20.109 Spring 2015 Module 2 – Lecture 5
System Engineering and Protein Foundations

   

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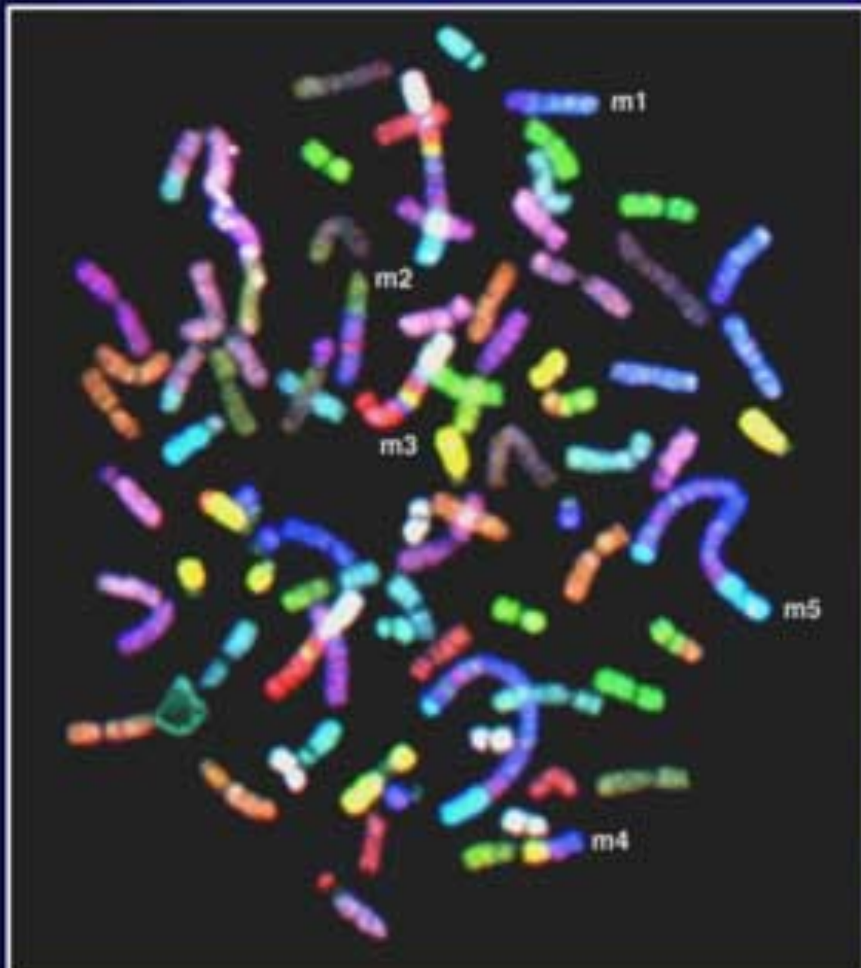
Leona Samson (Lectures)

Zachary Nagel (help with development) Alex Chaim

Large Deletions or Insertions

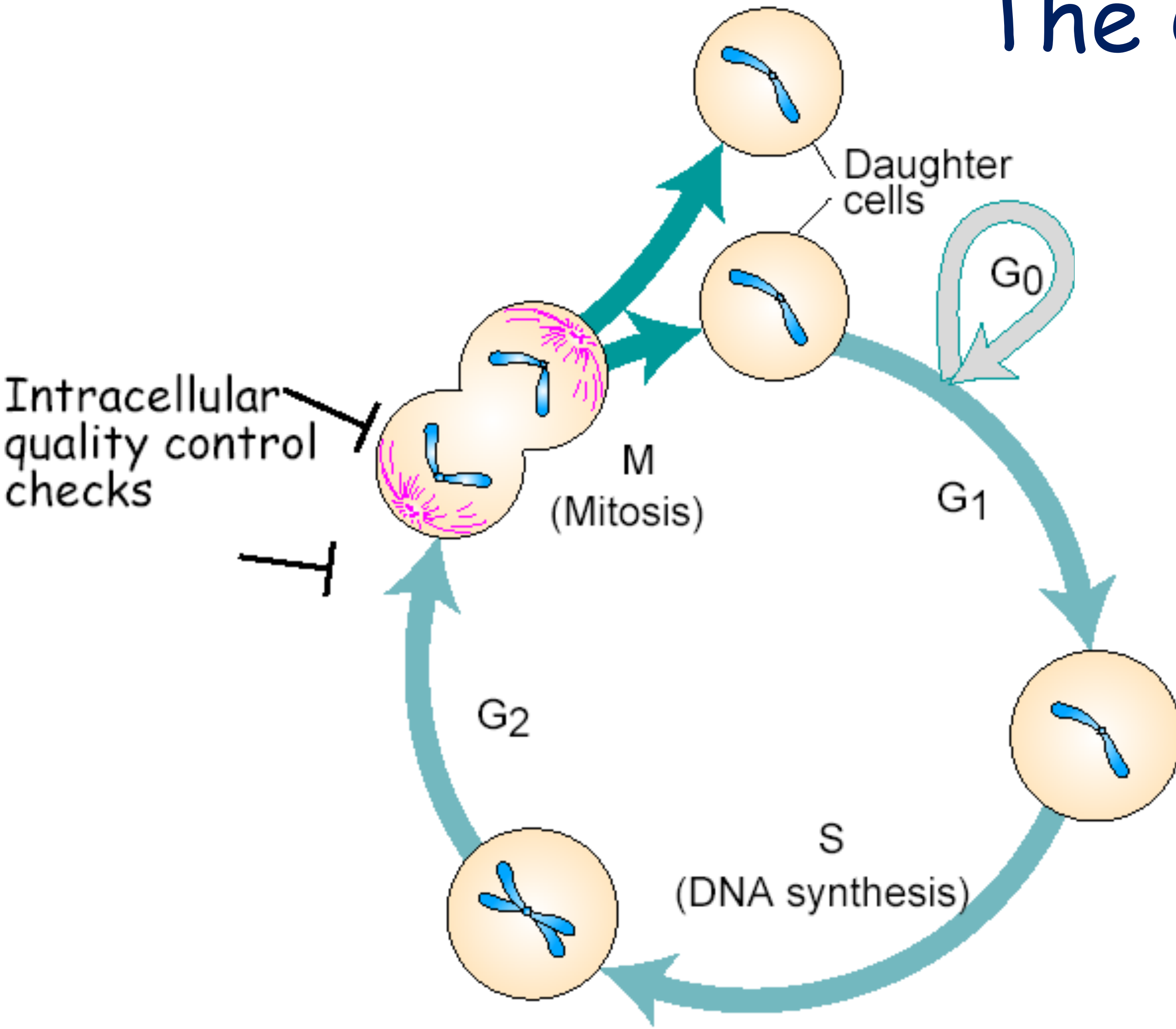
SKY chromosome painting: breast cancer



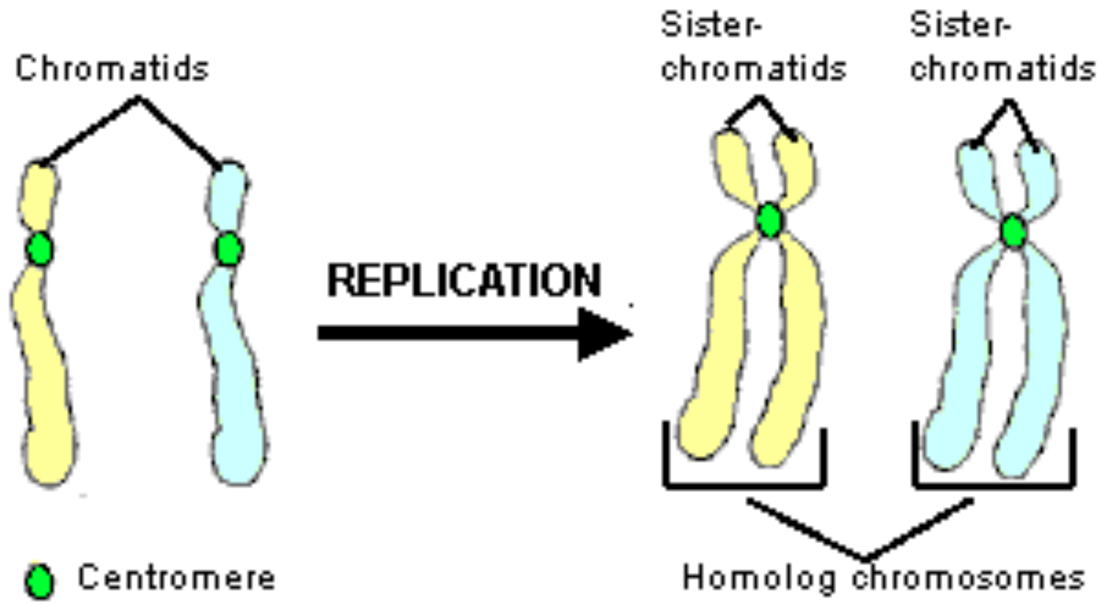
Normal SKY chromosomes are not multicolored.

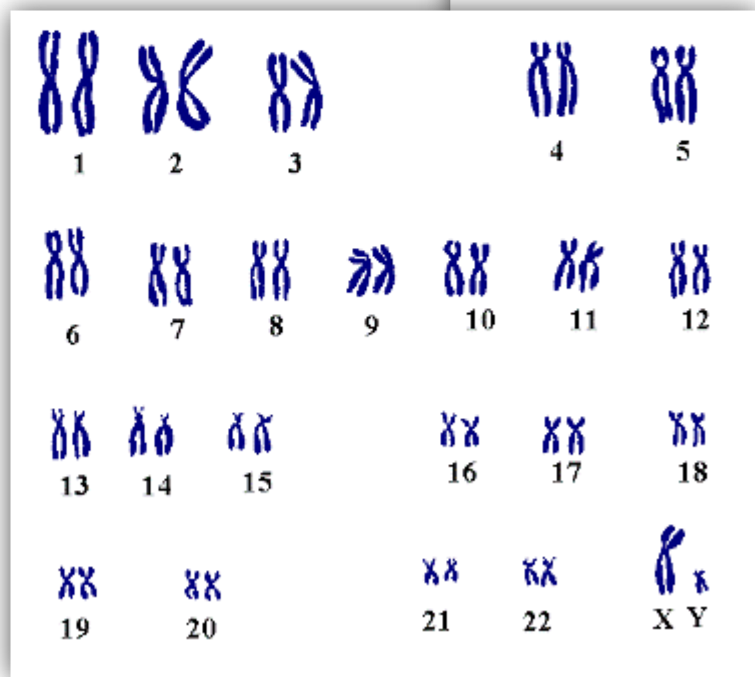
Chromosomes in breast cancer appear multicolored because they have exchanged genetic material.

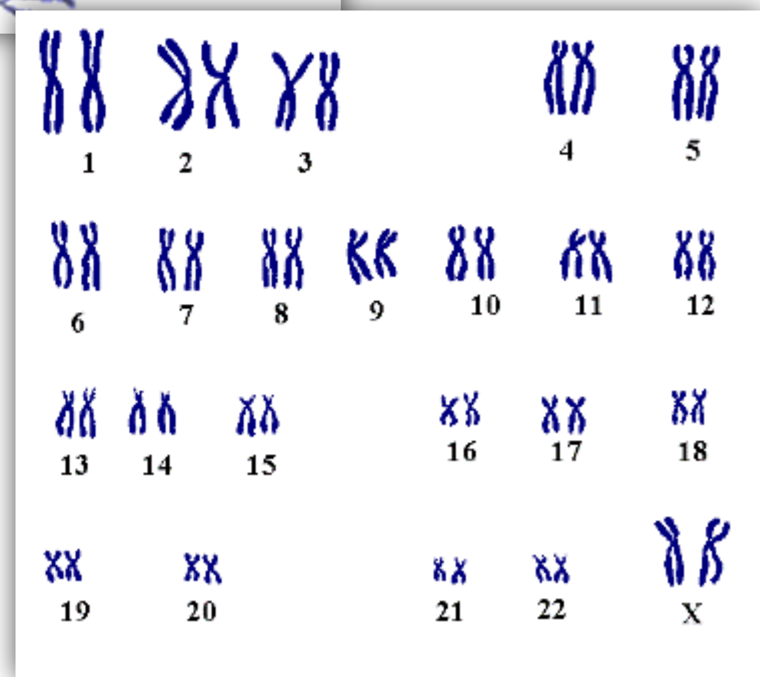
The Cell Cycle



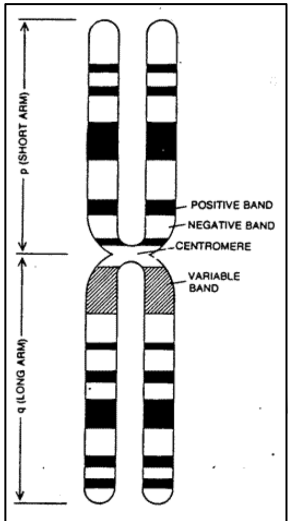
Duplication of chromosomes
DNA Replication


Chromatids
REPLICATION
Sister-
chromatids
Sister-
chromatids
Centromere
Homolog chromosomes

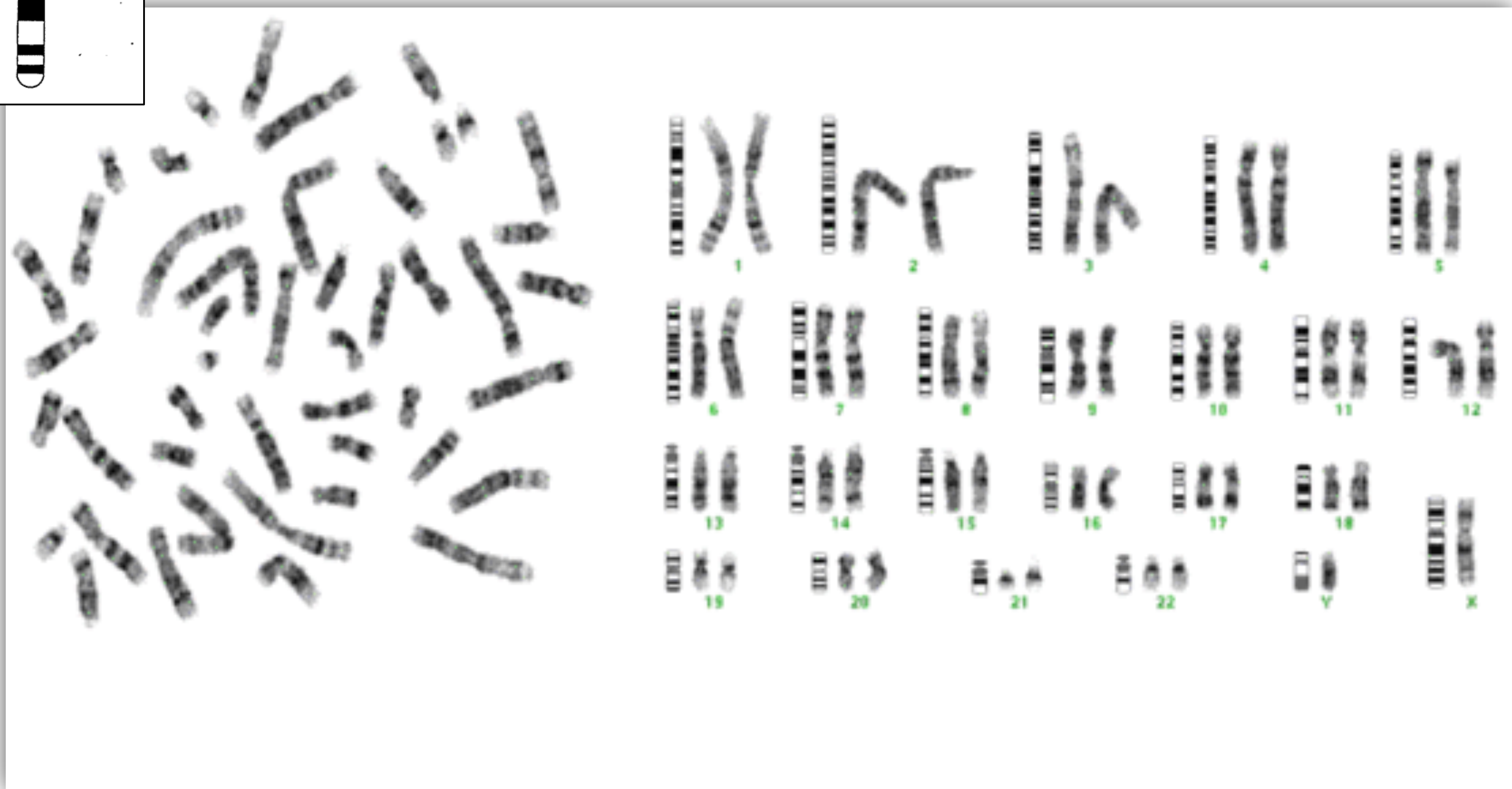




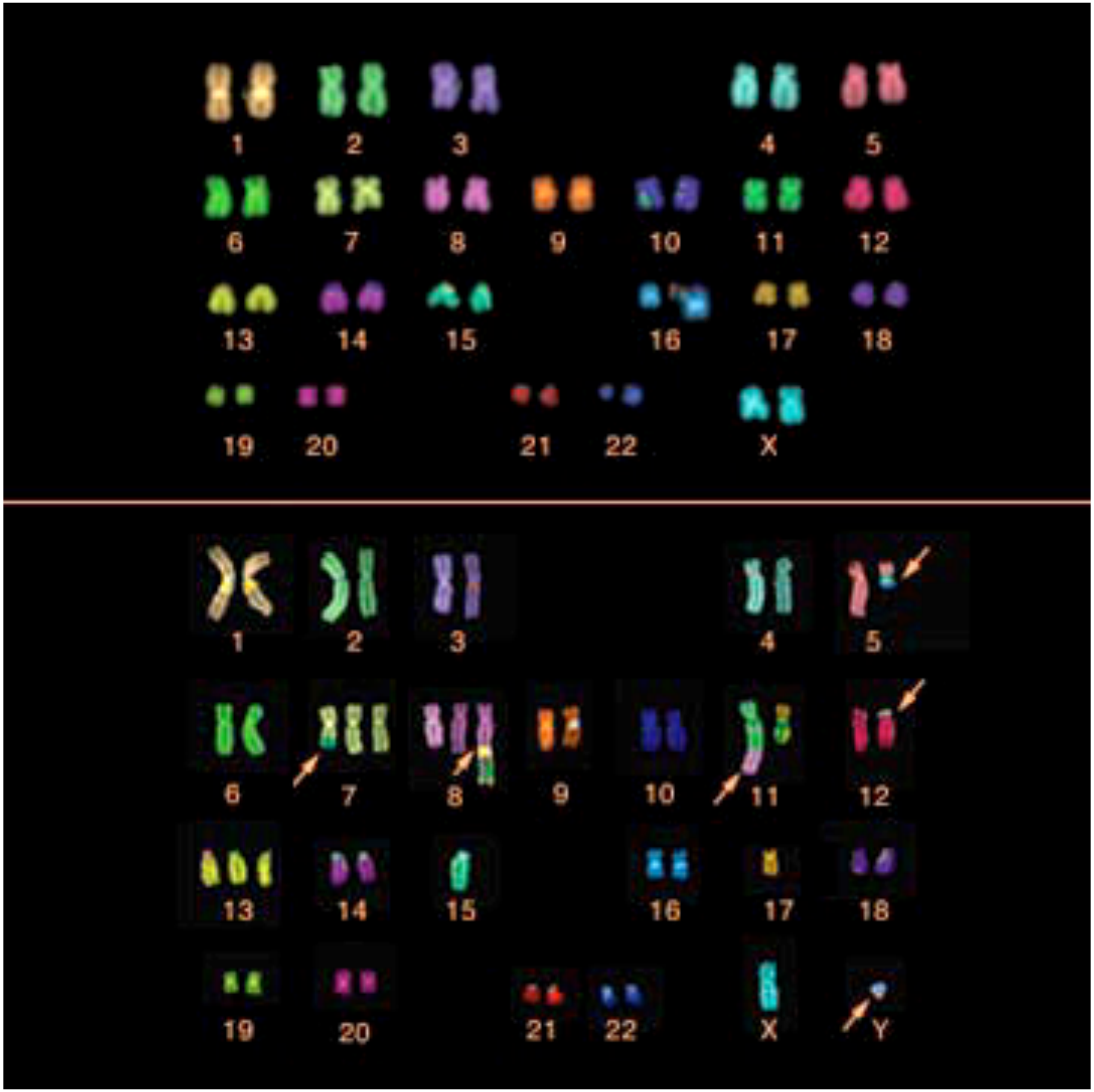
http://www.geocities.ws/genetrix4u/genetics/genetics2.html



Chromosome Banding - Karyotyping



http://geneticslab.wikispaces.com/Chromosomes

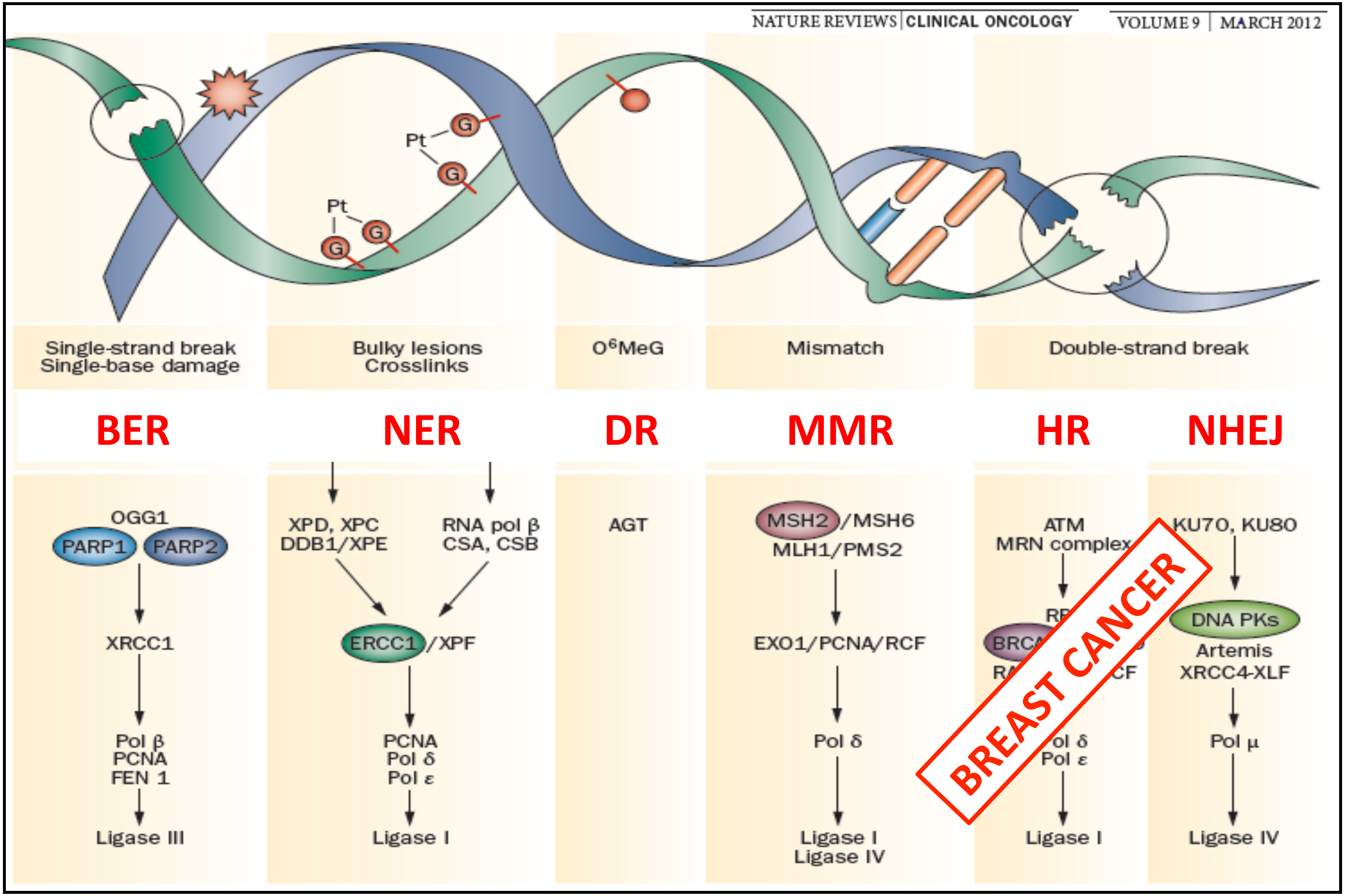


Chromosomes from a Normal cell

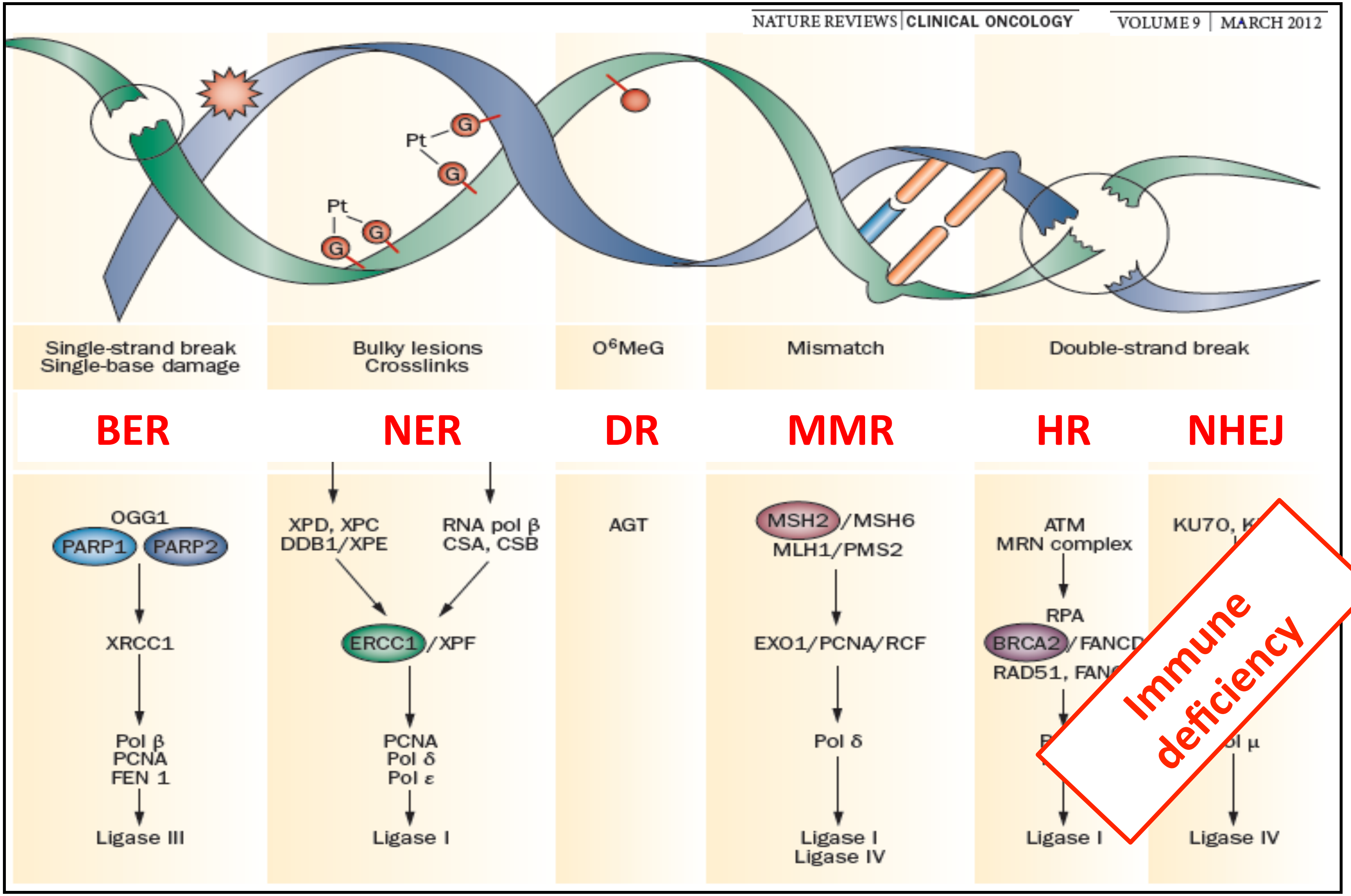
Chromosomes from a Tumor cell

Spectral Karyotyping (SKY)
“SKY Painted Chromosomes”

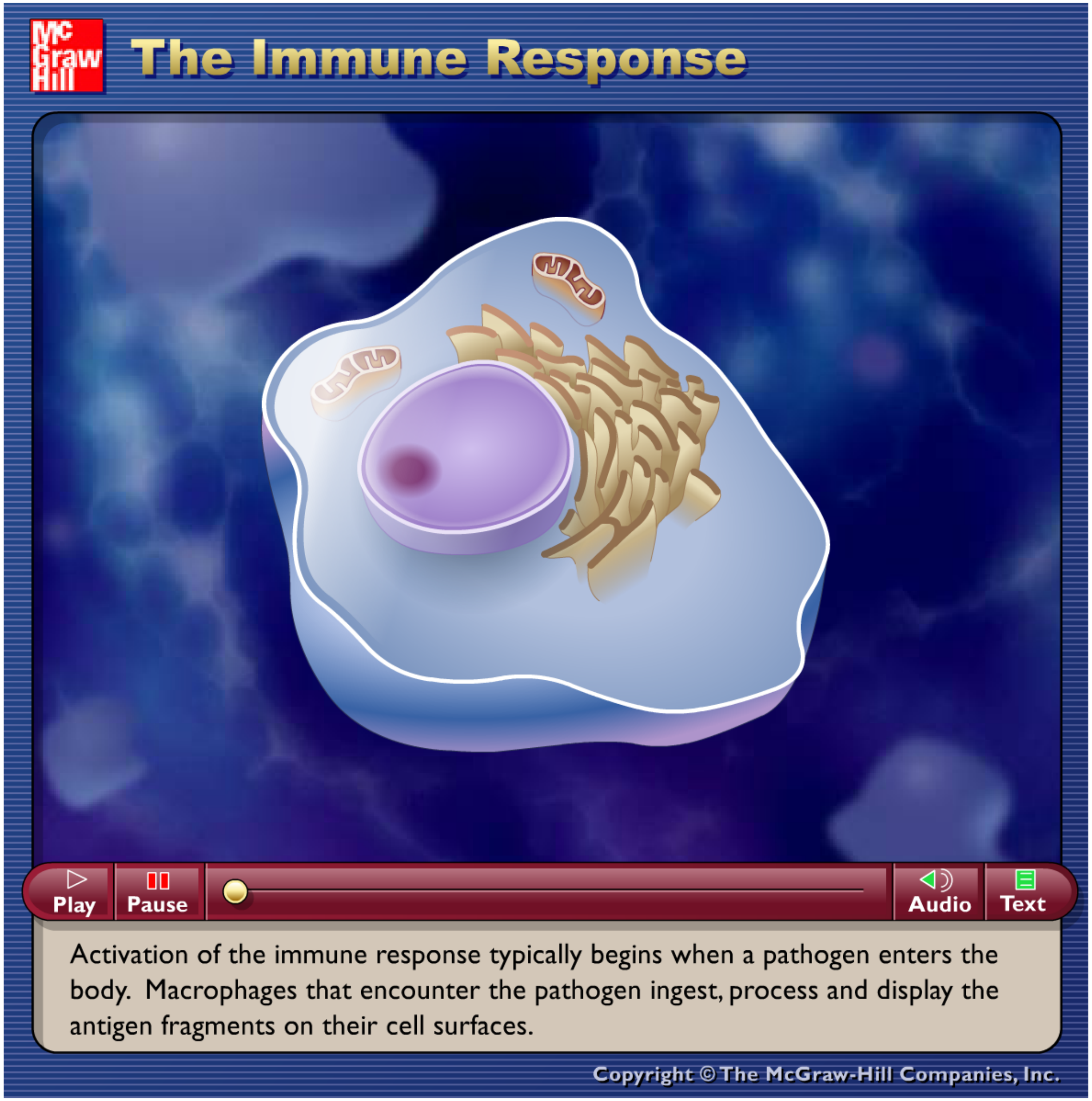
Six Major DNA Repair Pathways



Six Major DNA Repair Pathways

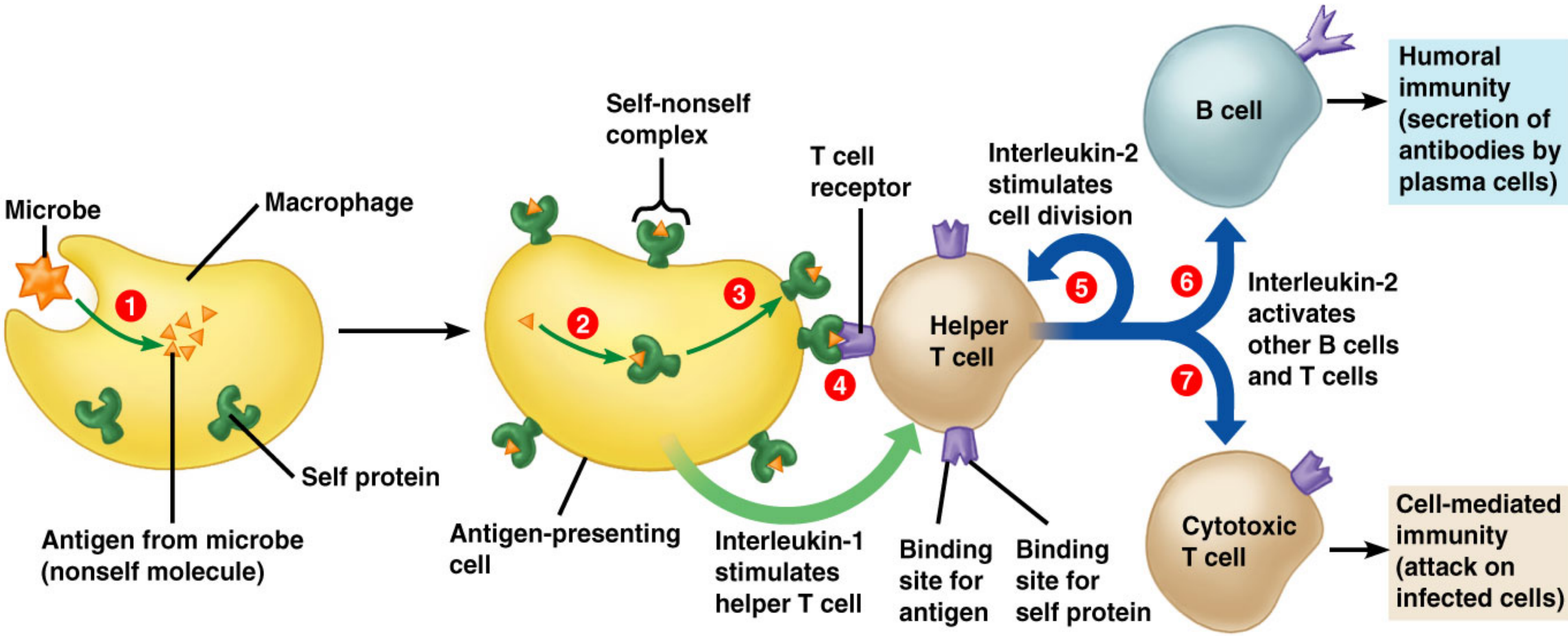


Non Homologous End Joining is REQUIRED for a functional immune system!



http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter24/animation__the_immune_response.html

The body contains millions of different T-cells and B-cells, each able to respond to one specific antigen.



http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

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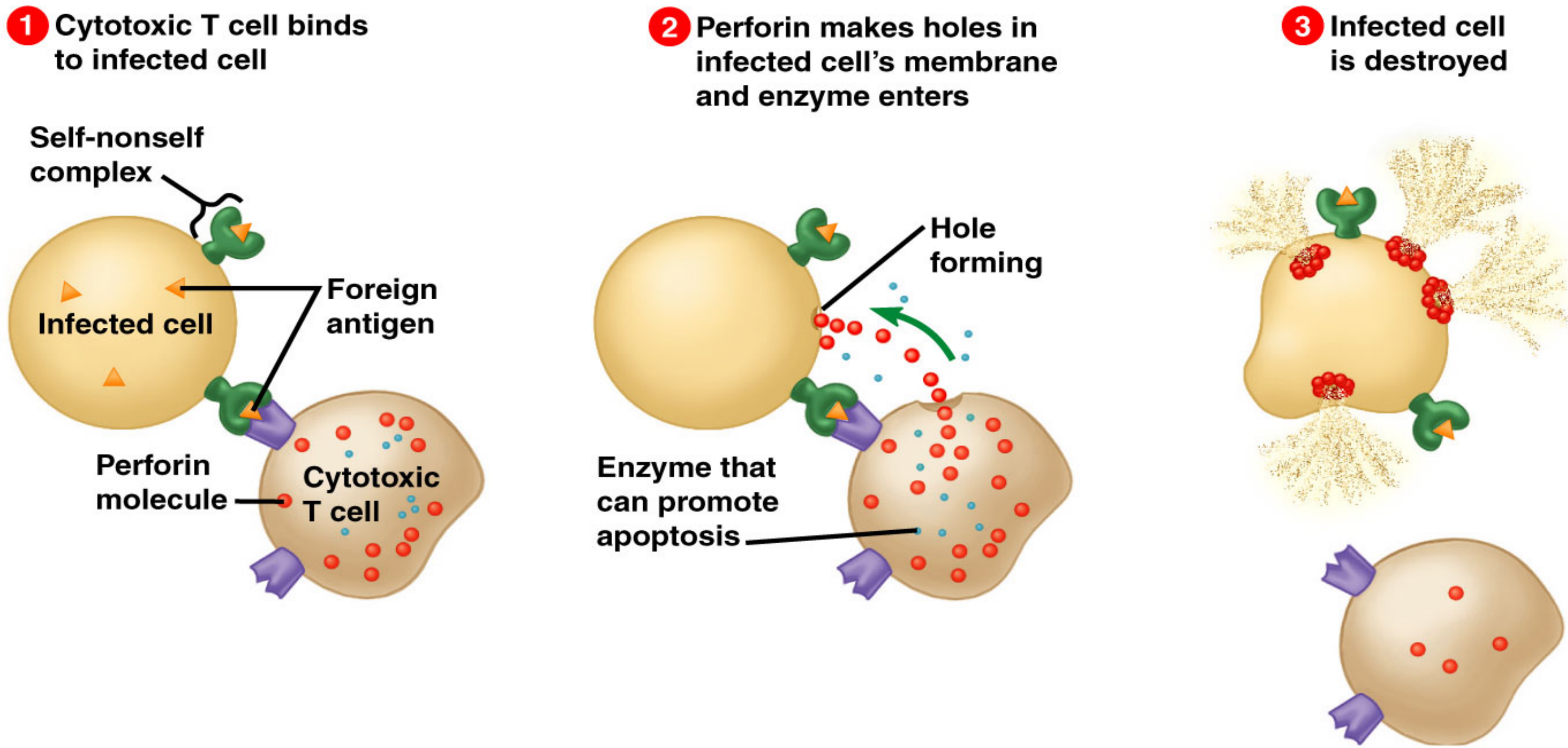
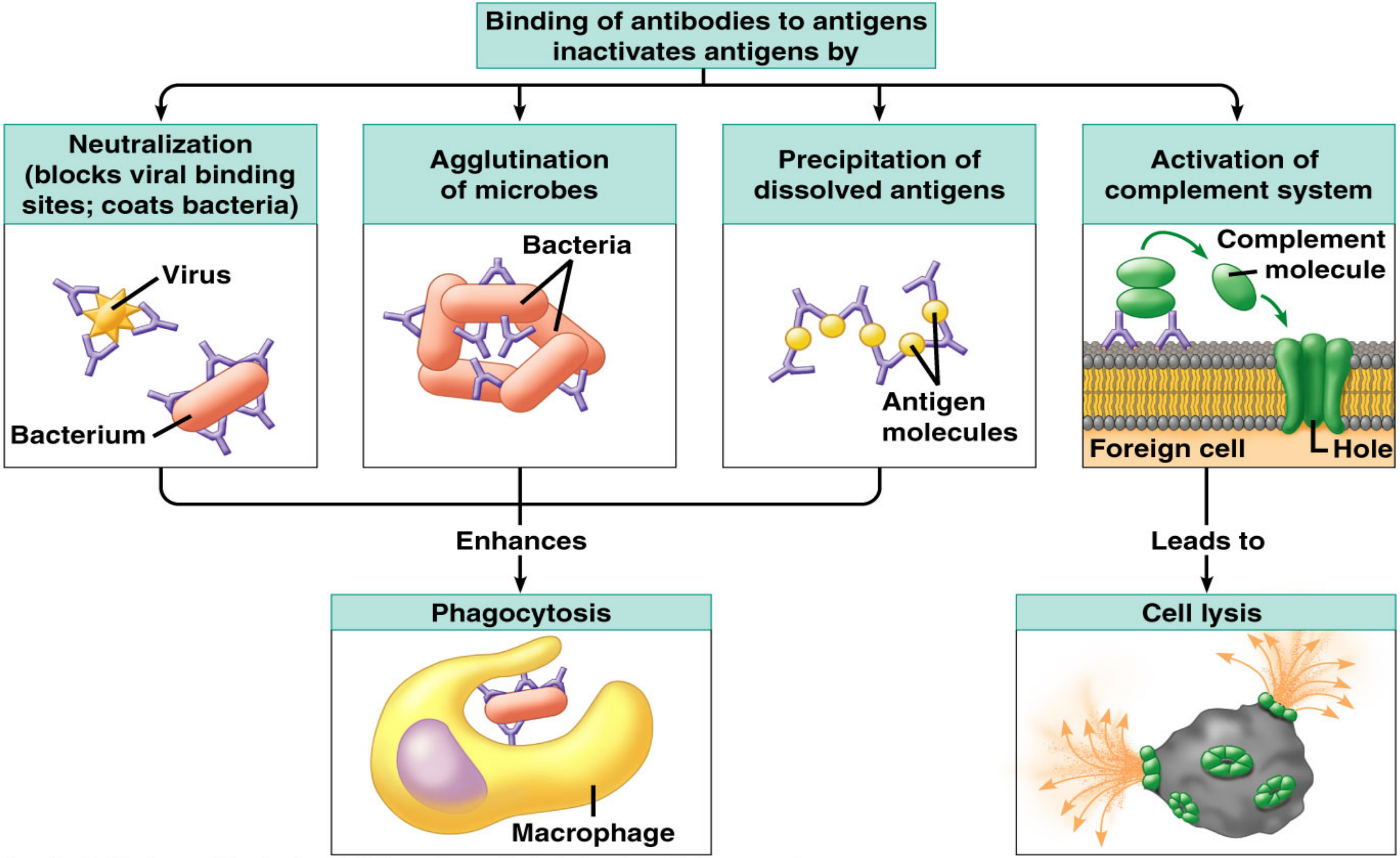


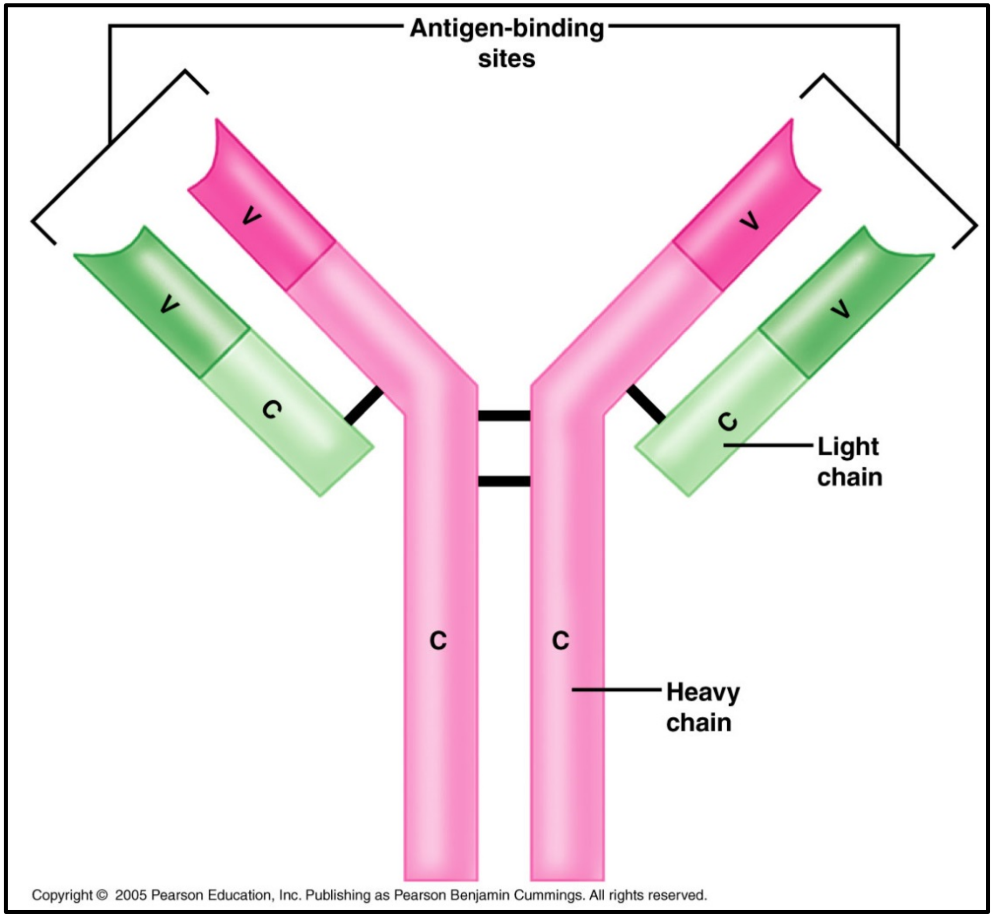
http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

Antibodies (secreted by B cells) work in several different ways

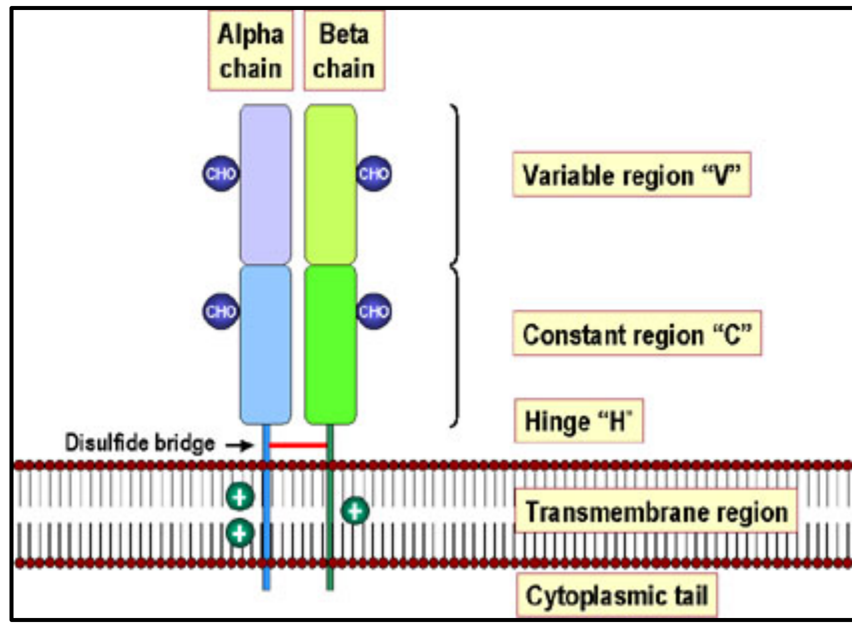


http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

"antigen" comes from **ANTI**-body **GEN**erating substances



Antigen Binding Site



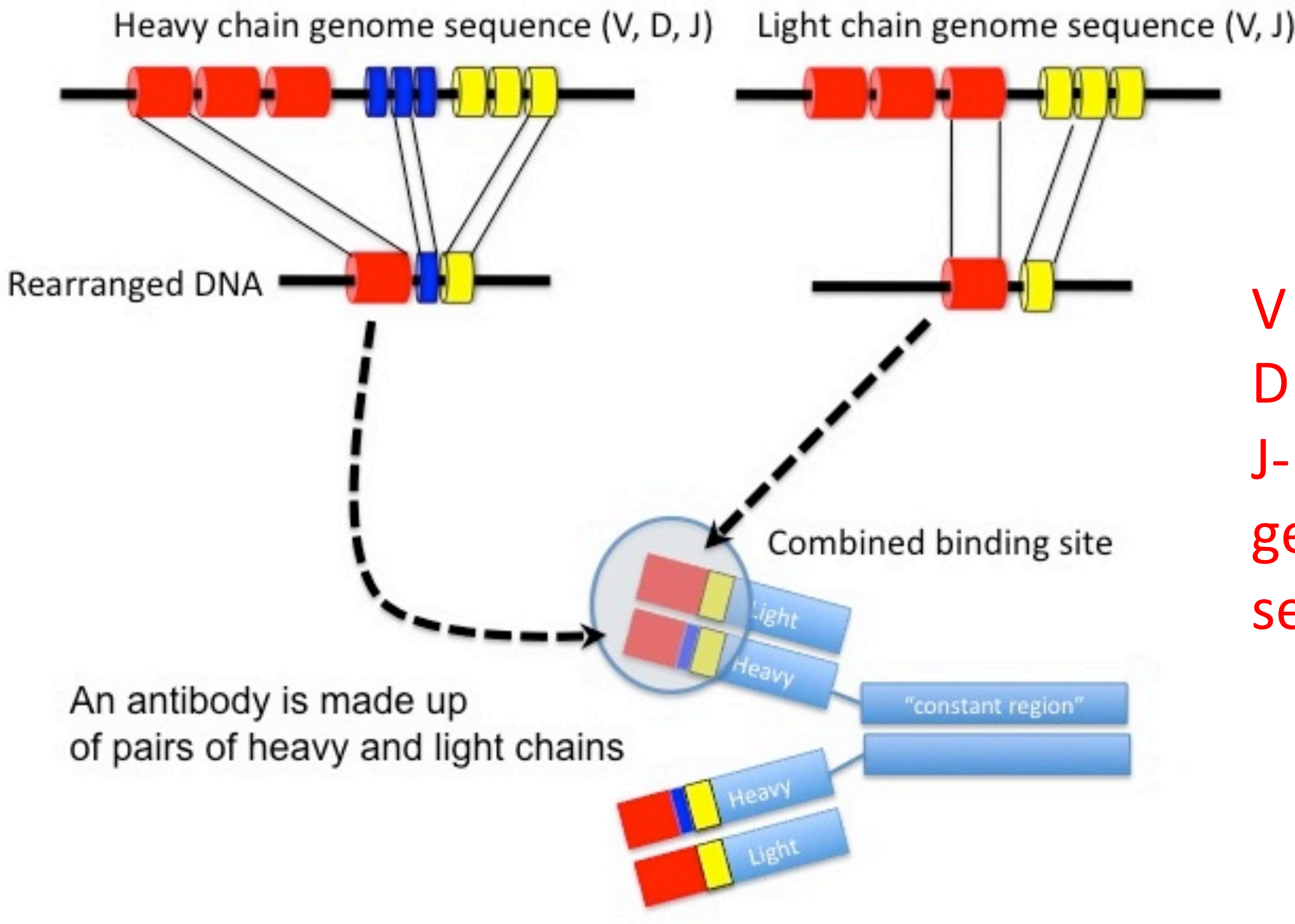
http://www.austincc.edu/apreview/EmphasisItems/Inflammatoryresponse.html#ANTIB

http://pathmicro.med.sc.edu/bowers/mhc.htm

B-cell Immunoglobulin

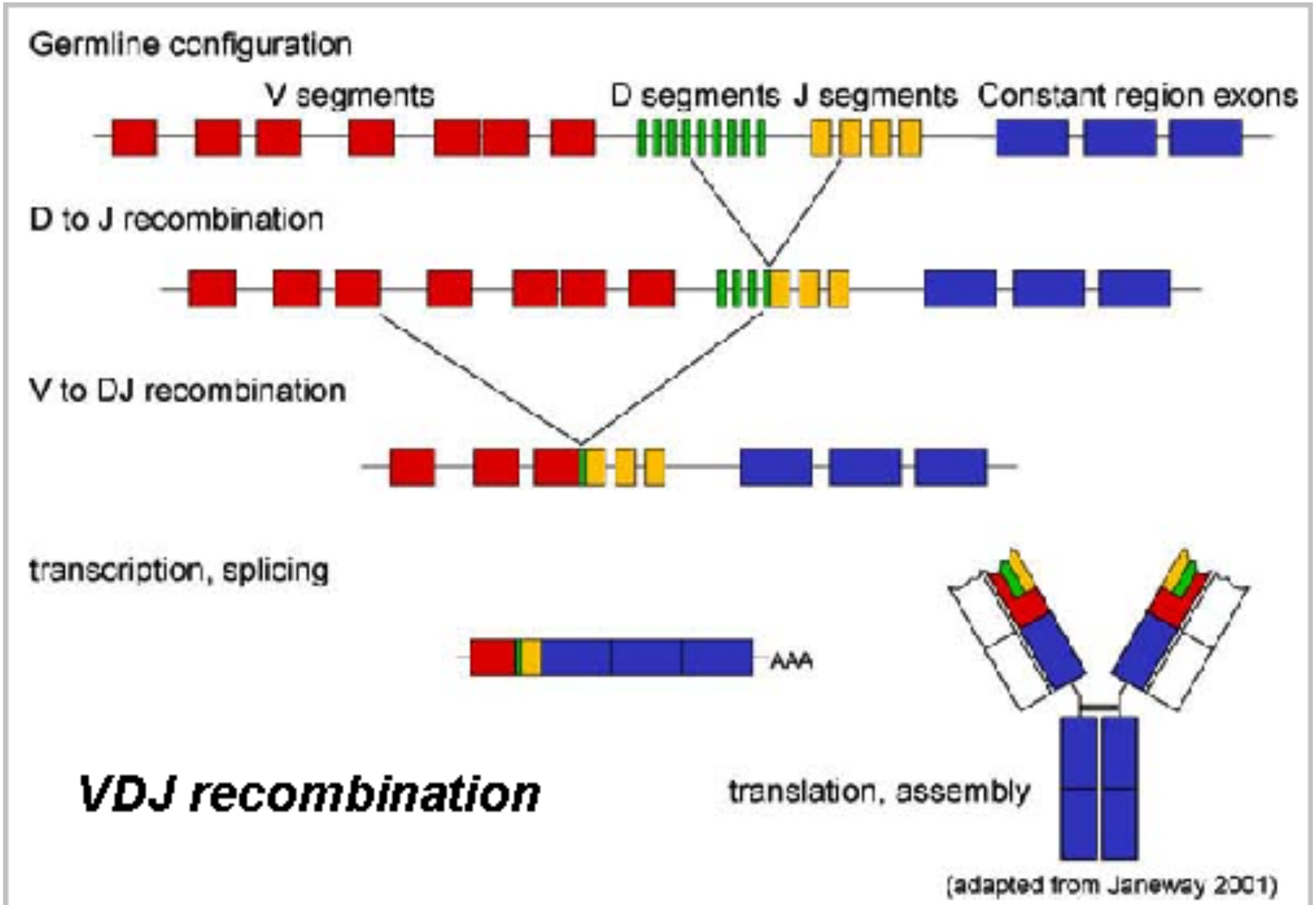
T-cell Receptor

How Do the Variable Regions become Variable? Through Programmed NHEJ!!



V -variable,
D - diversity
J- joining
gene
segments

How Do the Variable Regions become Variable? Through Programmed NHEJ!!



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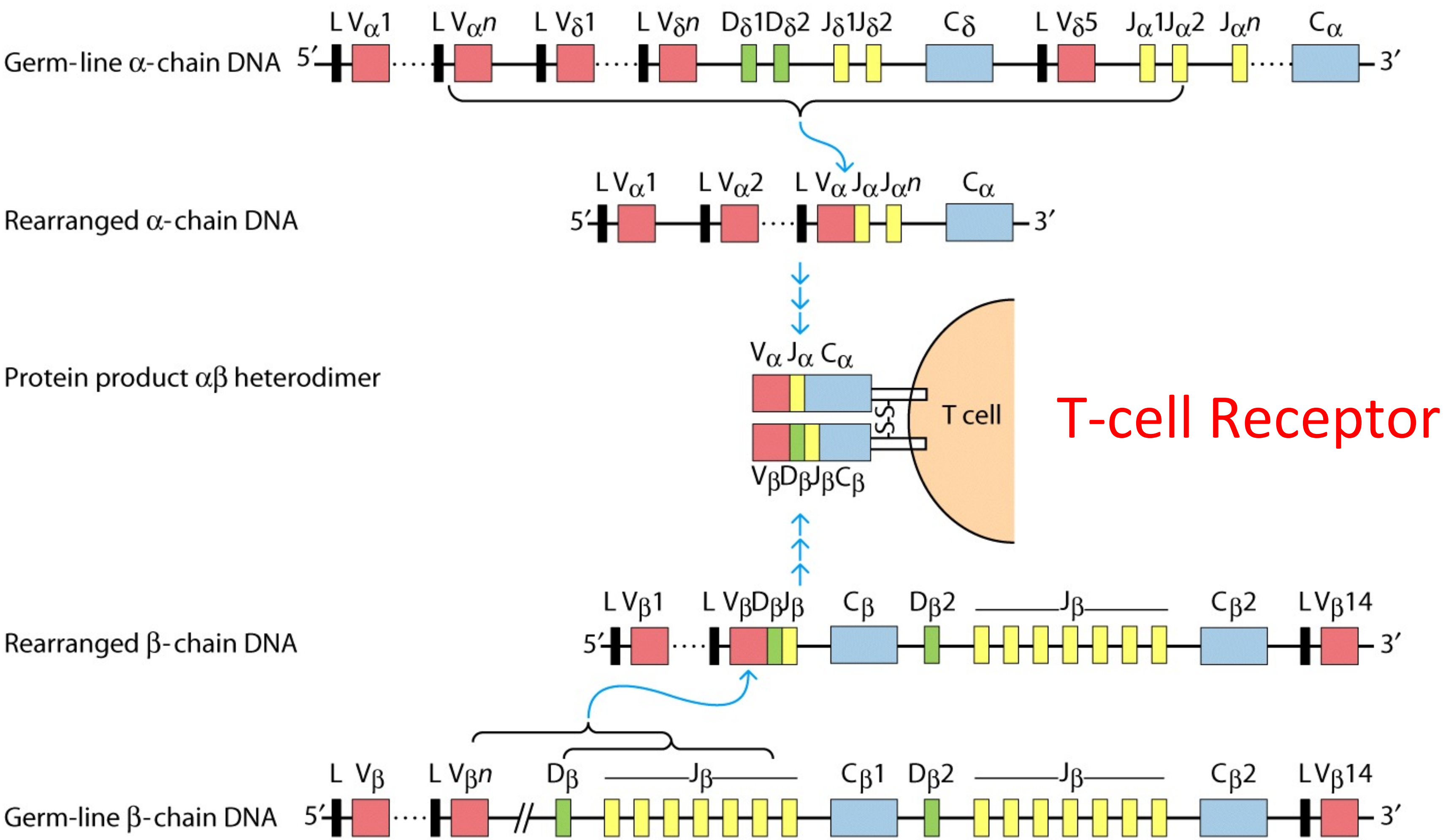
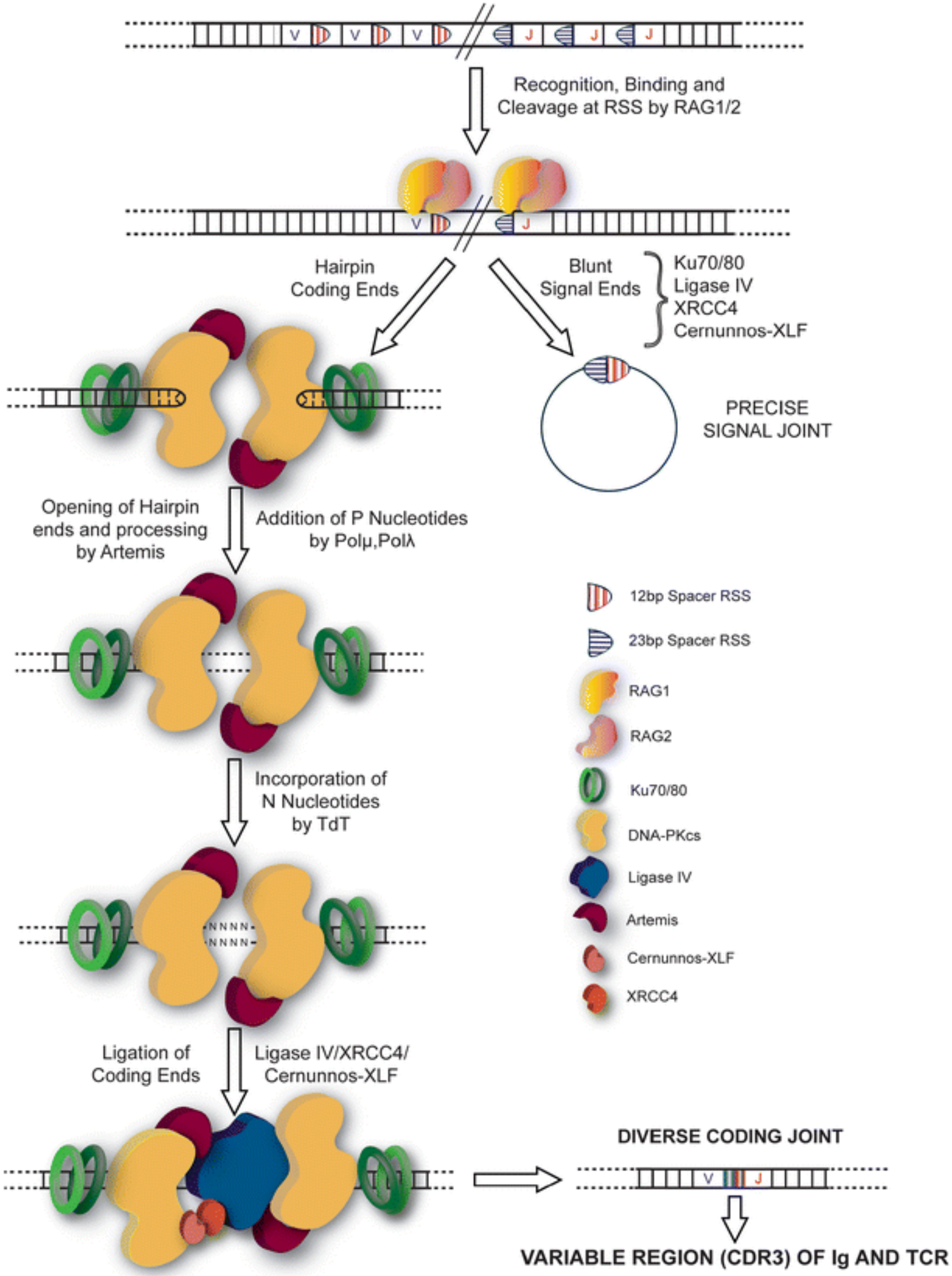


V(D)J Gene Recombination

http://www.youtube.com/watch?v=QTOBSFJWogE

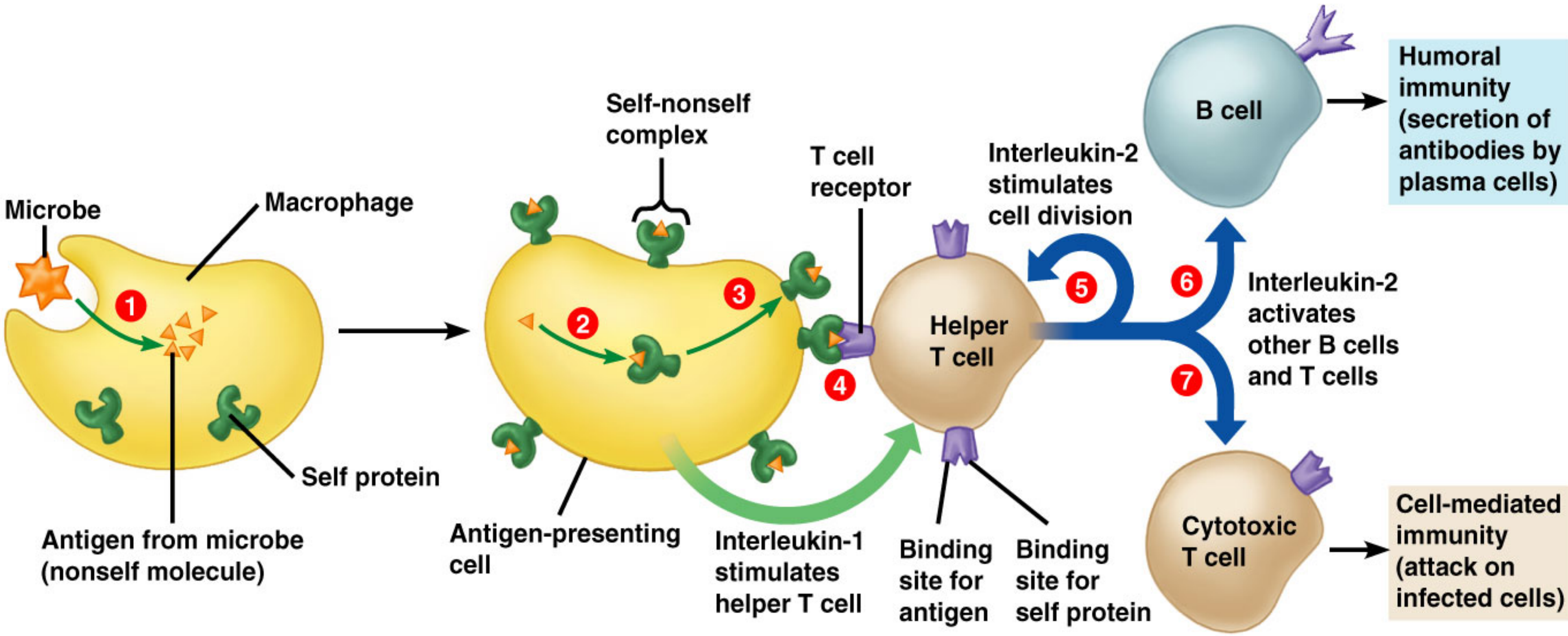


How Do the Variable Regions become Variable? Through NHEJ mediated DNA Recombination!

The rearrangement starts with the binding of products from recombination activating genes RAG1 and RAG2, whose expression is unique to lymphoid progenitor cells

Immunologic Research
December 2012, Volume 54, Issue 1-3, pp 233-246

The body contains millions of different T-cells and B-cells, each able to respond to one specific antigen.



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How Variable is Variable?

Number of functional gene segments in human immuniglobulin loci			
Segment	light chains		heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Over 15,000,000 combinations of variable, diversity and joining, V(D)J, gene segments are possible. Imprecise recombination and mutation increase the variability into billions of possible combinations.

How Variable is Variable?

	T cell receptor	
	α	β
Number of V gene segments	54	67
Number of diversity (D) gene segments	0	2
Number of joining (J) gene segments	61	4

Millions of combinations of variable, diversity and joining gene segments are possible. Imprecise recombination and mutation increase the variability into billions of possible combinations.

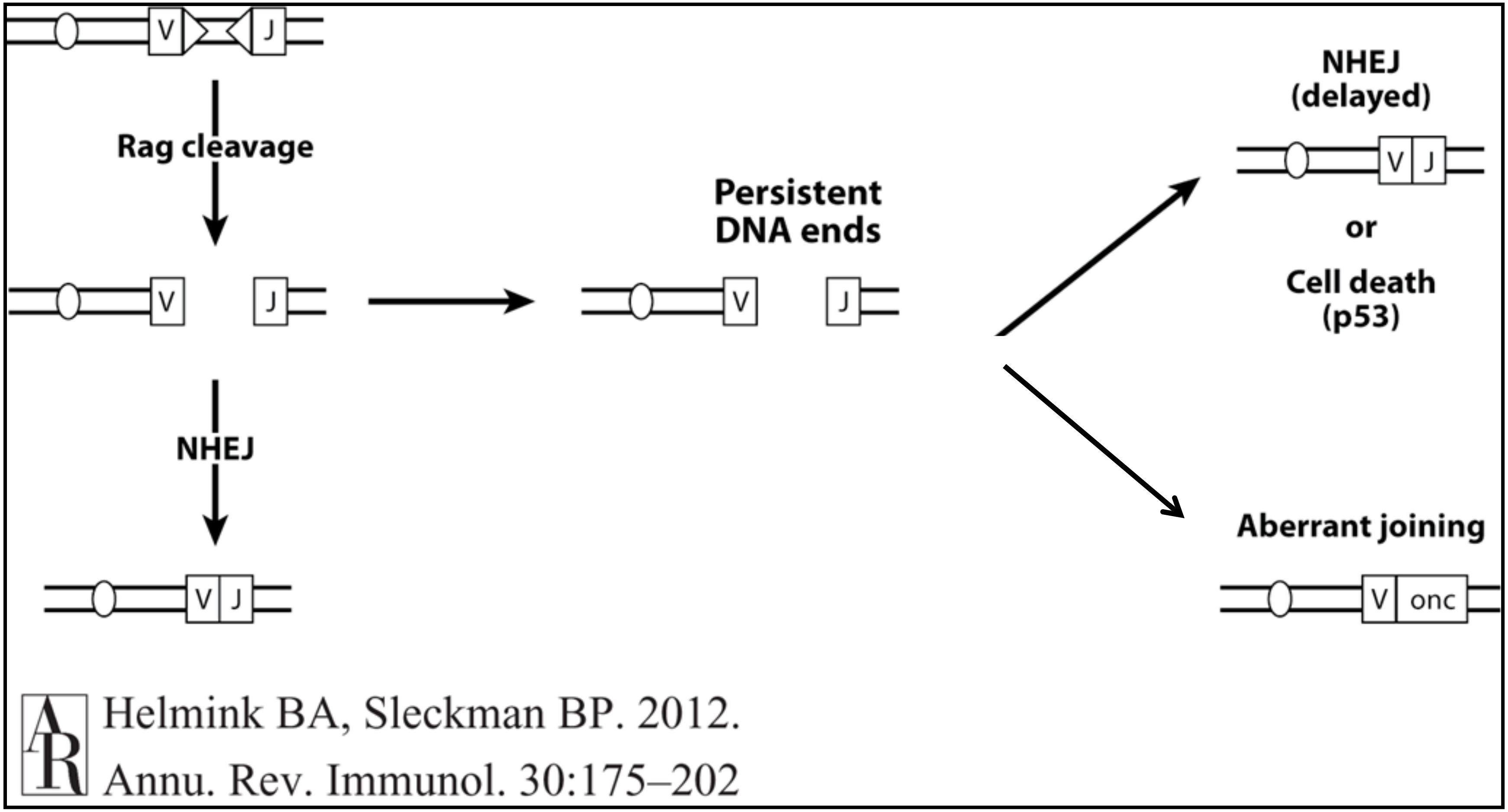
What happens if mice or people lose NHEJ capacity?

What happens if mice or people lose NHEJ capacity?

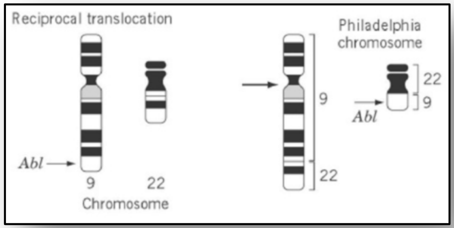
NHEJ gene	Mouse knockout phenotype	Patient phenotype
XRCC6 (encoding Ku70)	Viable, SCID, small size, radiosensitivity and thymoma[50,51]	None known
XRCC5 (encoding Ku80)	Viable, SCID, small size, radiosensitivity, genomic instability and tumours, especially with p53 deletion[47,52–54]	None known
PRKDC (encoding DNA-PKcs)	Viable, SCID, some genomic instability and tumours with p53 (REFS 55–57)	Human hypomorph has SCID and radiosensitivity[58]
DCLRE1C (encoding Artemis)	Viable, SCID, radiosensitivity and genomic instability[59]	Null results in SCID and radiosensitivity; hypomorph shows reduction in lymphocytes, genomic instability and lymphoma[60,61]
NHEJ1 (encoding XLF)	Mild lymphocytopaenia and radiosensitivity[62]	Cernunnos syndrome; immunodeficiency, developmental delay, microcephaly, reduced growth and genomic instability[63]
XRCC4	Null is lethal with neuronal apoptosis; rescue with p53 results in SCID, radiosensitivity, early B lymphoma and genomic instability[49,64]	None known
LIG4	Knockout is lethal with neuronal apoptosis; rescue with p53 results in pro-B lymphoma and radiosensitivity; hypomorph is small, lymphopaenic and has reduced haematopoietic stem cell function[65,66]	LIG4 syndrome; immunodeficiency, reduced growth, developmental issues, microcephaly and malignancy[67,68]

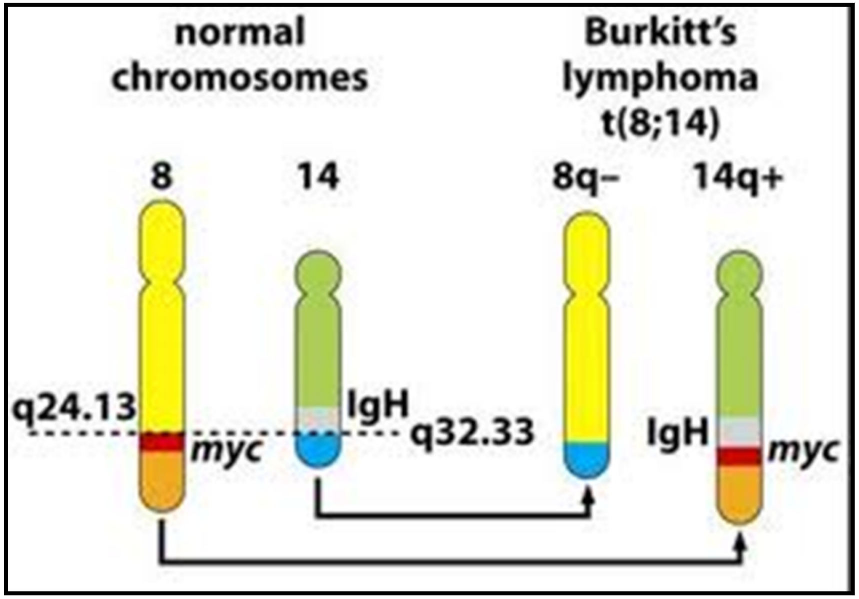
DCLRE1C, DNA cross-link repair 1C; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LIG4, DNA ligase 4; NHEJ, non-homologous end-joining; NHEJ1, NHEJ factor 1; PRKDC, protein kinase, DNA-activated, catalytic polypeptide; SCID, severe combined immunodeficiency; XLF, XRCC4-like factor; XRCC, X-ray repair cross-complementing protein.

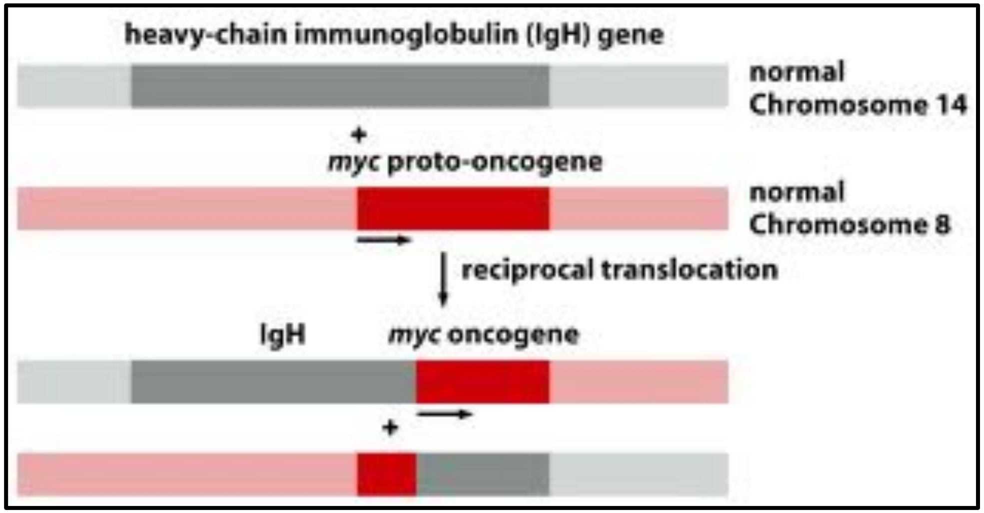
Can V(D)J Recombination Go Wrong?



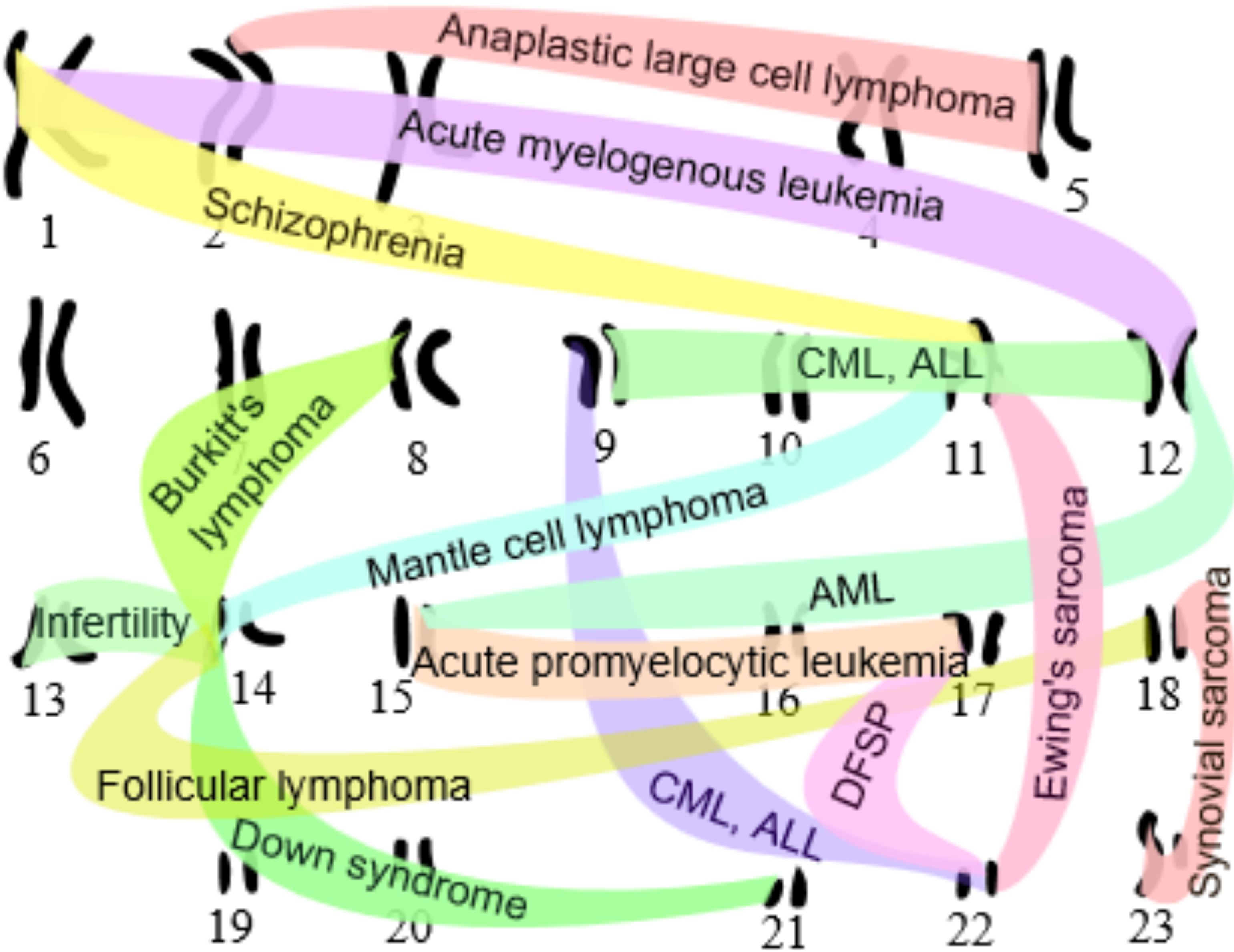
BURKITT's LYMPHOMA
B-cell Lymphoma



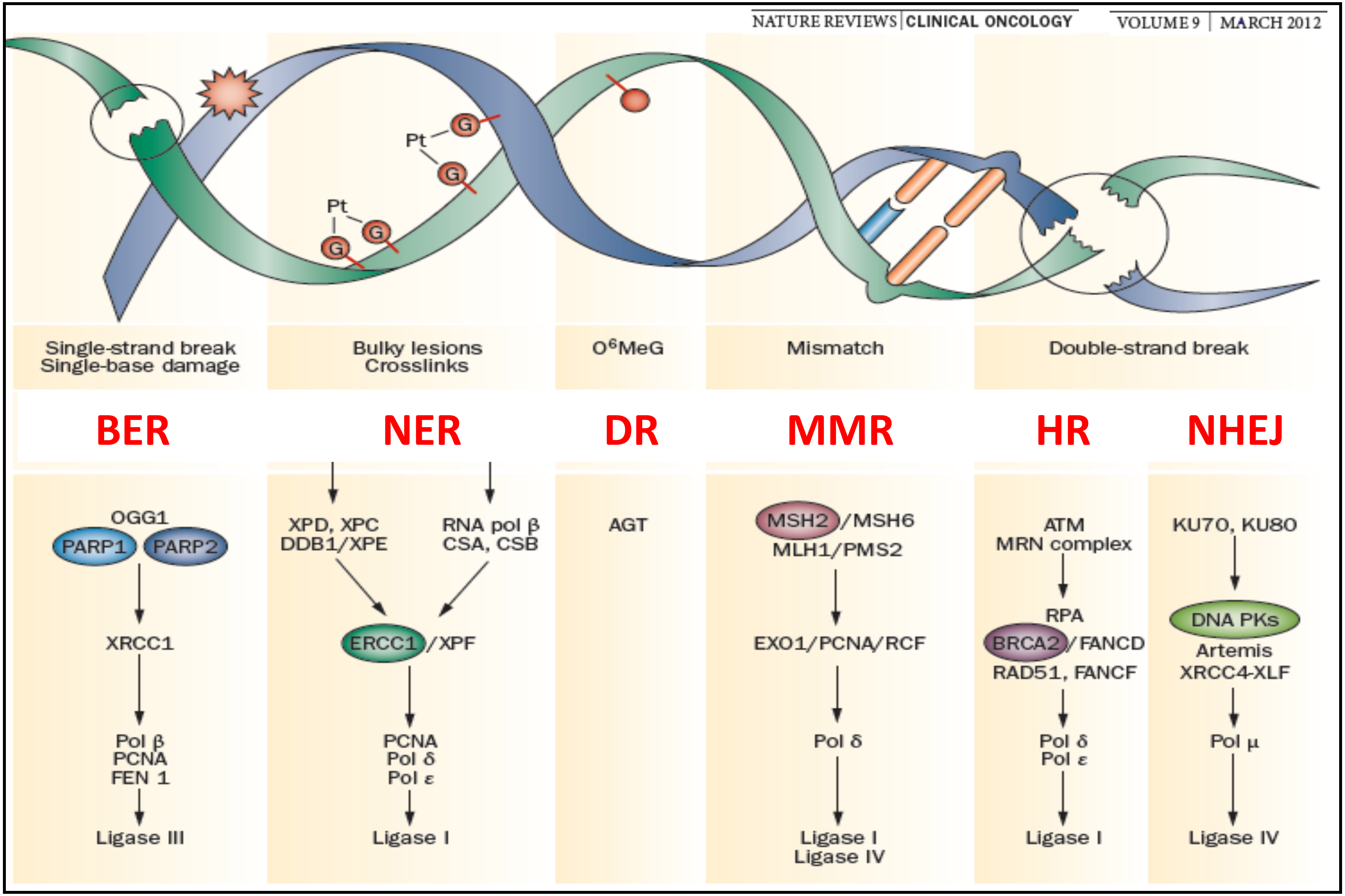


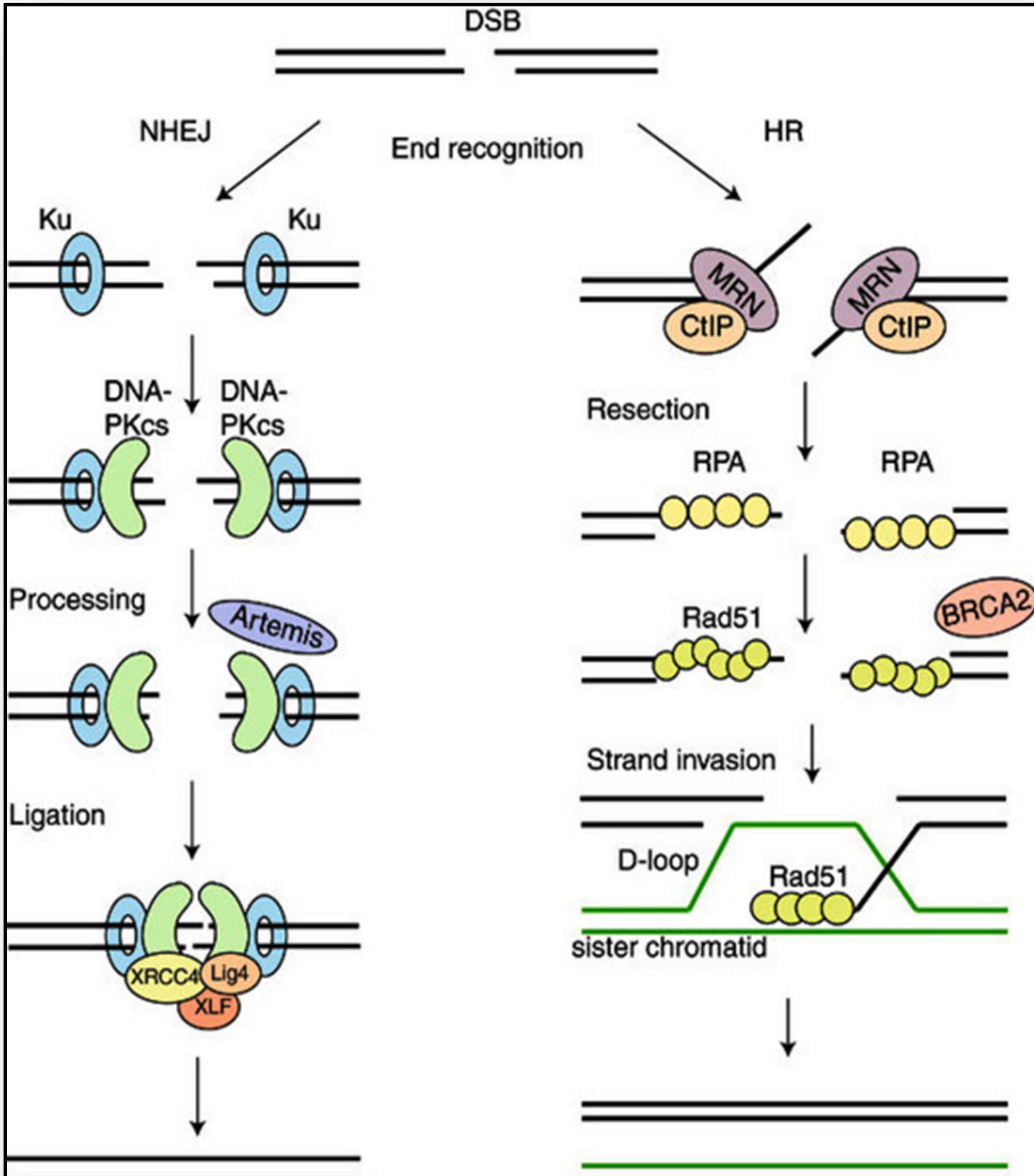


Diseases that involve Chromosome Translocations



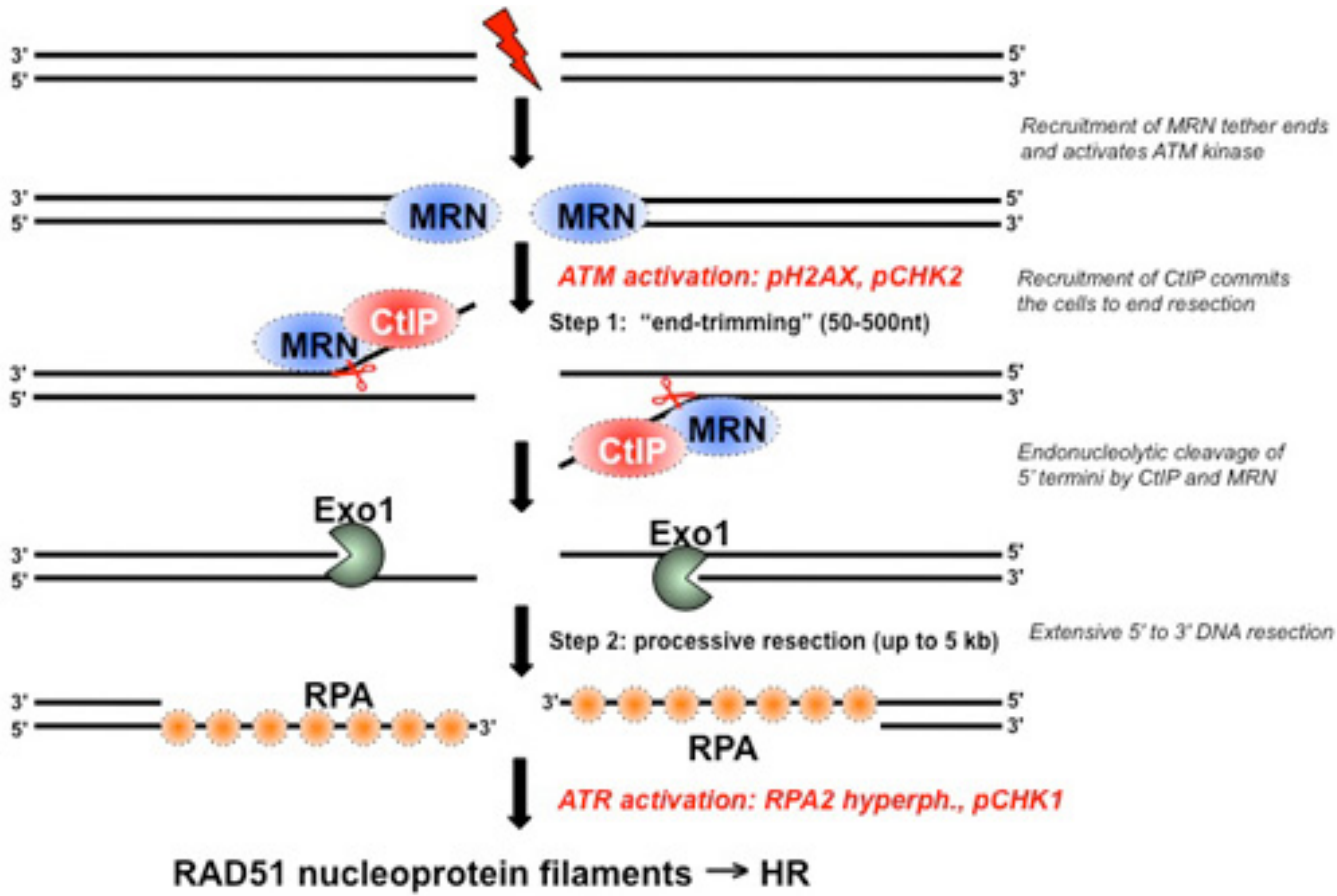
Six Major DNA Repair Pathways

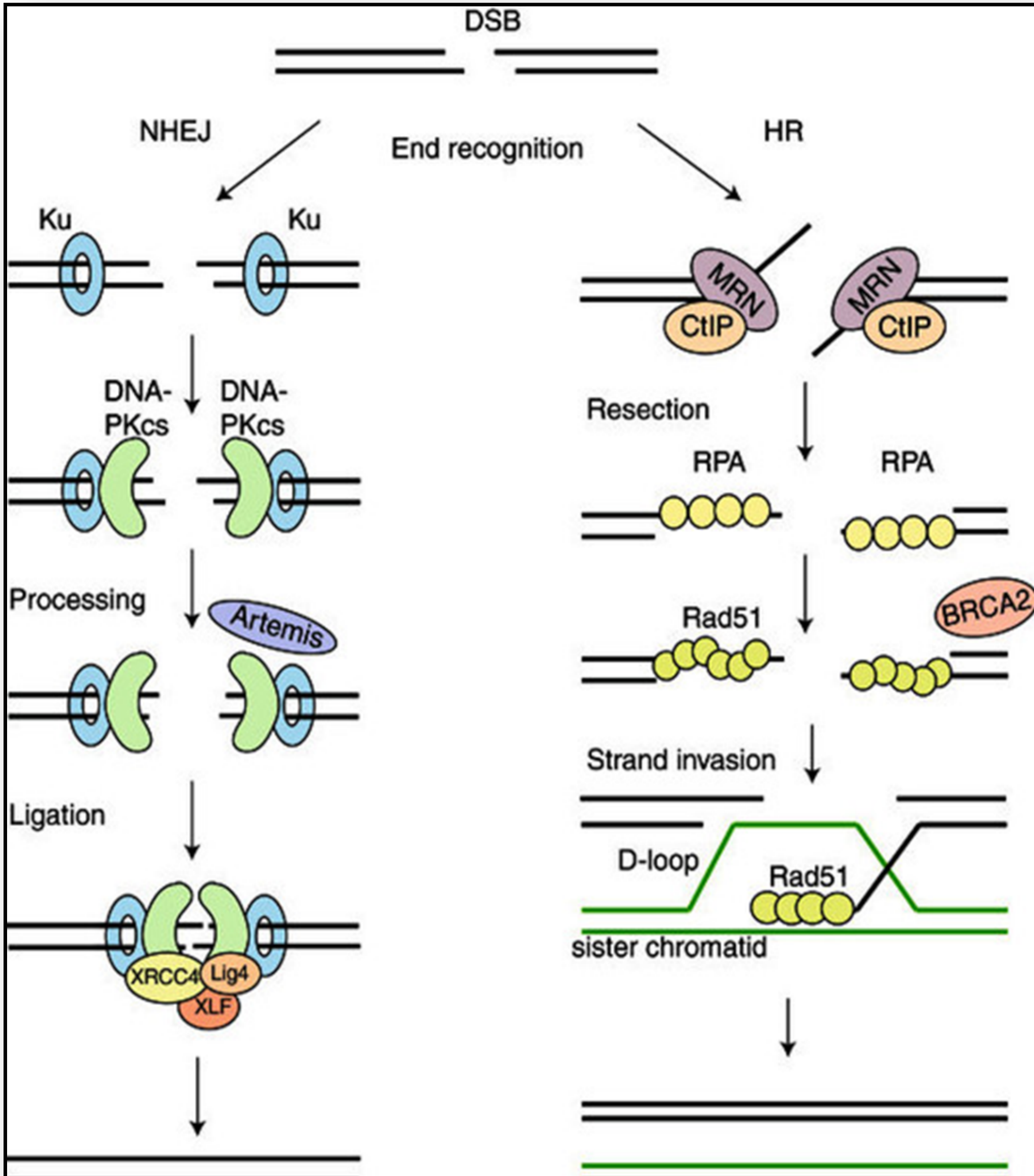




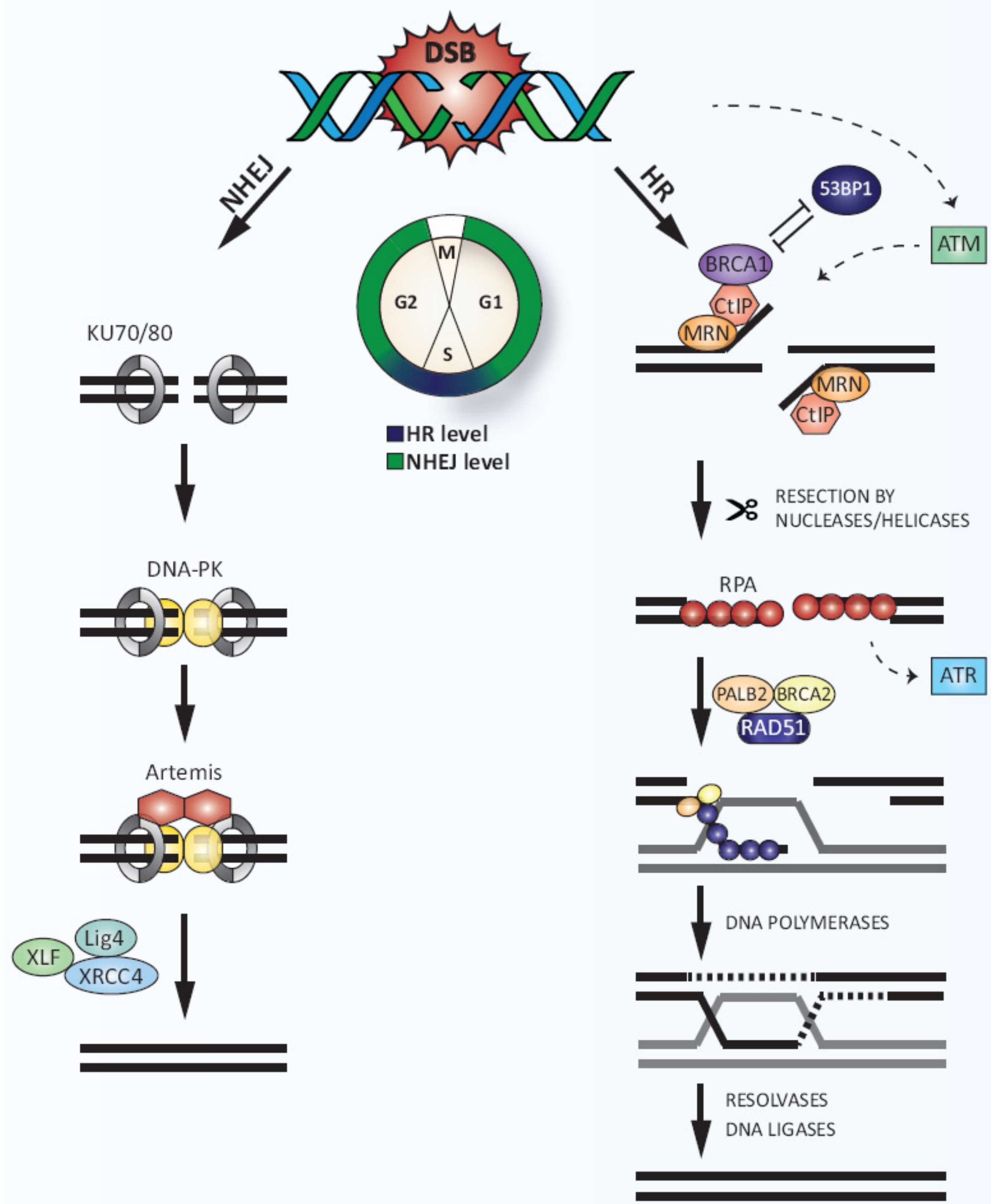
DNA Double Strand Break (DSB) Repair – What Determines Pathway Choice?

The MRN complex (Mre11/Rad50/Nbs1) competes with Ku for binding DSBs

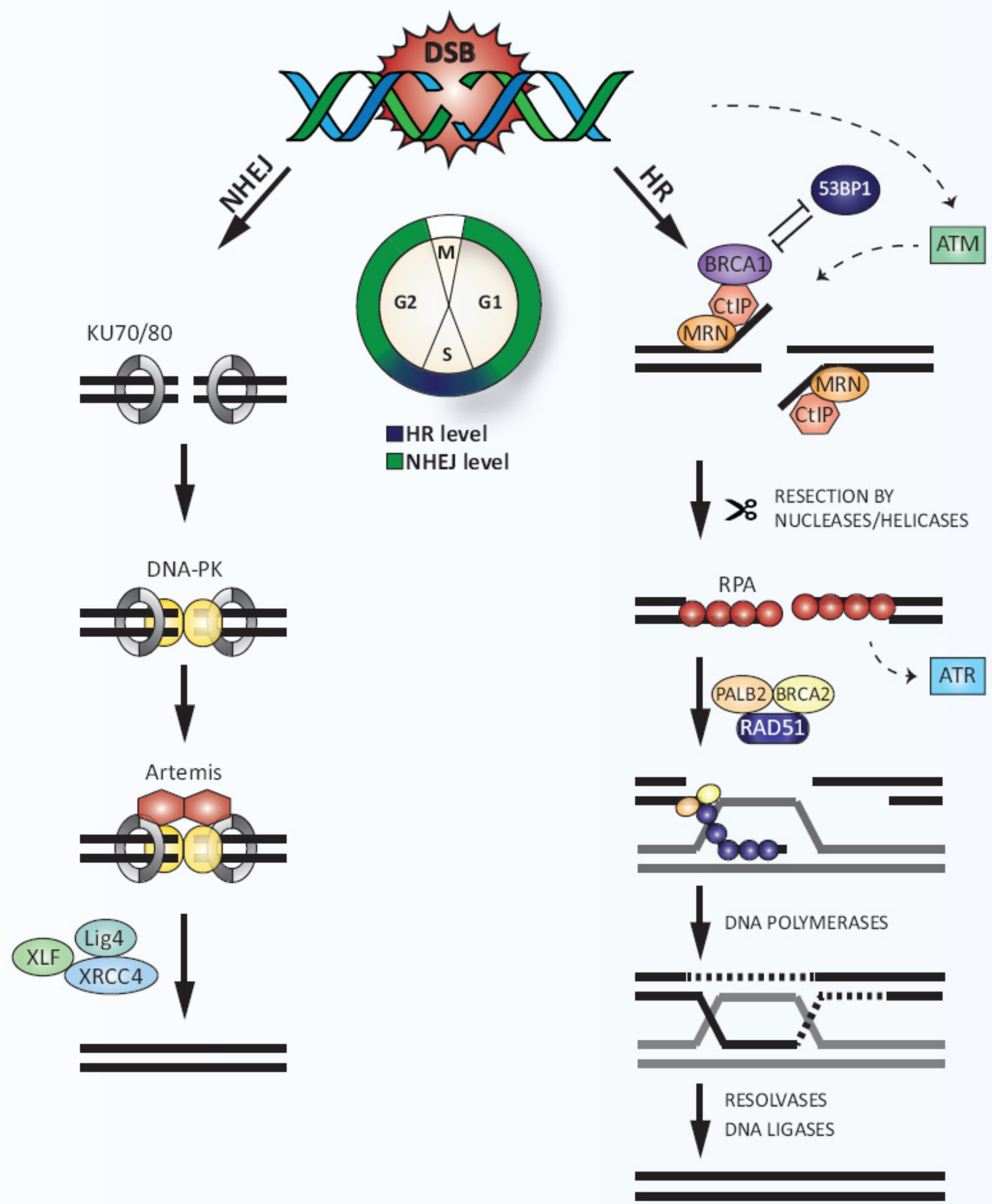




DNA Double Strand Break (DSB) Repair – What Determines Pathway Choice?

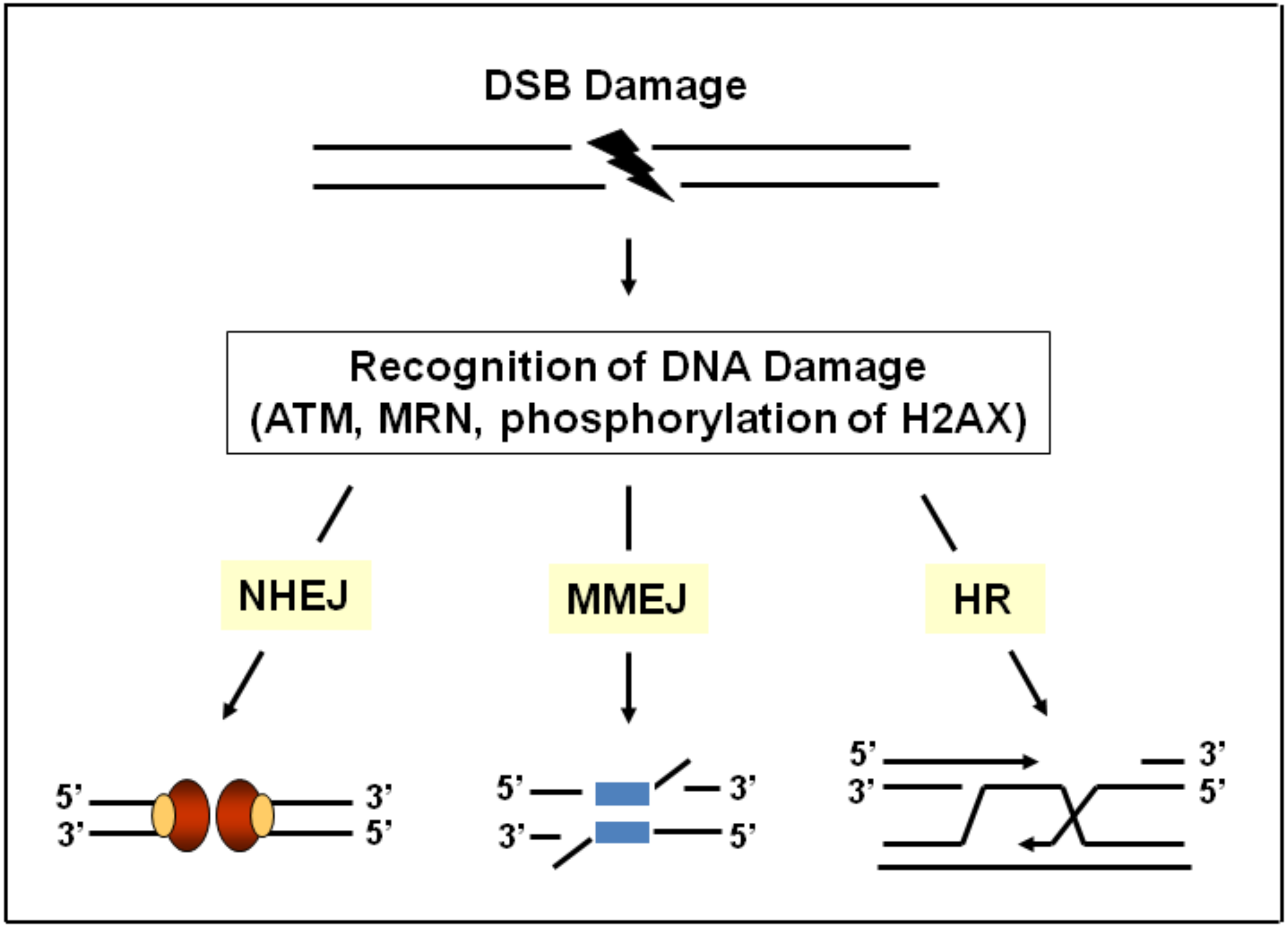


DNA Double Strand Break (DSB) Repair – What Determines Pathway Choice?



Are there really only two ways to repair DSBs?

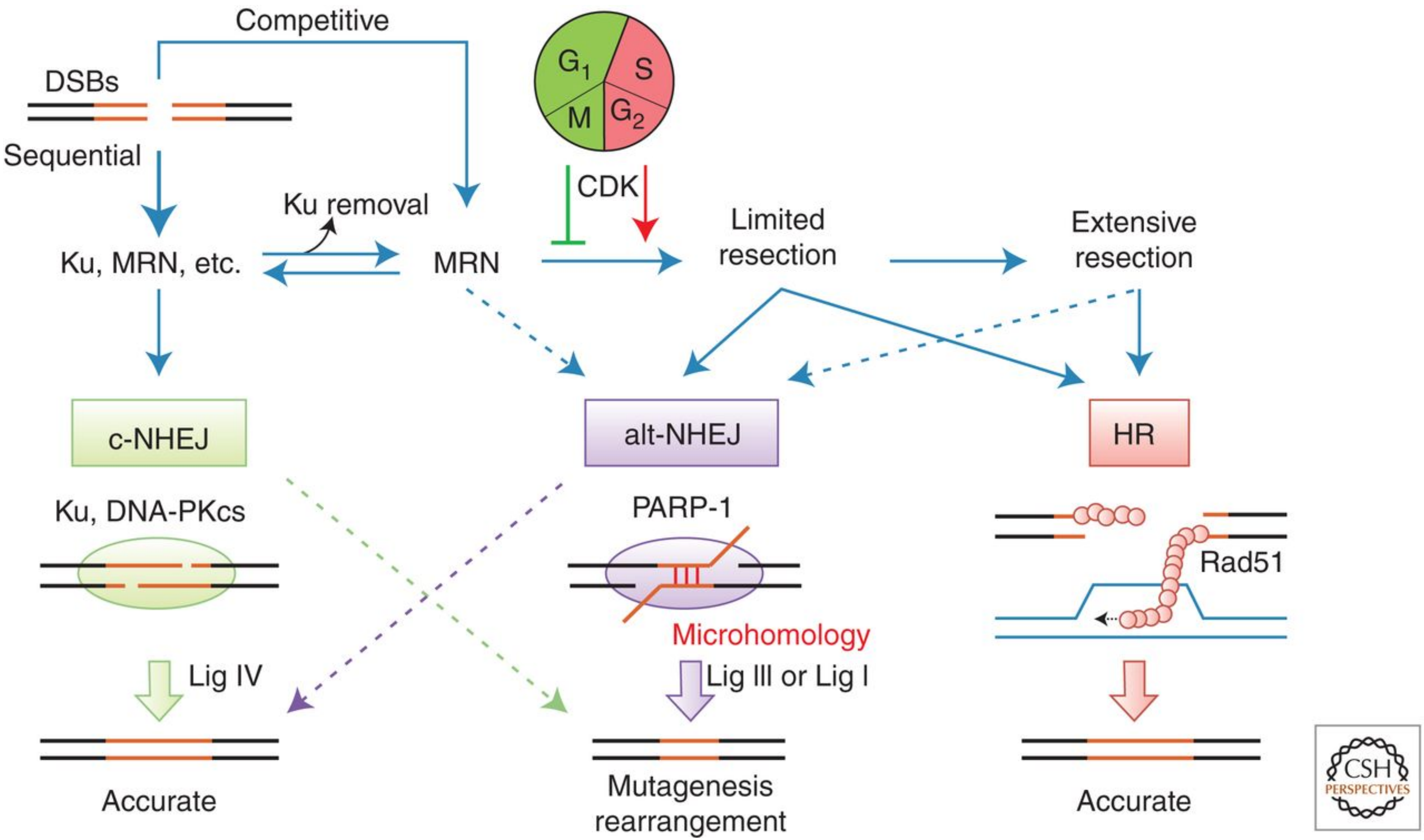
Sub-Pathway for NHEJ



MMEJ = Microhomology Mediated End Joining

http://www.intechopen.com/books/new-research-directions-in-dna-repair/emerging-features-of-dna-double-strand-break-repair-in-humans

Disposition of DSBs between repair pathways.



MMEJ = alternate-NHEJ, alt-NHEJ

Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

Non-Homologous End Joining

http://web.mit.edu/engelward-lab/animations/NHEJ.html

Double-Strand Break Repair via Single Strand Annealing - Alternate NHEJ - MMEJ

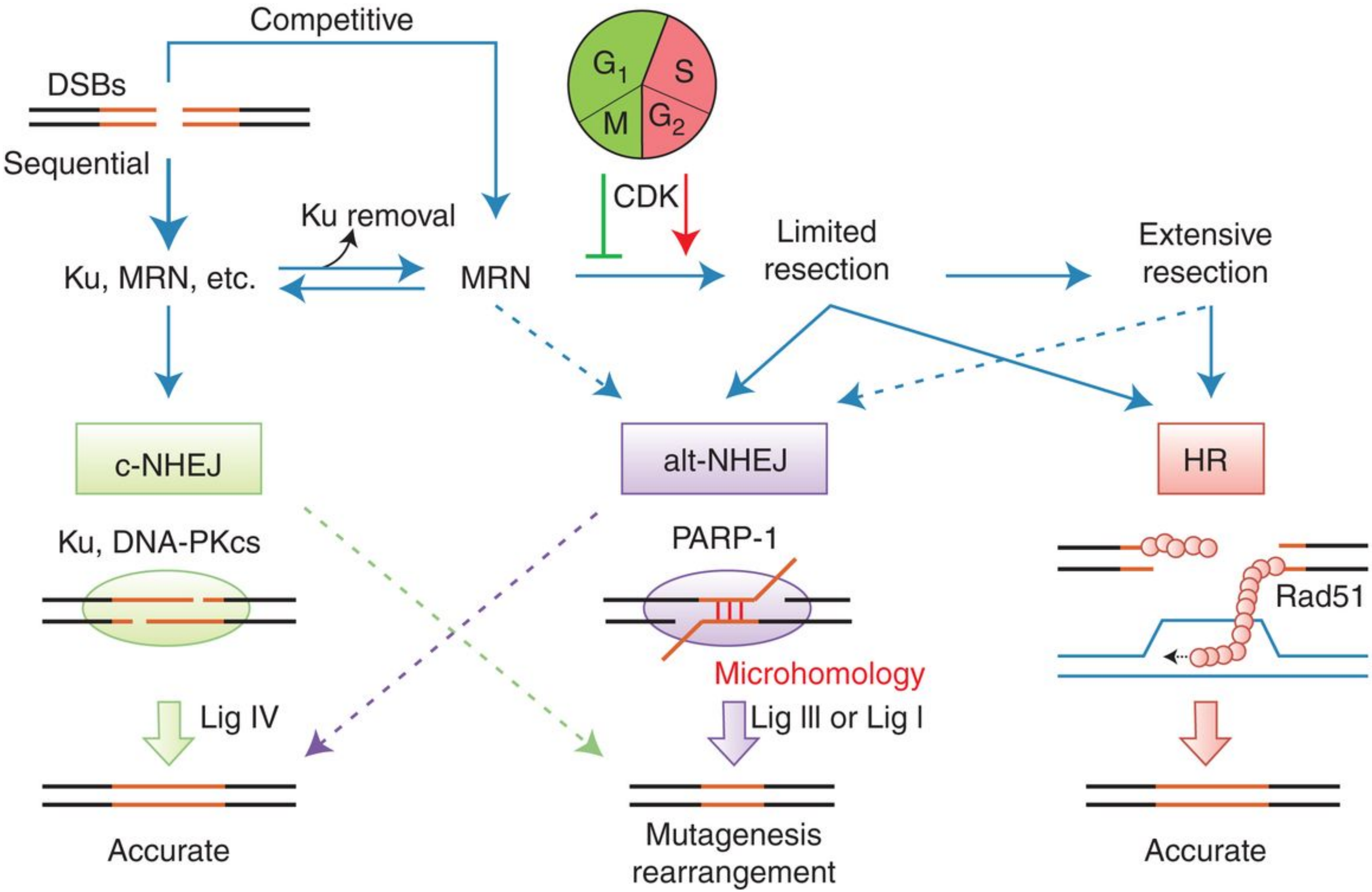
http://web.mit.edu/engelward-lab/animations/SSA.html

Synthesis-Dependent Strand Annealing (Homologous Recombination)

http://web.mit.edu/engelward-lab/animations/SDSA.html

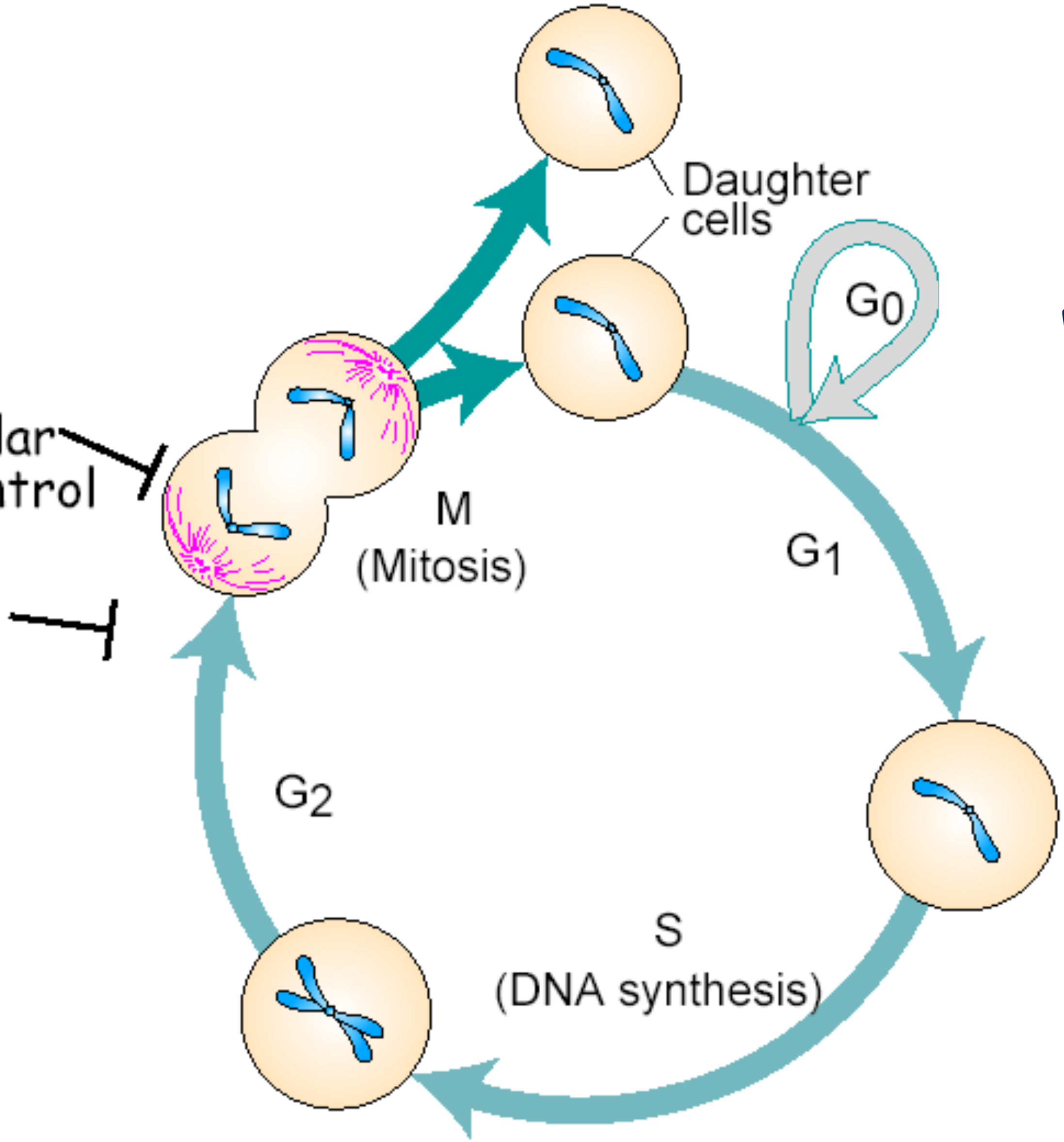
Engelward lab Animations

Disposition of DSBs between repair pathways.



Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

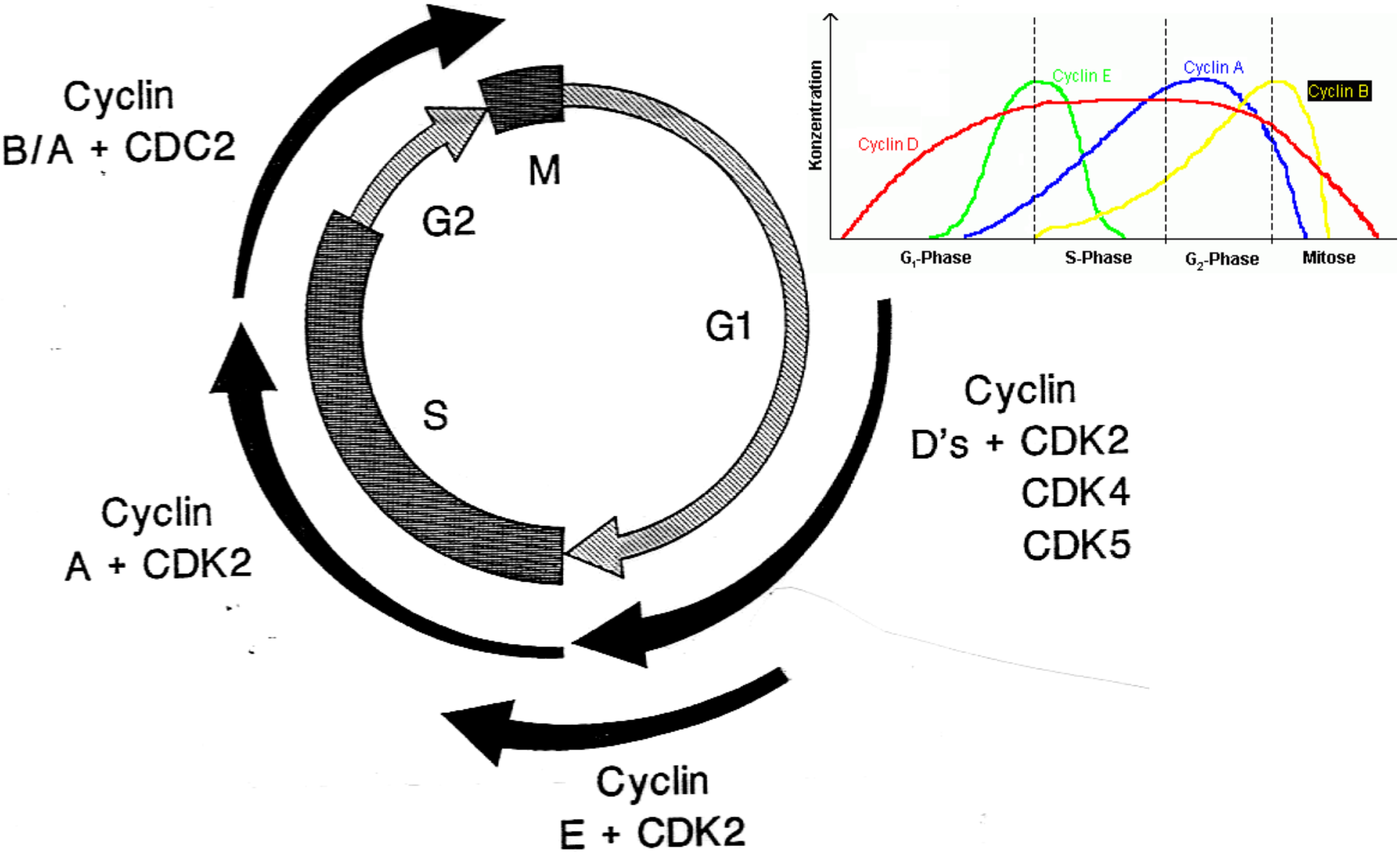
Intracellular quality control checks

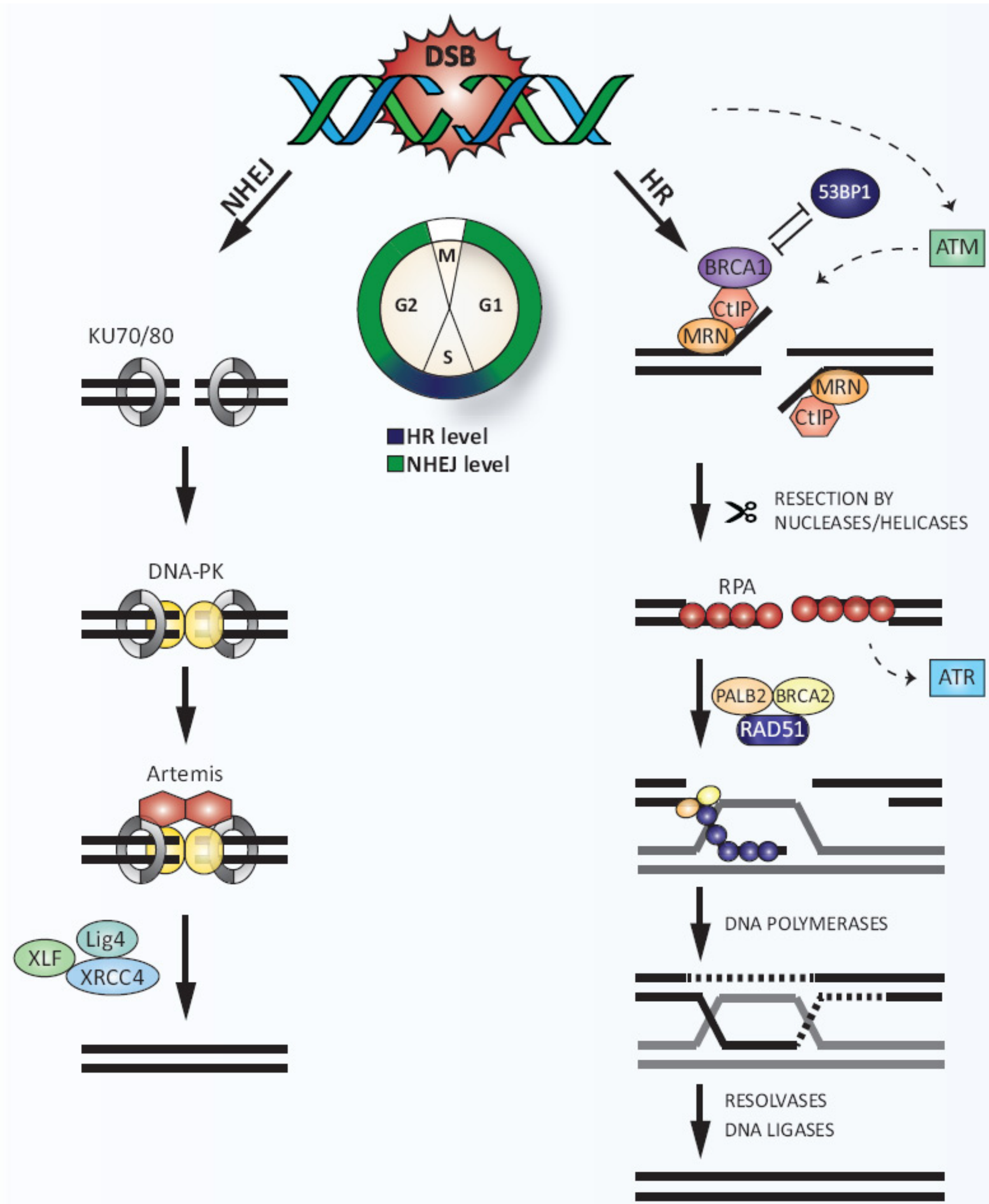


How does the cell "know" where it is in The Cell Cycle

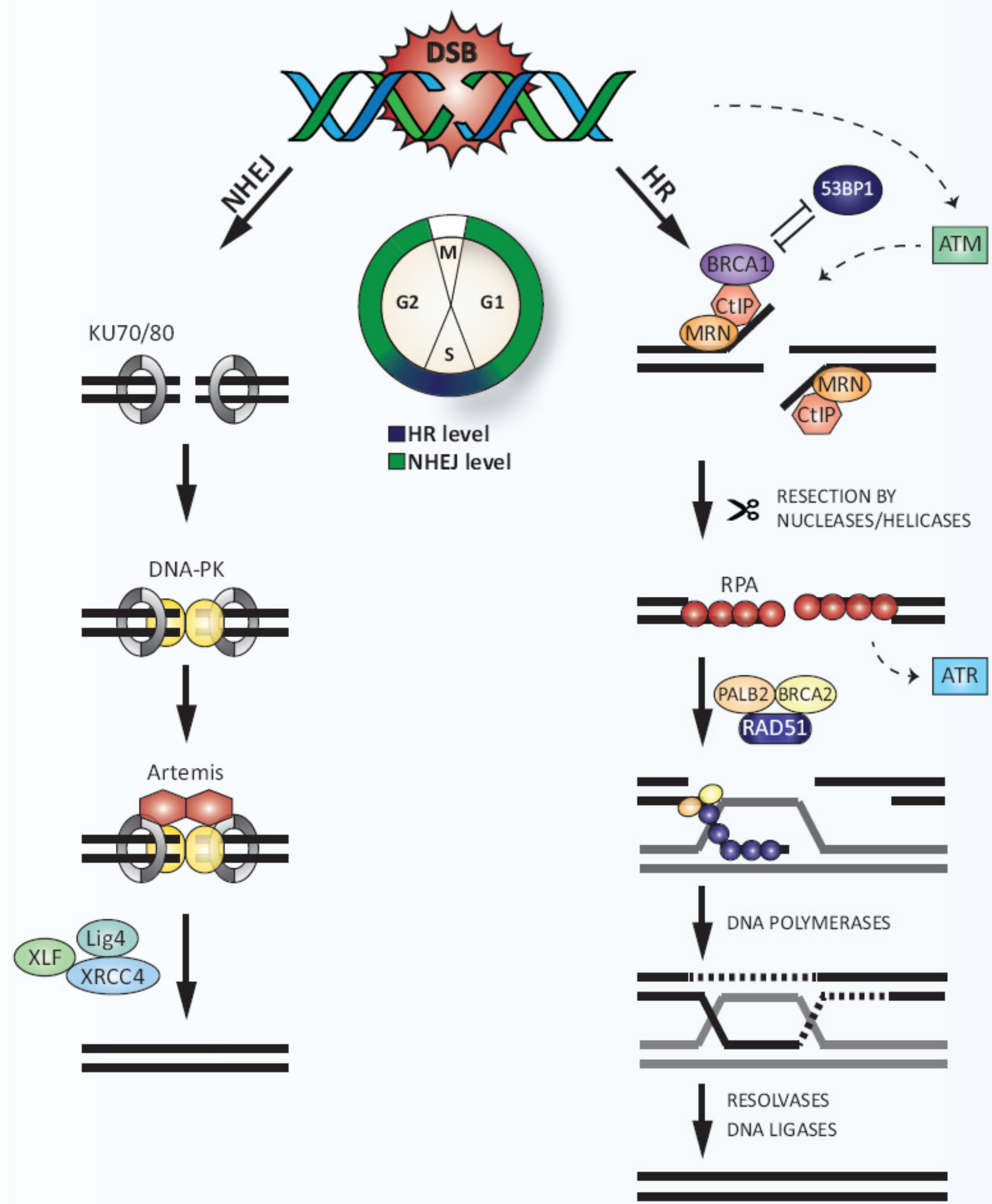
Duplication of chromosomes
DNA Replication

Progression through the Cell Cycle REQUIRES a series of cyclins and cyclin-dependent-kinases (CDKs)

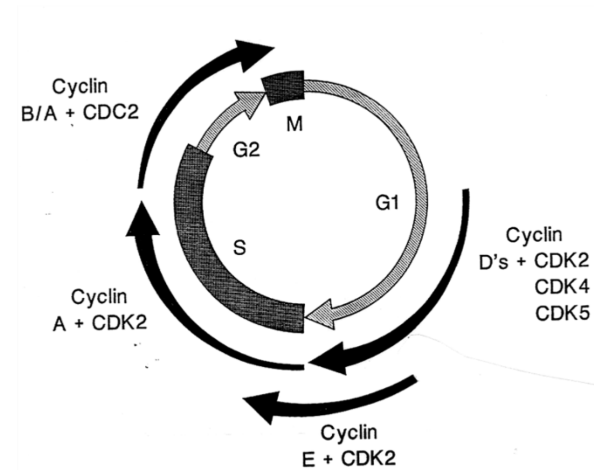


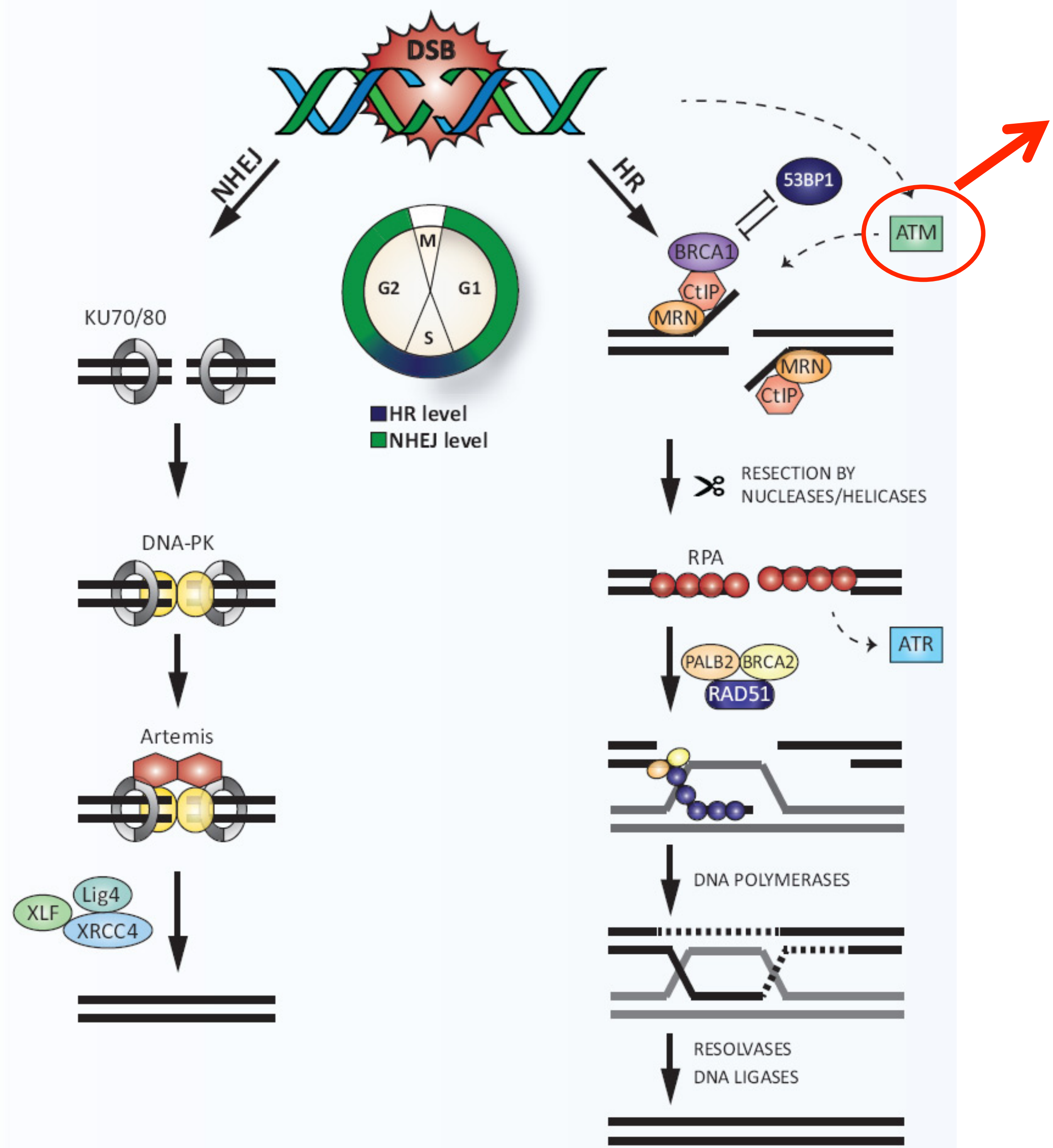


How does the cell decide which pathway to use?

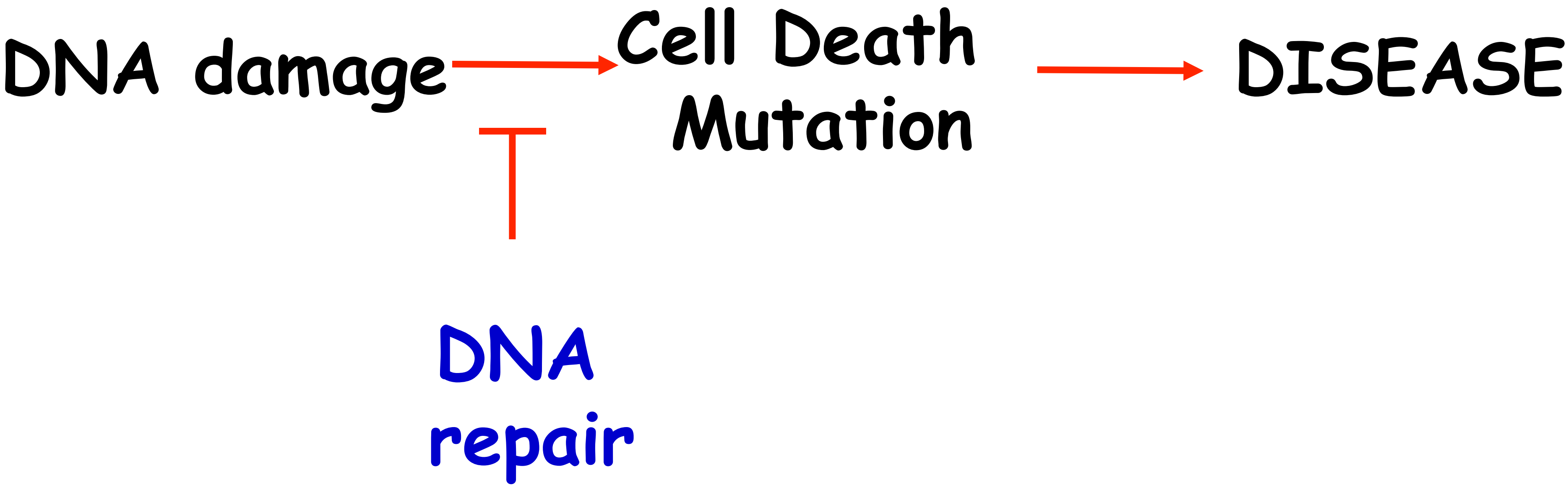


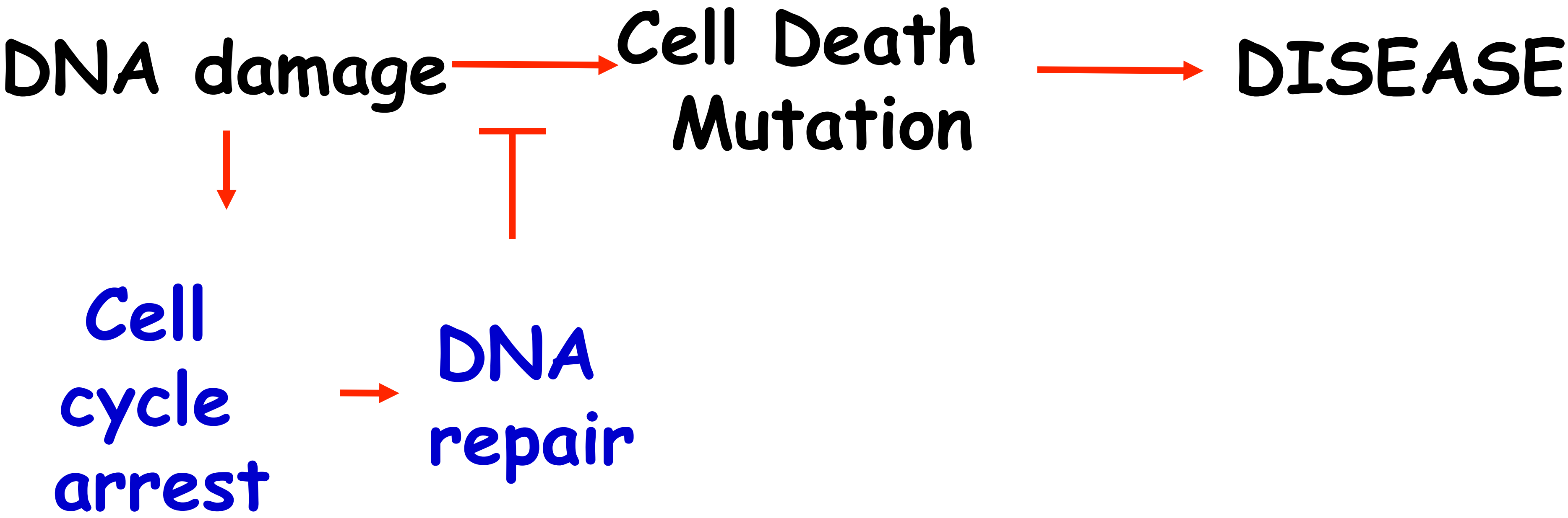
CyclinA-CDK2 targets the CtIP/BRCA1 complex





The ATM kinase also involved in activating the MRN complex and signaling for S-phase cell cycle arrest


DNA damage
Cell Death
Mutation
DISEASE
DNA
repair


DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair

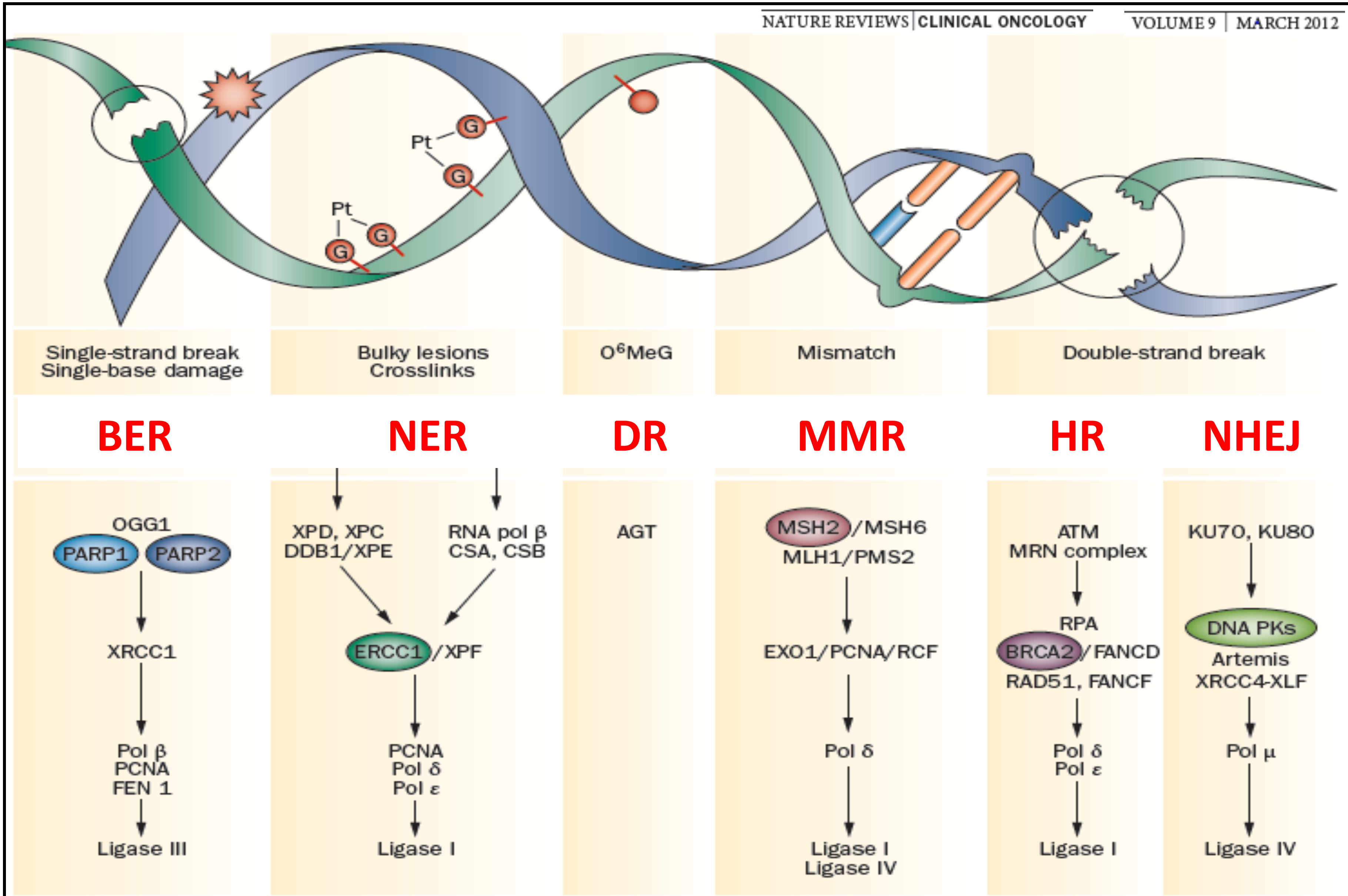
Ataxia Telangiectasia – Cancer Prone

Defective cell cycle arrest in response to DNA Damage affects neurodegeneration and cancer susceptibility

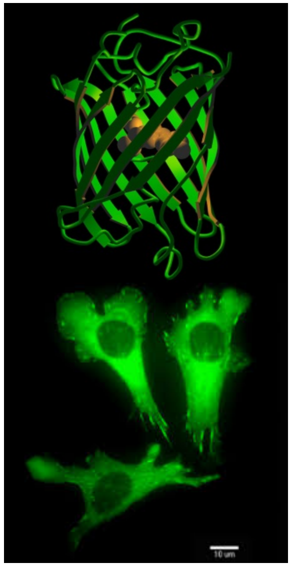
Ataxia Telangiectasia Mutated (ATM)

- Staggering gait
- Muscular un-coordination
- Mental retardation
- Dilation of small blood vessels
- Immune dysfunction
- Cancer prone…lymphomas
- Cells from AT patients have lost cell cycle checkpoints

Six Major DNA Repair Pathways

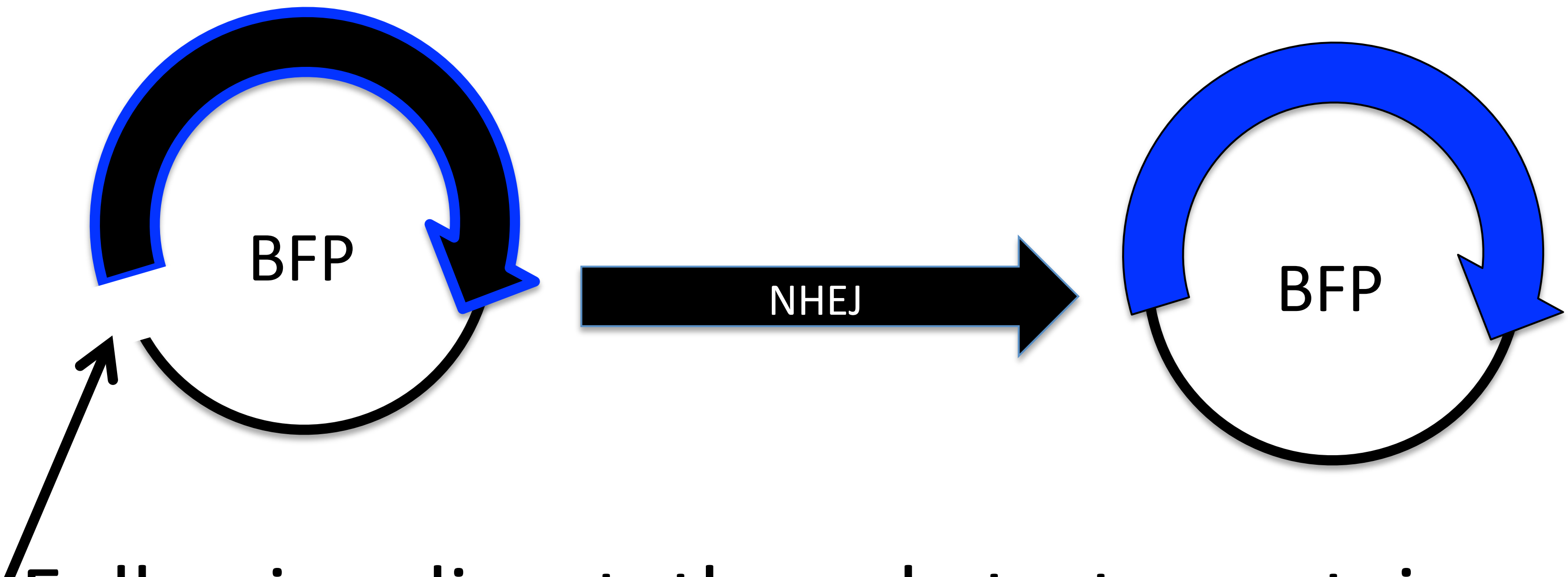


Key Experimental Methods for Module 2

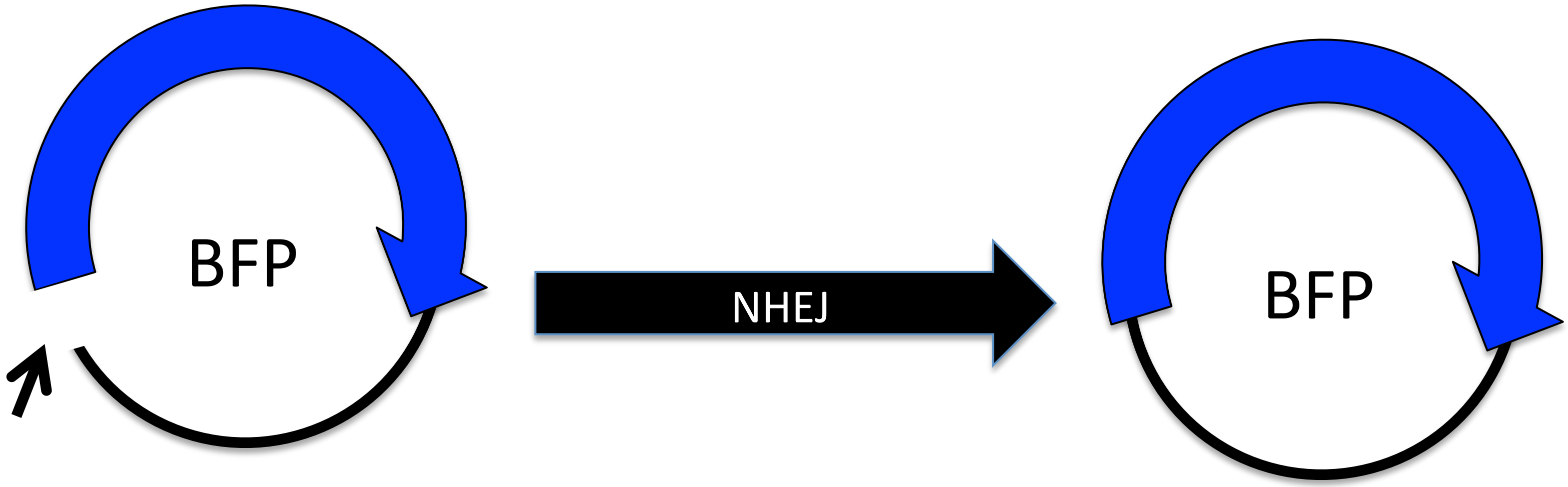


- Mammalian tissue cell culture
- Monitoring protein level by Western blot
- Generating plasmids with DNA damage
- Transfecting plasmids into mammalian cells
- Using fluorescent proteins as reporters of biological processes
- Flow cytometry to measure DNA repair
- Statistical analysis of biological data

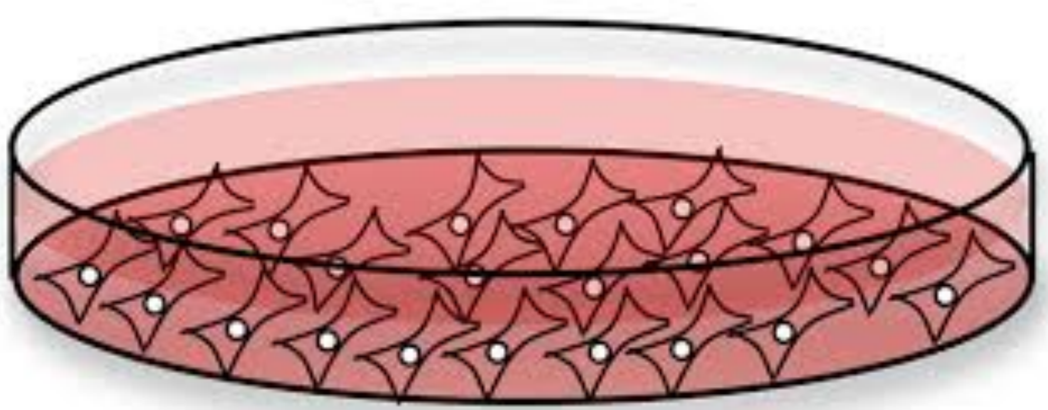
Basis for the fluorescent reporter assay:

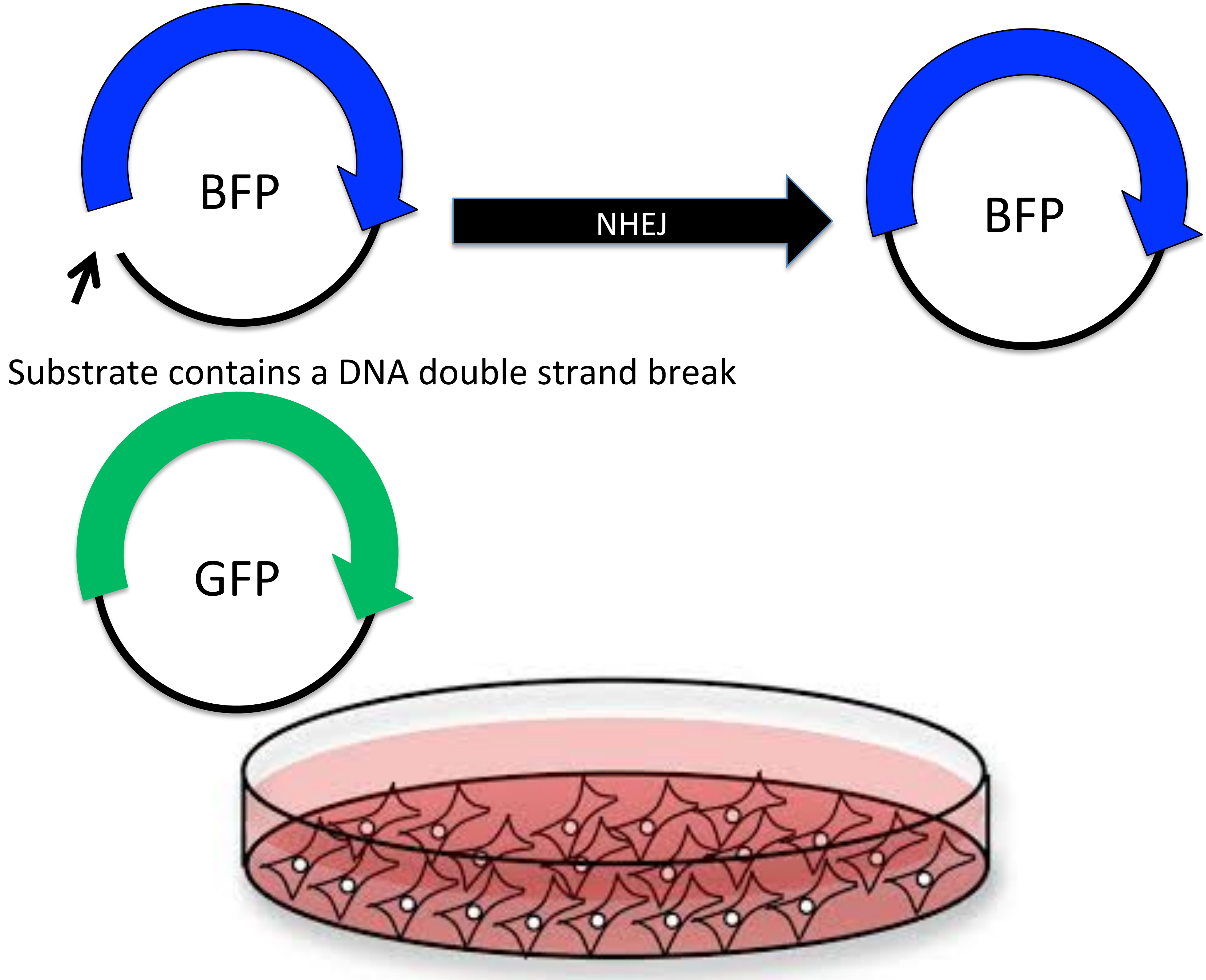


Following digest, the substrate contains a DSB in the 5' UTR that prevents fluorescent reporter expression

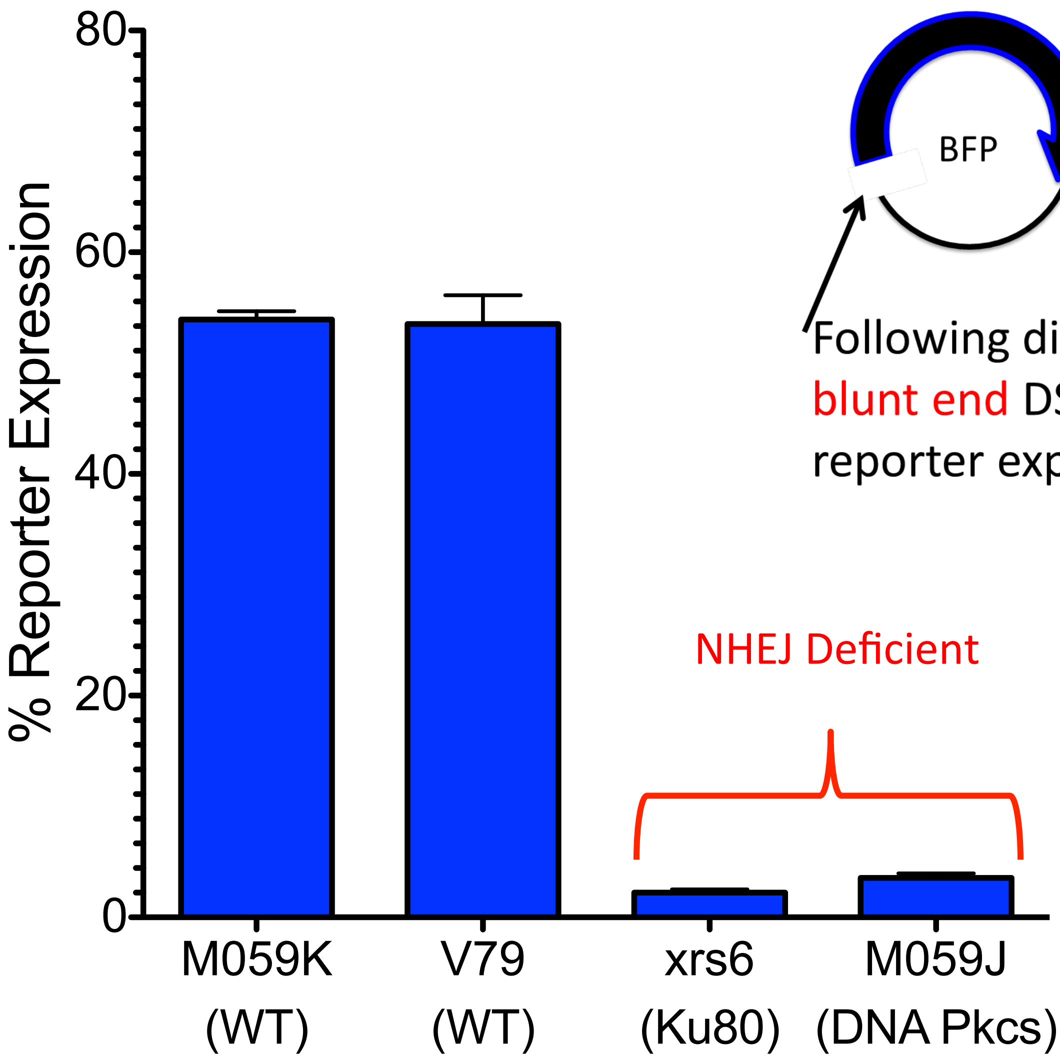


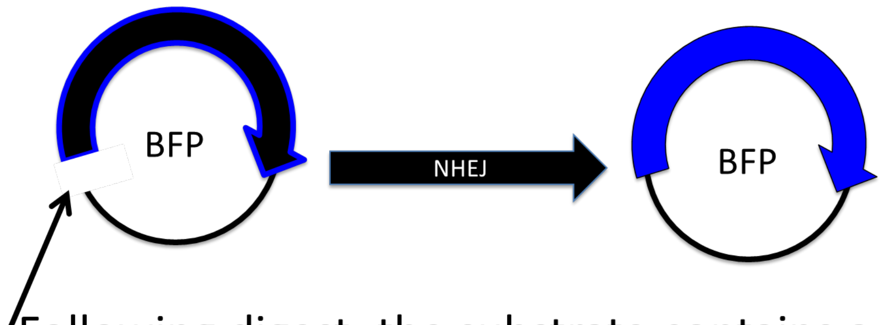
Substrate contains a DNA double strand break




BFP
NHEJ
BFP
Substrate contains a DNA double strand break
GFP

NHEJ HCR in WT and NHEJ defective cells at 18 hours post-transfection:

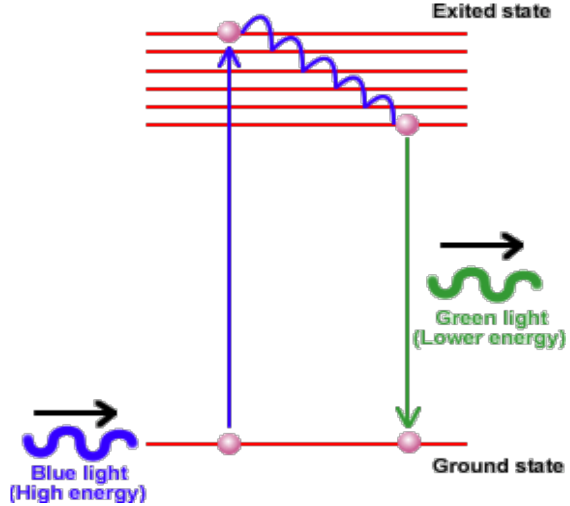


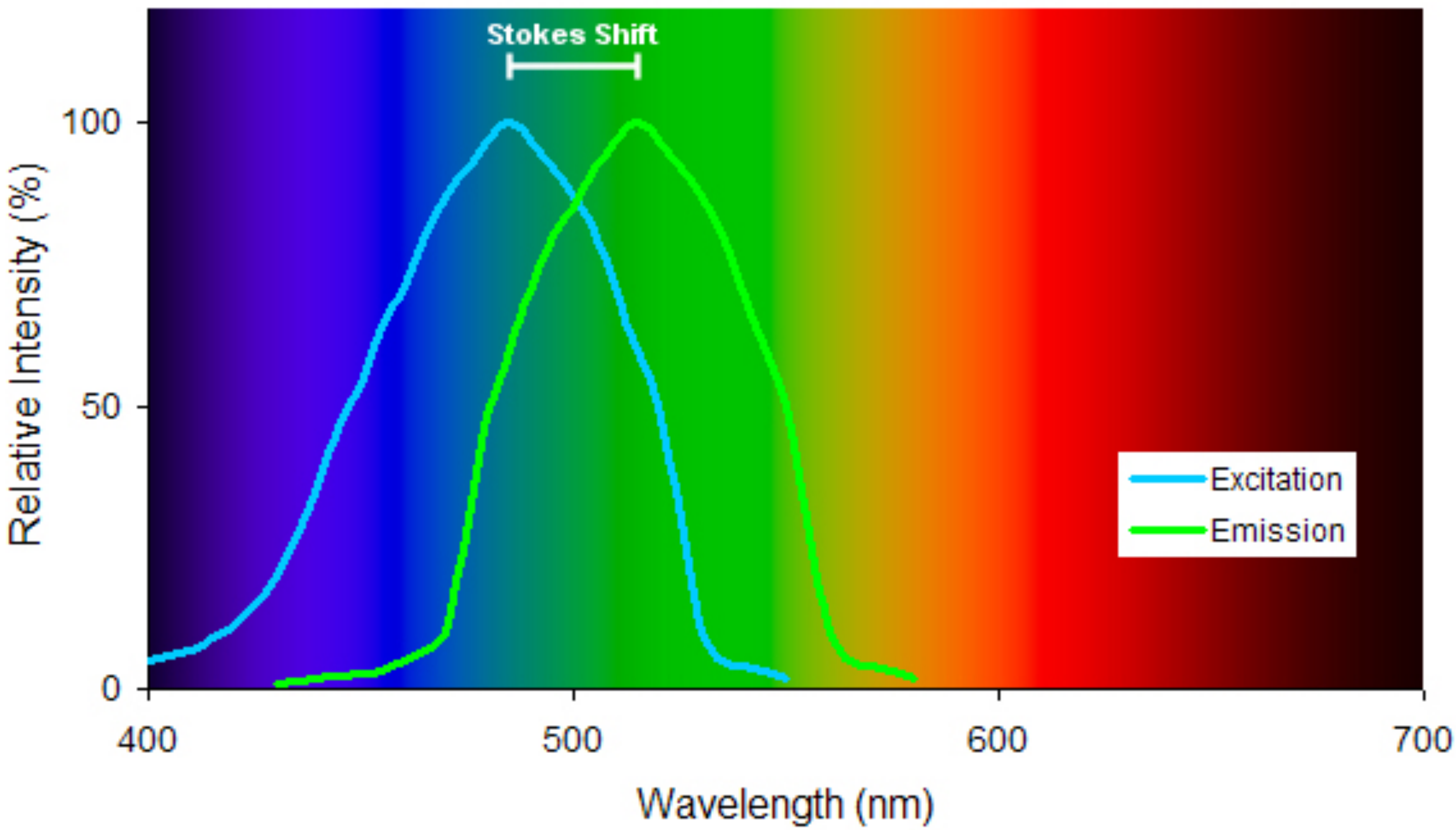


Following digest, the substrate contains a blunt end DSB that prevents fluorescent reporter expression

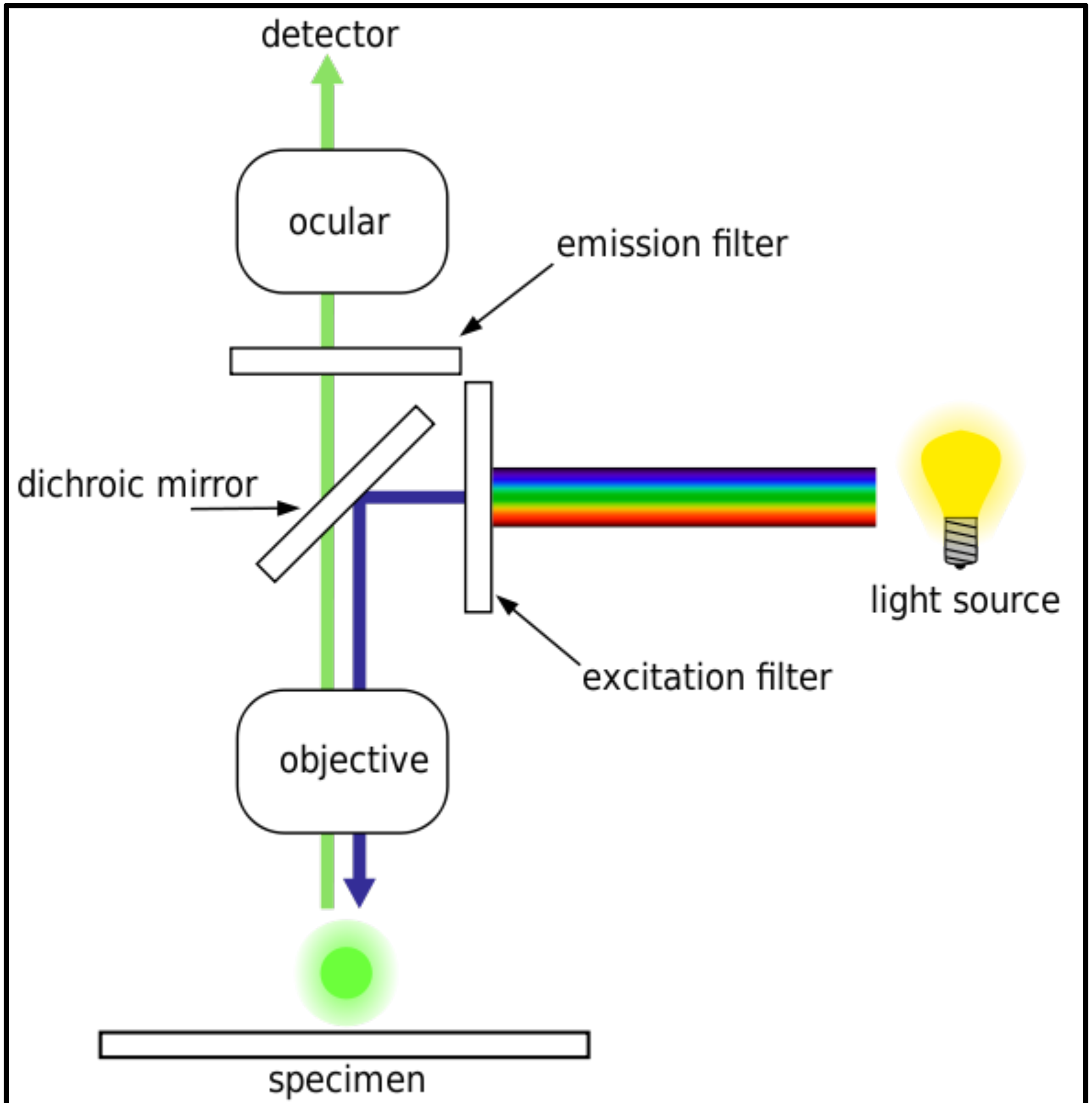
Emission

Excitation

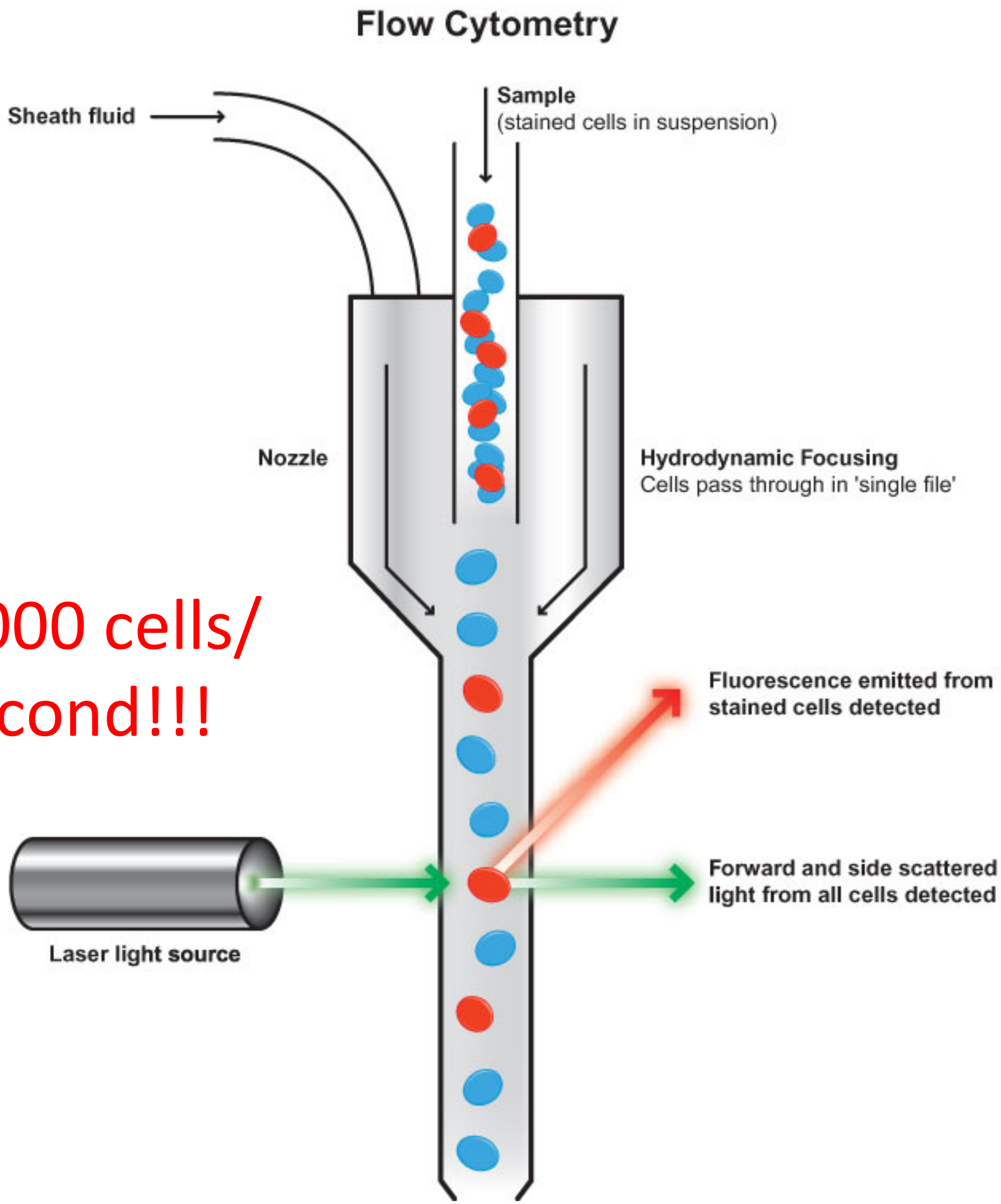




Fluorescence Detection

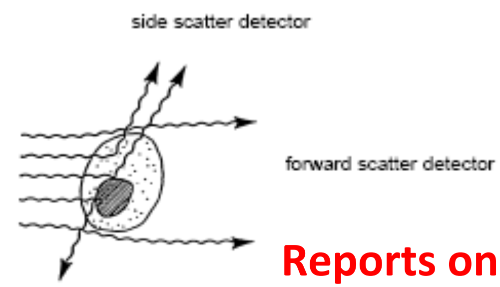


Flow Cytometry

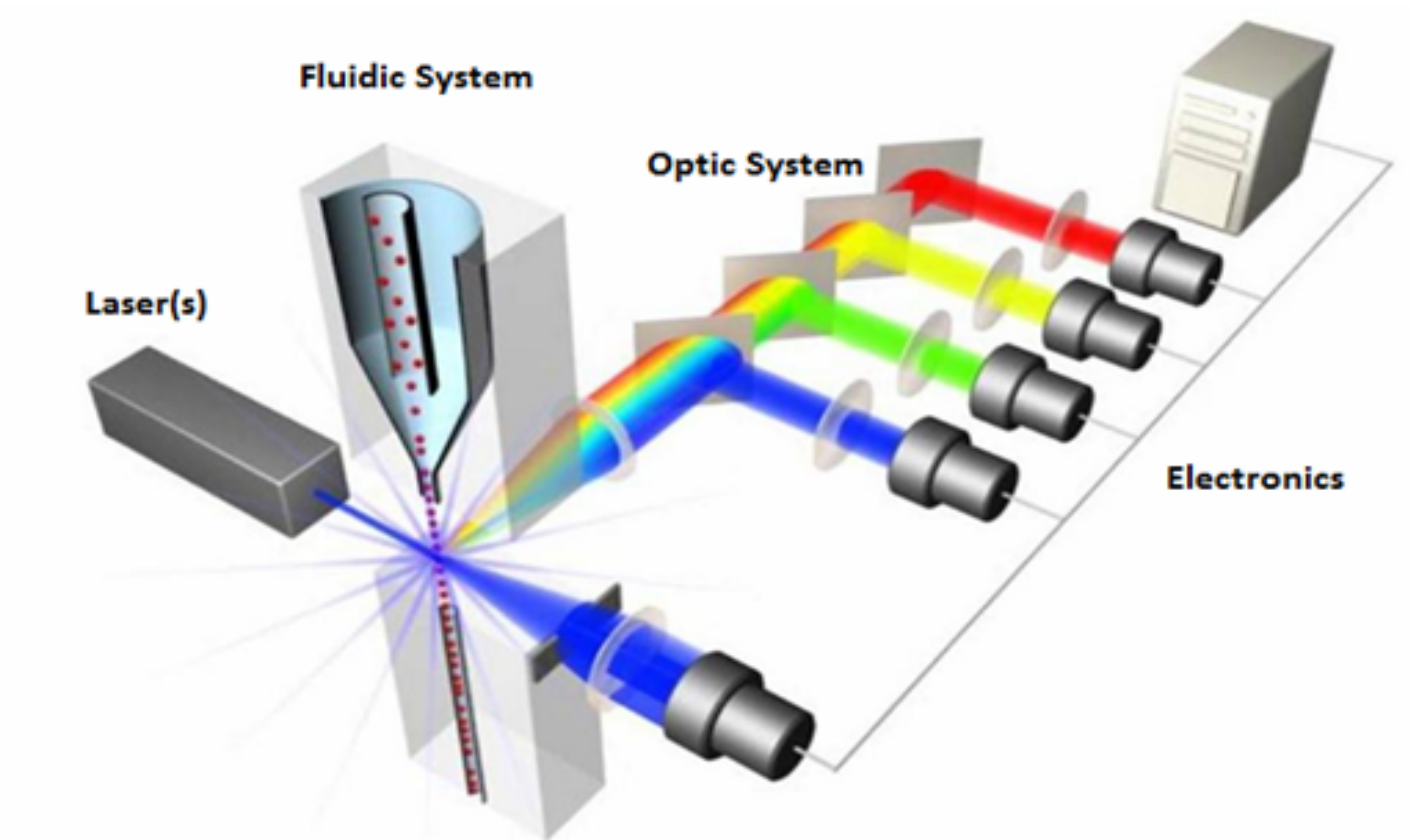


50,000 cells/ second!!!

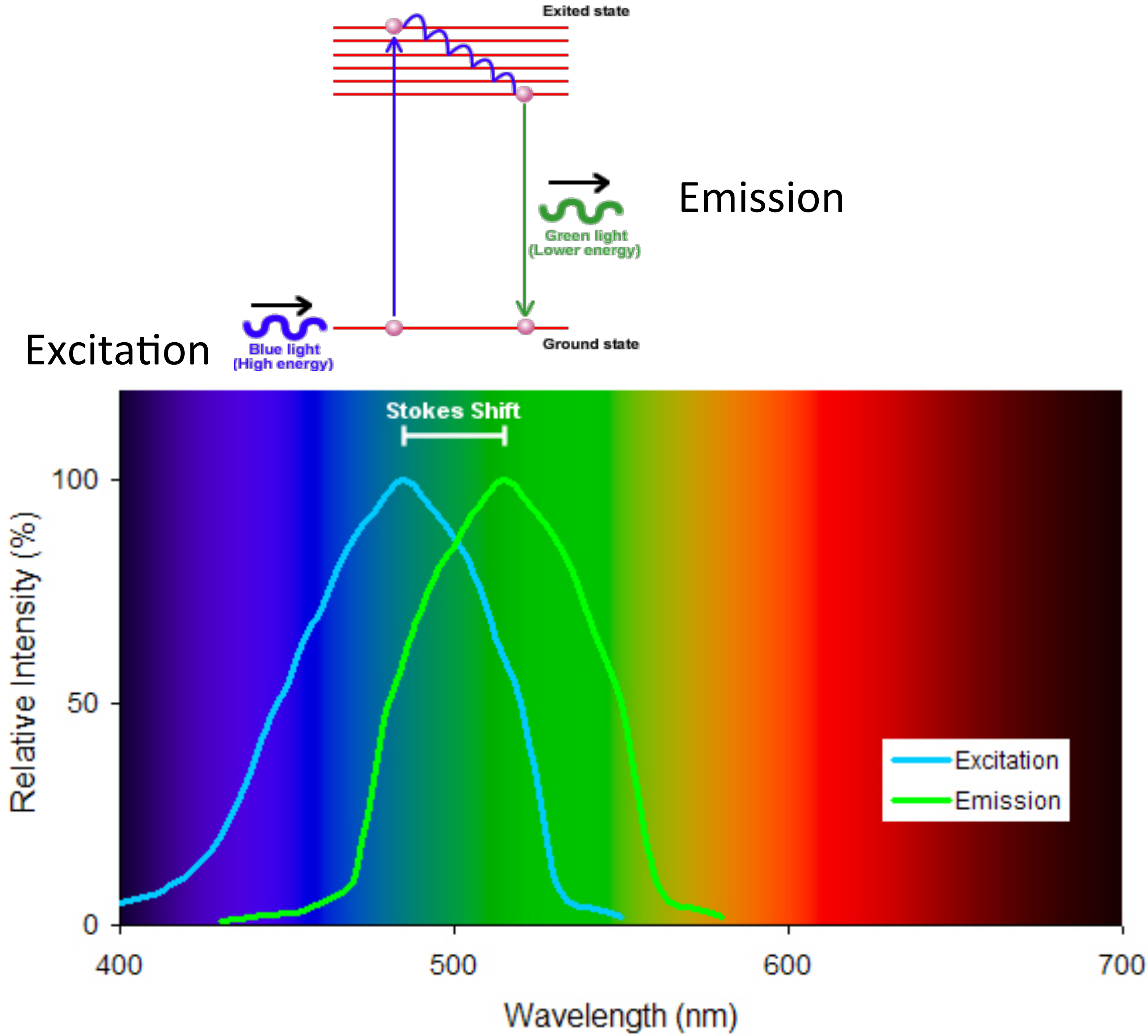
Reports on cell complexity



Reports on cell size

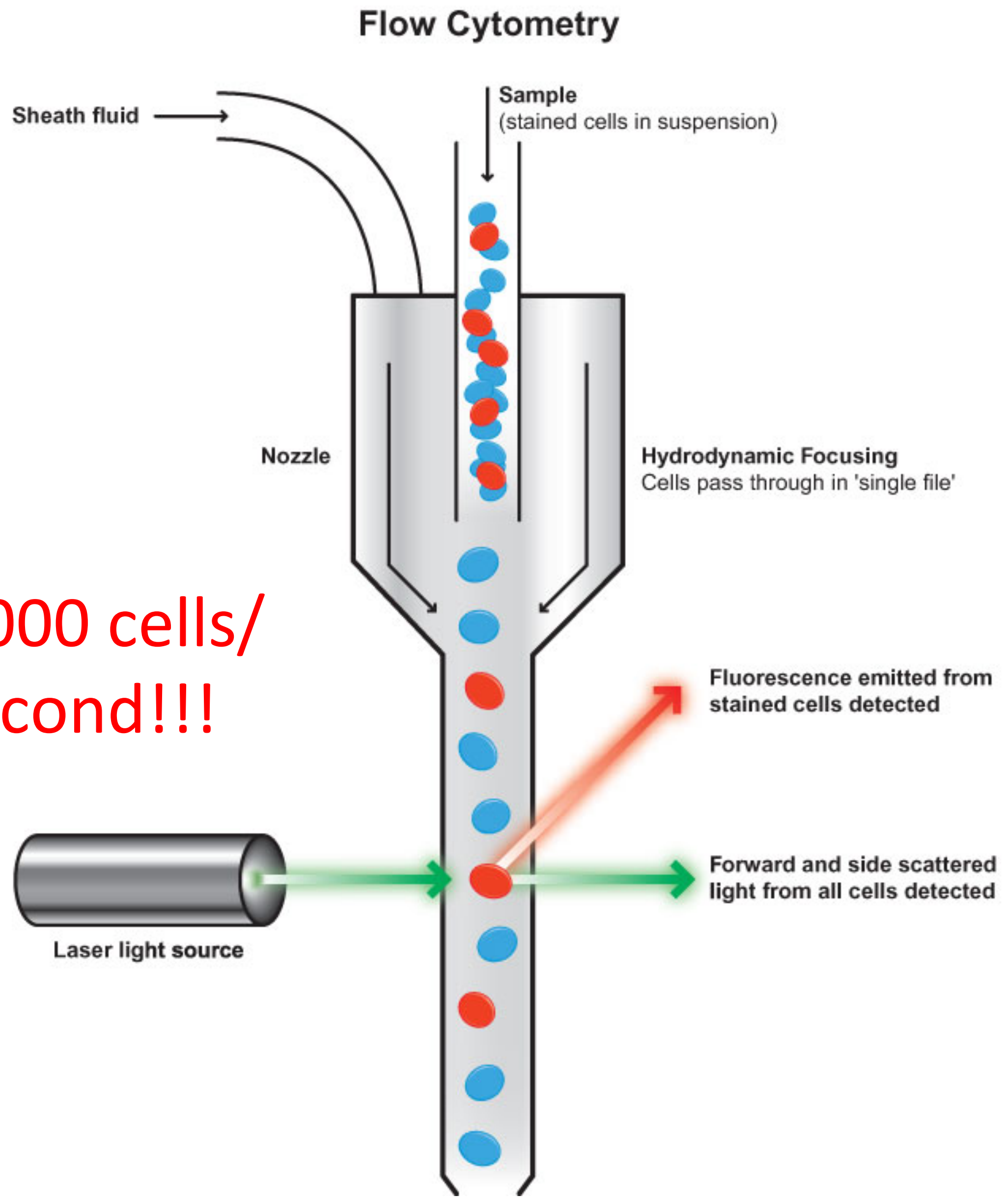


Fluidic System
Optic System
Laser(s)
Electronics



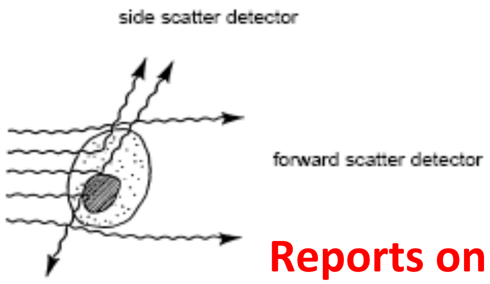
Exited state
Emission
Green light
(Lower energy)
Excitation
Blue light
(High energy)
Ground state
Stokes Shift
100
50
0
Relative Intensity (%)
400
500
600
700
Wavelength (nm)
Excitation
Emission

Flow Cytometry



50,000 cells/ second!!!

Reports on cell complexity



Reports on cell size

Flow Cytometry

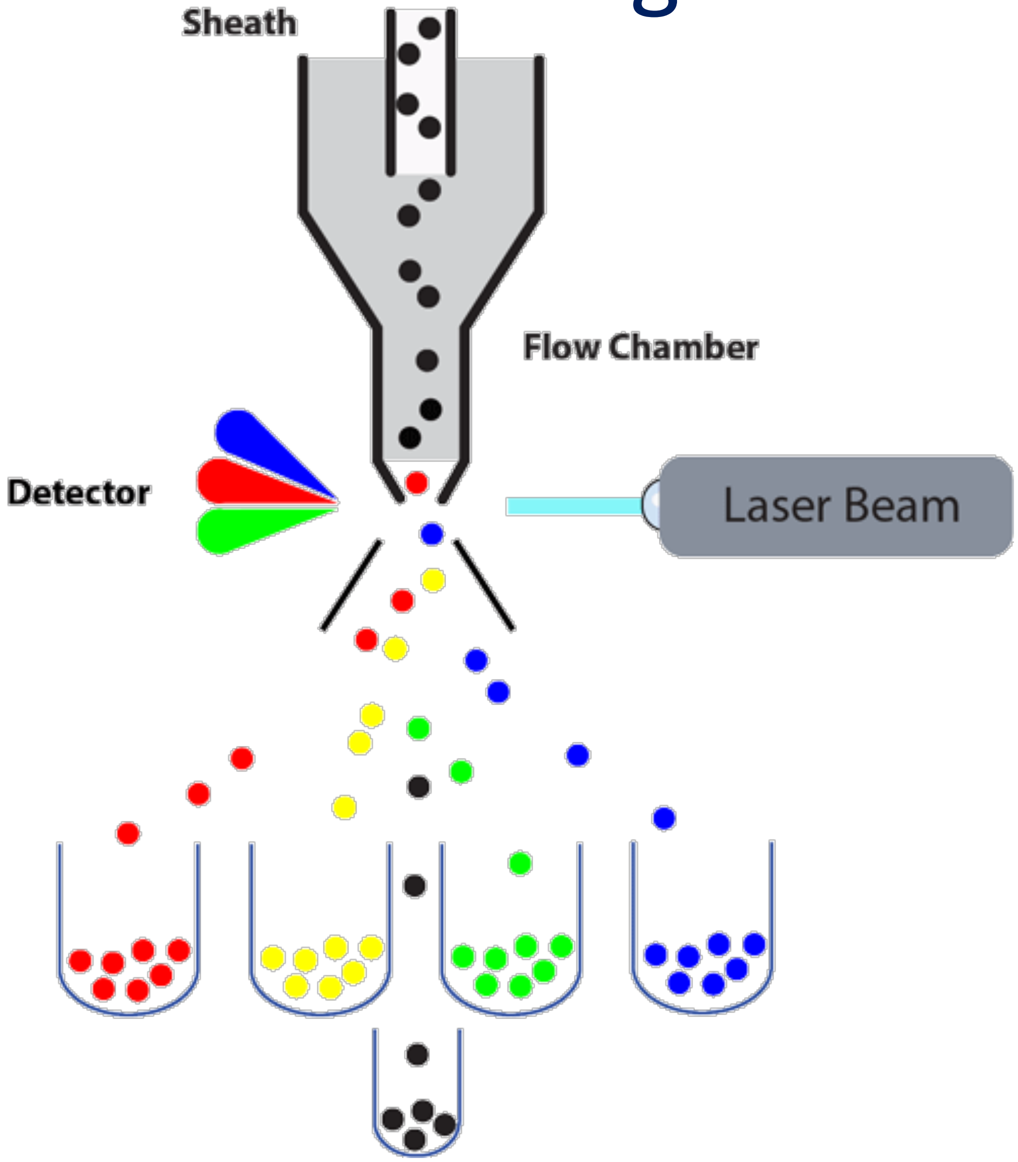
Flow cytometry analyzes cells one by one

Fluorescence, diffracted and reflected light can be measured for each cell

Multiple lasers and multiple colors can be analyzed at millions of cells per minute

Resulting plots show the relative level of fluorescence of each cell for specific wave lengths.

FACS – Fluorescence Activated Cell Sorting



20.109 Spring 2015 Module 2 – Lecture 5
System Engineering and Protein Foundations





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Nova Pishesha (TA)

Leona Samson (Lectures)

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