Patient Ascertainment Bias***
  - Current Best Clinical Indicators for performing subtelomeric FISH to obtain highest detection rate
    - Moderate to severe MR (not mild) with positive family history
    - Growth retardation (postnatal, prenatal)
    - One or more dysmorphic facial features
    - One or more nonfacial dysmorphic facial features including congenital anomalies such as cardiac defects
    - Normal chromosomes at high resolution (>550 bands)

- ***Related to Probe Characteristics***
  - Size and distance from the end of the chromosome
    - Long probes won't detect small deletions/imbalances
    - Probes that are a greater distance from the telomere will not detect abnormalities closer to the end

Distance of Commercial Probes from Chromosomal Ends

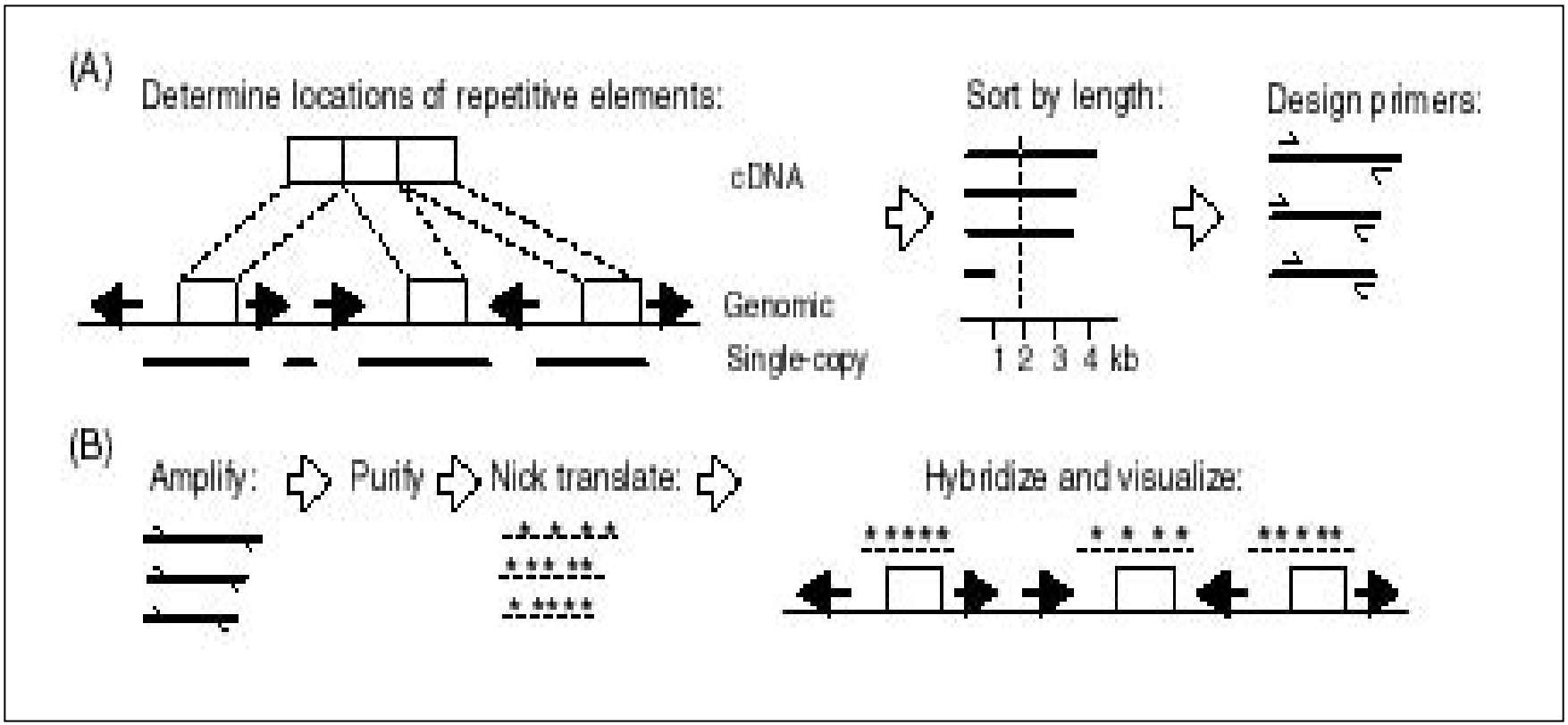
- All are estimated to be within 300,000 bp from end
- Of 42 telomeres, at least 10 are >300,000 bp and 7 cannot be positioned on genome sequence due to 'marker/anchor' designation
  - ie. probe for 8p is 1.2 x 10^6 bp from end; for 13q is 3 x10^6 bp from end; for 14q and 16p are ~4 x10^6 bp from end; for 17p is 590-910 x10^3 from end
  - ie. probe locations for 1p, 5p, 6p,11q, 19p, Yp, Yq cannot be determined with existing data

Structure of Chromosome Ends



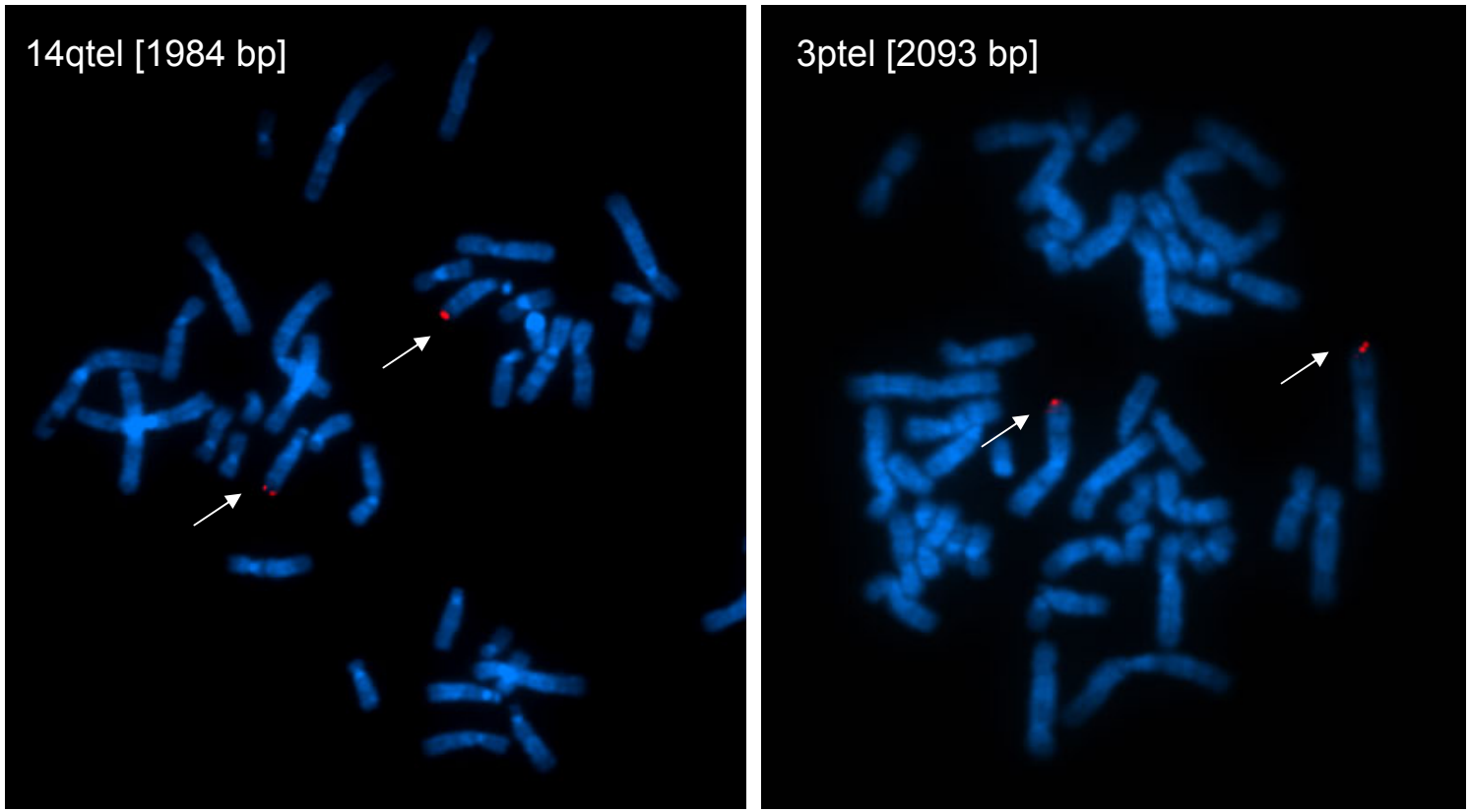
- TARs have interspersed unique sequences that can be utilized to derive single copy or scFISH probes

Single Copy Fluorescence *In Situ* Hybridization (scFISH): Concept and Basic Procedure

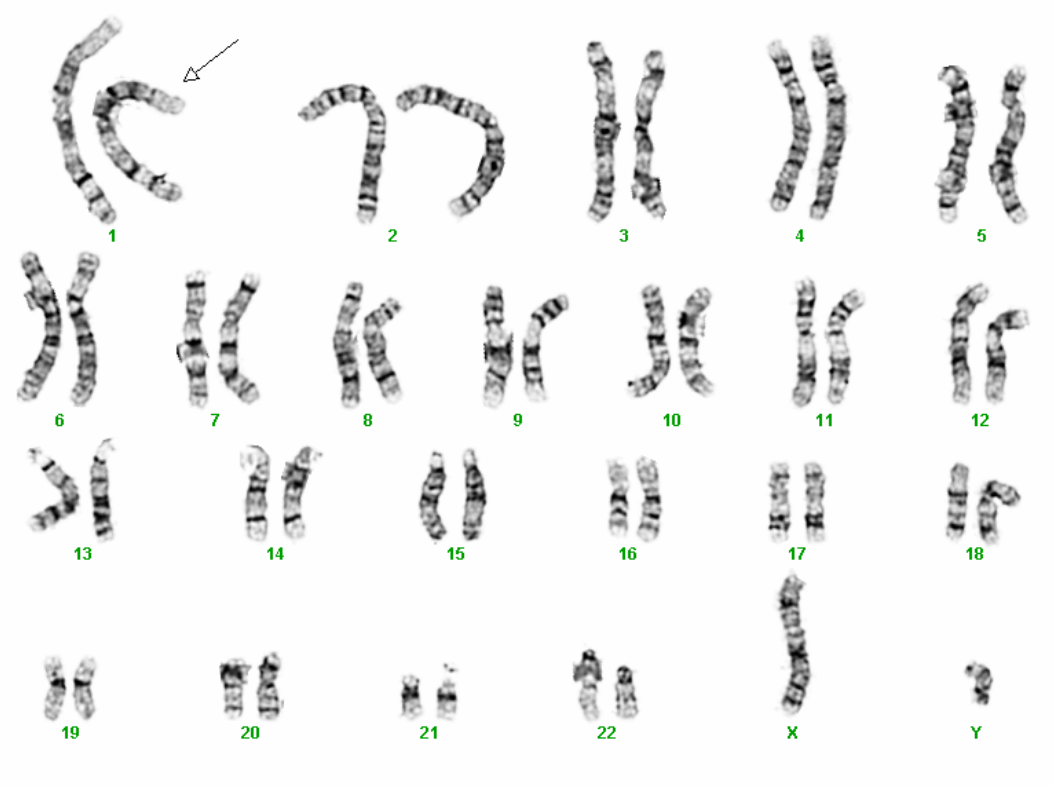


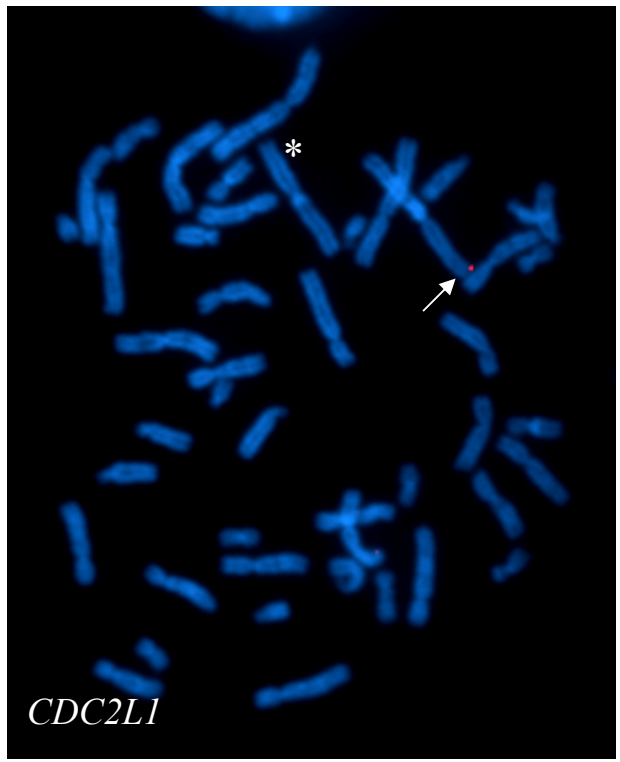
Steps in (A) are performed at the computer; steps in (B) are performed in the laboratory.

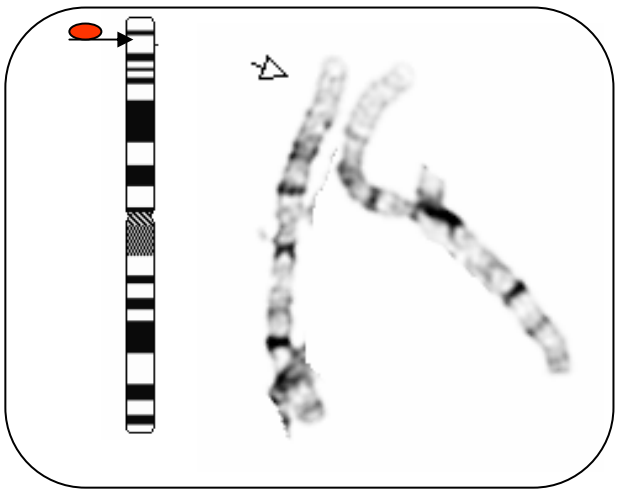
scFISH Subtelomeric Probes for Chromosomes 14q & 3p Hybridized to Normal Metaphase Cells



MONOSOMY CHROMOSOME 1P36 SYNDROME

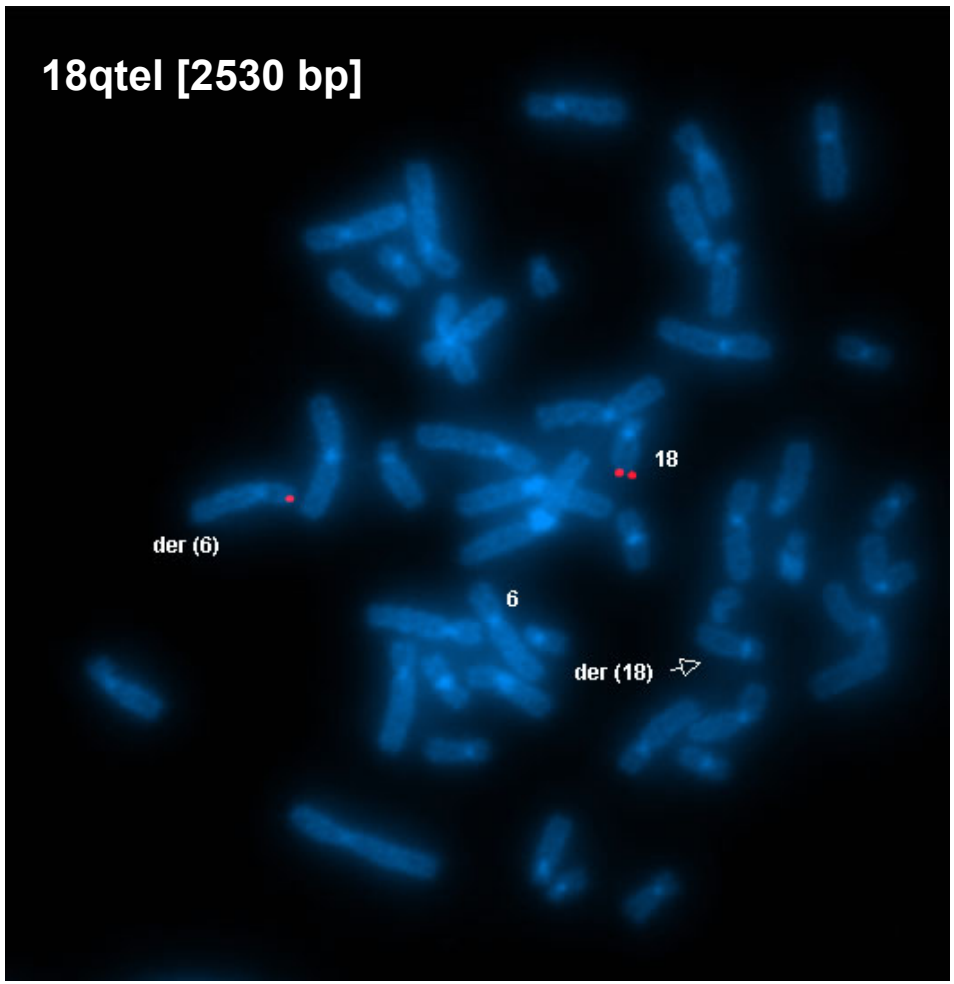


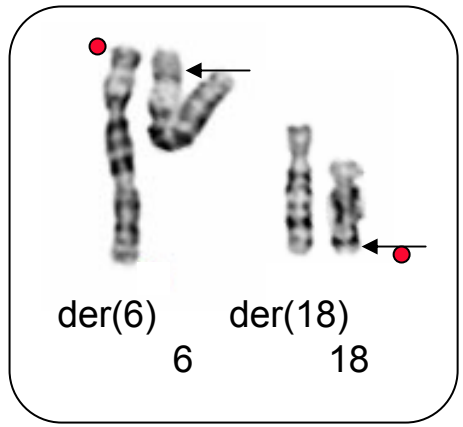




Karyotype: 46,XY,del(1)(p36.1).ish del(1)(p36.1)(CDC2L1-)

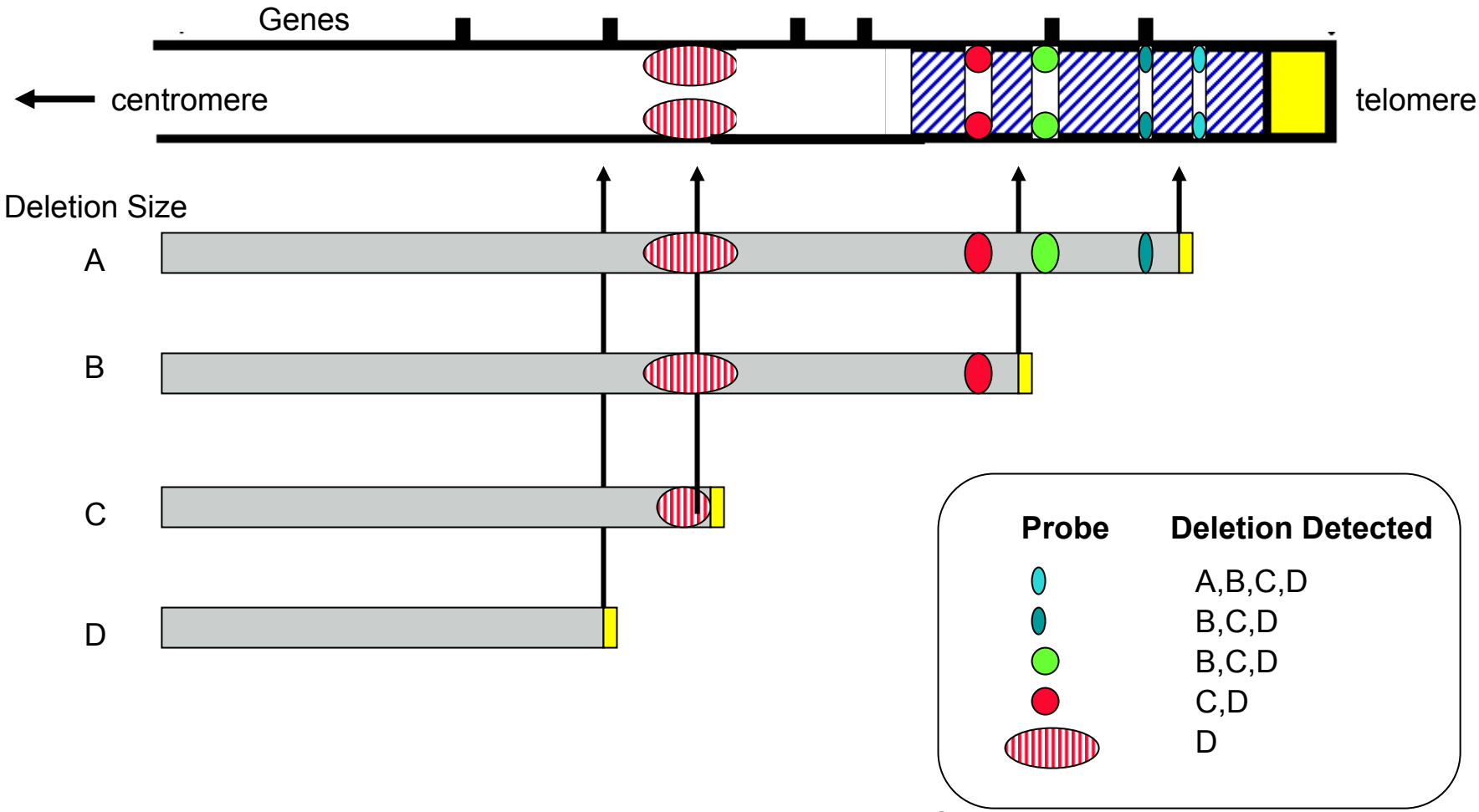
Chromosome18qtel scFISH Probe Hybridized to an Abnormal Metaphase Cell





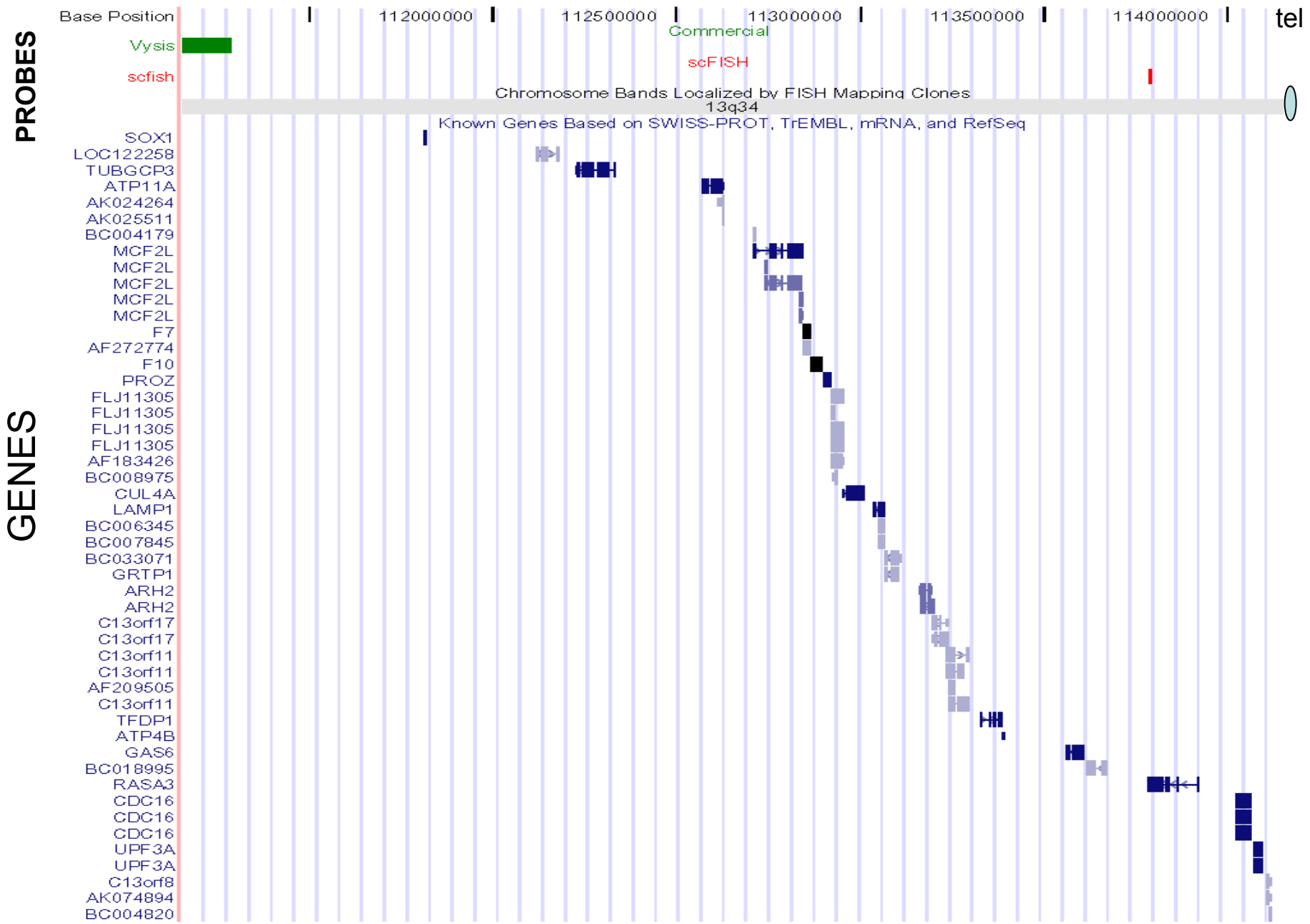
t(6;18)(p23;q23)

Detection of Different Sized Terminal Deletions

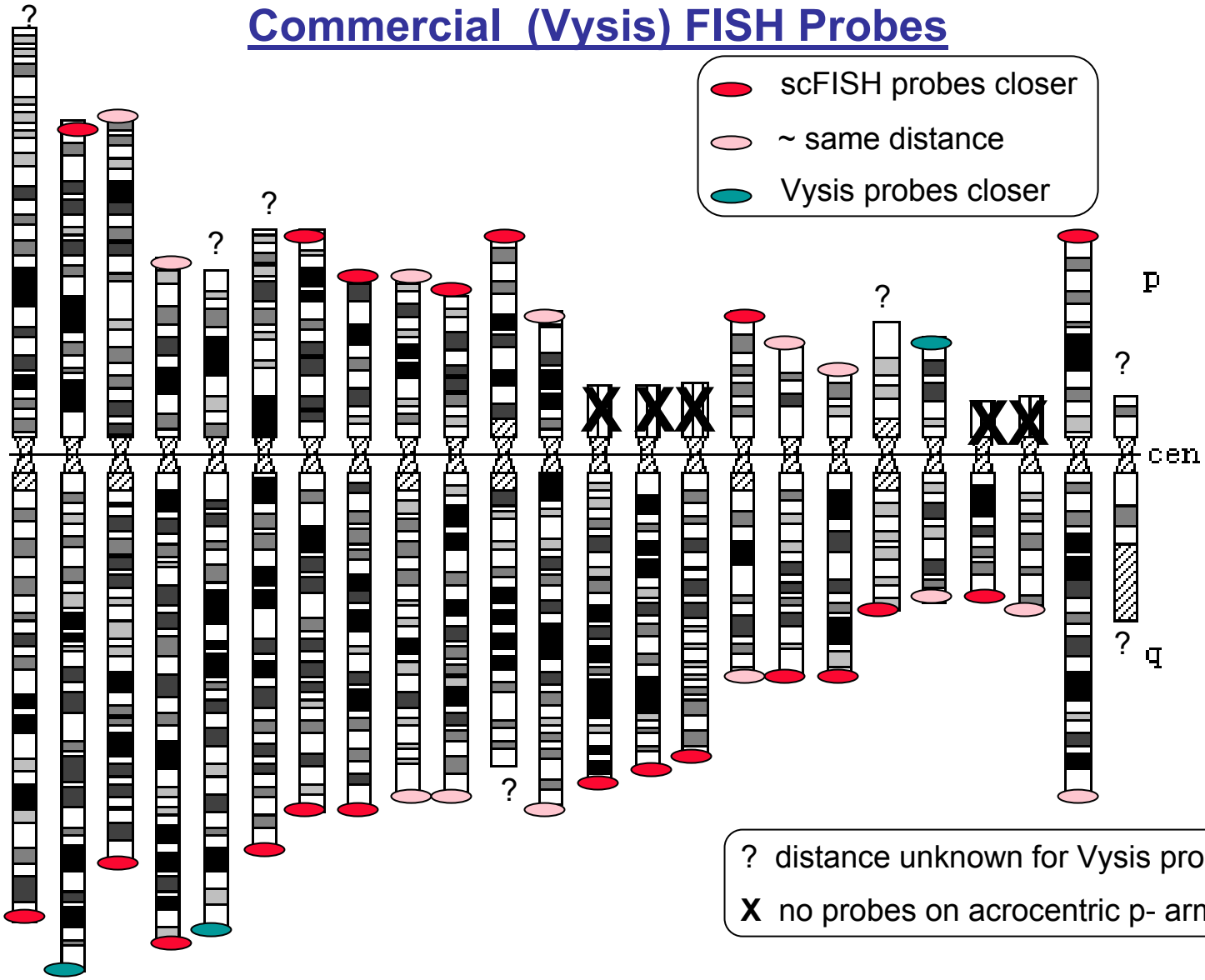


Probes that are shorter and closer to the telomere (ie. scFISH probes) will detect more terminal deletions than ones (conventional FISH probes) longer or more distal from the telomere.

Localization of Chromosome 13q tel Probes in Genome Sequence



Comparison of Distance from Chromosome End for scFISH and Commercial (Vysis) FISH Probes



Chr #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
# Probes (p/q)	4/2	1/1	2/2	1/1	1/4	1/1	2/4	2/2	1/4	4/1	2/3	1/1	1	4	1	3/1	4/2	1/1	4/2	2/4	1	2	2/2	1/-

Summary

- Telomeres
  - not chromosome specific
- Subtelomeric regions
  - chromosome specific (generally)
  - undergo rearrangements
  - generally enriched for genes
- Subtelomeric rearrangements
  - detected in ~4-5% patients (wide and variable range) with moderate to severe IMR and physical anomalies
  - better clinical indicators for patient selection are needed
- Commerical subtelomeric FISH probes
  - are not as close to the telomeres as previously thought
- Subtelomeric scFISH probes
  - most are closer to the telomeres and may detect a greater frequency of subtelomeric rearrangements in selected patient populations

Acknowledgments

- scFISH Research:
  - Dr. Peter Rogan
  - Patricia Walters
  - Patrick Angell
  - Camille Marsh
  - Angela Marion
- Conventional FISH/Routine Cytogenetics:
  - Clinical Cytogenetic Laboratory Staff