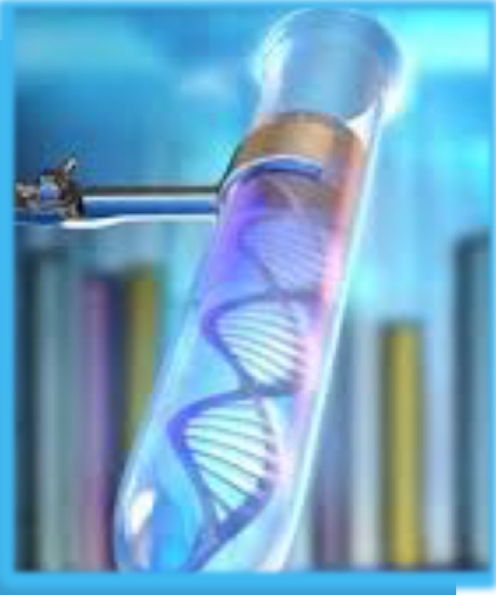
Mountains and Valleys of Preconception and Prenatal Screening Options

Karen E. Byrne-Essif, MS, CGC

"I have no financial disclosures.
I receive no funds from any of the laboratories."

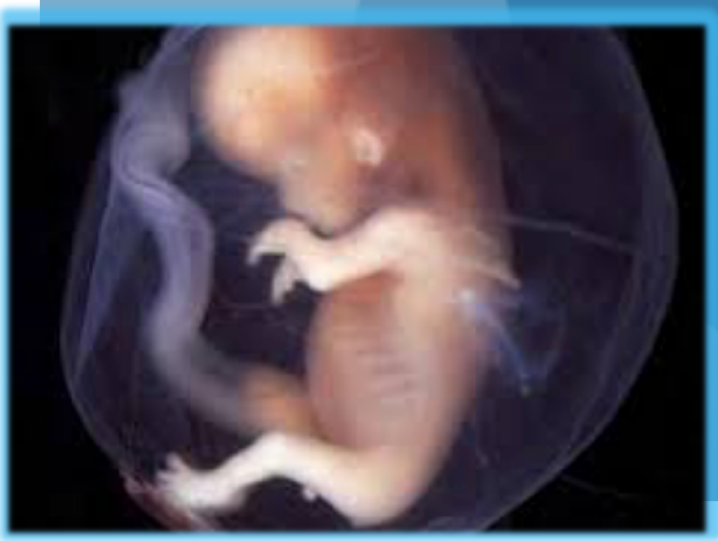


“Prenatal Screening” begins before conception

Expanded DNA Carrier Screening

Direct-to-Consumer Screening

Preimplantation Genetic Screening



“First” Genetic Counselors were Matchmakers

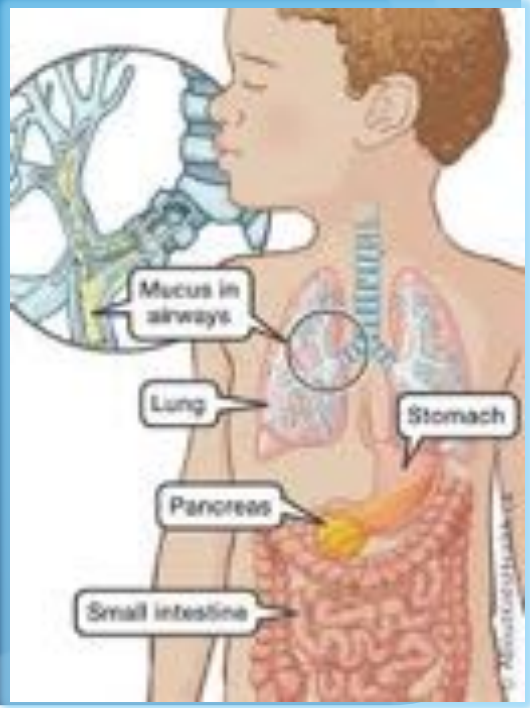
Israel's "Dor Yeshorim" Program: Ultra Orthodox Ashkenazi Jewish

- Purpose: Avoid marriage of individuals at risk for same Autosomal Recessive Disease
- Belief-based objection to pregnancy termination
- Prevents marriage of two heterozygotes with mutations in same gene
- All potential spouses tested but not told results
- Exception: Fragile X "because a 'positive' result

could preclude marriage of a carrier woman"

DNA carrier screening: Cystic Fibrosis

- Standard to offer cystic fibrosis DNA carrier screening
- Affects more than 25,000 American children and young adults
- Risk for lung congestion, pneumonia, diarrhea and poor growth
- Many have severe medical problems
- Does not affect intelligence
- Treatments are improving
- Those with cystic fibrosis are living longer



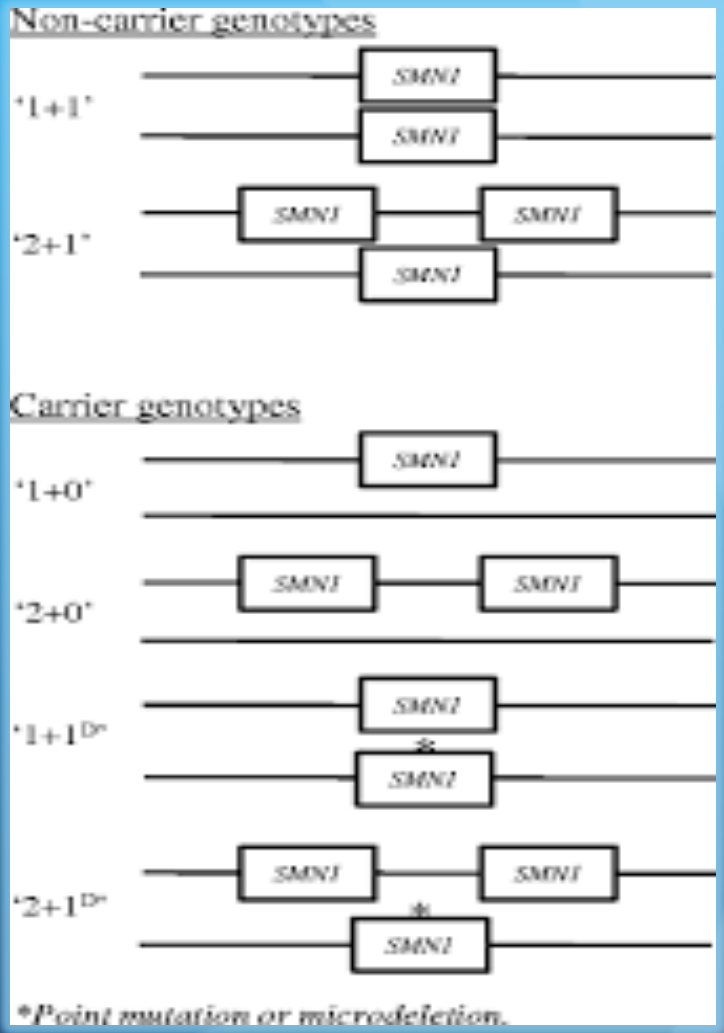
Spinal muscular atrophy:

- Most common inherited cause of infant mortality
- Affects approximately 1 in 6,000 to 1 in 10,000 live births
- Muscle weakness affects head and neck control
- Most severe form leads to inability to swallow and breath
- Many die by age of 2
- There is now medication that if started early may improve long term outcome

Spinal Muscular Atrophy

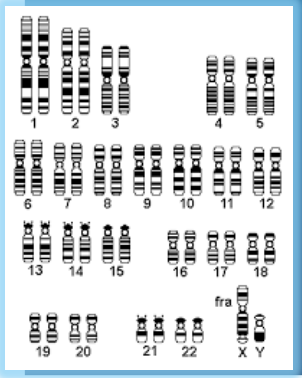
"Silent" carriers occur

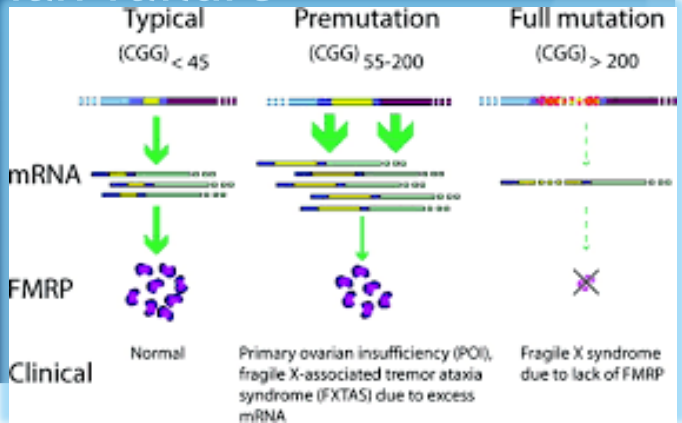
Screening reduces odds of being a carrier



Fragile X carrier screening:

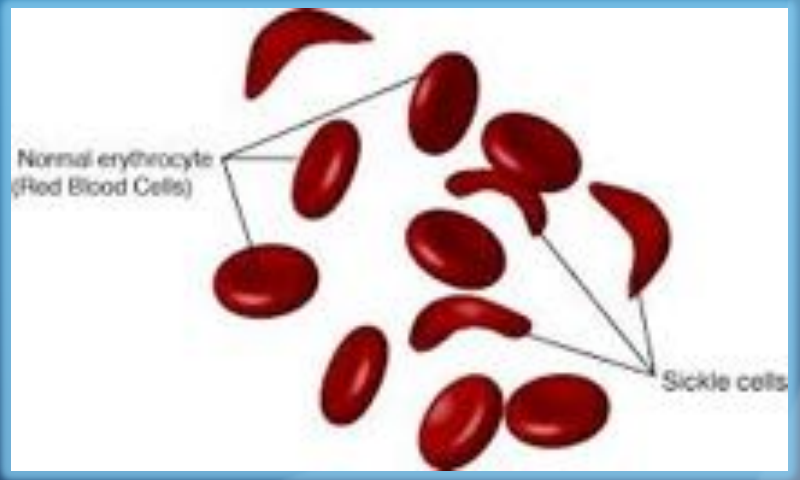
- Should be offered to those with confirmed or suspected family history of intellectual disabilities
- Most common inherited cause of intellectual disabilities
- Approximately 1 in 260 women in the US is a carrier
- No treatment
- Carriers can have early onset of ovarian failure





Hemoglobinopathy Carrier Screening

- ACOG suggest all pregnant women be screened
- Sickle cell prep alone not recommended
- Hemoglobin electrophoresis and DNA testing
- In utero treatment using gene therapy for alpha-thalassemia major
- Many do not know ancestry



Expanded Genetic Screening Panels

- Comprehensive testing
- Some labs offer screening for over 270 autosomal recessive and x-linked conditions
- Several genetic labs have assistance programs for those with no insurance or high deductable
- Cost range from $99 to $250
- EOB will show much higher cost

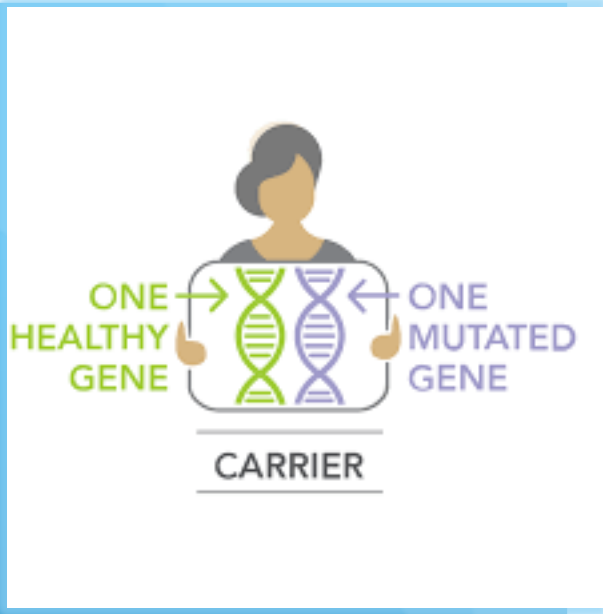
Cases to Consider Offering:

- Adopted with no known history
- History of Stillborn or Neonatal Death of Unknown Etiology
- Family History of SIDS or Unexplained Deaths
- Ethnic Background
- Couple Related to Each Other
- Known family history of genetic disorder
- Patient Request
- More cost effective

Where are the patients? G. Lazarin, et al.

- Identifying more carriers than would expect based on population incidences
- Suggest in utero mortality
- Smith Lemli Opitz: 50% fetal loss rate
- Disorder of Glycosylation 1a: fetal hydrops
- MCAD: medium chain-CoA dehydrogenase deficiency: 5% die within 72 hours before a diagnosis is made

Carrier Screening:



- Does not have phenotype
- Have one variant allele (gene mutation)
- Ideal time is before pregnant
- Does not replace newborn screening
- Not all States offer same panels for newborn screening
- More cost effective to do expanded carrier screening
- Woman first or both at same time
- Discount for partner if “positive”

Implications if “positive”

- Other relatives at risk
- Reproductive partner needs to be offered screening
- Residual risk (will not eliminate all risk)
- Preconception testing
- Prenatal testing

Residual risk: Non-syndromic hearing loss

- General Population: 1 in 43 are carrier
- 1/1 x 1/43 x 1/4 = 1 in 172
- Gene detection: greater 95%
- Residual risk: 1 in 841 for screened negative partner
- Risk to fetus: 1 in 3,364
  - 1/1 x 1/841 x 1/4 = 1 in 3,364

  - General Population risk:
  - 1 in 7,396

Cost: performed only once in a person's lifetime (ACOG)

- Cystic fibrosis 97 mutations: $447.50
- Cystic fibrosis 32 mutations: $306.50
- Fragile X syndrome, DNA: $214.50
- Spinal Muscular Atrophy, DNA: $437.50
- "Jewish Panel" (16 conditions): $915
- Expanded Genetic Panels: $99 to $250

SHOULD HAVE INFORMED CONSENT

EVERYONE IS A CARRIER FOR ABNORMAL FUNCTIONING GENES

Carrier Screening does NOT replace Newborn Screening

Direct-To-Consumer Labs: market directly to consumers

23 and ME

De Code

My Heritage DNA

Dante Labs

Helix

Navigenics

Counsyl

Living DNA

Tribe Code

Genos Research

Gene by Gene

Family Tree DNA

Ancestry by DNA

Genographic Project

24 Genetics (Spanish Market)

Roots for Real

2003 Human Genome Project Completed

- 2005/2006: DTC companies formed
- 2006: US Govn't Accountability Office investigates
- 2010: GAO report and FDA starts regulatory proceedings
- 2013: FDA sends cease and desist letters
- 2015: FDA approves DTC carrier screens
- 2017: FDA approves DTC genetic health risk test

Why popular?

- 2007 cost was $1000
- 2019 cost $99 or less
- Testing initiated by patient
- Interpretation used for multiple purposes, eg. ancestry, paternity, health, type of wine, fitness, chocolate or vanilla
- Product price not tied to healthcare payment model
- Information not in health record

Accuracy of DNA Ancestry

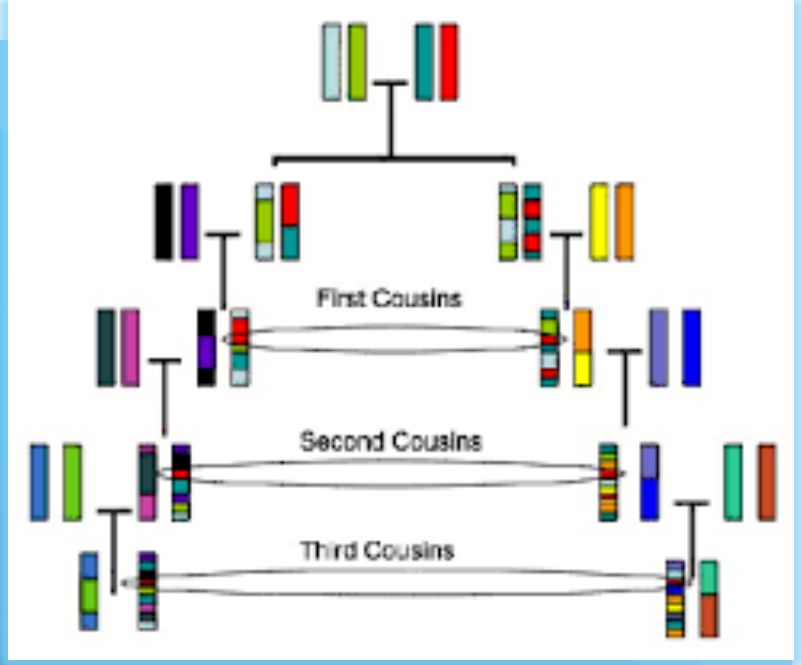
Pair of identical twins
Sent DNA to 5 companies
Got 10 different assessments

More than 15 million DNA kits have been sold

60% of white Americans can be identified

Able to match at 3rd and 4th cousin level
Have ~190 3rd cousins

Golden State Killer



- FBI tested DNA from crime screen
- Uploaded to free site: GED Match
- Made match to relative
- DNA from Joseph de Angelo's trash and car door
- More than 50 other suspects apprehended
- Family Tree DNA in agreement to do testing and uploading for FBI without subpoena
- Use their lab Gene to Gene
- Forensic databases have ~20 markers compared to SNP chips with ~700,000 markers

Caution:

- Largely unregulated
- Information given without clinical support
- May not have option to decline certain information
- Safeguards for sample and data
- For-profit selling of data for secondary use
- Sold or goes out of business
- Warrants and court orders

Scammers:

- Offer Medicare beneficiaries cheek swabs for genetic testing
- Steal personal information
- Done at health fairs, booths at public events, door-to-door visits
- Louisville: claimed to work with Medicaid insurer
- Nebraska: go to senior living and assisted living centers
  - Offer cheek swab for DNA carrier testing
  - “It’s nothing scary. No craziness. It’s just going to tell her if she has the cancer gene.”

Challenges for Healthcare Providers:

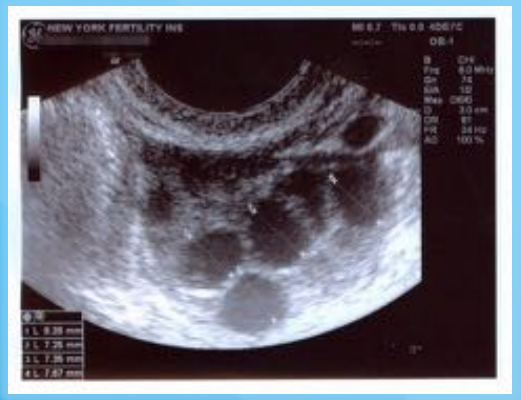
- Feel stuck sorting out mess
- Need to decide when and how to incorporate test results into medical management decisions
- Should DTC report be made part of medical record?
- Concerns with collection and chain of custody
- When to repeat
- What lab to use

Genetic Data Protection Act: Internationally

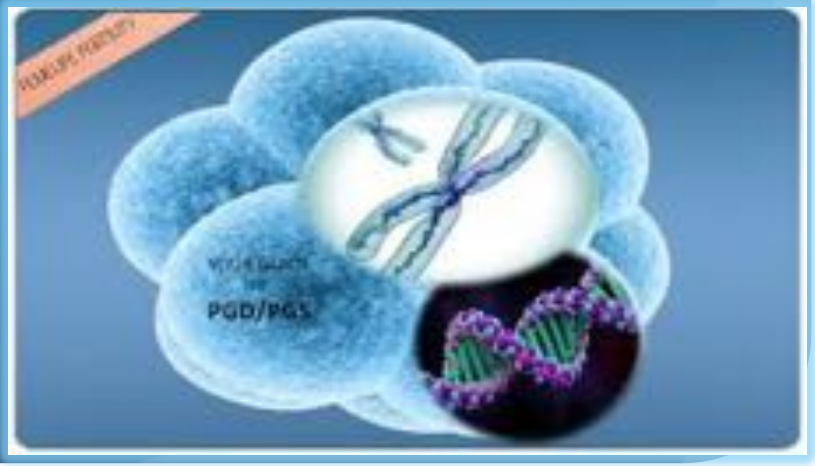
- Would provide governance over access, storage, use of genetic data
- If compromised, cannot get new genome
- Affects not just us, but our relatives
- Individual ownership of digital genetic data
- Genetic data should be nontransferable
- Protects against patenting of human genes

Preimplantation Genetic Screening/Diagnosis:

Preimplantation



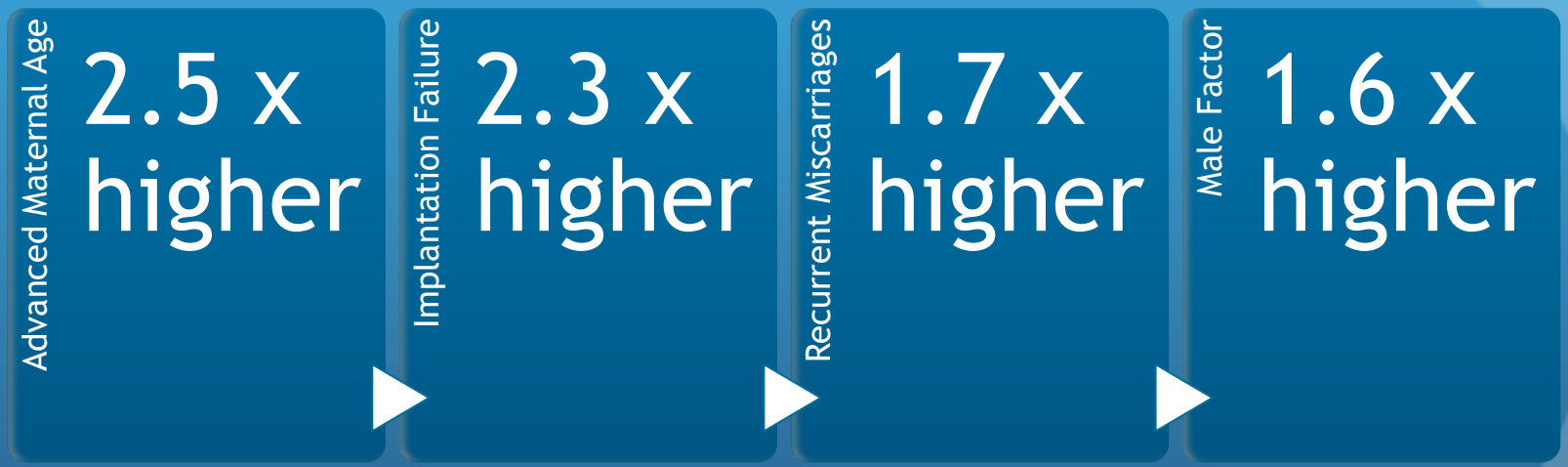
- PGT-M (PGD): monogenic-single gene
- PGT-A/PGT-SR(PGS): preimplantation genetic screening: chromosome for aneuploidy or structural rearrangements
- Biopsy on day 5 as have more cells
- Freeze embryo
- Implant following month
- Do SNP's, FISH, microarray



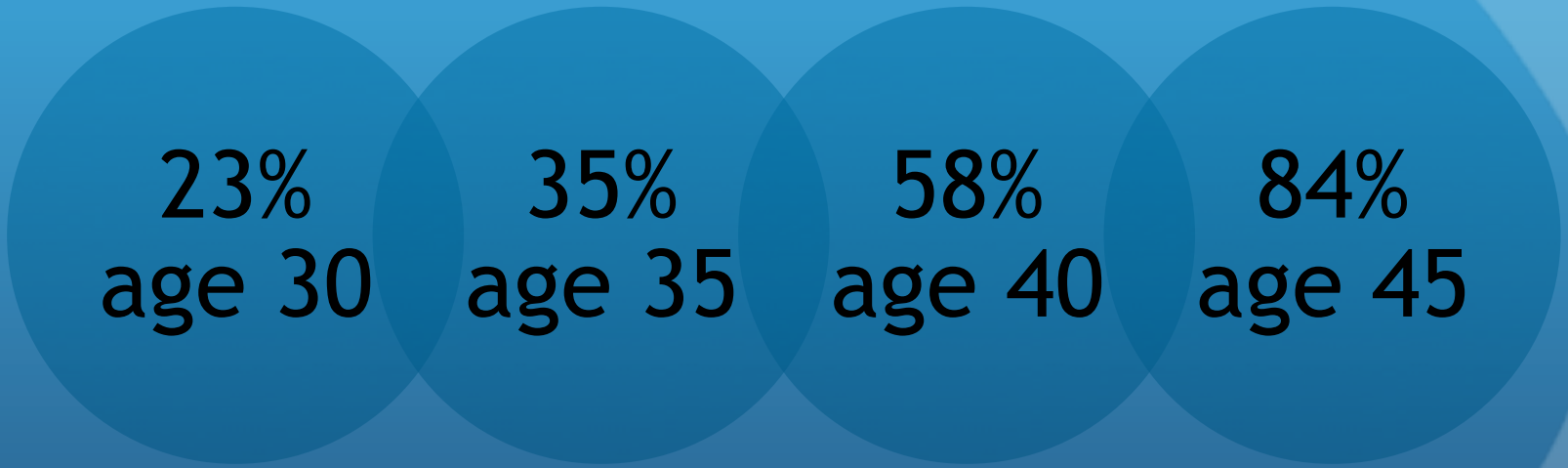
Indications for Preimplantation Genetic Screening for Aneuploidy

- Advanced maternal age
- Failure to conceive after transfer 10 or more embryos
- Failure to conceive after 3 IVF cycles
- Repeat miscarriages—2 or more consecutive spontaneous abortions
- Severe male factor using ICSI
- All having IVF

Increased success rates:



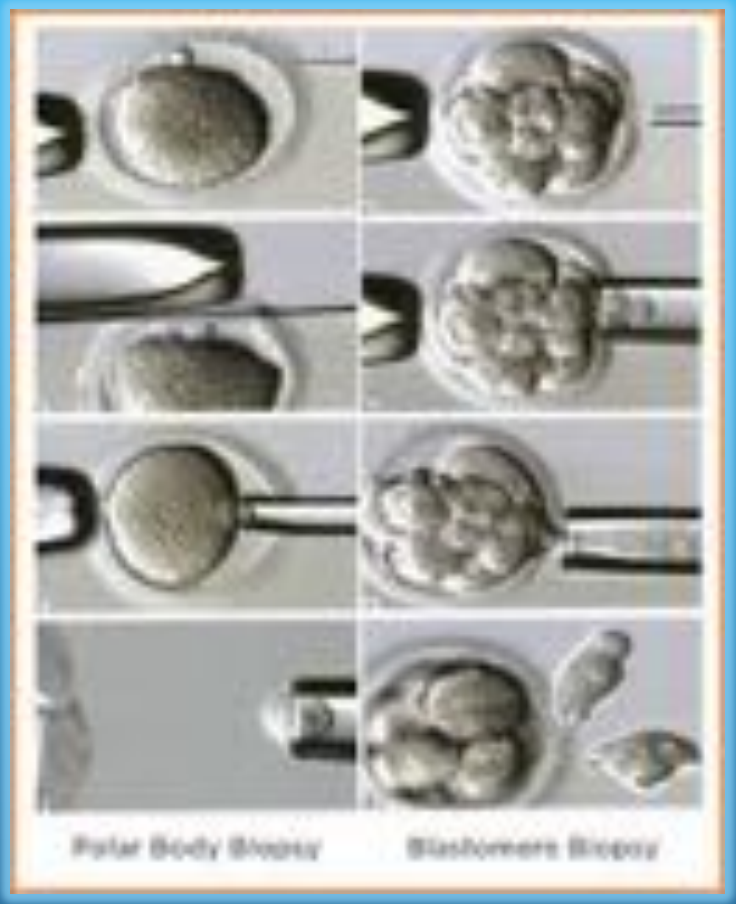
Maternal Age and Aneuploidy: percentage of embryos with an abnormal number of chromosomes

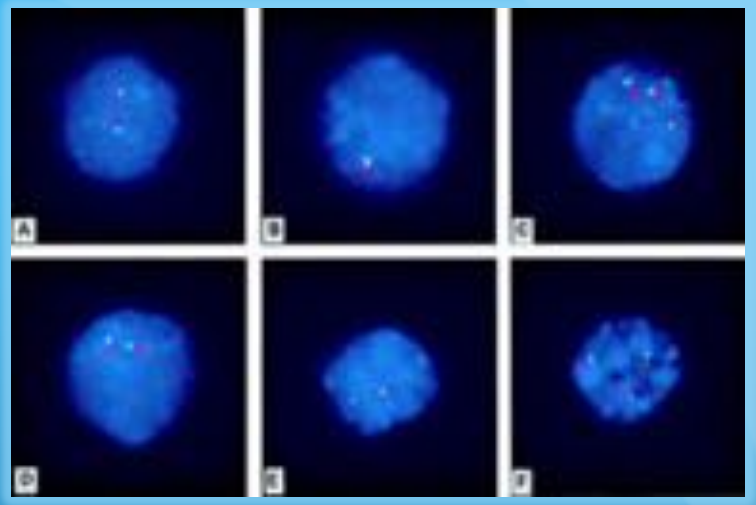


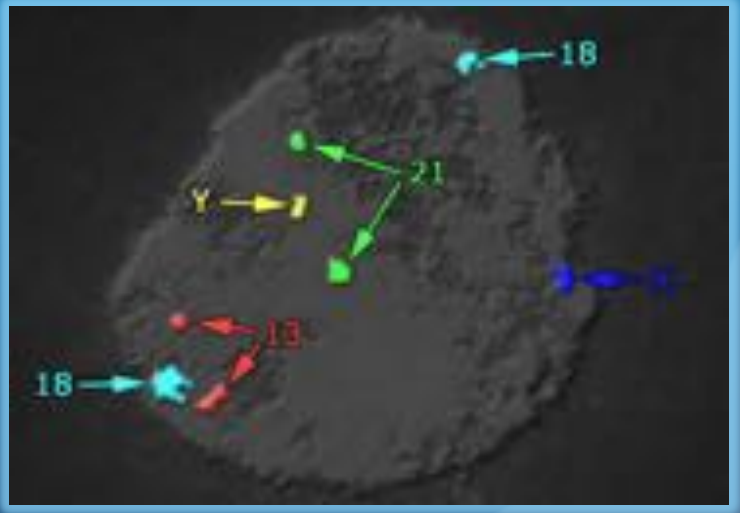
Adapted from Franasiak, JM, et al. Fertil Steril 2014:101(3):656.

Unproven:

- Recurrent IVF implantation failure
- Advanced maternal age
- Recurrent early pregnancy loss-UNLESS DUE TO ANEUPLOIDY
- To improve implantation rates in women under 35
- When using donor eggs from a younger donor

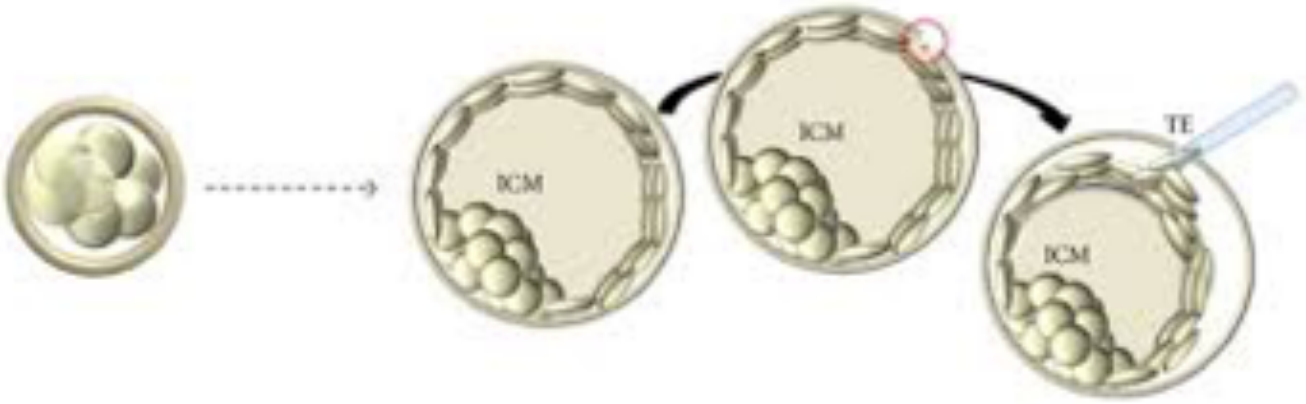






Aspiration of 1st polar body from oocyte

One to two cells from 5- 8 cells of 3 day old embryo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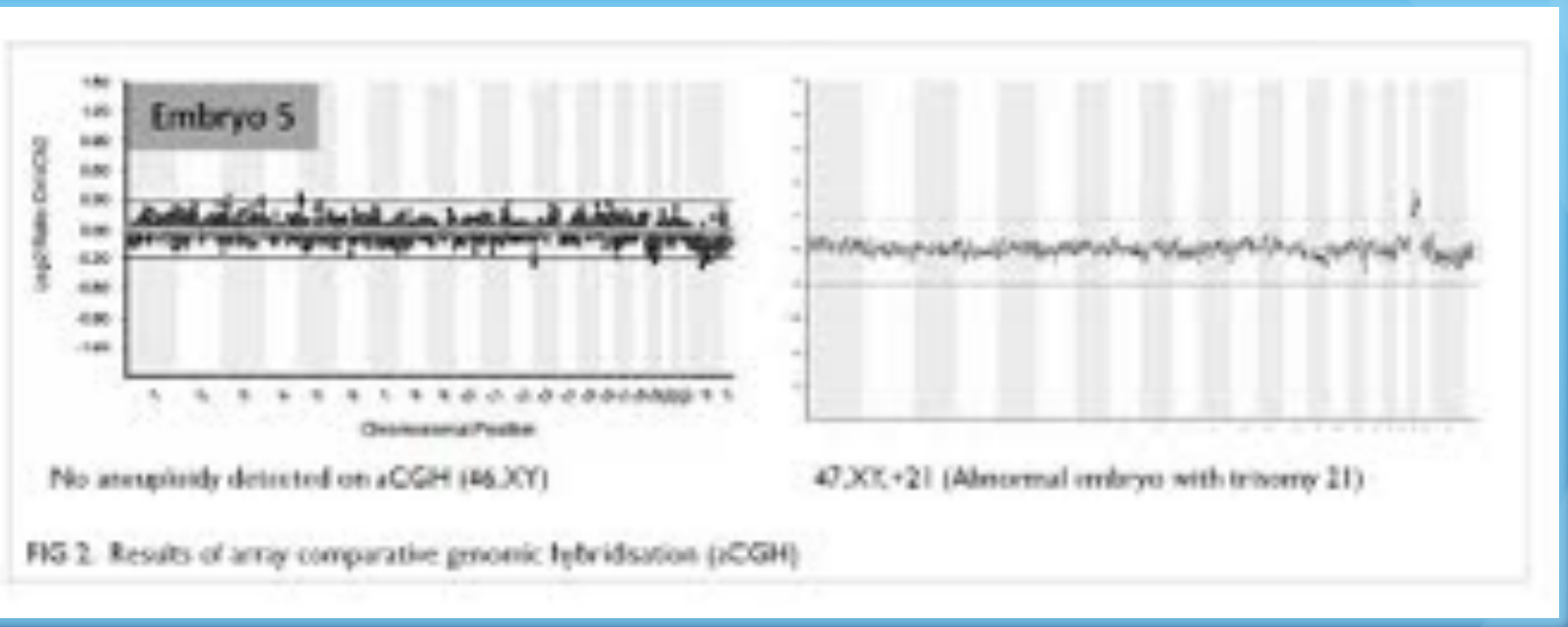
Array Comparative Genomic Hybridization (aCGH)



No aneuploidy detected on aCGH (46,XY)

47,XY,+21 (Abnormal embryo with trisomy 21)

FIG 2. Results of array comparative genomic hybridisation (aCGH)

It is not perfect

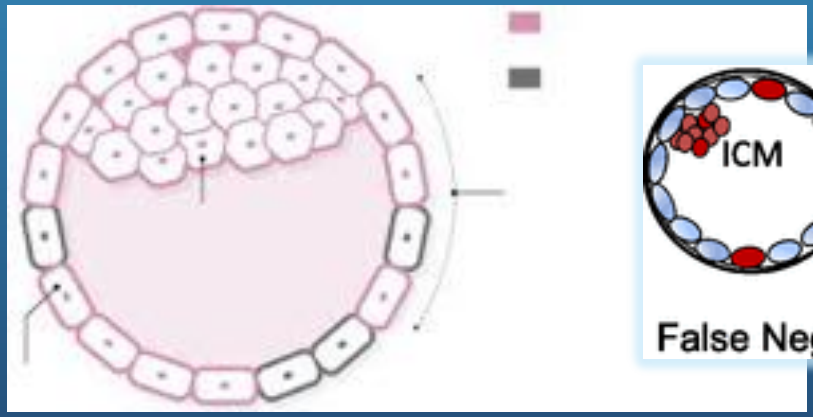
- False negative: 1% to 5%
- False positive: 20%
- Mosaicism most likely explanation

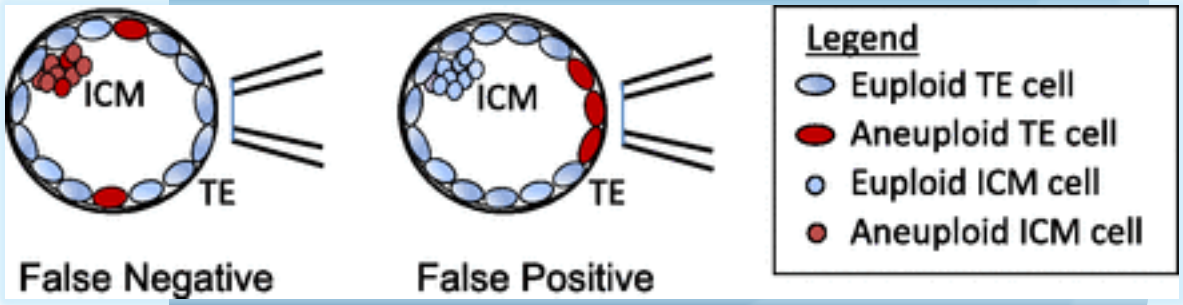
Rate of Mosaicism as High as 20%

Euploidy

Aneuploidy

Mosaic





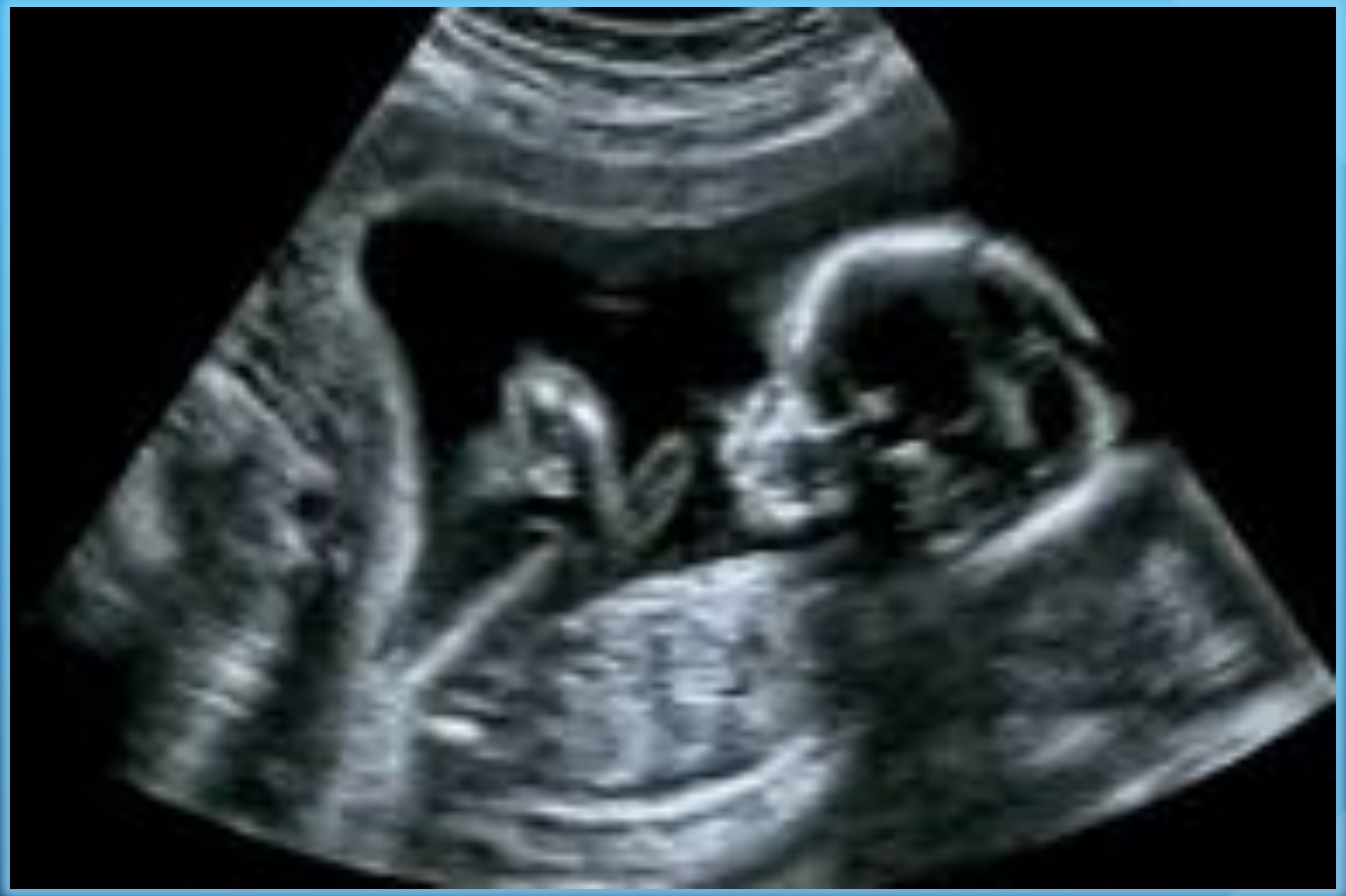
- Maternal age 43 and older: 12% euploidy
- Under age 30: 60% euploidy
- Chromosomes avoid:
  - 2, 7, 16---IUGR
  - 14, 15—UPD

Our Cases:

- 41 years old
- 46, XX
- Abnormal NT and NIPT
- CVS: 47,XY,+21
- Confirmed on POC

- 44 years old
- 46, XX
- 47,XX, +21

Post-conception Screening

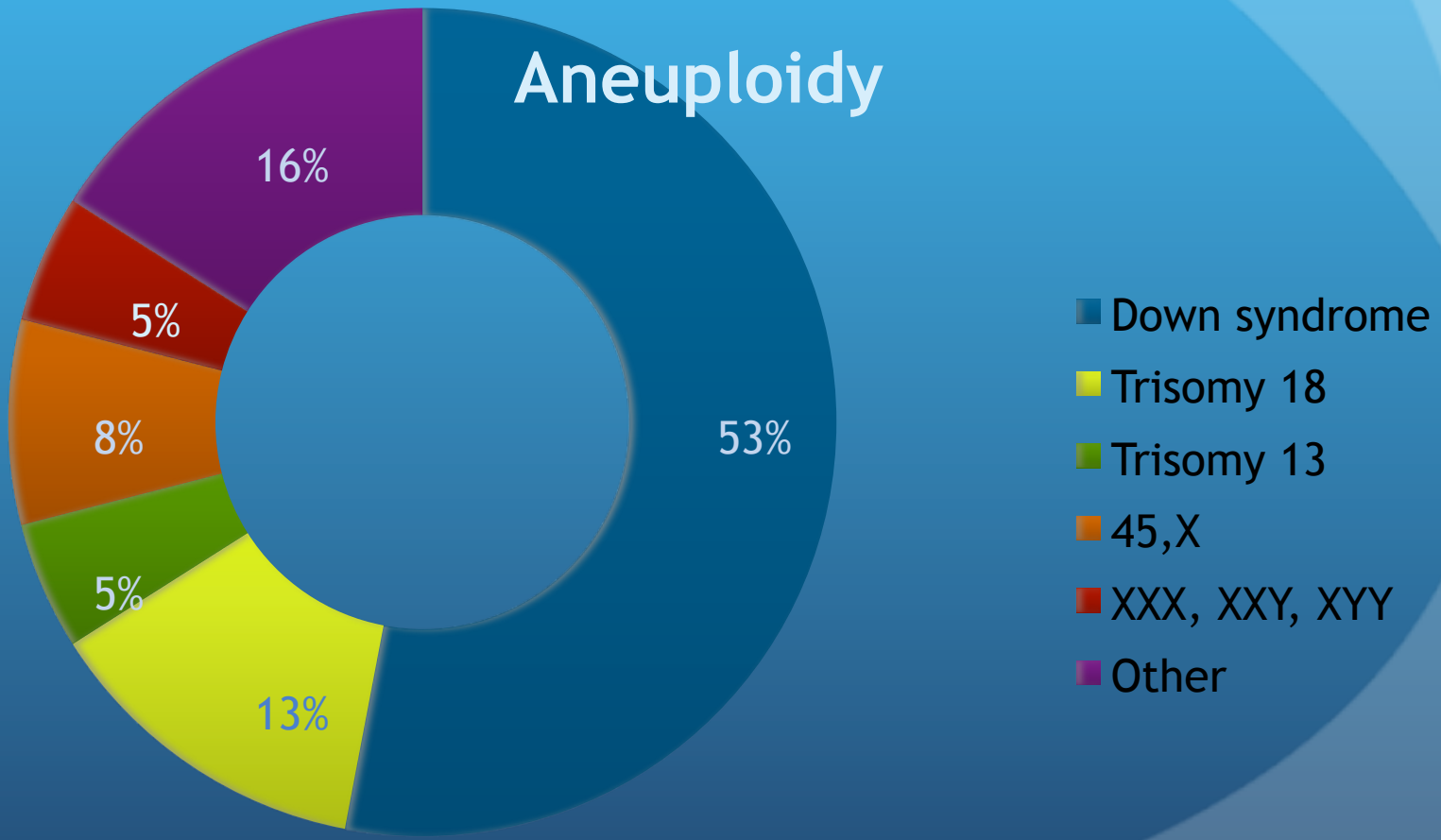


Timeline for Maternal Blood Screening

1970's:
NTD & Abdominal Wall Defects Screening

2015:
"Whole Genome"

Prevalence: 1 in 228 (0.4%) if include livebirths, IUFD, TAB



FALSE "POSITIVE" Rates with Maternal Age Using Multiple Marker Screening:

Maternal age group (years)	Probability of a FALSE screen "POSITIVE" for Down syndrome
Under 25	1 in 35
25—29	1 in 25
30—34	1 in 15
35—39	1 in 7
40—44	1 in 3
45 and over	Greater than 1 in 2
ALL	1 in 20 (5%)

Non-Invasive Prenatal Screening: NIPT

Blood test that uses cutting edge technology to screen pregnant women for chromosome problems, as early as 9 weeks in pregnancy

2011

- NIPT for Chromosome 21 offered by Sequenom Lab

2012

- Addition of Chromosome 18 followed by Chromosome 13

Timeline: Continued

2012

- Add Sex Chromosome

2013

- Late 2013--
- 22q del
  - DiGeorge
- 15q del
  - Prader Willli/ Angelman
- 1p36 del
- 5p del
  - Cri-du-Chat

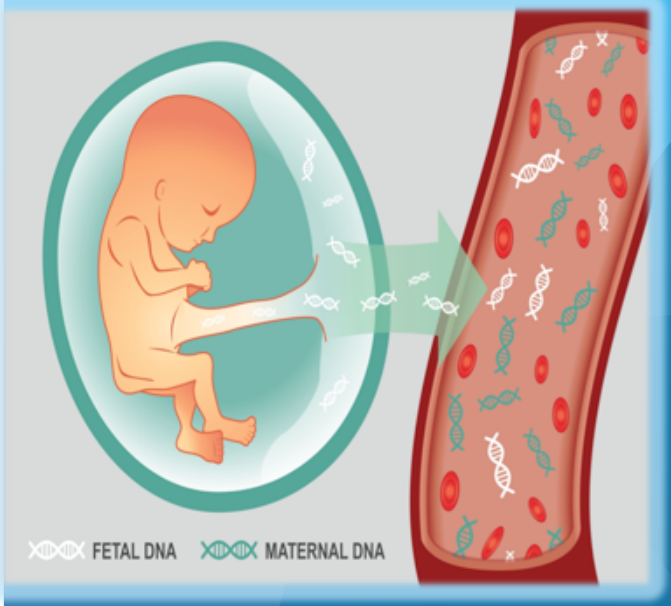
2014

- Chromosome 4p del
  - Wolf S.
- Chromosome 11q del
  - Jacobsen S.
- Chromosome 8q del
  - Langer-Giedion S.

August 31, 2015: Whole Genome

All chromosome aneuploidy	Duplication/ Deletions to 7 Mb	Less than 7 Mb for 22q, 15q, 5p, 1p36, 4p, 11q & 8q

cfDNA comes from Apoptotic cells in Maternal Circulation



Maternal adipocytes (lipocytes & fat cells)

Maternal white blood cells

Fetal:

Placental cell (trophoblasts)

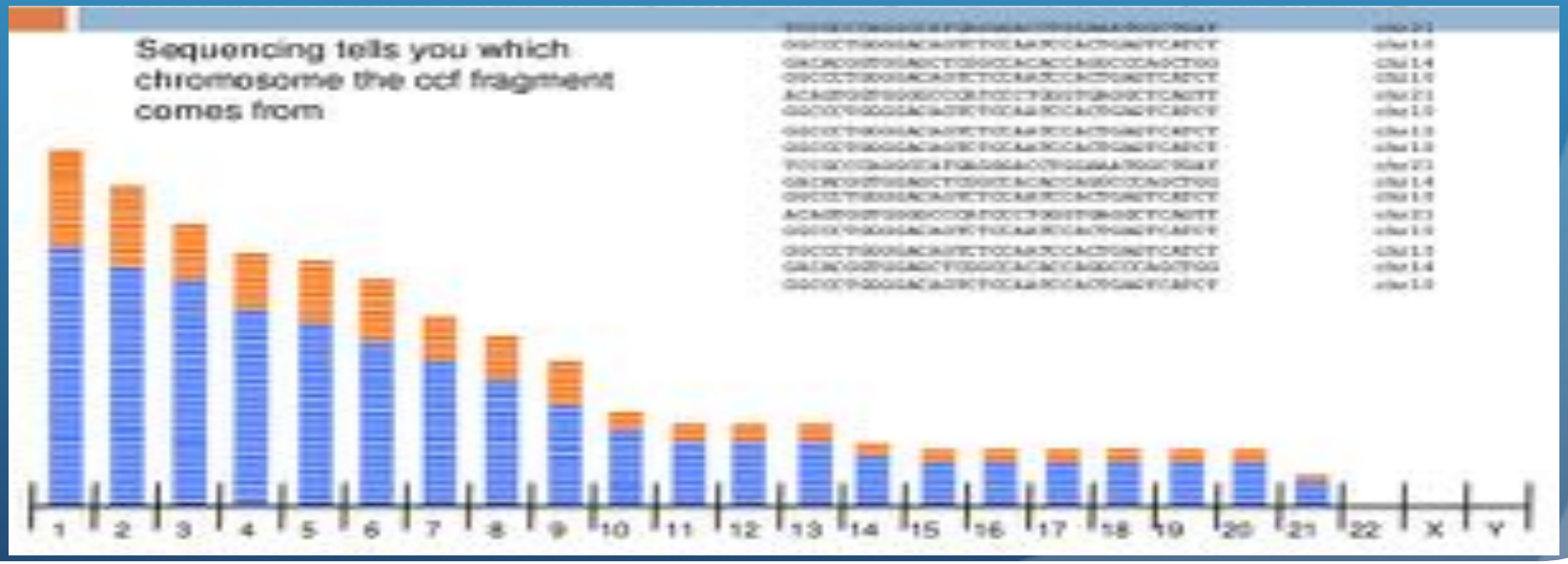
By 10 weeks gestation:

~90% of total is maternal

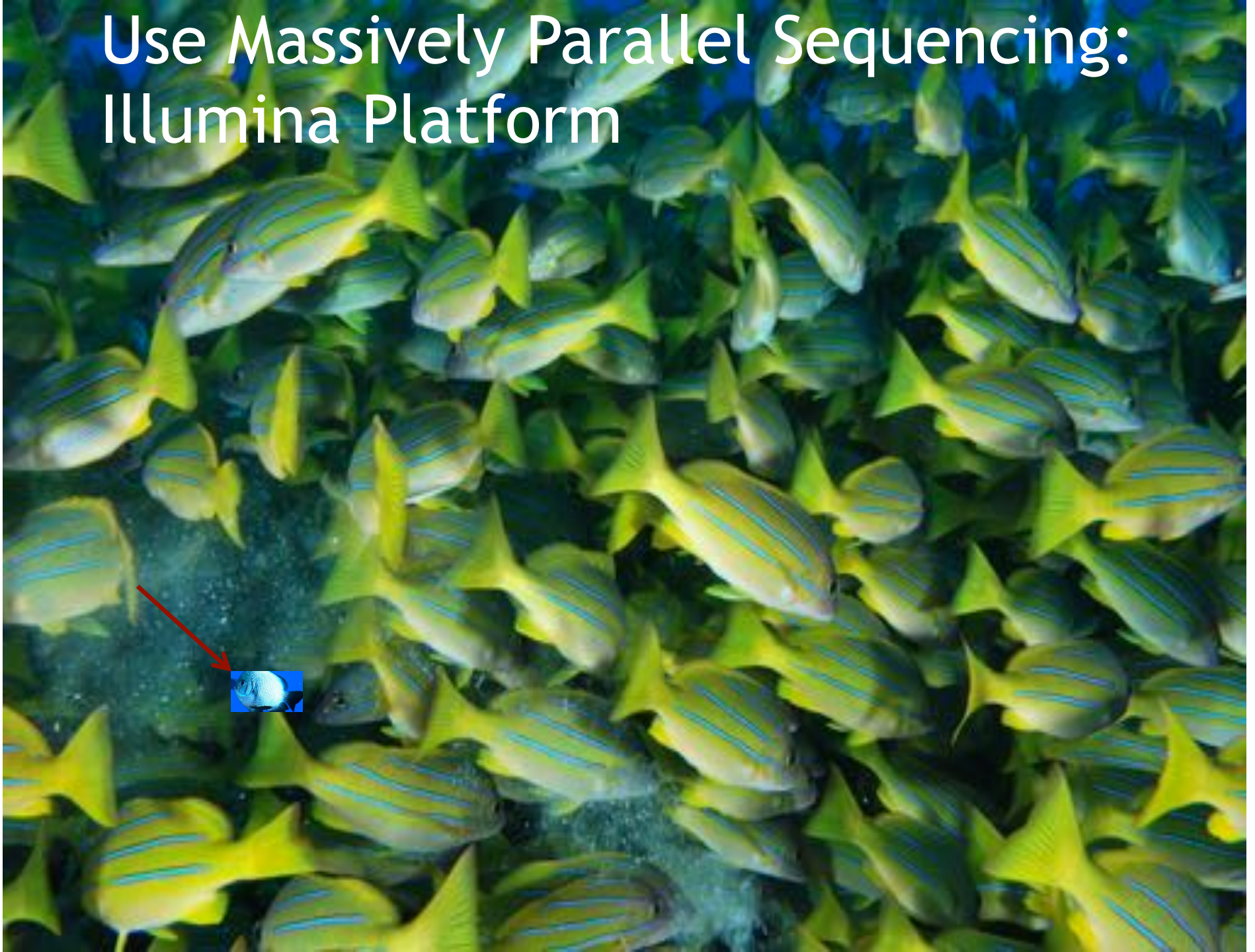
- Primarily from apoptosis of blood cells

~10% is from pregnancy

- Primarily from apoptosis of placental cells



Use Massively Parallel Sequencing: Illumina Platform




Clusters are sequenced simultaneously.
One by one, fluorescently-labeled reversible terminator nucleotides are incorporated and fluoresced by laser


Each base (A,T,C,G) has its Own Color
Each cycle is captured digitally

LABS & NAMES FOR NIPT:

Harmony
ARISOSA
NIPS
INVITAE

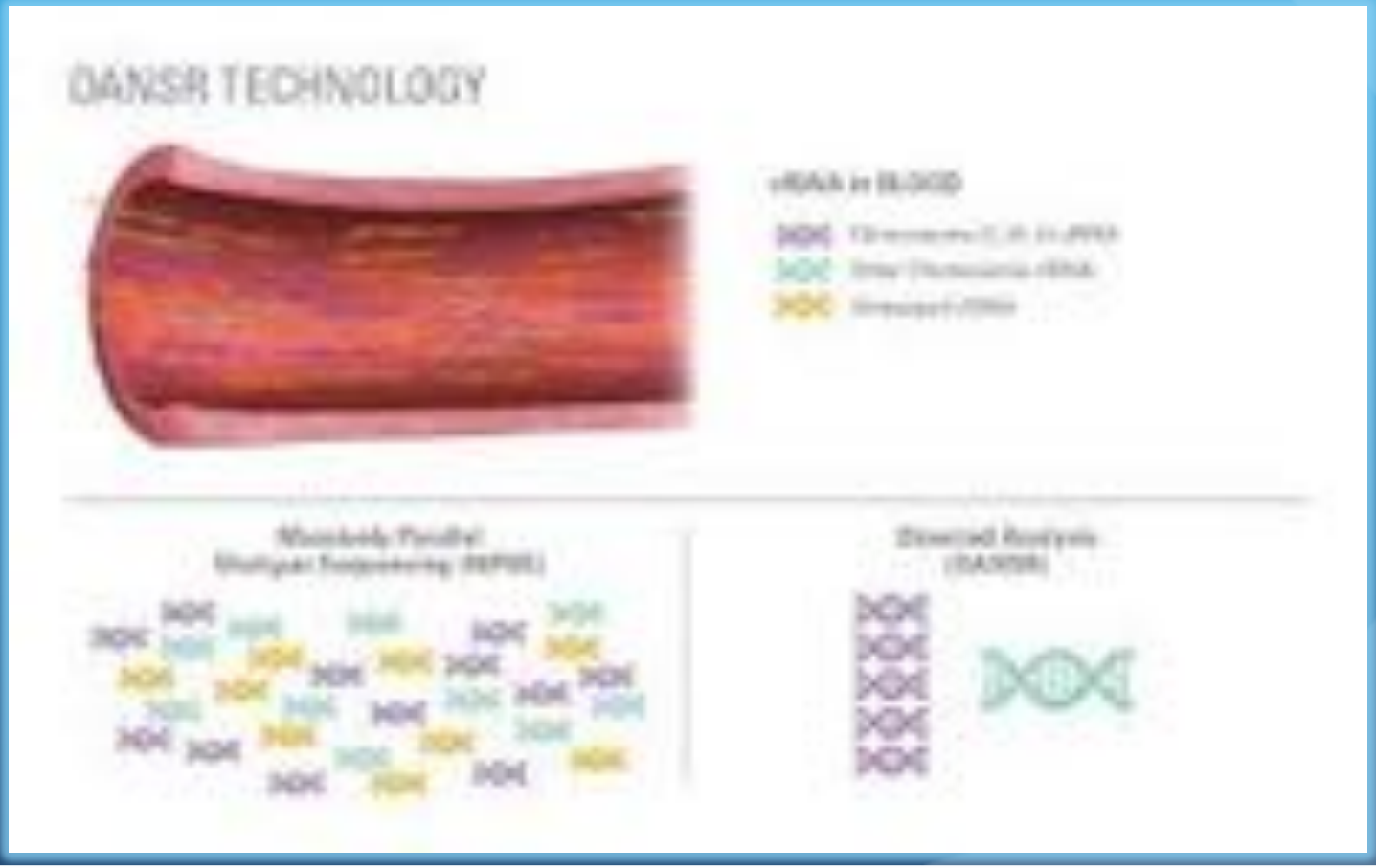
informaSeq
LabCorp/
INTEGRATED GENETICS

Panorama
NATERA
Prequel
MYRIAD

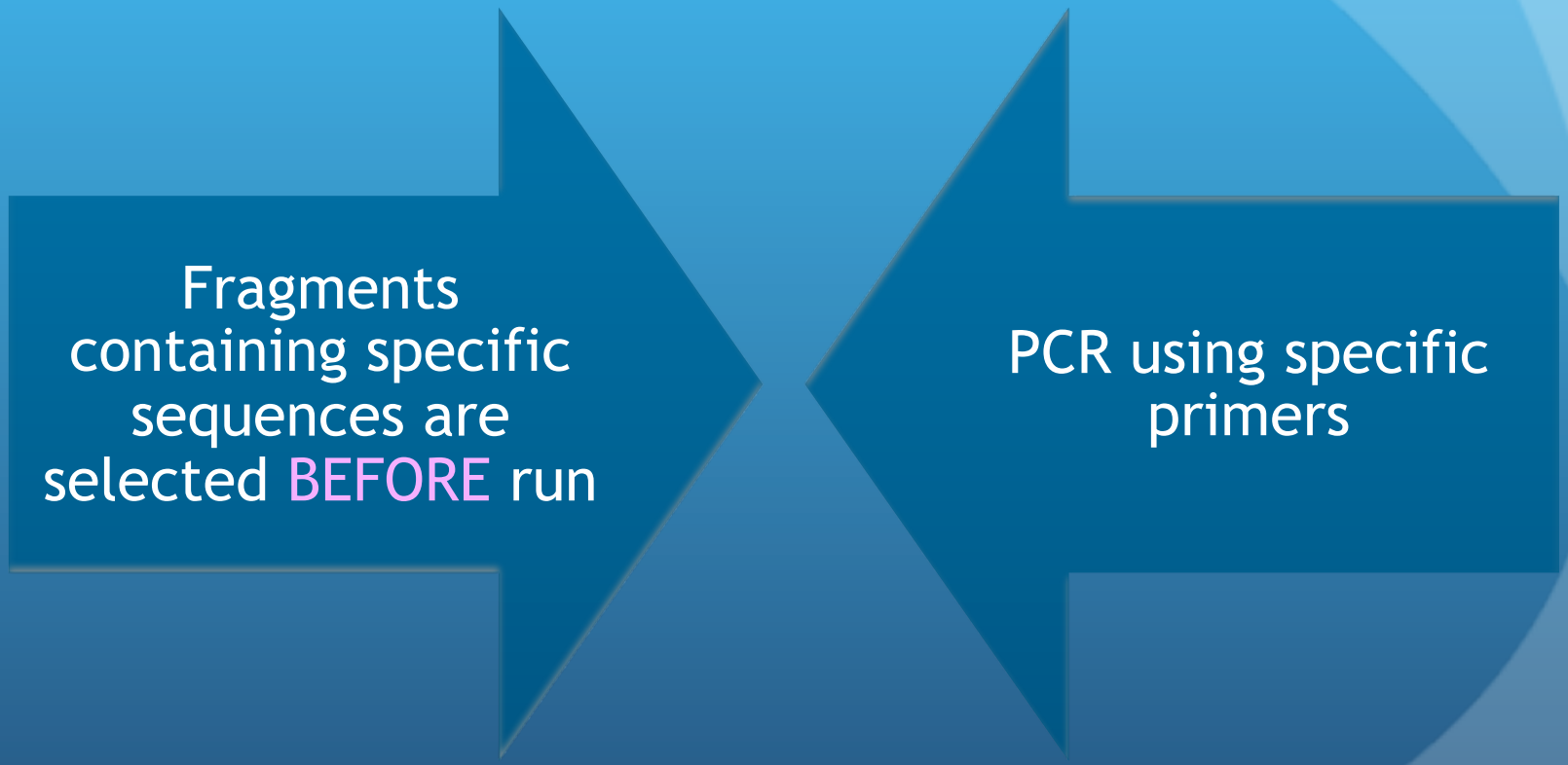
Innatal/Verify
PROGENITY

MaterniT 21 Plus
MaterniT
Genome
SEQUENOM

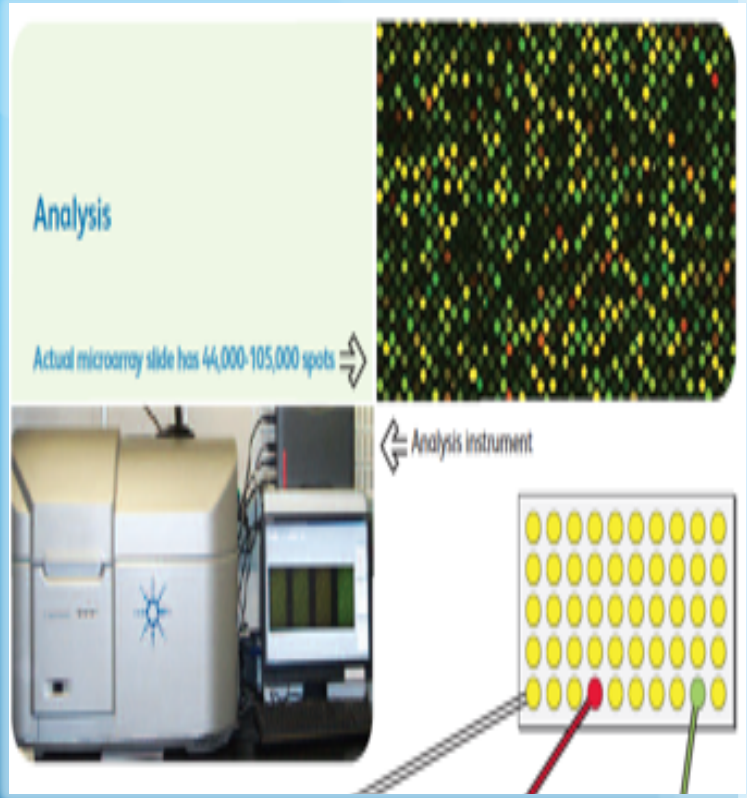
TARGETING CHROMOSOMES OF INTEREST
or
TARGETING ALL CHROMOSOMES



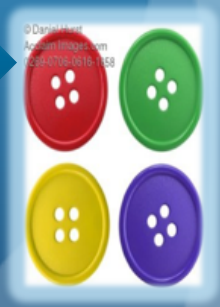
Targeted sequencing



- Selection of Nonpolymorphic Loci
- ~400 loci per chromosome of interest
- Each 56 base pairs in length



Select ONLY From Chromosomes of interest



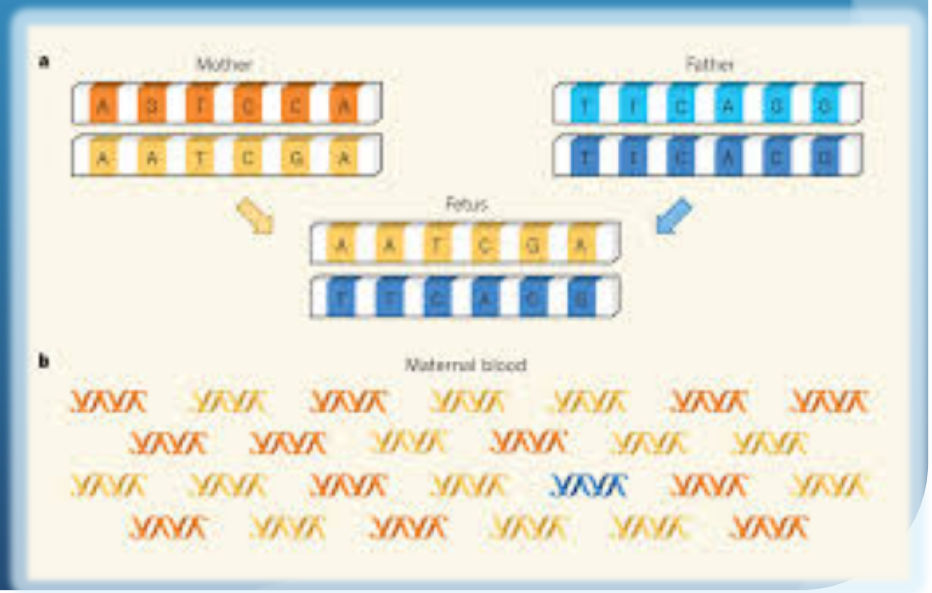
SNPs: single nucleotide polymorphisms

Algorithm assigns risk category to each chromosome of interest

Selection of Polymorphic SNPs

- SNP= single nucleotide polymorphism
- Common type of genetic variation among people
- >10,000 total SNPs over all chromosomes of interest
- “looking at the fetal DNA”
- Find unique SNPS

  FETAL in Origin



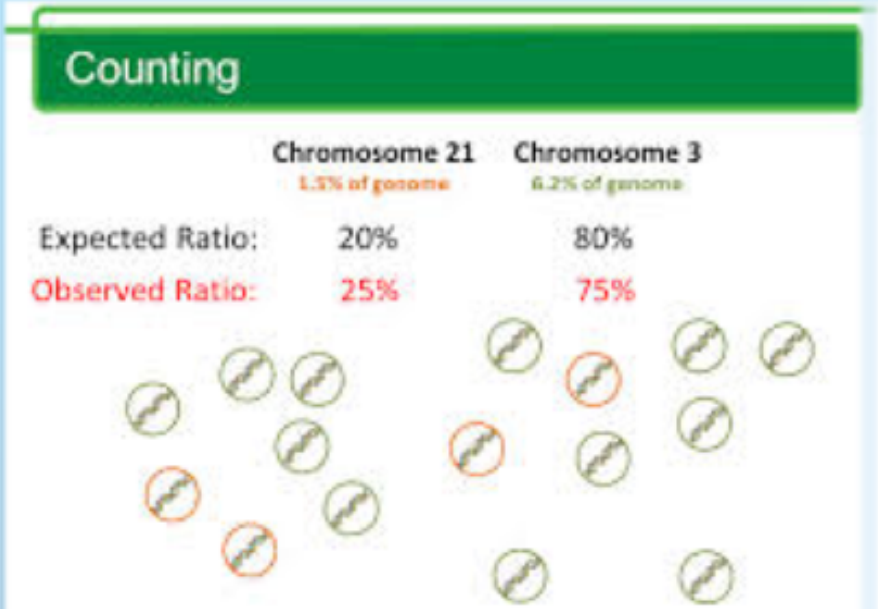
Shotgun sequencing

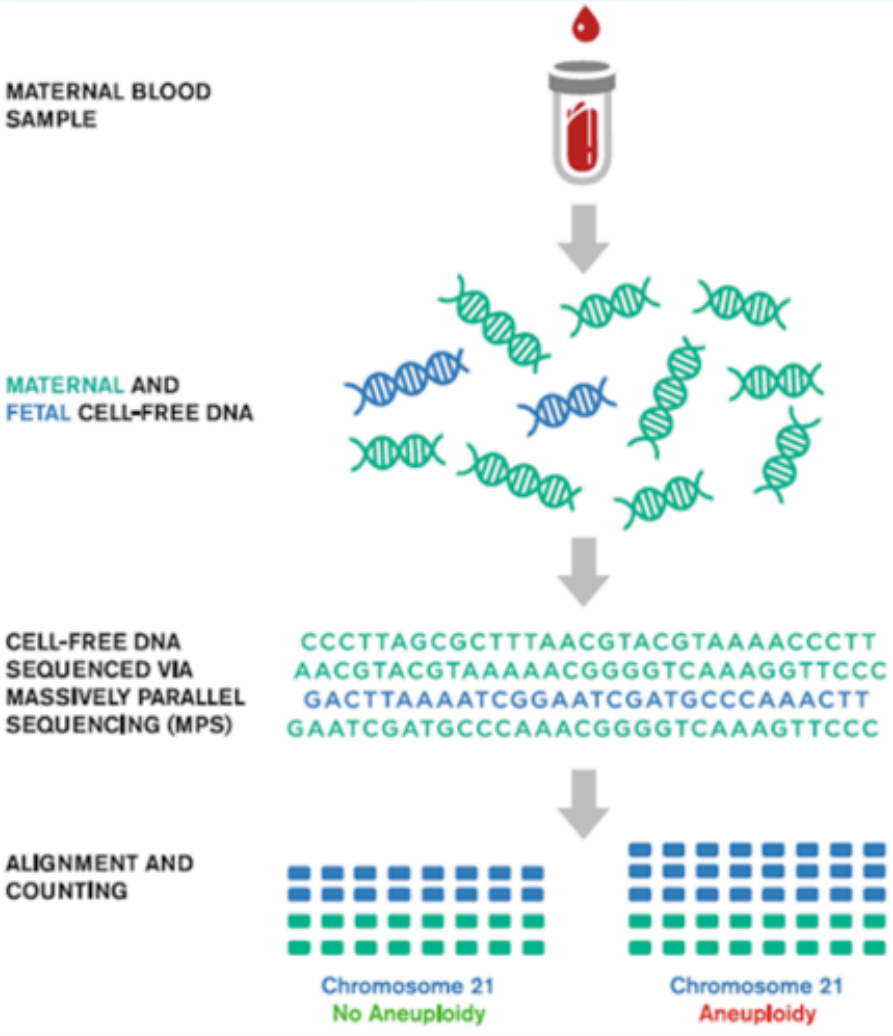
- All fragments are run
- Several million total
- Doesn’t restrict amount of information that can be found
- Enabled whole genome reporting

QUANTIFYING FRAGMENTS:

Each fragment is put into its chromosome “bucket”

Know the expected ratio for each chromosome





HOW NIPT RESULTS ARE REPORTED:

- "Positive"
- "Negative"
- "Uninformative"

- Aneuploidy Detected
- No Aneuploidy
- Aneuploidy Suspected

- "High Risk"
- "Low Risk"
- With Number of Risk

LESS NIPT OPTIONS IF MULTIPLES

And…if offered…Unable To Determine WHICH One is AFFECTED

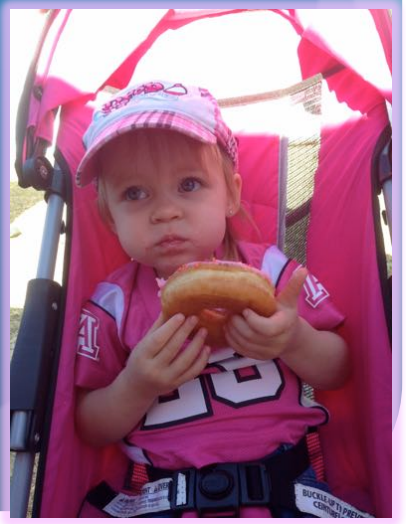
Factors Influencing Sequencing

- GC content:
  - Varies with each chromosome
  - G-C nucleotide pairs held together by 3 hydrogen bonds
  - A-T nucleotide pairs held by 2 hydrogen bonds
  - When G-C content high or low
    - DNA forms hairpins
    - Polymerase does not bind well

Only 80% of Trisomy 13 will be a “Positive” NIPT result

Reasons for low fetal fraction: Perinatal Quality Organization

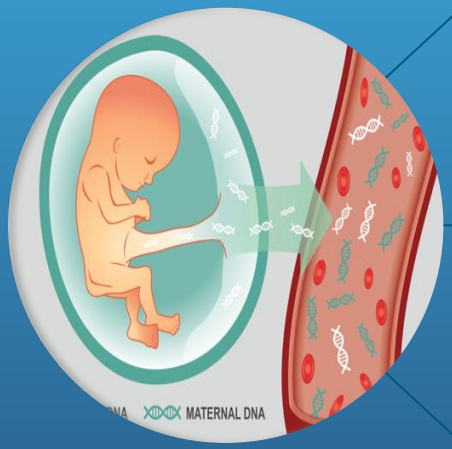
- Difference seen between women is unknown
- Should be at least 4%
- With Trisomy 21 fetal fraction INCREASES
- With Trisomy 13, 18 and XO fetal fraction DECREASES
- 14% risk aneuploidy if TWO failed results due to low fetal fraction
- Overall failure rate was 3 to 5%
  - 20% if maternal weight greater than 250 pounds
  - 50% if maternal weight greater than 350 pounds



Retrospective Study: Aug. 2014 to Oct. 2015

- 172,131 samples
- Under 175 pounds: success rate greater than 99%
- 175 to 250 pounds: success rate greater than 96.9%
- 250 to 300 pounds: success rate ~93%
- Over 300 pounds: 92.7% or less
- According to ACOG 14.8% of women are over 200 pounds

“False” Positives: Mosaicism



True positive

- Generalized mosaicism
- Trisomy cells in placenta & fetus

False positive

- Confined placental mosaicism
- Trisomy cells only in placenta

False negative

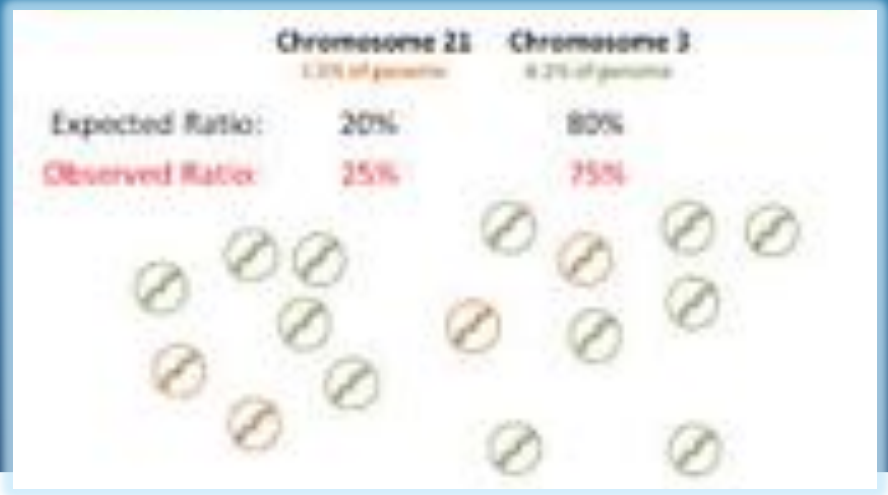
- Fetal mosaicism
- Trisomy cells only in fetus

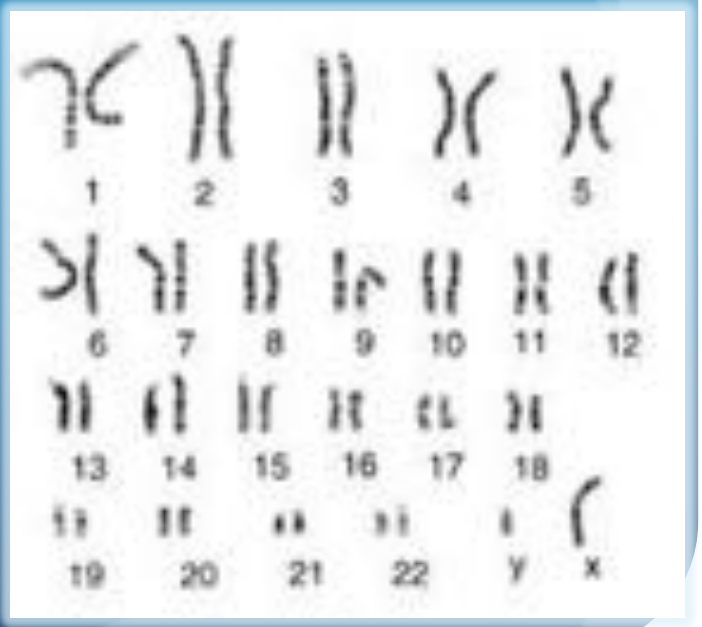
“False” Positive:

- Maternal Mosaicism
- Maternal Sex Chromosome Abnormality
- Maternal 22q deletion
- Maternal cancer
- Unrecognized or vanishing twin

Maternal Cancer:

- Results are abnormal due to “Monosomy 21, 13, or 18”
- Due to the reference chromosome being Trisomy
- Liver, breast, ovarian, leukemia, cervical, colon, malignant melanoma
- Tumor sheds DNA
- 20% cancer risk (JAMA 2015:314)





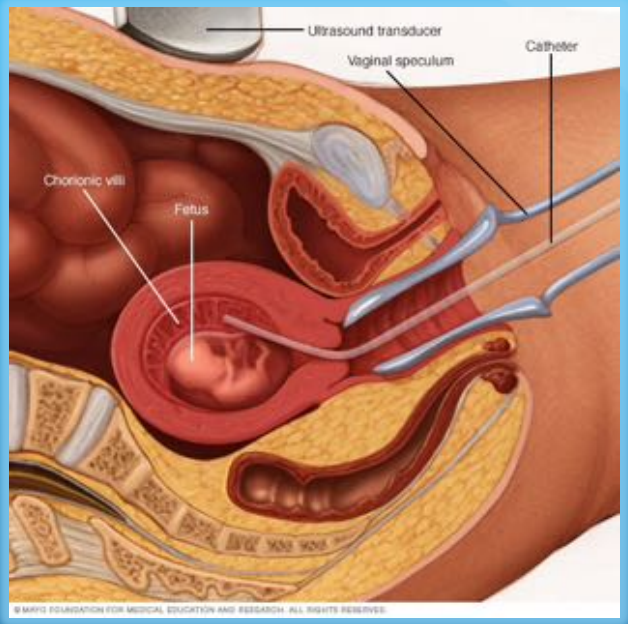
NIPT: Not Diagnostic Testing!!!

NIPT/Cell Free DNA Screening Predictive Value Calculator

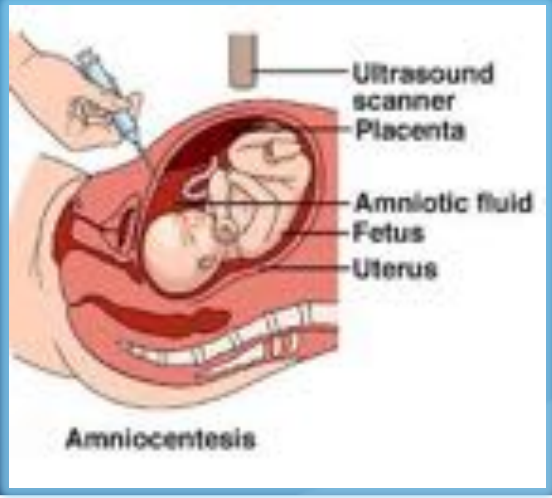
- http://perinatalquality.org
- Free On-line tool
- PPV and NPV Calculator
- Gives the probability that results is a TRUE NEGATIVE: the fetus is NOT AFFECTED
- Gives the probability that is is FALSE NEGATIVE: the fetus is AFFECTED

Best Diagnostic Test Based on Likelihood of Placental Mosaicism

CVS:
Trisomy 21: 2%
Trisomy 18: 4%



Amniocentesis:
Trisomy 13: 22%
Turner S (X): 59%

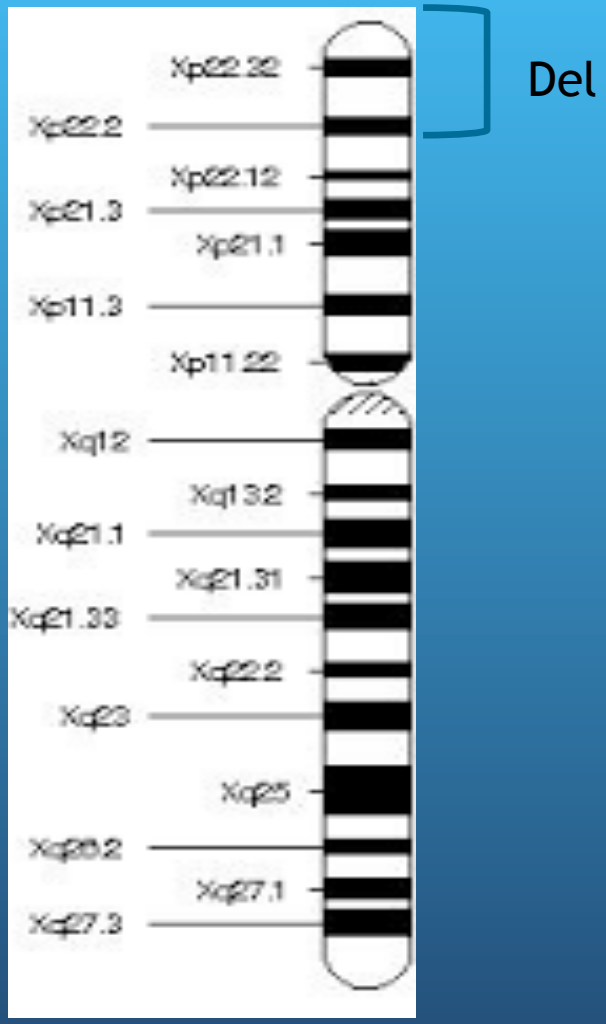


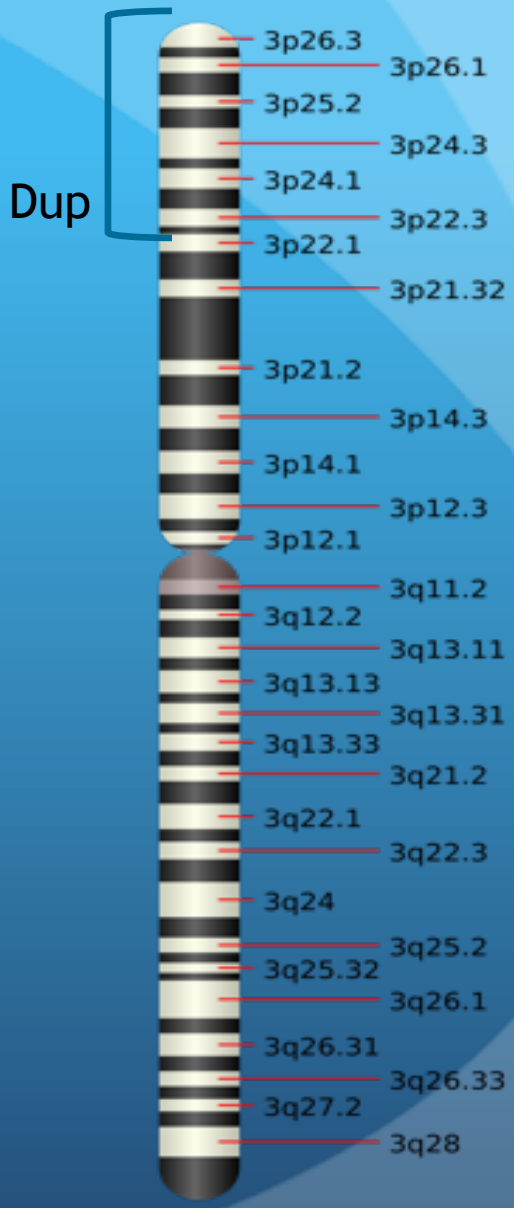
Genome

- Reportable Fetal Fraction Threshold is equal to or greater than 4% (8% for 22q11 deletion)
- Sensitivity:
  - Genome-wide events
  - Greater than or equal to 7 Mb
  - 95.9%
- Specificity:
  - Genome-wide events
  - Select microdeletions of 22q, 15q, 11q, 8q, 5p, 4p & 1p
  - Greater than 99.9%

“This is a complex case.”

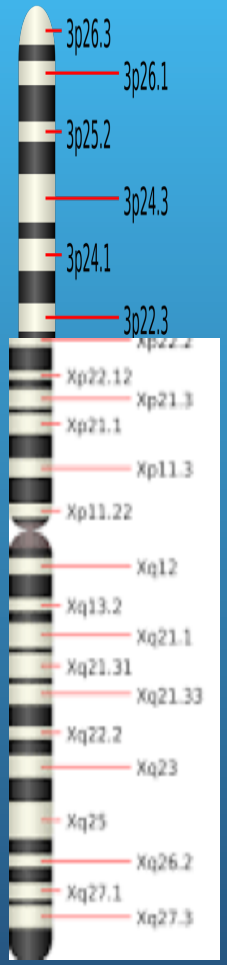
Chromosome X and 3





Power of Lyonization

Gain of Chromosome 3 Translocated to Chromosome X: Partial Trisomy 3/ Monosomy X



Missing X

22q11 deletion Syndrome:

- Incidence: 1 in 2000
- NIPT is for LOW risk. If family history offer CVS/amnio
- Most common deletion is 3 Mb
- If “positive” there is 1 in 19 risk that true positive
- Some labs will not call unless fetal fraction at least 8%

DiGeorge Syndrome
Velocardiofacial Syndrome
Opitz G/BBB

- Haploinsufficiency of ~50 genes
- Leads to abnormal neural crest cell migration
- Outflow track of heart:
  conotruncal cardiac anomalies
- Thymic hypoplasia: immunodeficiency
- Parathyroid gland hypoplasia: hypocalcemia
- Palate anomalies

Whole Genome: 8-31 to 12-31-15
Total of 234 cases that were “positive”

- 53% Trisomy 13/18/21
- 17% Sex Chromosome
- 1% Microdeletion

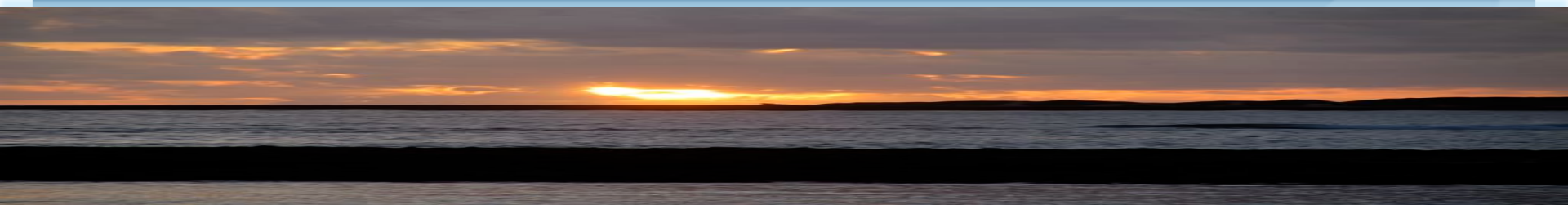
- 29% Abnormal Genome:
  - 7% Complex deletions/duplictions
  - 8% Isolated deletions/duplications
  - 14% Esoteric Trisomic

  Have seen “positive” in every chromosome

  Genome “Positive” rate: 6.2%

VALIDATION TRIALS:

- NEXT study was large-scale: for Trisomies 21, 18, 13
  - High sensitivity: between 98% and 99%
  - High specificity: between 99.5% and 99.8%
- Sub-chromosomal abnormalities have NOT been validated by any large-scale study
  - Will be difficult as rare events
  - Do not have same selection of high risk groups
- "Expansion to SCA requires availability of genetic counselors" *Genetics in Medicine, Dec 2015*



LOOKING TO THE FUTURE:

Should we offer NIPT to Everyone?

Just because you want NIPT,
can you get it?

Screening for Trisomy 21 with Population Rate of 1 in 500:

100,000	Pregnancies			
		Trisomy 21:	Normal:	
		N= 200	N= 99,800	
Method of Screening:	Detection Rate	Detected	Percent False "Positive"	# of False Positives
Serum AFP+ @ 16 wks gest	70%	140	5%	4990
NT @ 12 wks	90%	180	5%	4990
Sequential	97%	194	3%	2994
Cell-free DNA @ 9+ wks gest	>99%	>199	<0.1%	<100

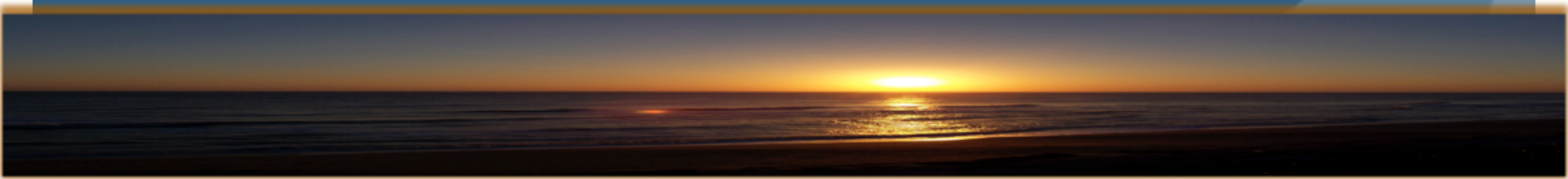
Bill H.R. 3441: *Accurate Education for Prenatal Screenings Act*

- Introduced Aug 2015
- House Energy and Commerce Committee's Subcommittee on Health gave it legislative hearing
- NIPS is screening tool, not diagnostic
- Need accurate pre-test, and if needed, post-test counseling

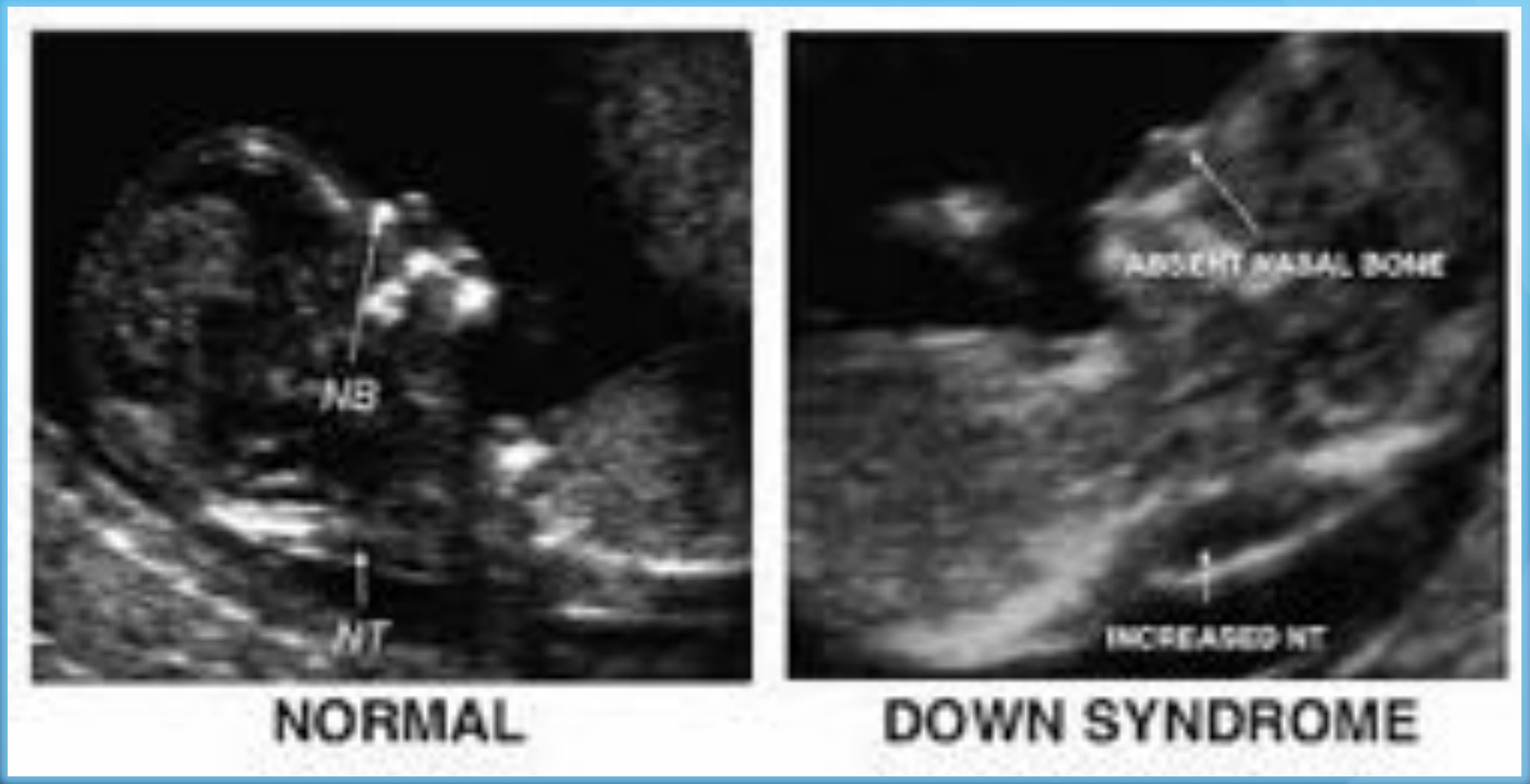
Cost to implement education and training programs that are in bill have NOT been addressed

Per AGOG, Sequential Screen still standard of care.

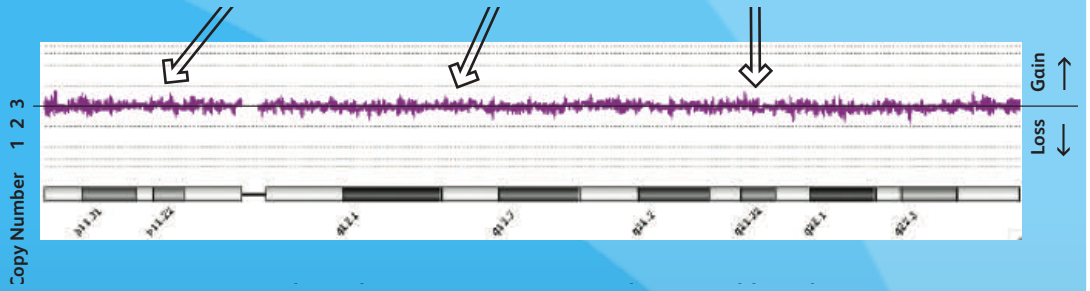
- **NIPT SHOULD NOT BE USED IN PLACE OF ROUTINE ULTRASOUND OR DIAGNOSTIC TESTING FOR CHROMOSOME ANEUPLOIDIES**
- Patients should receive counseling about limits and potential unexpected findings with NIPT.
  - Still need MS-AFP b/n 16-18wks, and, ultrasound @ 18 wks
- MaterniT Genome is currently offered by MFM practices
- "No call" and "low fetal fraction" in 2nd trimester should be offered consult for diagnostic testing
- Understand some "low risk" patients receiving 'direct marketing' for NIPT
  - Sample acceptance and cost differ with labs
  - Still needs to be ordered by Healthcare Professional



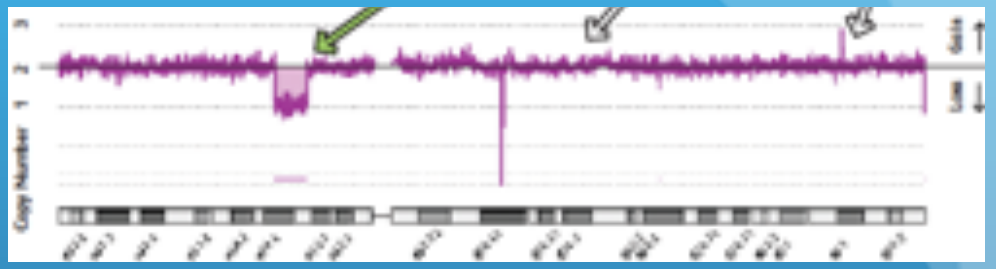
NT Ultrasound: Does It Still Have Value?



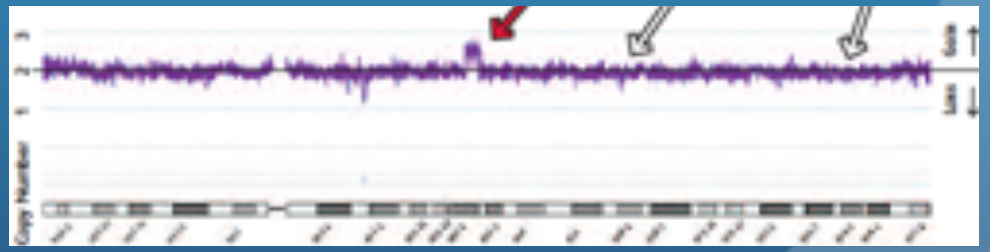
Normal Results:



Deletion Result:

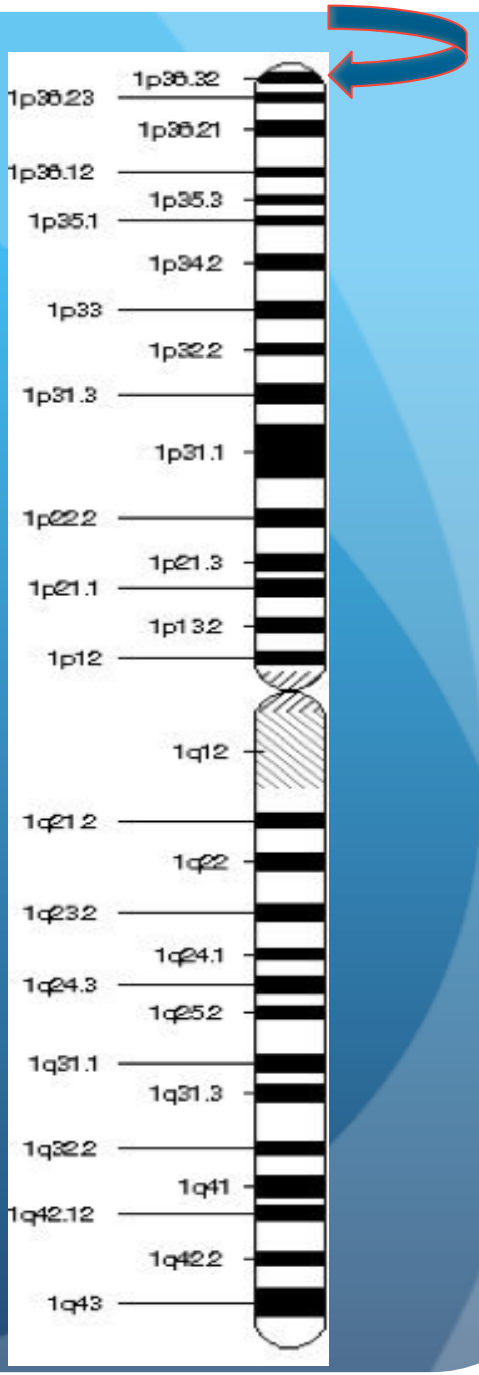


Duplication Result:

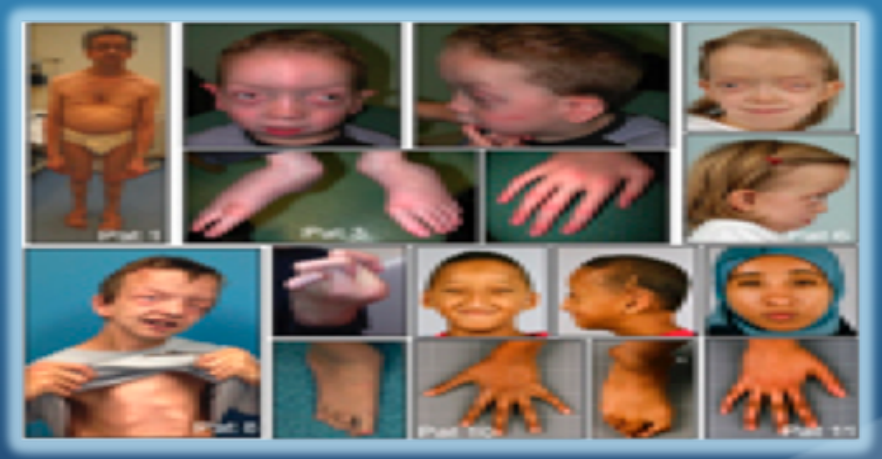


Variant Uncertain Significance

- Chromosome 1p36.33-p36.32
- Gain of 2.76 Mb
- No reports
- Area of SKI gene
- Shprintzen-Goldberg Syndrome



Shprintzen Goldberg Syndrome



Assume nothing!

Questions?