Fundamentals of Molecular Ecology I:

Heredity & The Nature of Heritable Information

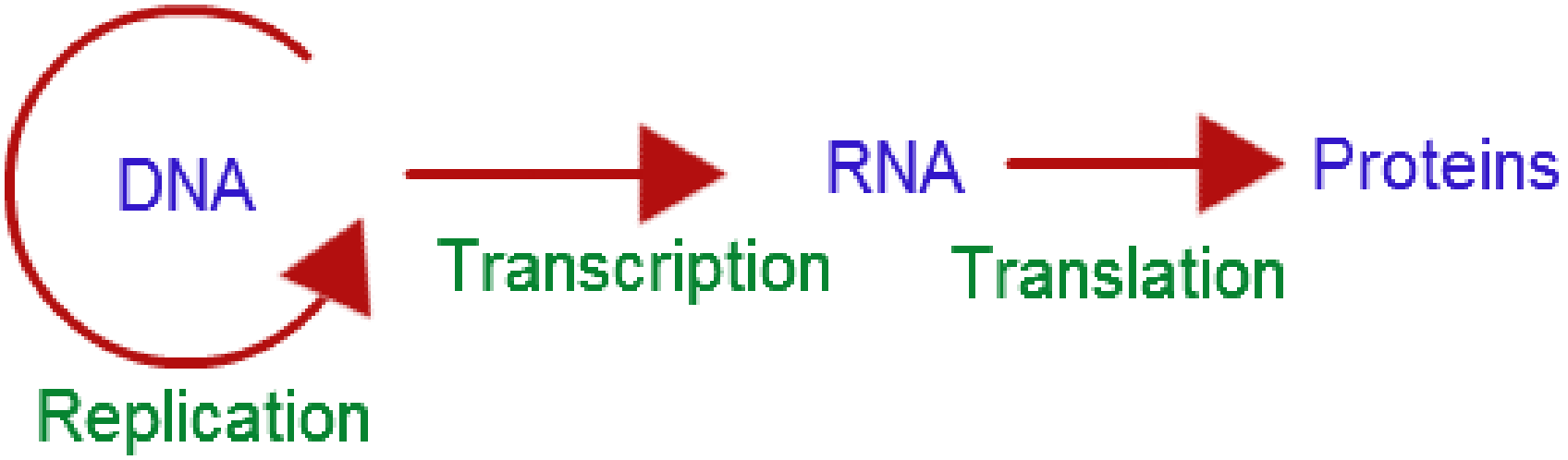
John Whalen

Lecture credit: Dr. Christopher E. Bird

Learning Objectives

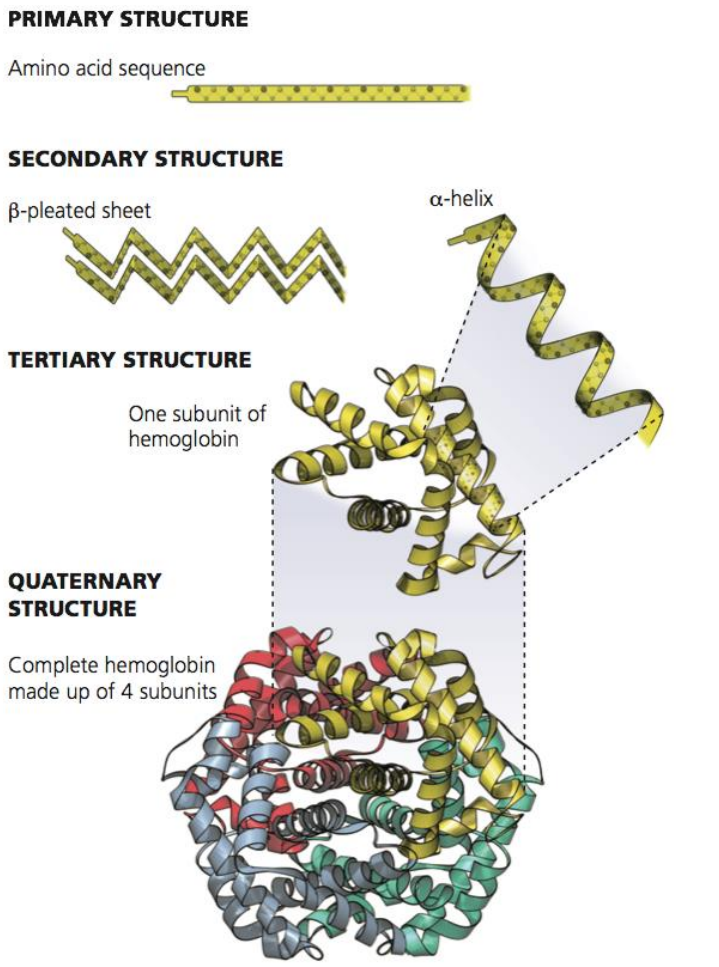
- Describe the structure of proteins
- Describe the organization of DNA and its connection to heritable traits, evolution
  - Coding vs noncoding
  - Somatic vs germline mutations
  - Independent assortment & genetic recombination
- Describe mechanisms of gene expression
  - Role of environment
- Discuss complex relationship between phenotype and genotype

Central Dogma of Biology

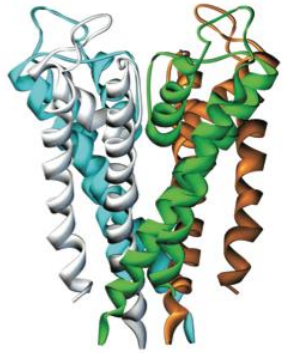


Proteins are chains of amino acids

- Cells mostly composed of proteins
- Critical components of life's chemical reactions
- 100,000 proteins in humans
  - Made from how many amino acids?
- Amino acid sequence determines structure and function



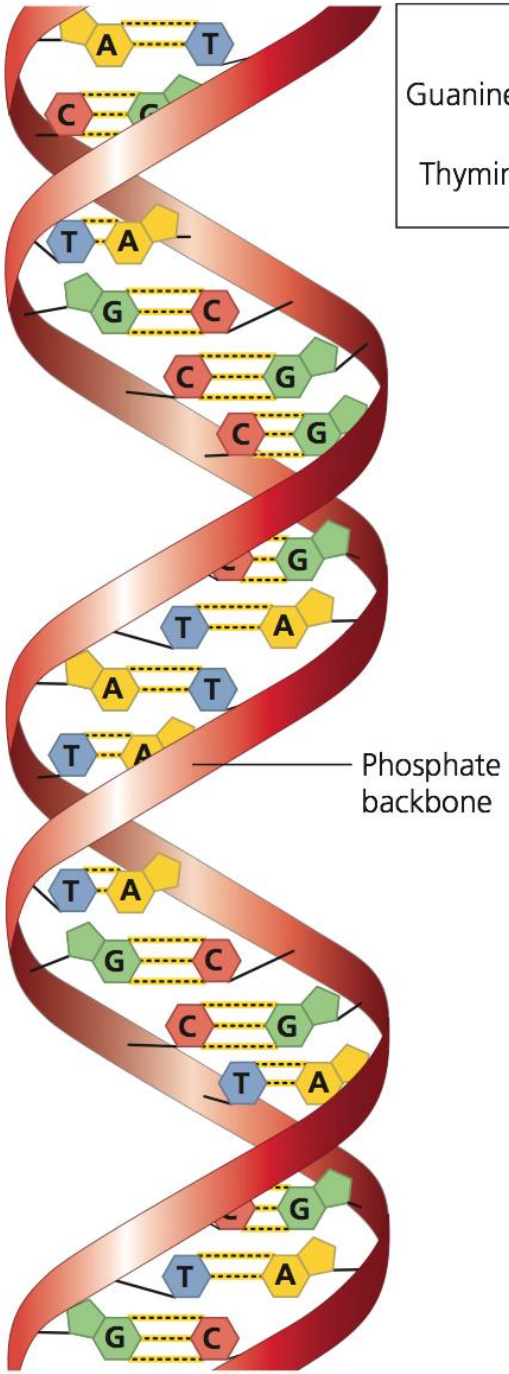
Hemoglobin with iron-bound heme groups shown in place



Ion channel

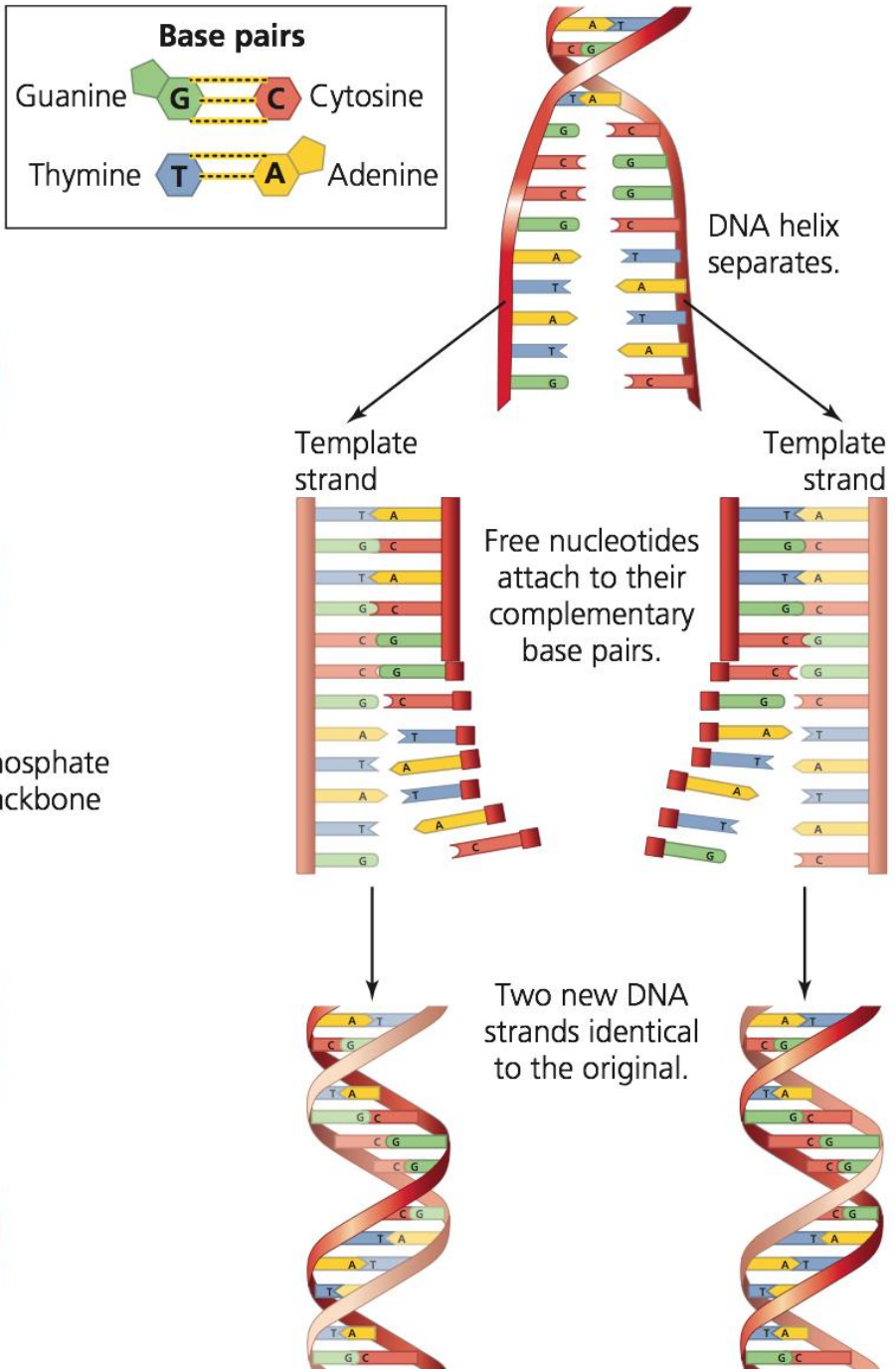
Human protein-coding genes and gene feature statistics in 2019 (Piovesan et al 2019)

	Protein-coding genes[a]	mRNAs[b]
Number		
Total entries	19,116	49,632
Median	N/A	N/A
Mean	Per chr: 797	N/A
SD	N/A	N/A
Min	chrY: 47 chr21: 228	N/A
Max	chr1: 1952	N/A
Length		
Median	26,018 bp	2938 bp
Mean	66,646 bp	3522 bp
SD	131,781 bp	2557 bp
Shortest	189 bp (*KRTAP6-2*, chr21)	186 bp (*DEFB133*, chr6)
Longest	2,473,592 bp (*RBFOX1*, chr16)	109,224 bp (*TTN*, chr2)
Total	1,274,002,474 bp	174,797,813 bp



Amino acid sequences encoded in DNA

- DNA composed of nucleotide chains
  - Phosphate Backbone
  - Bases encode information
    - ATGC
- Double Helix
  - AT-GC rule
- All life uses DNA, except some viruses
  - SARS-CoV-2

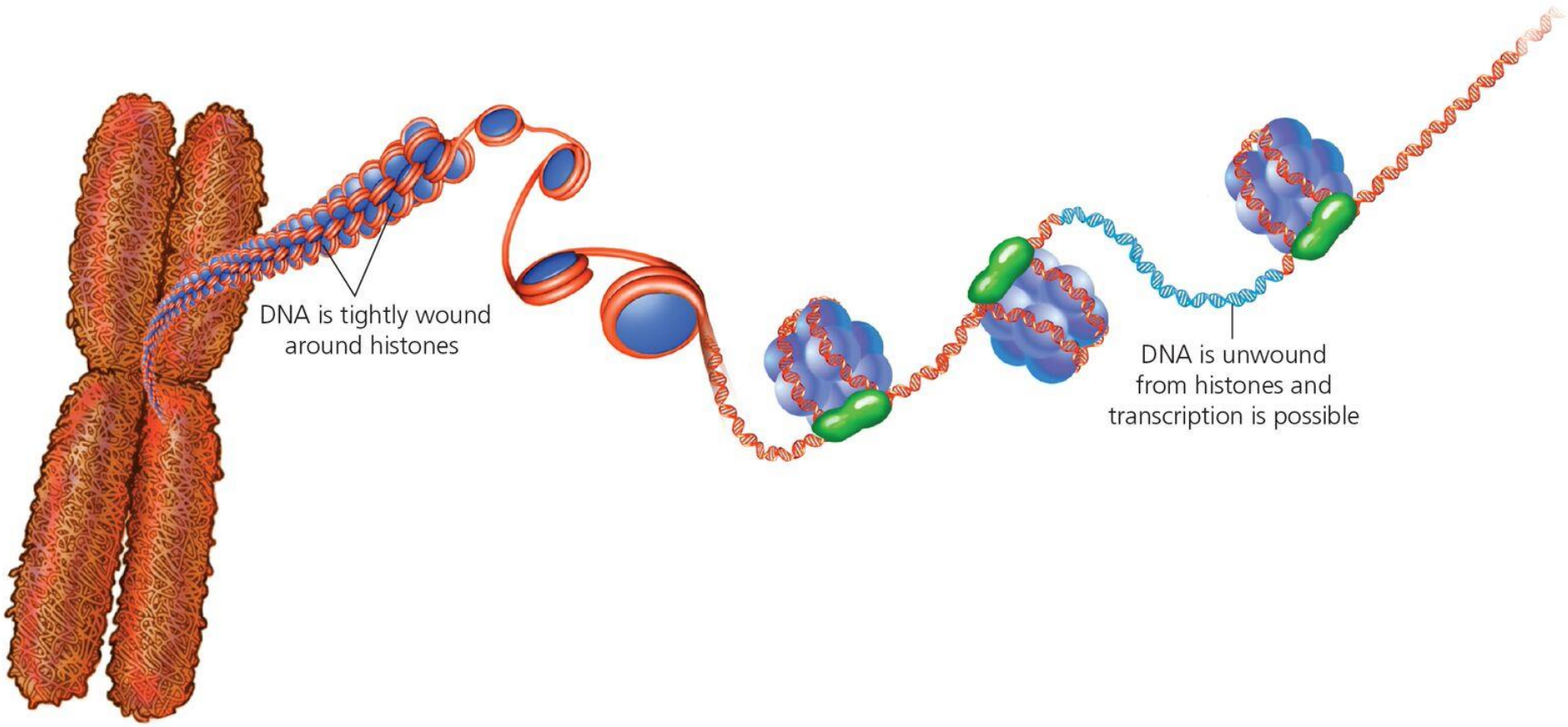


DNA Replication:

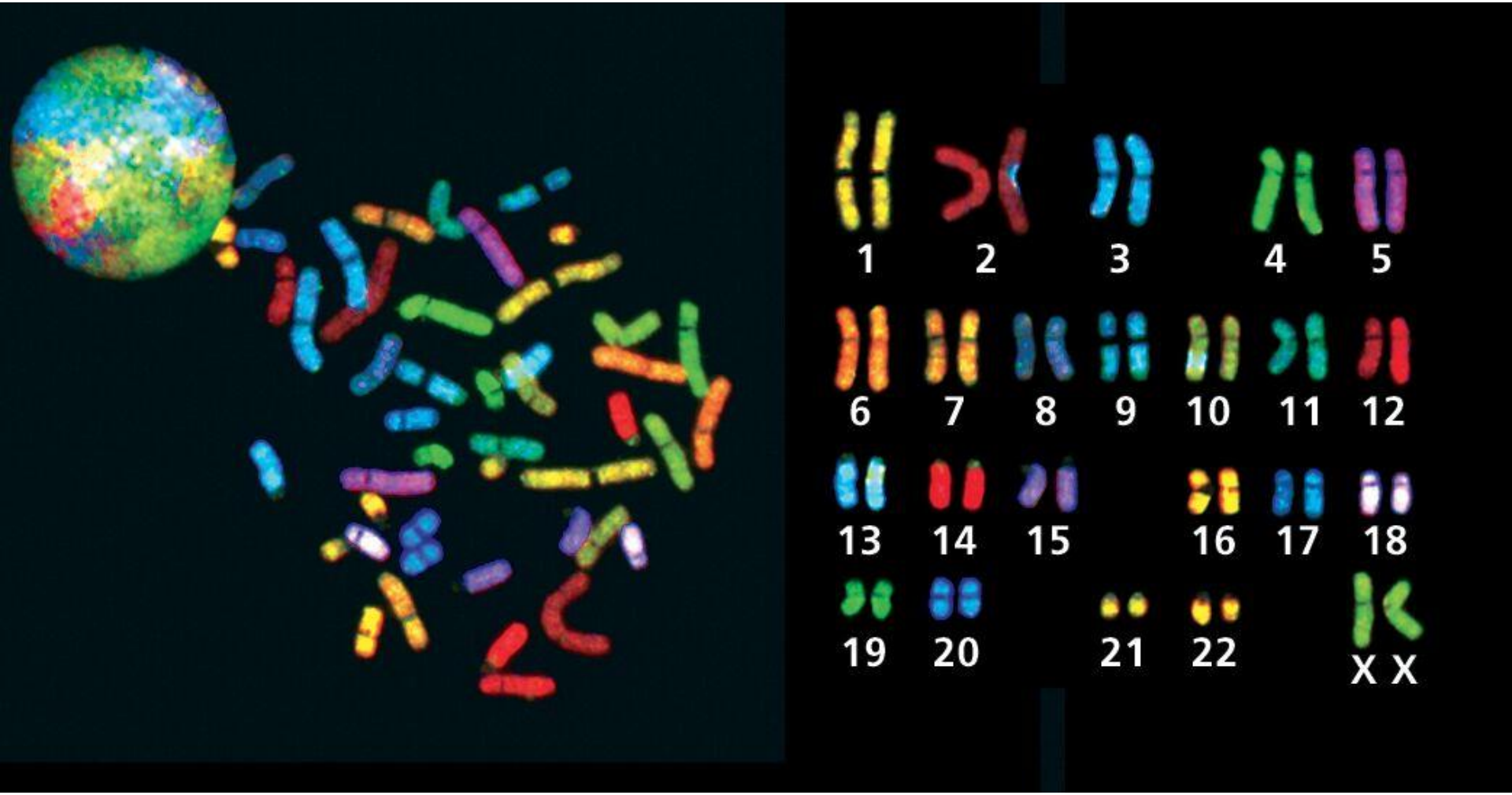
Passing on heritable information

- DNA sequence replicated during cell division
  - template strand
- Mutation: any change to the genomic sequence
  - Can occur during DNA replication

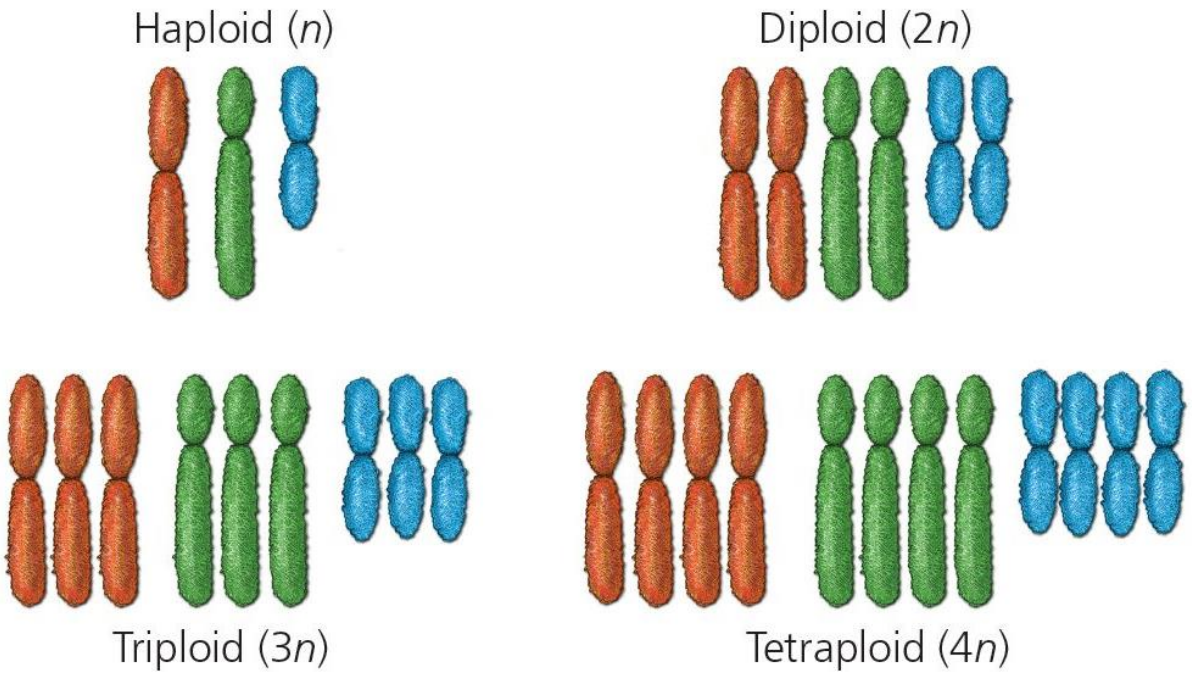
Eukaryotic DNA is organized into chromosomes

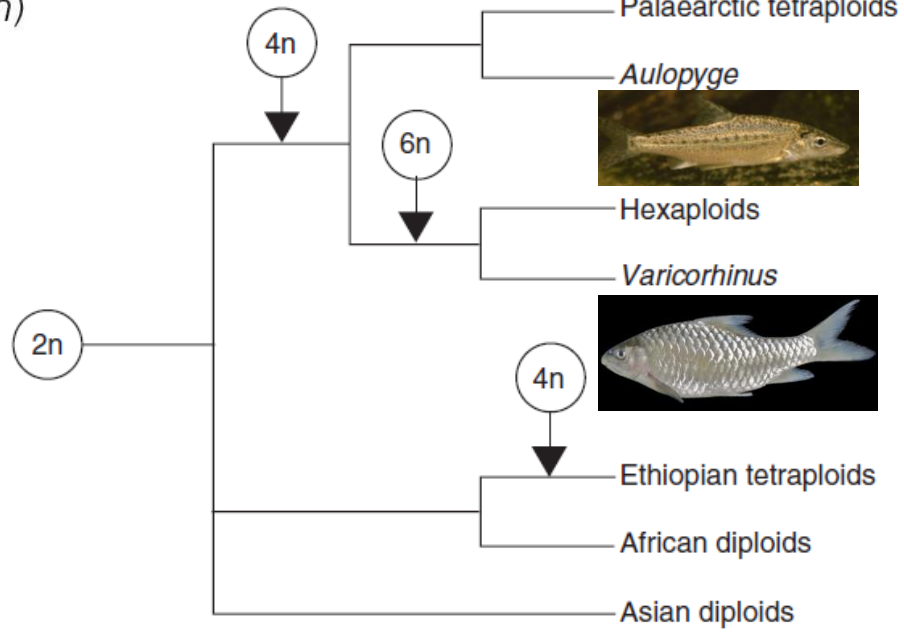


In diploids, chromosomes come in homologous pairs

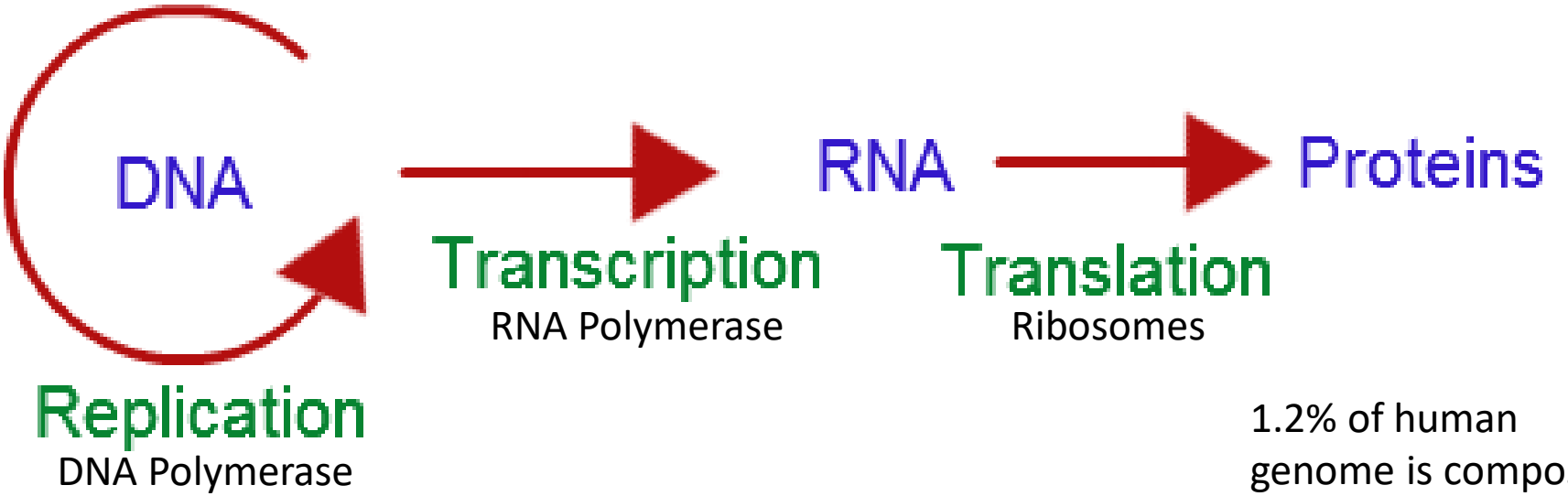


Ploidy: Number of copies of unique chromosomes in a cell





Central Dogma of Biology

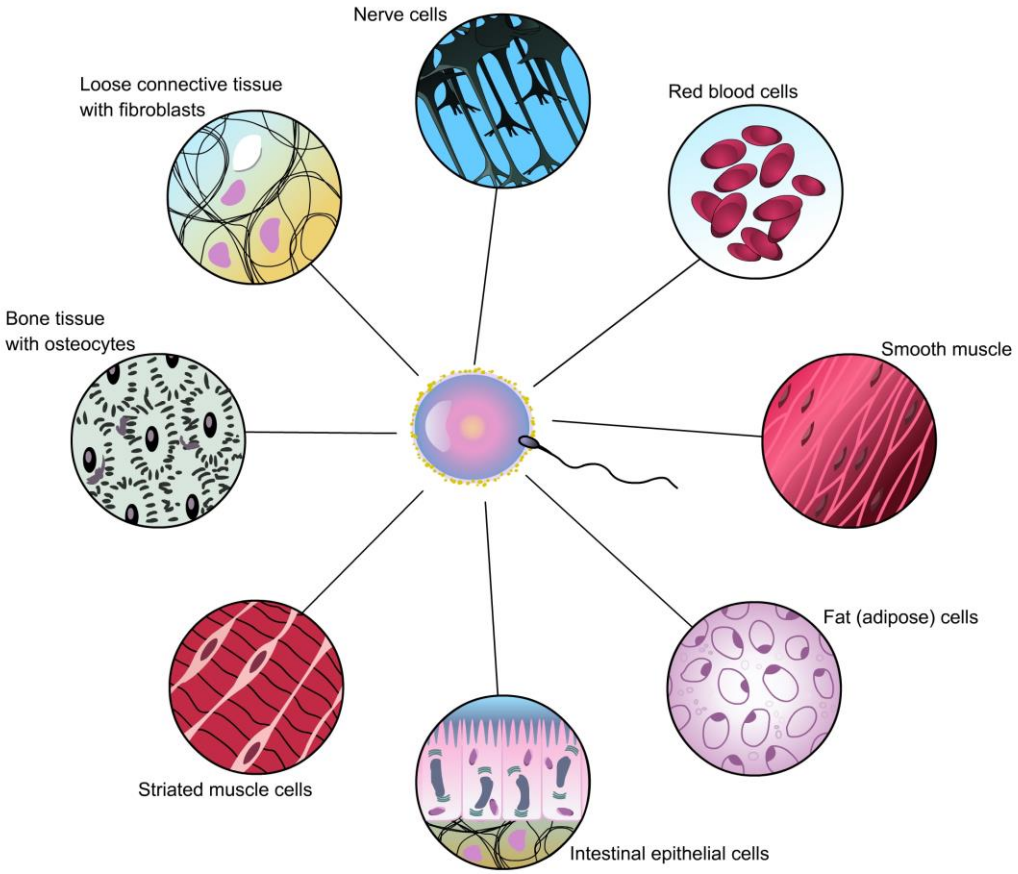


1.2% of human genome is composed of genes

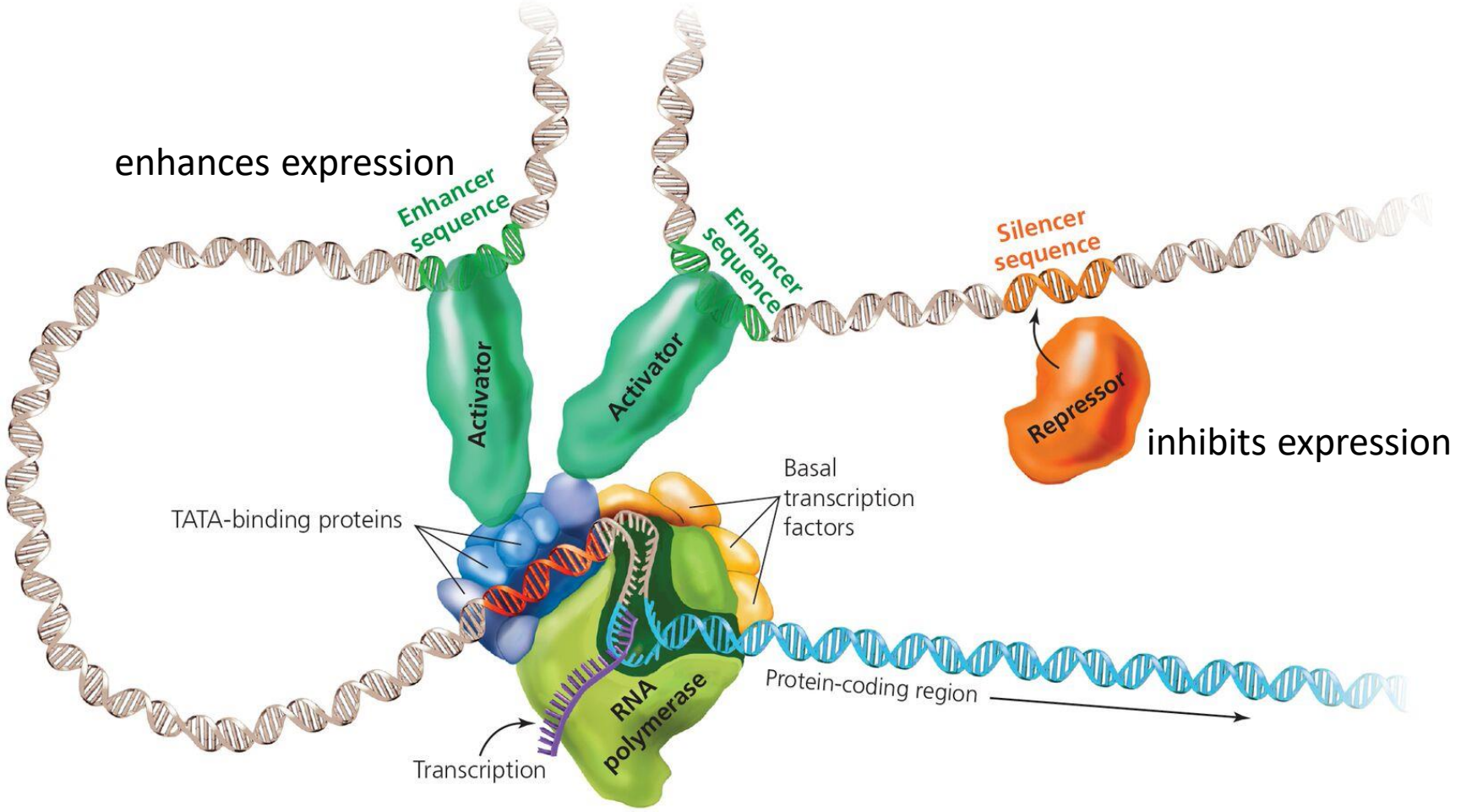
- Proteins are encoded in genes
  - make up small portion of most eukaryotic genomes
- DNA must be transcribed to RNA before the RNA is translated to a Protein
  - Thymine is transcribed to Uracil

Gene Regulation: process that modulates frequency, rate, or extent of gene expression

- Most genes are not continuously expressed
  - **expression**: conversion of genes into a product
  - all cells in organism have same genome but can have different form and function due to gene expression



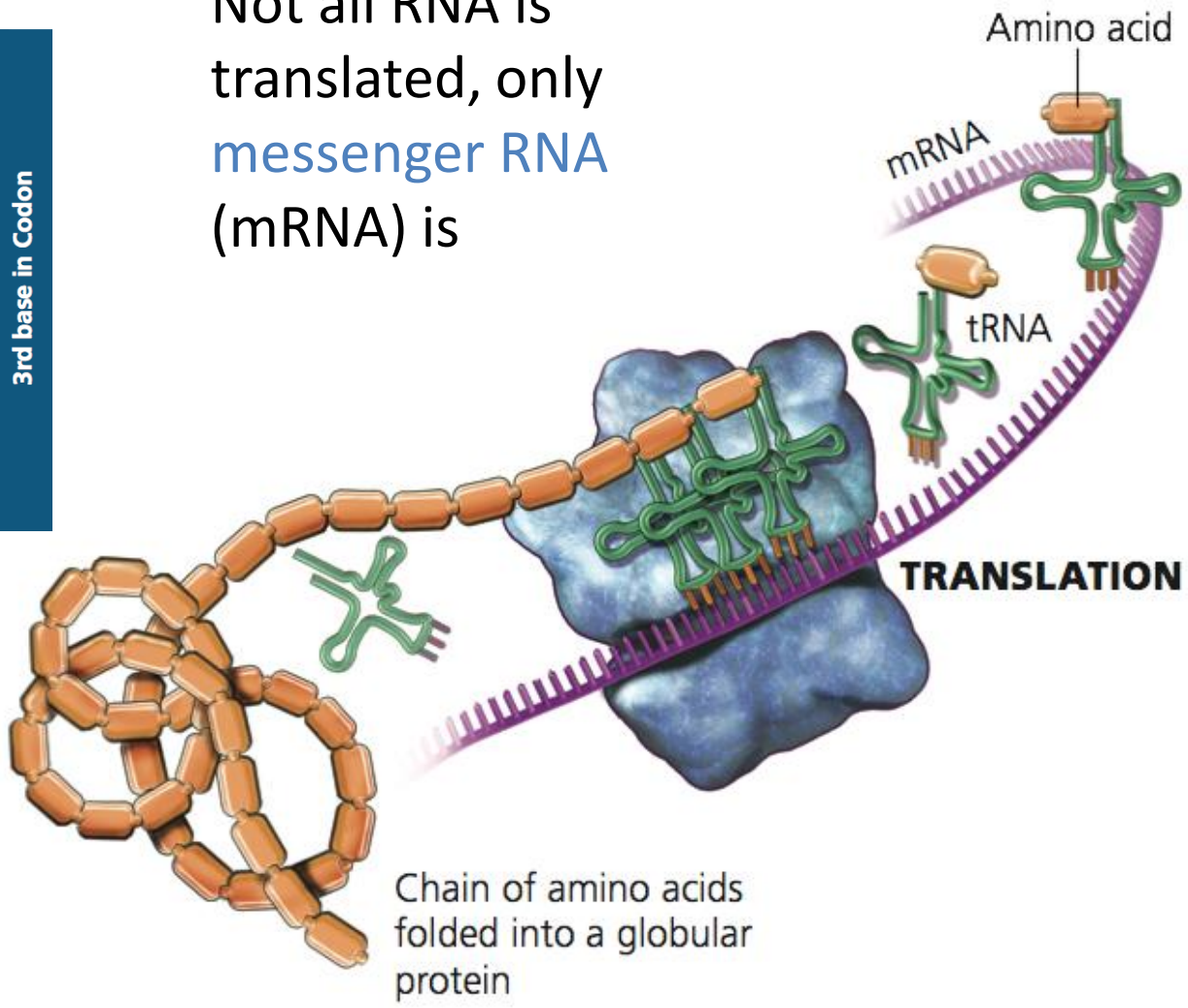
Several proteins are necessary for RNA transcription, any one could regulate transcription



Ribosomes translate mRNA into protein

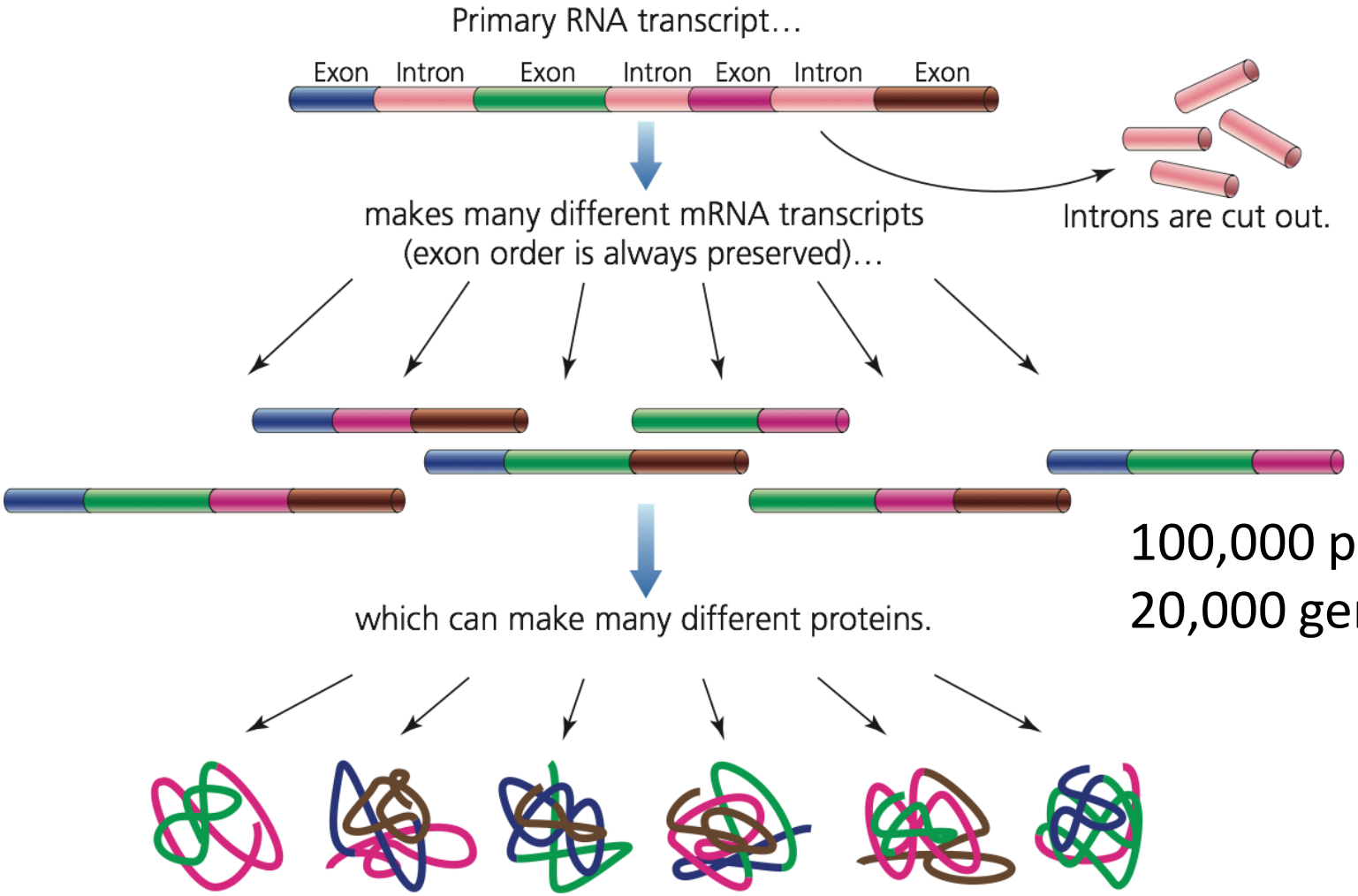
1st base in Codon	2nd base in Codon: U	2nd base in Codon: C	2nd base in Codon: A	2nd base in Codon: G	3rd base in Codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Not all RNA is translated, only messenger RNA (mRNA) is



RNA codons encode particular amino acids within 3 ribonucleotides

Alternative RNA splicing can result in multiple proteins from a single gene



100,000 proteins
20,000 genes

Genomes vary in size and complexity

TABLE 5.2 Variation in Genome Size and Complexity

Organism	Number of Chromosomes	Megabases in Genome	Approximate Number of Protein-Coding Genes
N. deltocephalinicola (bacteria)	1	0.112	137
E. coli (bacteria)	1	4.6	4300
S. cerevisiae (yeast)	16	12.1	6700
C. elegans (nematode)	6	100	20,000
A. thaliana (Thale cress)	5	120	27,000
D. melanogaster (fly)	4	180	14,000
N. vectensis (sea anemone)	15	450	27,000
C. familiaris (dog)	39	2400	20,000
M. musculus (mouse)	20	2600	19,900
H. sapiens (humans)	23	3000	20,000
P. abies (Norway spruce)	12	20,000	28,300
A. mexicanum (axolatl)	14	32,000	23,251
N. forsteri (lungfish)	17	43,000	31,120

Most variation in size is due to differences in mobile genetic elements: DNA segments that can move (ex: transposons) and replicate themselves within genome

Key Concepts

- Proteins have diverse functions and changes that can affect
  - Cell structure
  - Ability to catalyze enzymatic reactions
  - Cell-cell signaling
  - Ability to respond to other molecules

- Because of its stability, DNA forms the basis of a system that encodes and replicates information essential for life

Key Concepts

- Mutations to DNA can alter the structure of proteins

- Genomes typically contain a diversity of noncoding elements that reflect the evolutionary history of the organism

MUTATIONS CREATE VARIATION

Key Concepts

- Germ-line mutations are rare, but accumulate in populations over time
  - These mutations are the raw material for evolution
- In diploids, deleterious mutations may be masked by the presence of a functional copy of the gene on the other chromosome
- Changes in gene expression affect where, when, and how much a gene is transcribed
- Changes in gene expression add another component to heritable genetic variation

Types of mutation

Point mutation

TGCATTGCGTAGGC

TGCATTCCGTAGGC

Insertion

TGCATTTAGGC

TGCATTCCGTAGGC

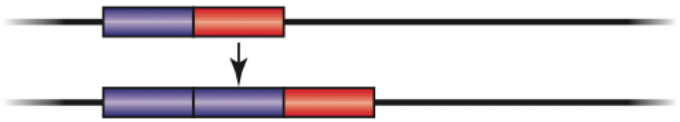
CCG

Deletion

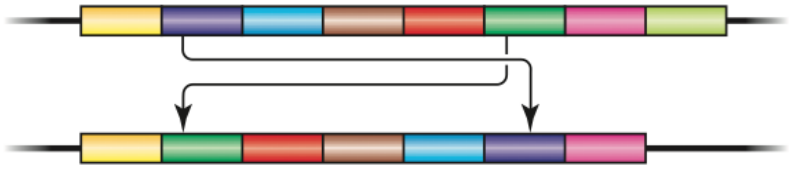
TGCATTCCGTAGGC

TGCATTTAGGC

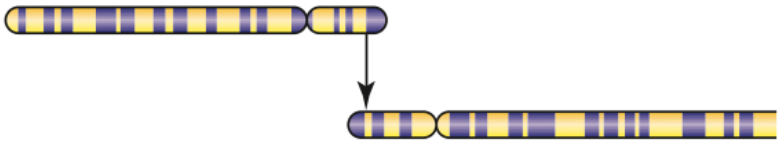
Gene duplication



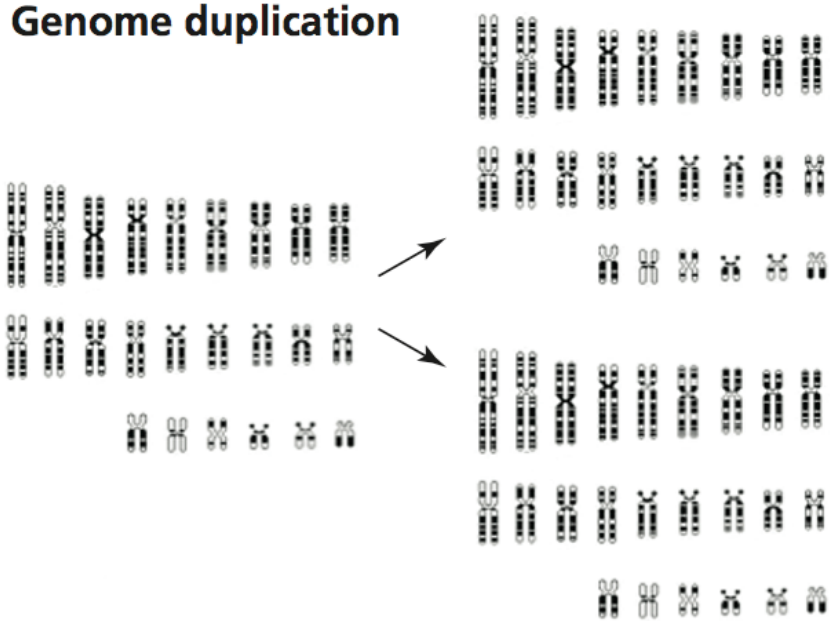
Inversion



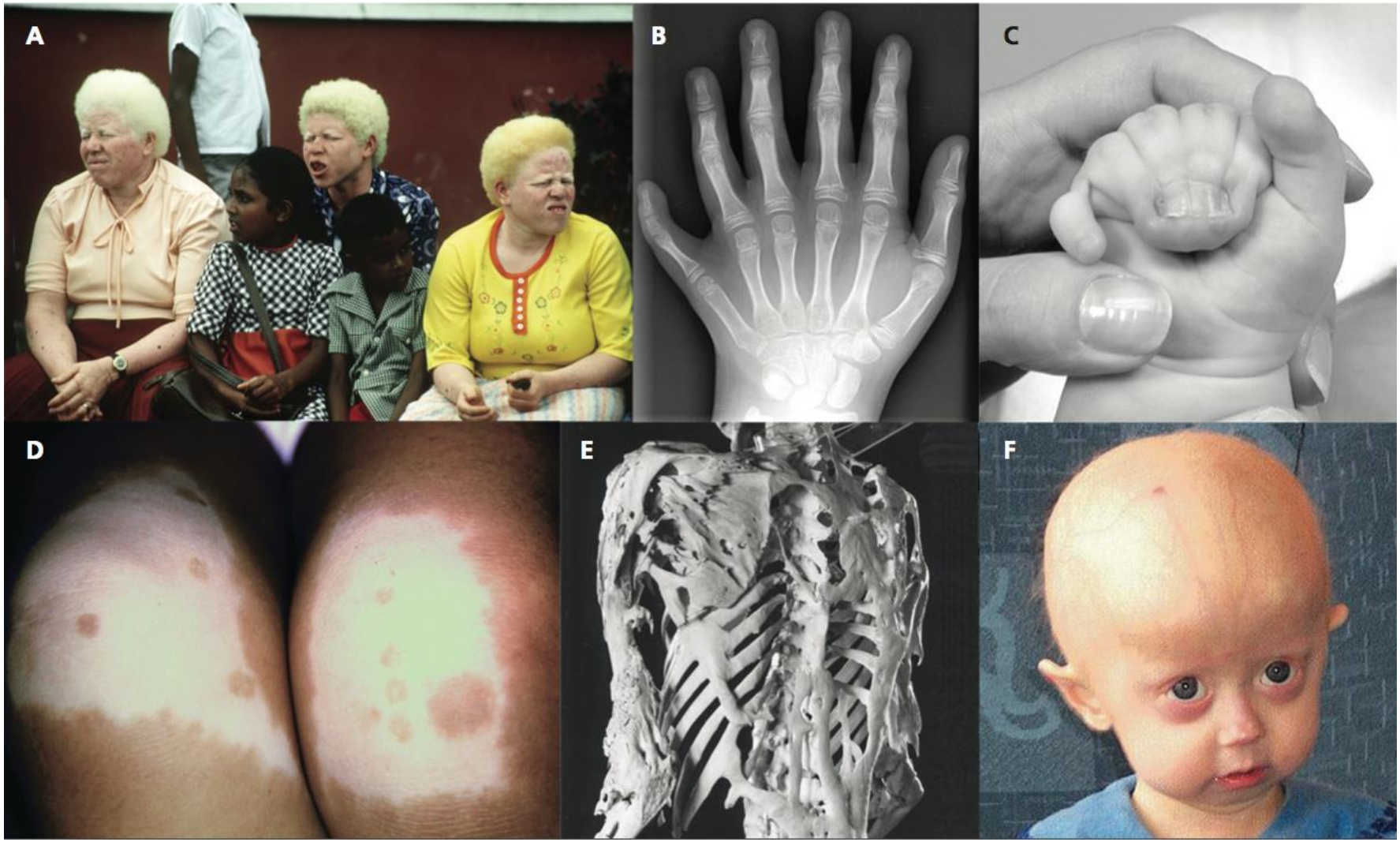
Chromosome fusion



Genome duplication

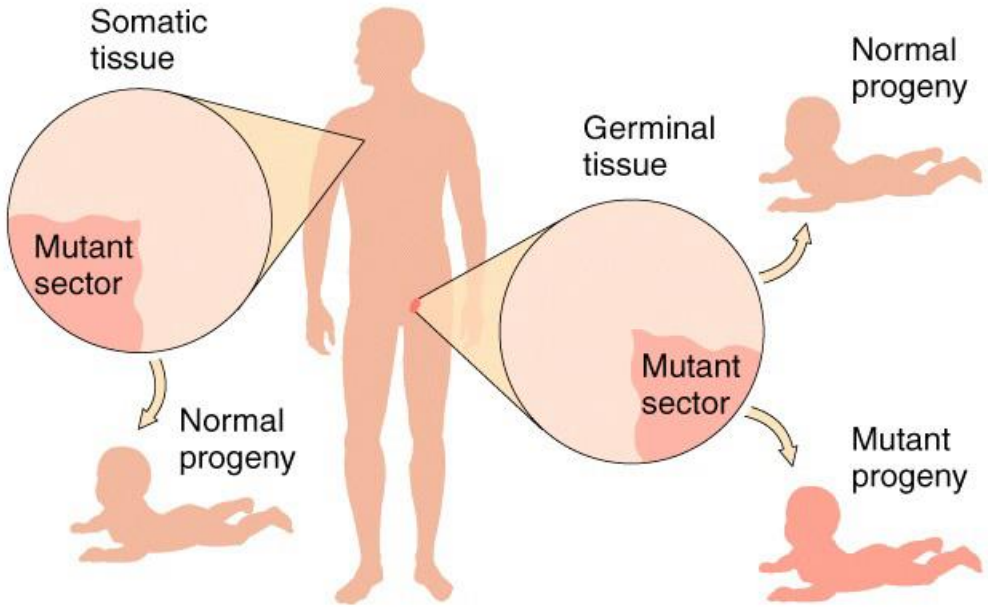


Point mutations can affect phenotype

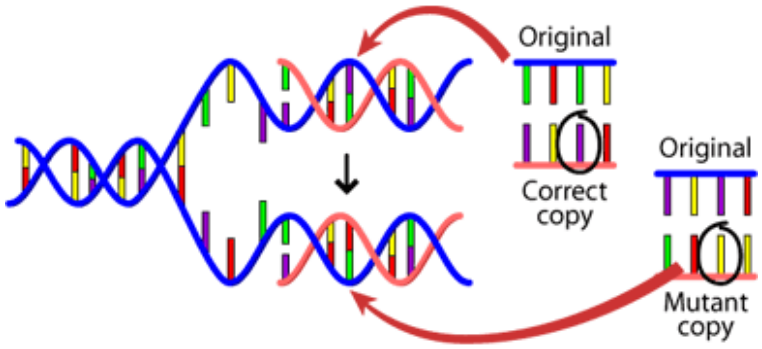


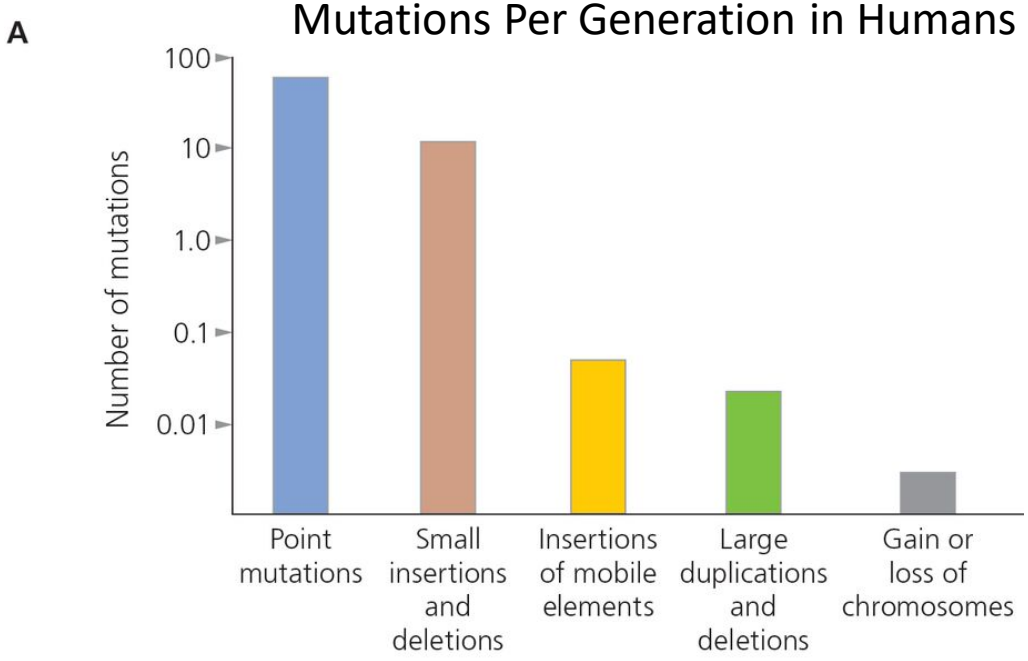
Germ line mutations are heritable

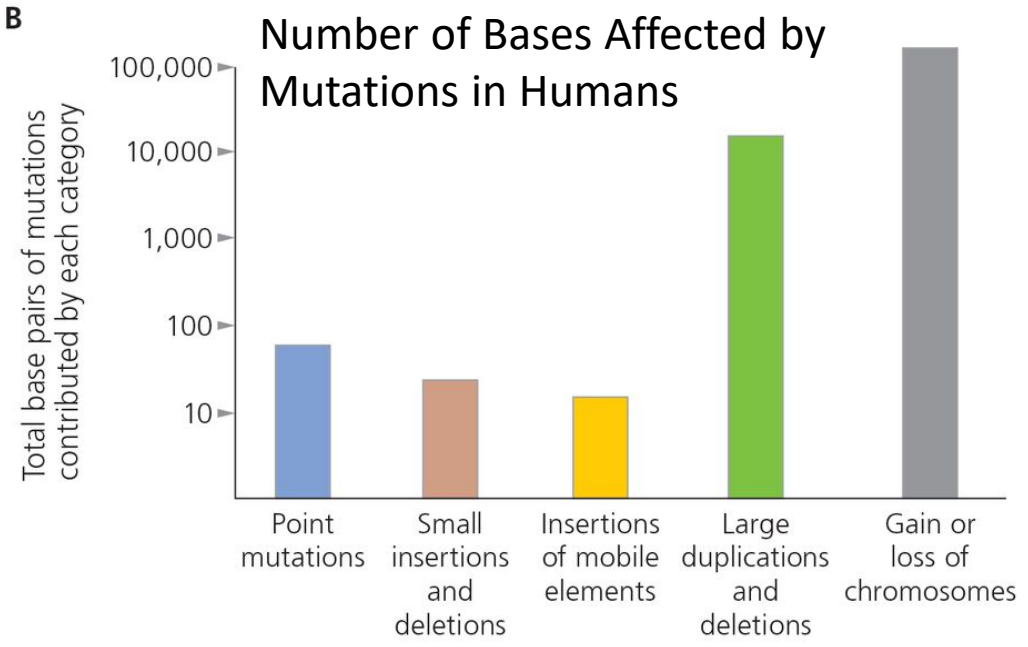
- **Somatic mutations:** affect cells in the body of an organism; not heritable
- **Germ-line mutations:** affect gametes; heritable and relevant to evolution



Mutations arise at different rates





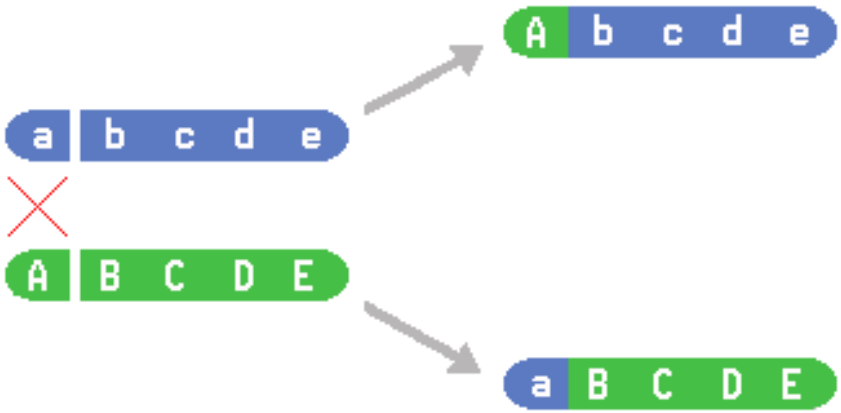


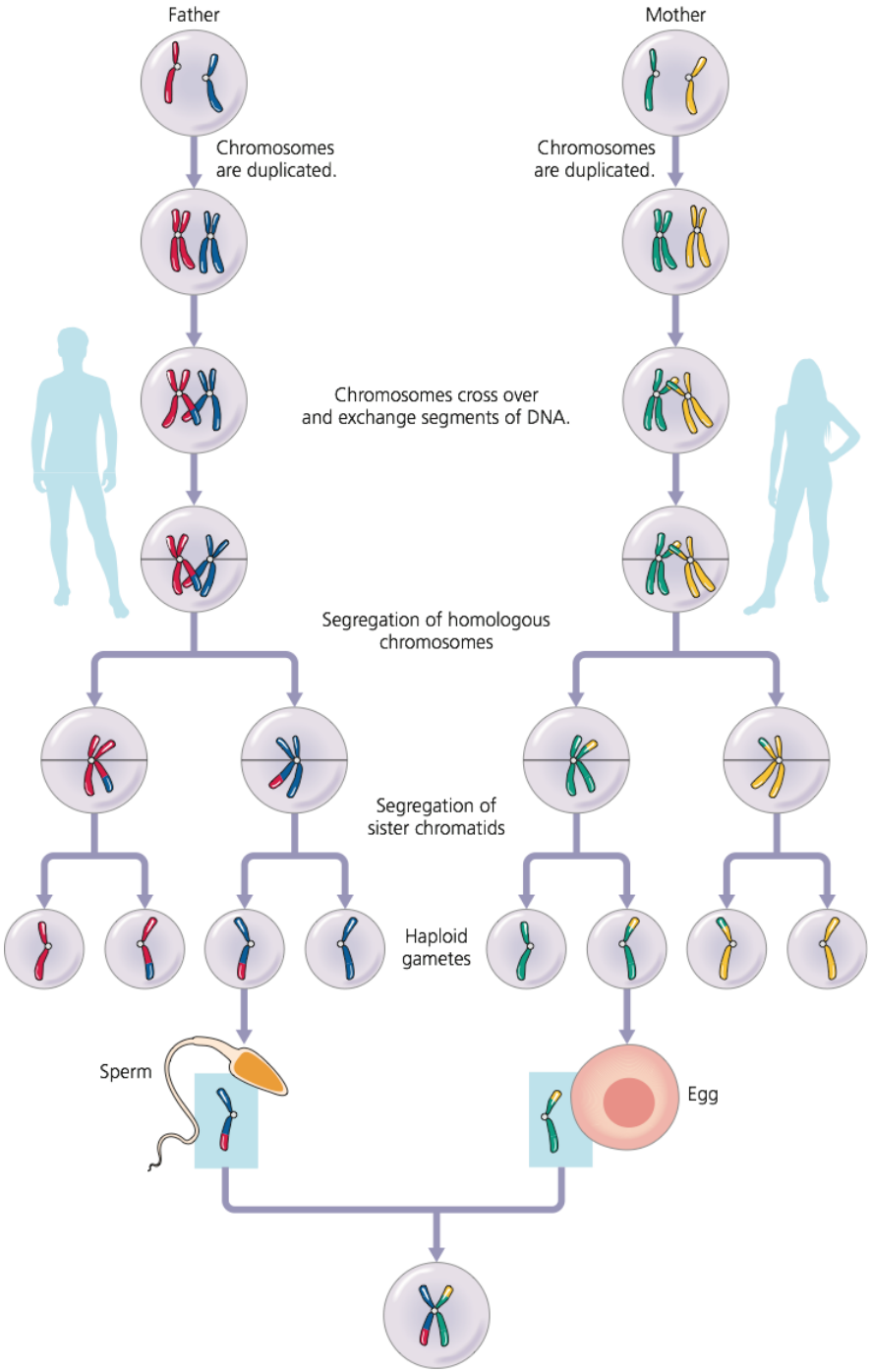
HEREDITY

Key Concepts

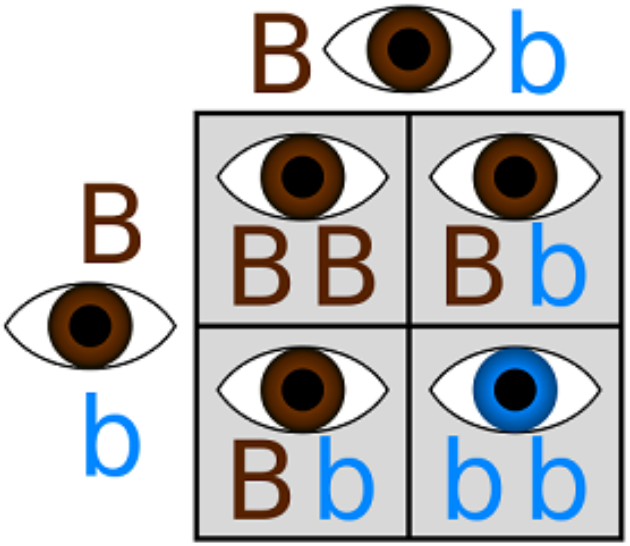
- Meiosis generates considerable genetic variation
  - Recombination
  - Independent assortment
- Fusion of egg and sperm results in great genetic diversity among offspring

Recombination causes novel combinations of alleles among loci

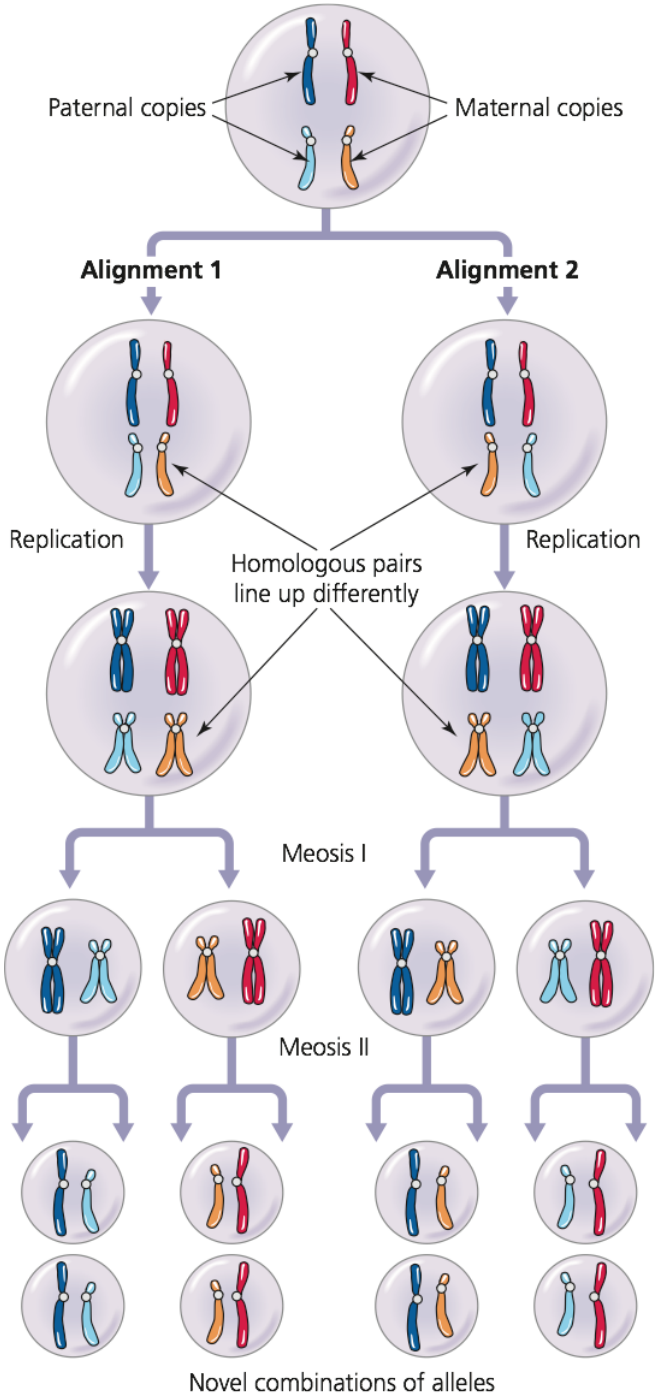




Independent assortment ensures novel combinations of alleles among loci



In humans, there are 2^{23} different possible combinations of chromosomes in the offspring of two parents

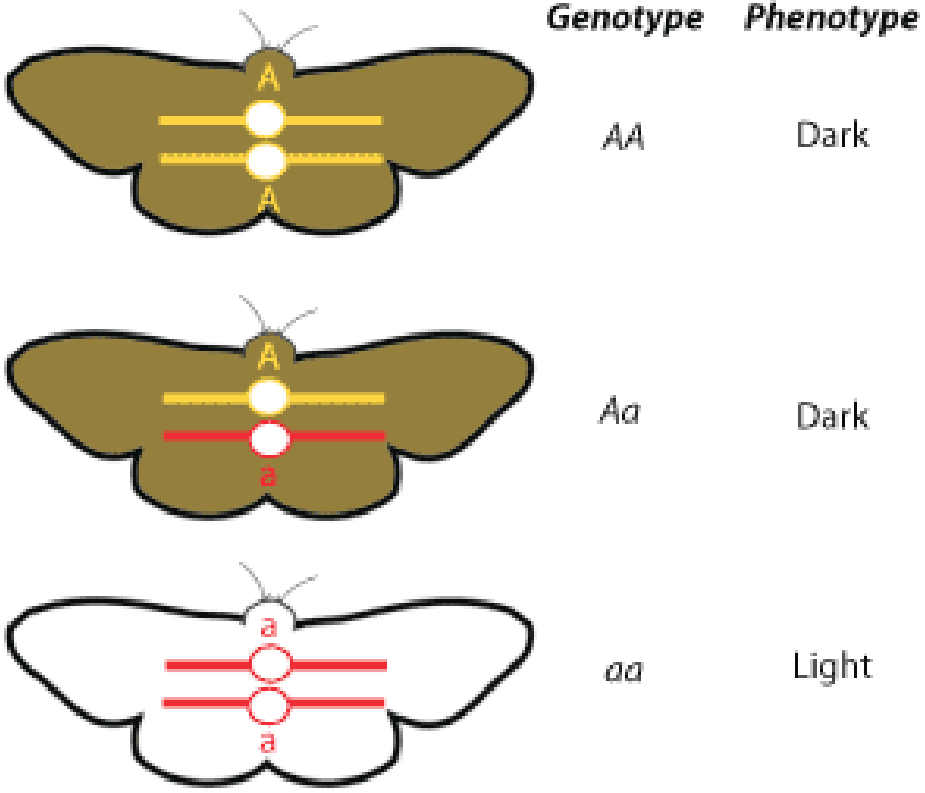


Learning Objectives

- Describe the structure of proteins
- Describe the organization of DNA and its connection to heritable traits, evolution
  - Coding vs noncoding
  - Somatic vs germline mutations
  - Independent assortment & genetic recombination
- Describe mechanisms of gene expression
  - Role of environment
- Discuss complex relationship between phenotype and genotype

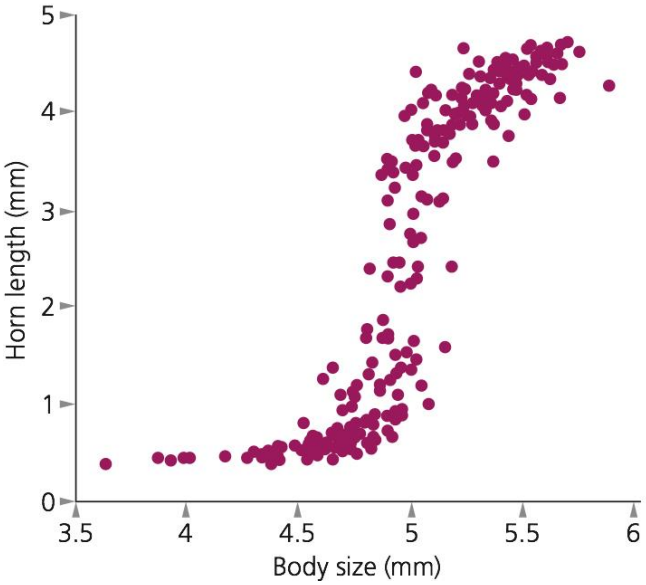
Linking genotype and phenotype

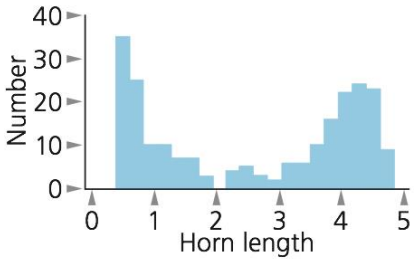
- **Genotype**: the genetic make-up of an individual
- **Phenotype**: an observable measurable characteristic of an organism

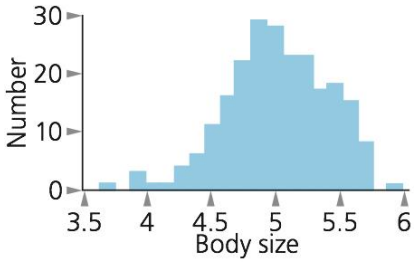


Sometimes a single genotype can produce multiple phenotypes

Polyphenic trait: single genotype produces multiple phenotypes depending on environment



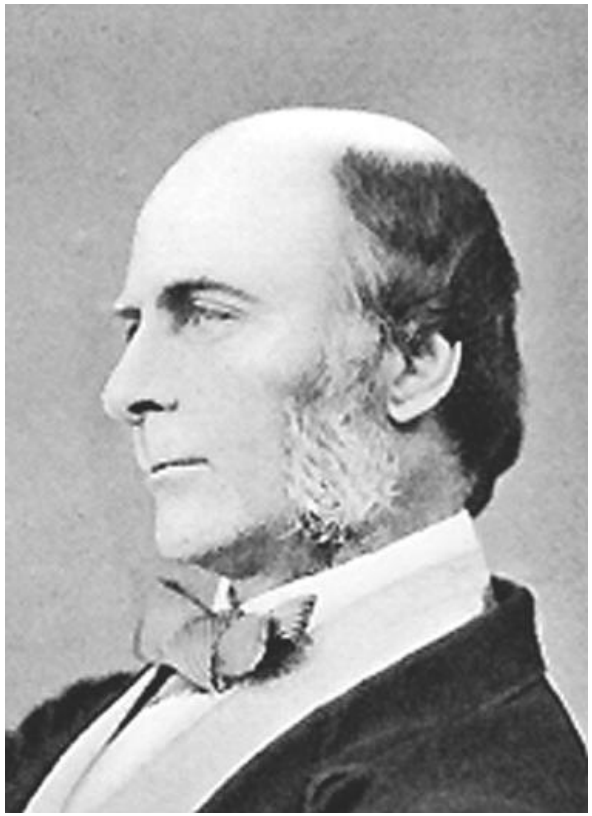




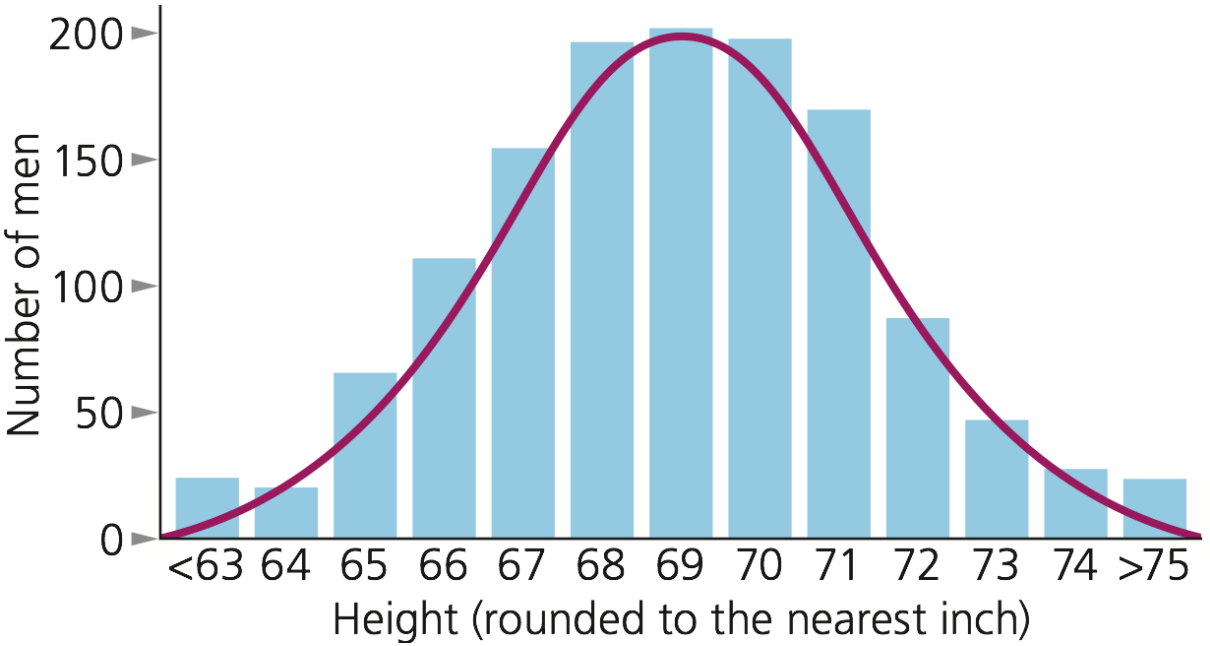
Phenotypic Plasticity: phenotypic changes in response to a unique environment

Horn length affected by nutrition in beetles

Quantitative traits influenced by genes and the environment

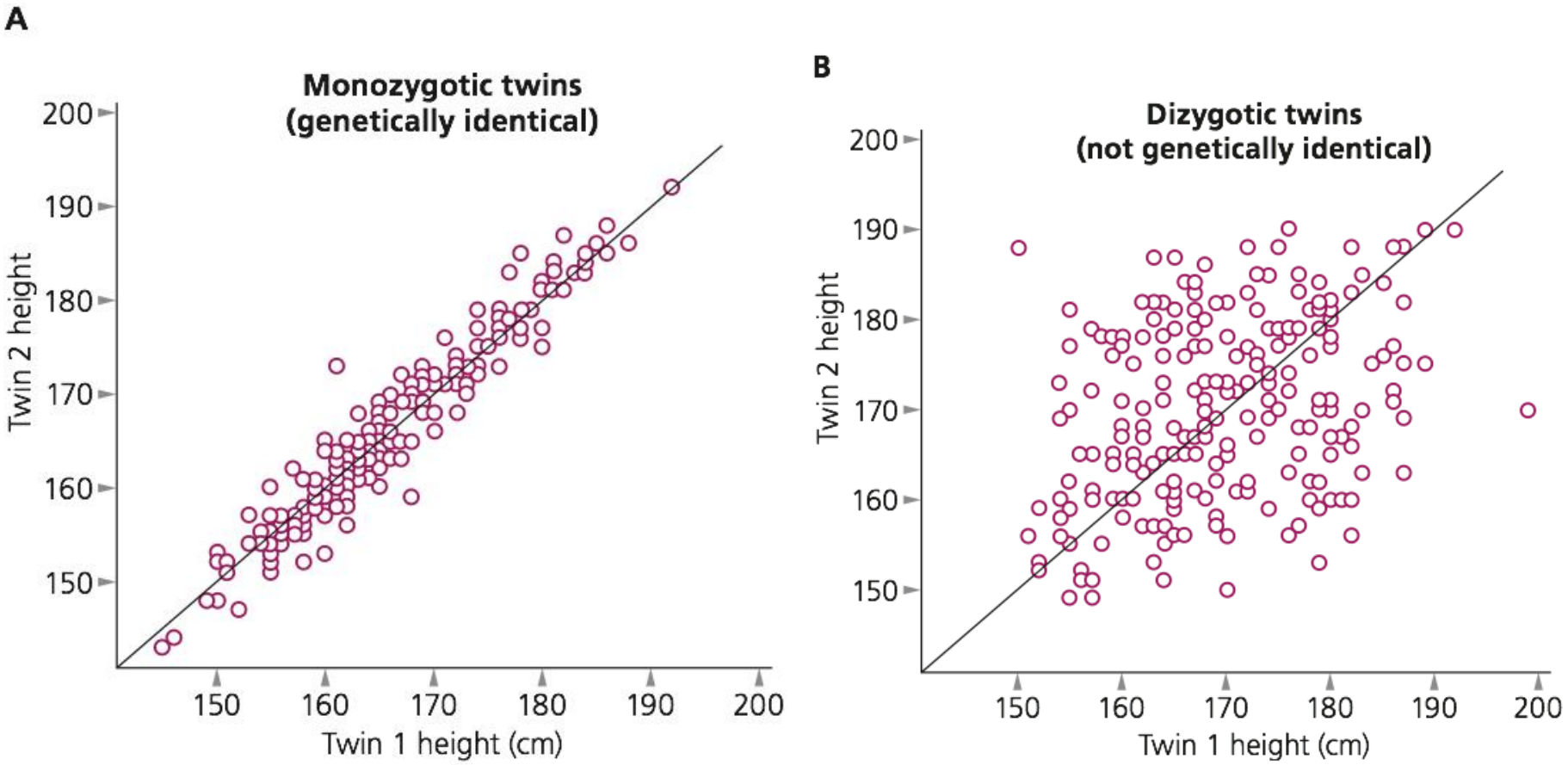


Francis Galton (1822-1911)



Quantitative traits influenced by multiple genes; generate a normal distribution

Human height has genetic component



Human height also has environmental component (nutrition)

- Children of more affluent families are taller
  - Guatemalan Ladino (tall & rich) and Maya (short & poor)
  - American Maya 10cm taller

So What Does All of this Have to do With Evolution?

- Heritability is a key component of evolutionary theory
- A correlation between genotype and phenotype is required for evolution to be a viable theory
  - Natural selection can drive phenotypic changes but the phenotypes are not directly heritable
  - Natural selection can drive genotypic changes

- While Darwin had no specific knowledge of the laws of heritability, his theory of evolution via natural selection is consistent with these laws

DNA Preservation & Stabilization

John Whalen

Lecture credit: Dr. Christopher E. Bird

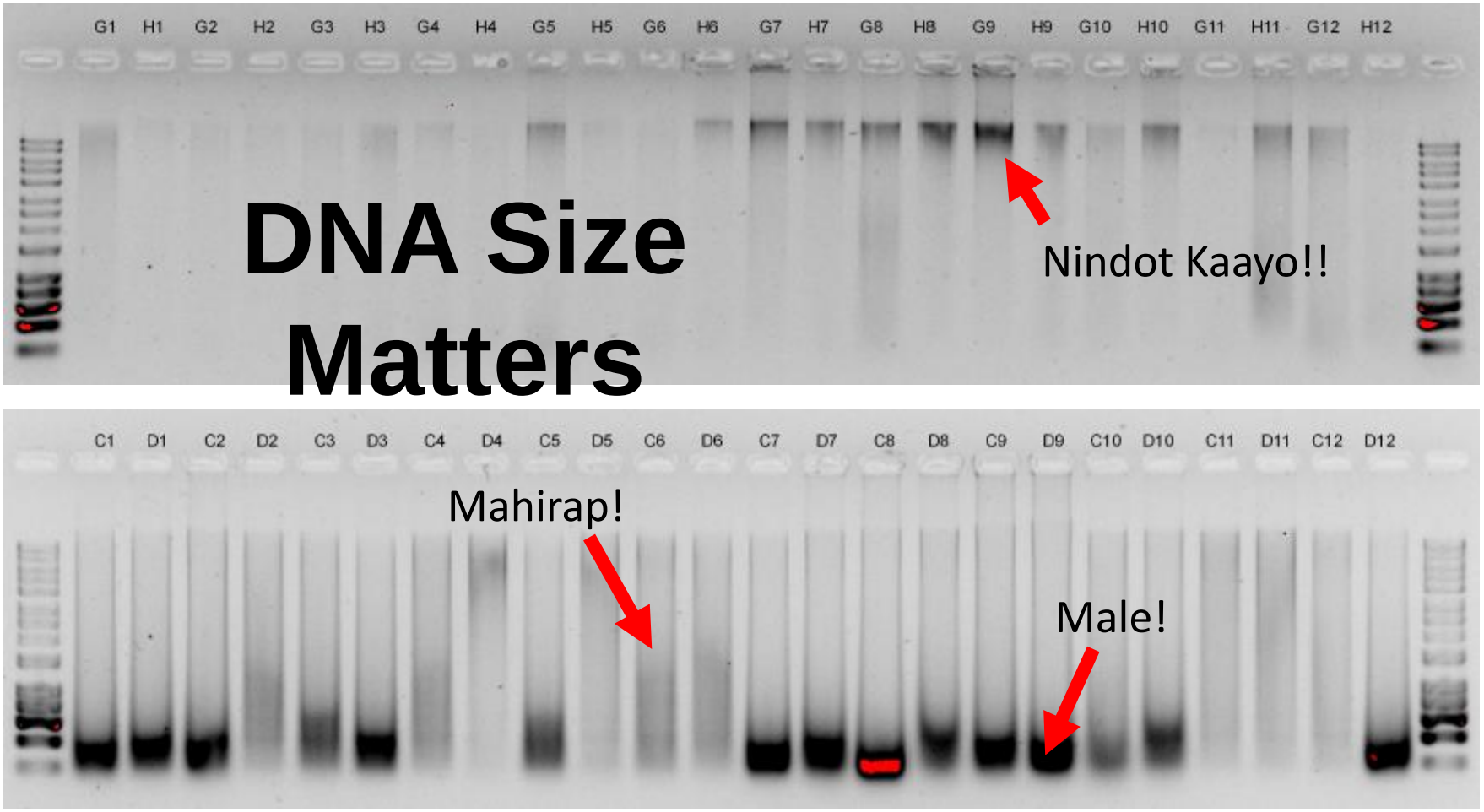
GenomicsCoreLab
Texas A&M University - Corpus Christi

Sample Collection & Preservation Should Be Planned

- Minimize time between collection/tissue death & preservation
- Test effectiveness of different preservation methods

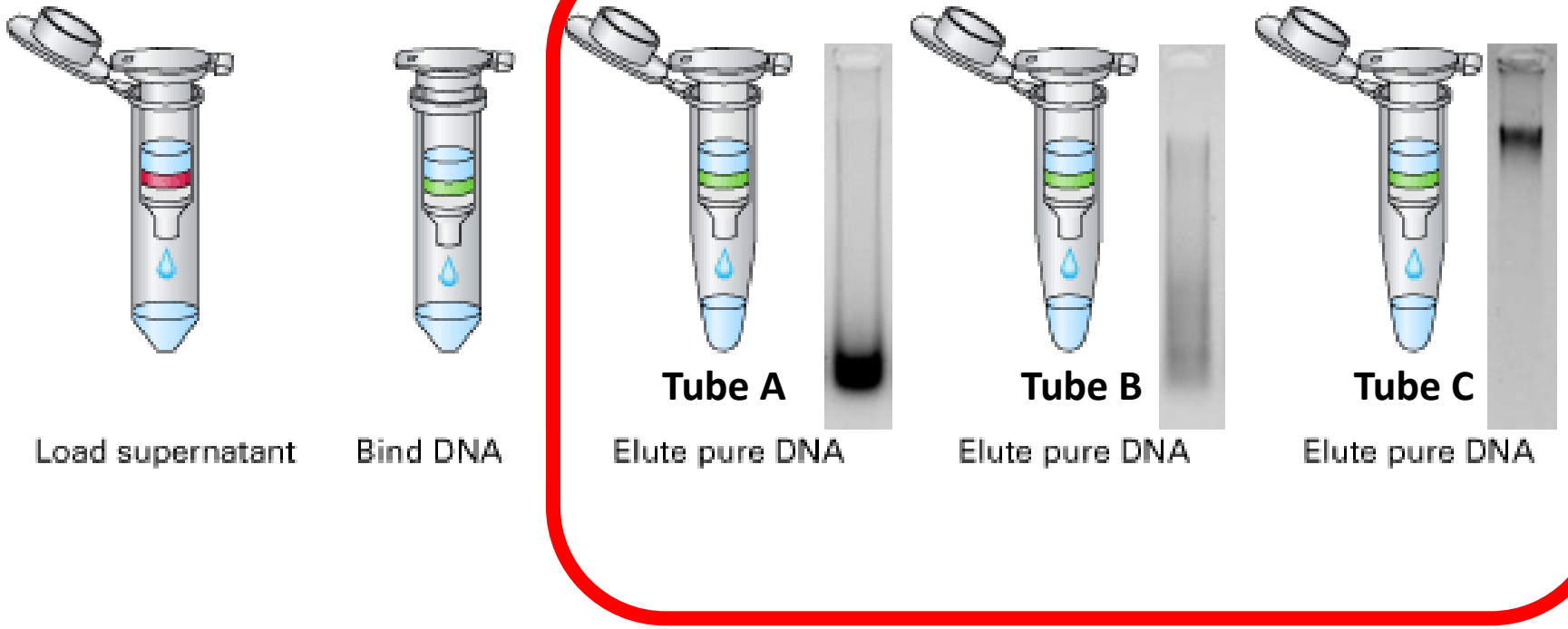
Collection Methods For DNA or RNA analysis

- Fish:
  - Electrofishing
  - Nets: Seine, dip, gill
  - Rotenone
  - Trawling
  - Line fishing
  - Blast fishing
  - Market collections


G1 H1 G2 H2 G3 H3 G4 H4 G5 H5 G6 H6 G7 H7 G8 H8 G9 H9 G10 H10 G11 H11 G12 H12
DNA Size Matters
Nindot Kaayo!!
C1 D1 C2 D2 C3 D3 C4 D4 C5 D5 C6 D6 C7 D7 C8 D8 C9 D9 C10 D10 C11 D11 C12 D12
Mahirap!
Male!

We Can Enrich for Long Strands of DNA

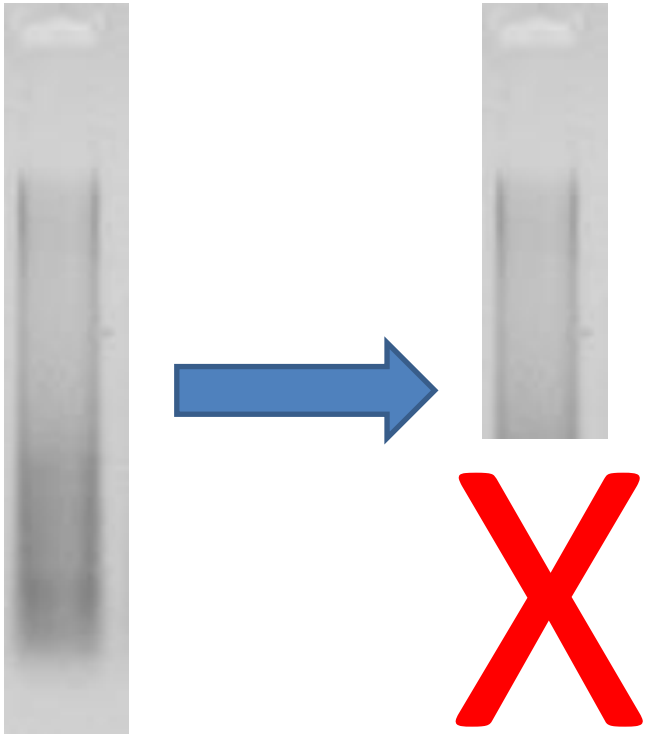
DNA Extraction: Separate Ligations



We Can Enrich for Long Strands of DNA

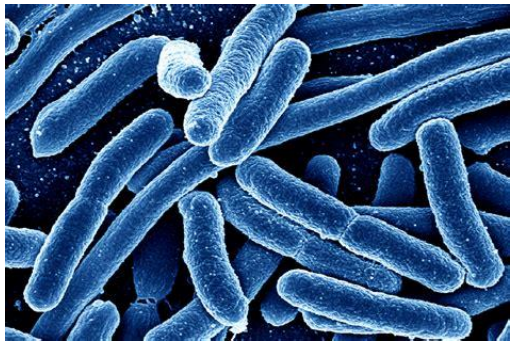
SPRI - Select Beads-based Size Selection

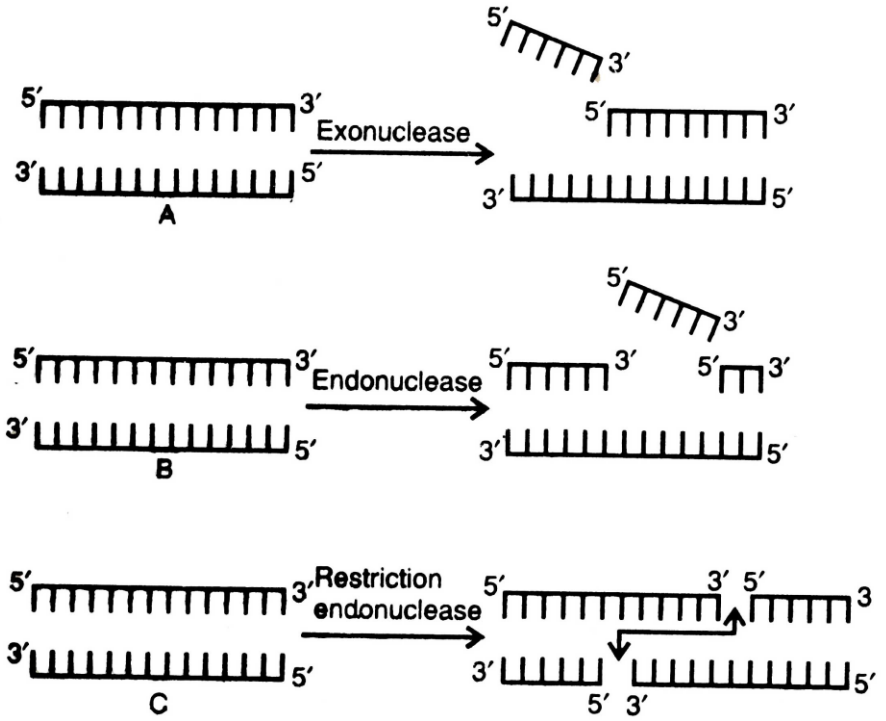
But it would be better if we didn't have to rescue a sample



Causes of DNA Degredation

- Autolysis
  - DNases
    - Exonucleases
    - Endonucleases
- Microbes

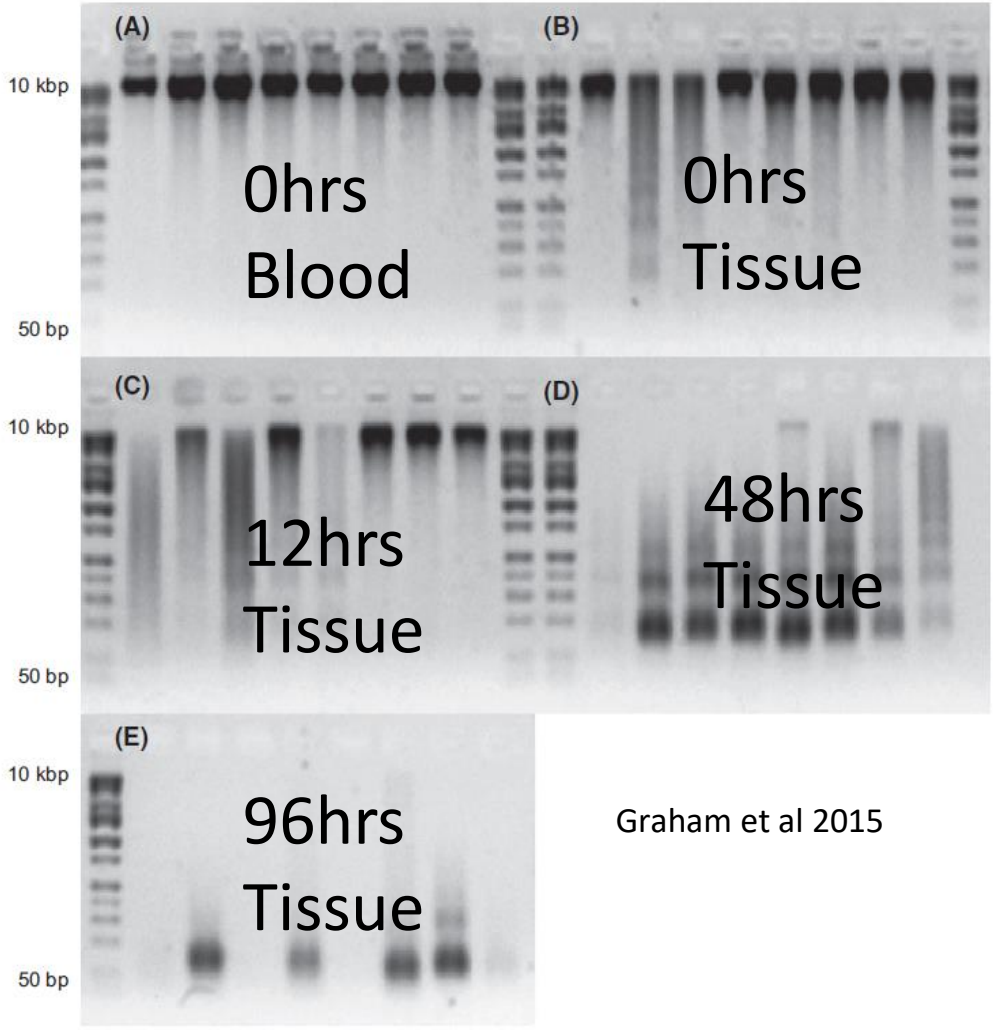




DNA Degrades Following Death

- DNA extracted following euthanasia
- Degradation increases with time

- When in doubt, get tissue into preservative as soon as possible

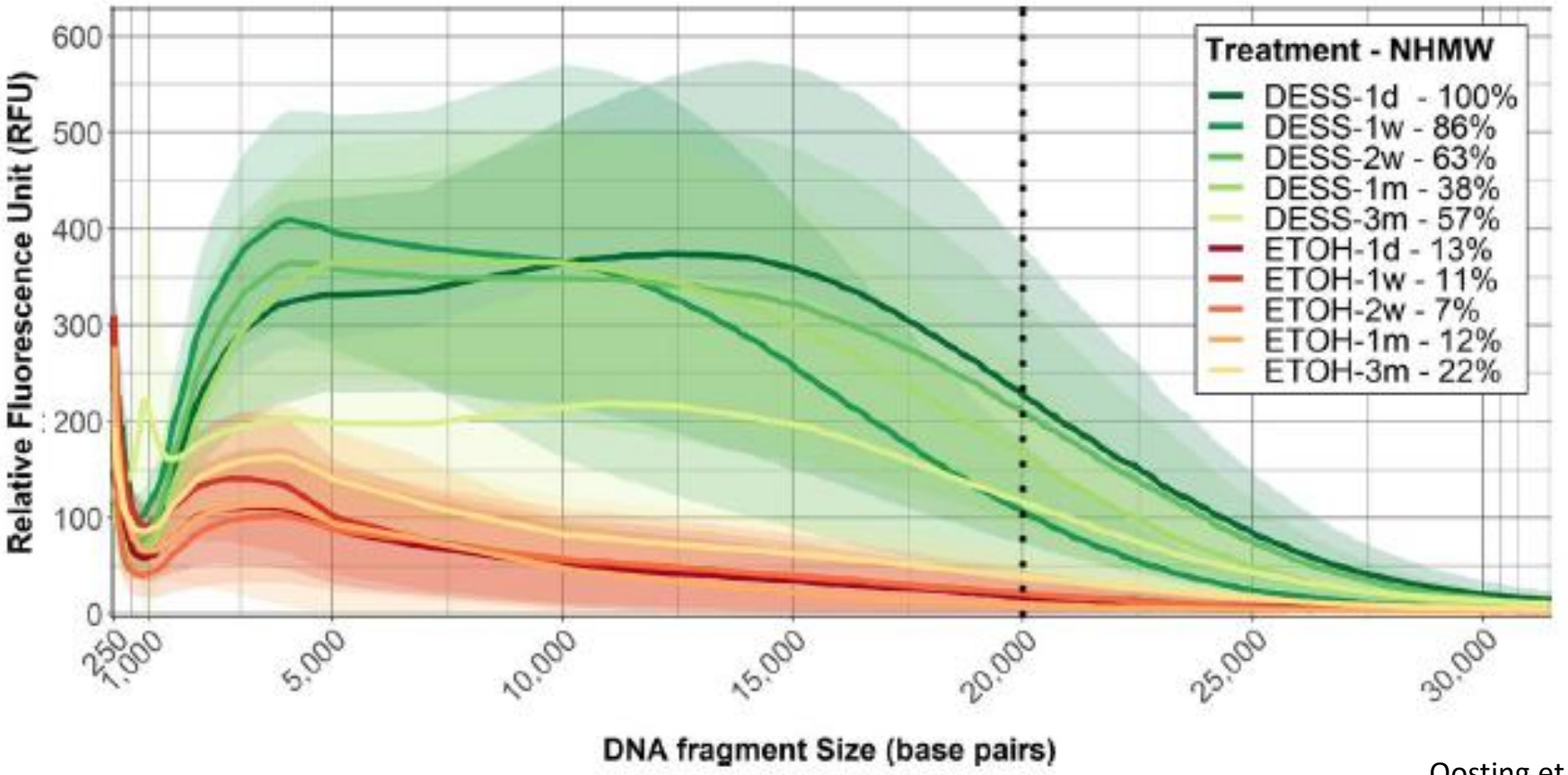


Graham et al 2015

DNA Size Matters

- Preservation solution: DESS > EtOH
- DNA degrades following death

HMW DNA



Oosting et al 2020

Common Preservation Methods for Marine Animals

- Extract Live Tissue
- Liquid Nitrogen
- Ethanol (95% or higher)
- DIY RNA Later
- DIY Salt-saturated DMSO
- Commercial products
- Guanidine

Pros & Cons

- Field Extraction of Live Tissue
  - Pros:
    - It works
  - Cons:
    - Cumbersome equipment transport
    - Silica membrane clogs with vacuum
    - Generally inconvenient to collect and extract in same day
- Equipment List
  - Thermomixer
  - Vacuum pump & manifold
  - Pipettors
- Supplies & Reagents
  - Tips
  - Gloves
  - Extraction kit

Pros & Cons

- DIY RNA Later
  - Pros:
    - Works with everything
    - Very inexpensive
    - Components are common
    - Not flammable
  - Cons:
    - Must be frozen (-20C) after 24hrs
    - It takes a while to make
    - Tissue gets soft
- Liquid Nitrogen
  - Pros:
    - Doesn’t introduce chemicals
    - Works for everything
  - Cons:
    - Bulky dewars
    - Expensive
    - Samples very sensitive to temperature changes
    - Accidental exposure
    - Looks like a bomb

Pros & Cons

- Commercial Preservatives
  - Pros:
    - They work
    - Not flammable
  - Cons:
    - Expensive
- 4M GuHCl / Qiagen Buffer AL
  - Pros:
    - Works with everything
    - Not flammable
    - Denatures proteins
  - Cons:
    - Dissolves tissue
    - I’m not sure how long samples can be preserved, I figured out that this works by mistake

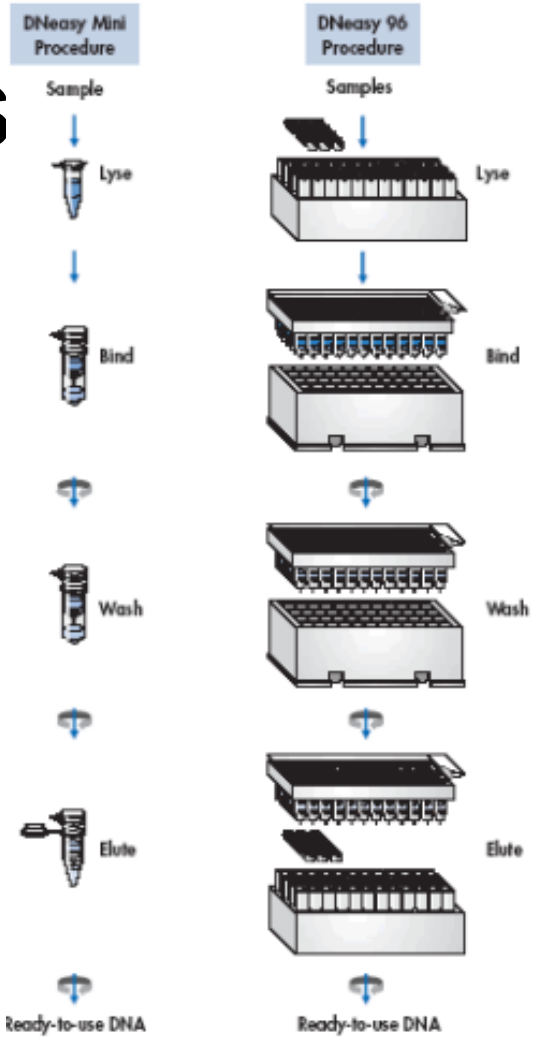
Pros & Cons

- Ethanol
  - Pros:
    - Good for fish
    - Common
    - Not too expensive
  - Cons:
    - Flammable
    - Not great for most inverts

- DESS (DMSO, EDTA, saturated salt)
  - Pros:
    - Works with everything
    - Inexpensive
    - Components are common
    - Not flammable
  - Cons:
    - Takes time to make
    - Tissue turns to mush

Extraction Tips

- Clean workstation/tools with bleach, DNase, DI
  - Ethanol kills microbes but preserves DNA
- Rinse tissue before digestion
- Use Proteinase K and RNase A for digestion
- Use a thermomixer or equivalent to incubate tissue digest
- Minimize digestion time
  - Incubate at temp, >1000rpm, until tissue is dissolved
- Separate Elutions
- Save and freeze columns



Recipes Available Online

Salt-Saturated DMSO Buffer

- 1L 0.5M EDTA:
  - 900 ml nanopure H20
  - EDTA (FW 292.24) 146.12 g
  - NaOH pellets to pH EDTA to 8.0
  - Bring final volume to 1L
- Then for the salt saturated DMSO:
  - 0.5M disodium EDTA 1L
  - Dimethyl Sulfoxide (DMSO) 400mL
  - Nanopure water 500mL
  - NaCl – Enough to saturate the solution.

RNA-Later-Like Buffer

- For 1.5 liters:
  - 935 ml of autoclaved, MilliQ water
  - 700 g Ammonium sulfate
  - Stir until dissolved
  - Add 25 ml of 1 M Sodium Citrate
  - And 40 ml of 0.5 M EDTA
  - Adjust to pH 5.2 using concentrated H2SO4 (about 20 drops= 1 ml)
  - Store at RT

Happy Fil-Am Friendship Day!