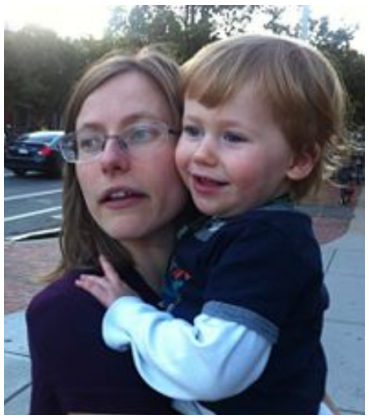
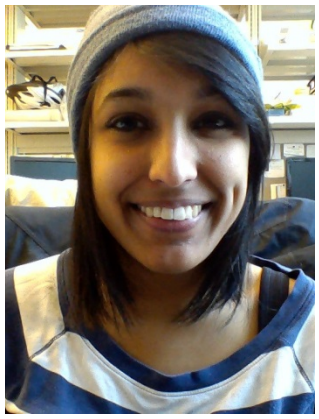
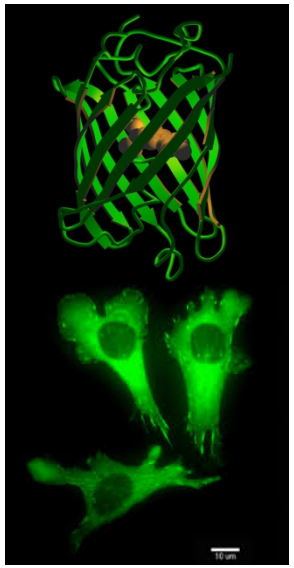
20.109 Spring 2014 Mod 2 – Lecture 5
System Engineering and Protein Foundations



Agi Stachowiak
Shannon Hughes
Aneesh Ramaswamy
Suhani Vora (TA)
Leona Samson (Lectures)
Zachary Nagel (help with development)

What experimental question will you ask in Module 2?

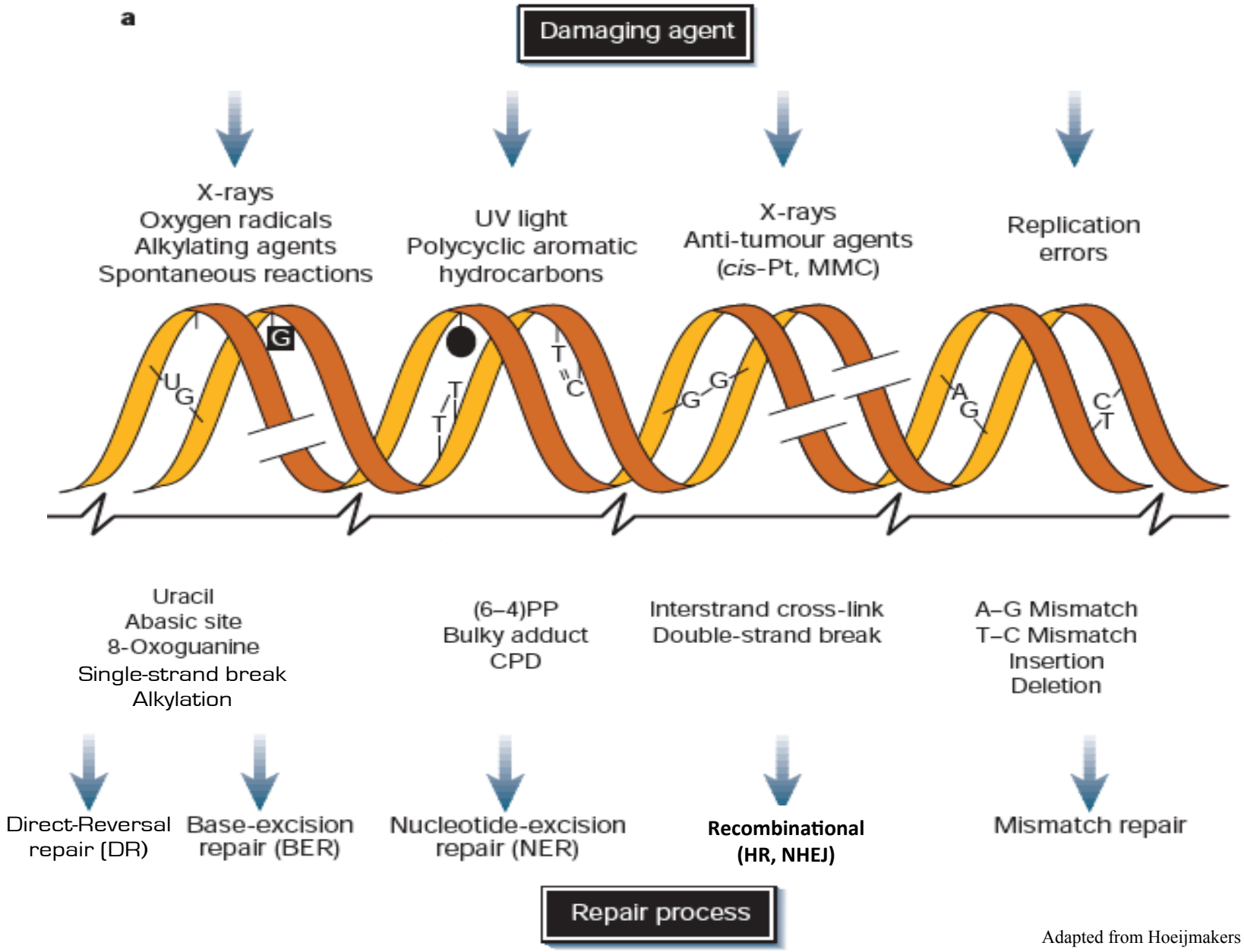


How efficiently does DNA repair by the Non Homologous End Joining (NHEJ) pathway act on DNA damage with different topologies?

This raises the following questions

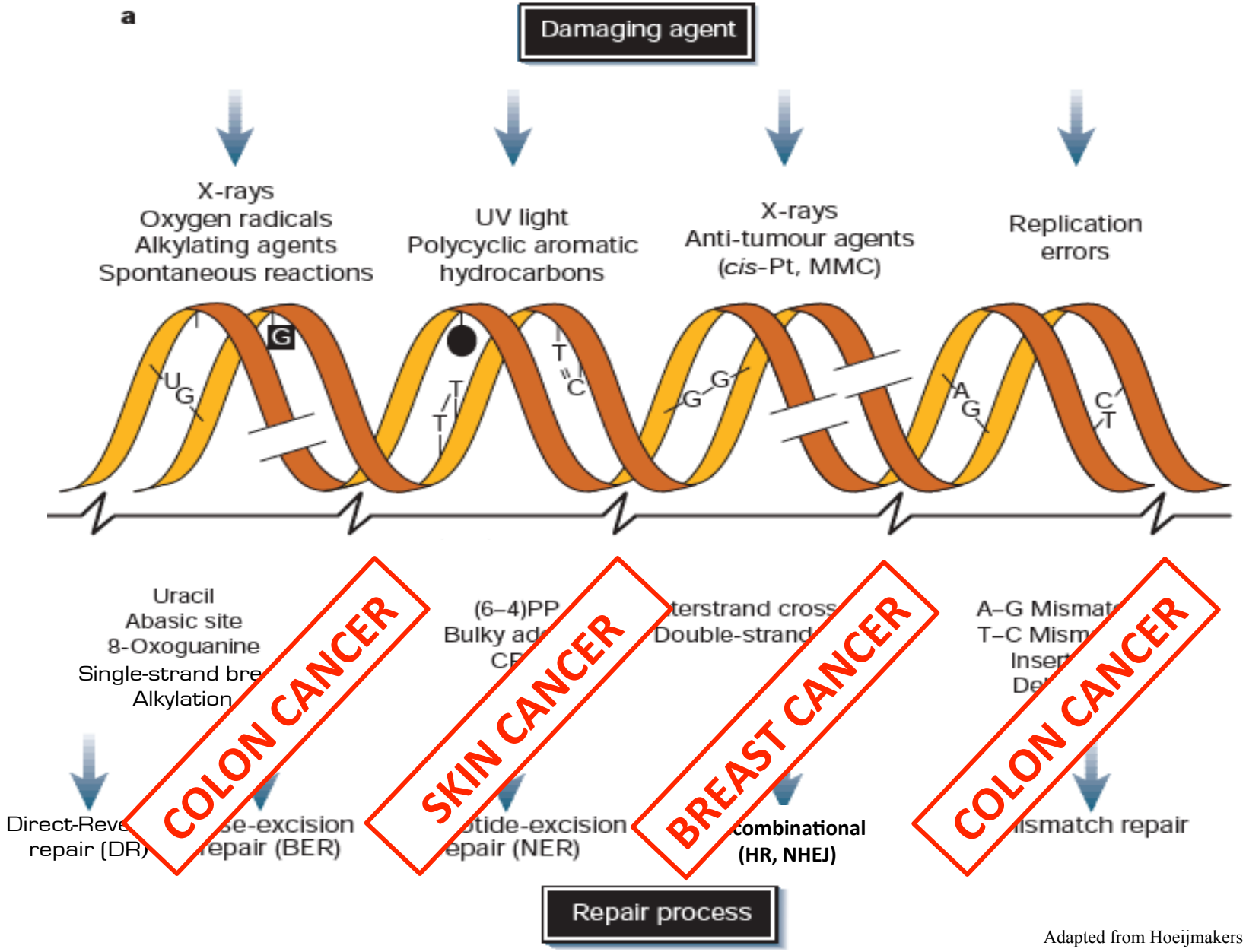
- How does DNA get damaged?
- What is DNA repair?
- Why does DNA repair exist?
- Why do we care about how efficient DNA repair is?
- How does one actually measure DNA repair efficiency?

DNA Damage and Repair



Adapted from Hoeijmakers **2001** *Nature*

DNA Damage and Repair



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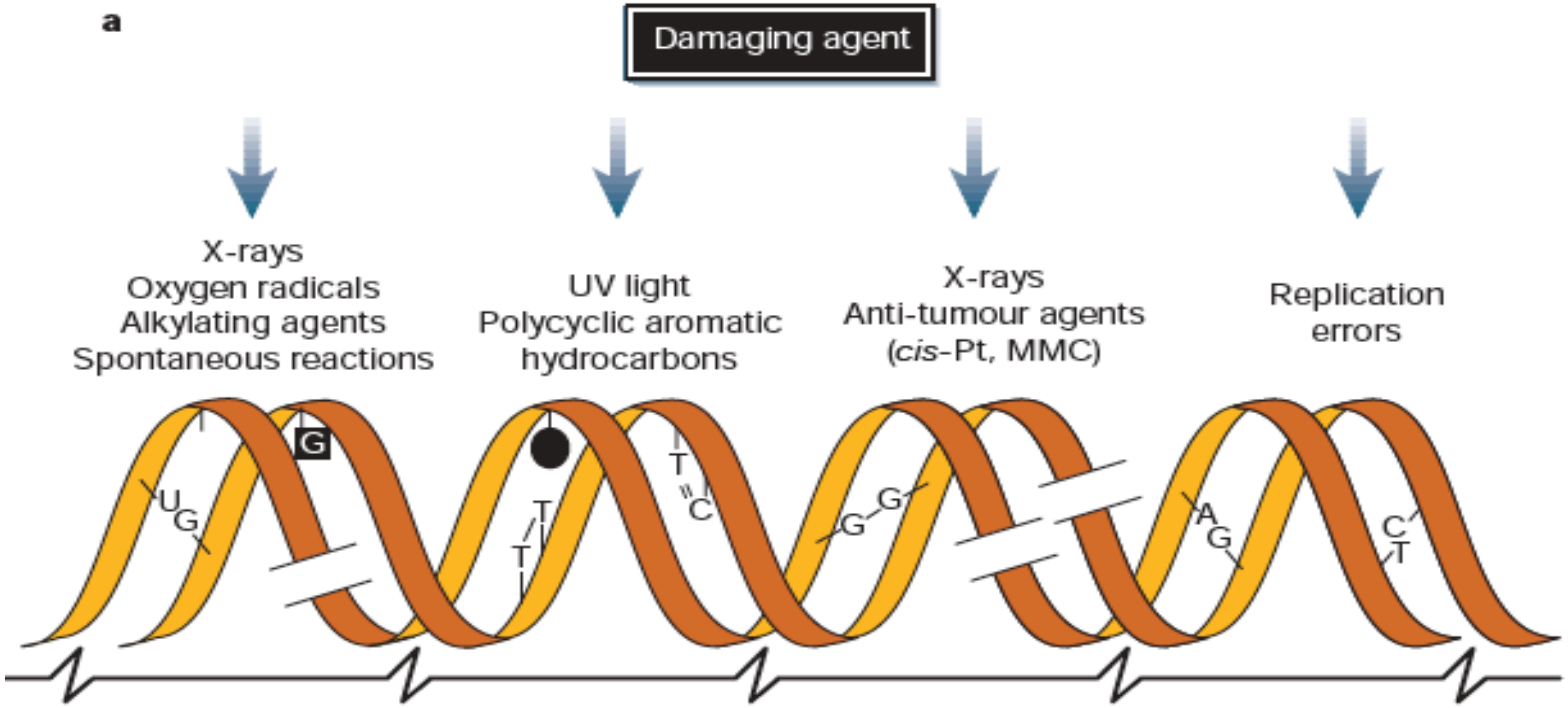
DNA Damage and Repair



RESPONSES of TUMOR and NON-TUMOR CELLS to CANCER RADIOTHERAPY and CHEMOTHERAPY

Direct-Reversal repair (DR)

Base-excision repair (BER)

Nucleotide-excision repair (NER)

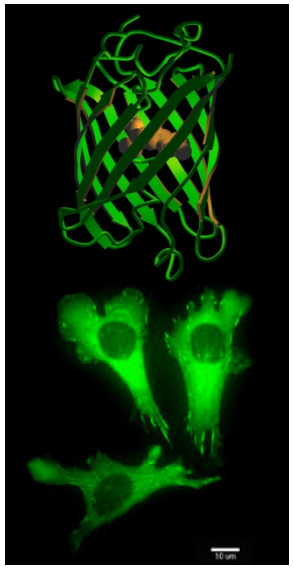
Recombinational (HR, NHEJ)

Mismatch repair

Repair process

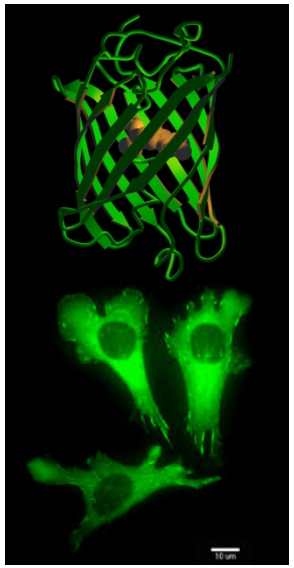
Adapted from Hoeijmakers **2001** *Nature*

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

Key Experimental Methods for Module 2



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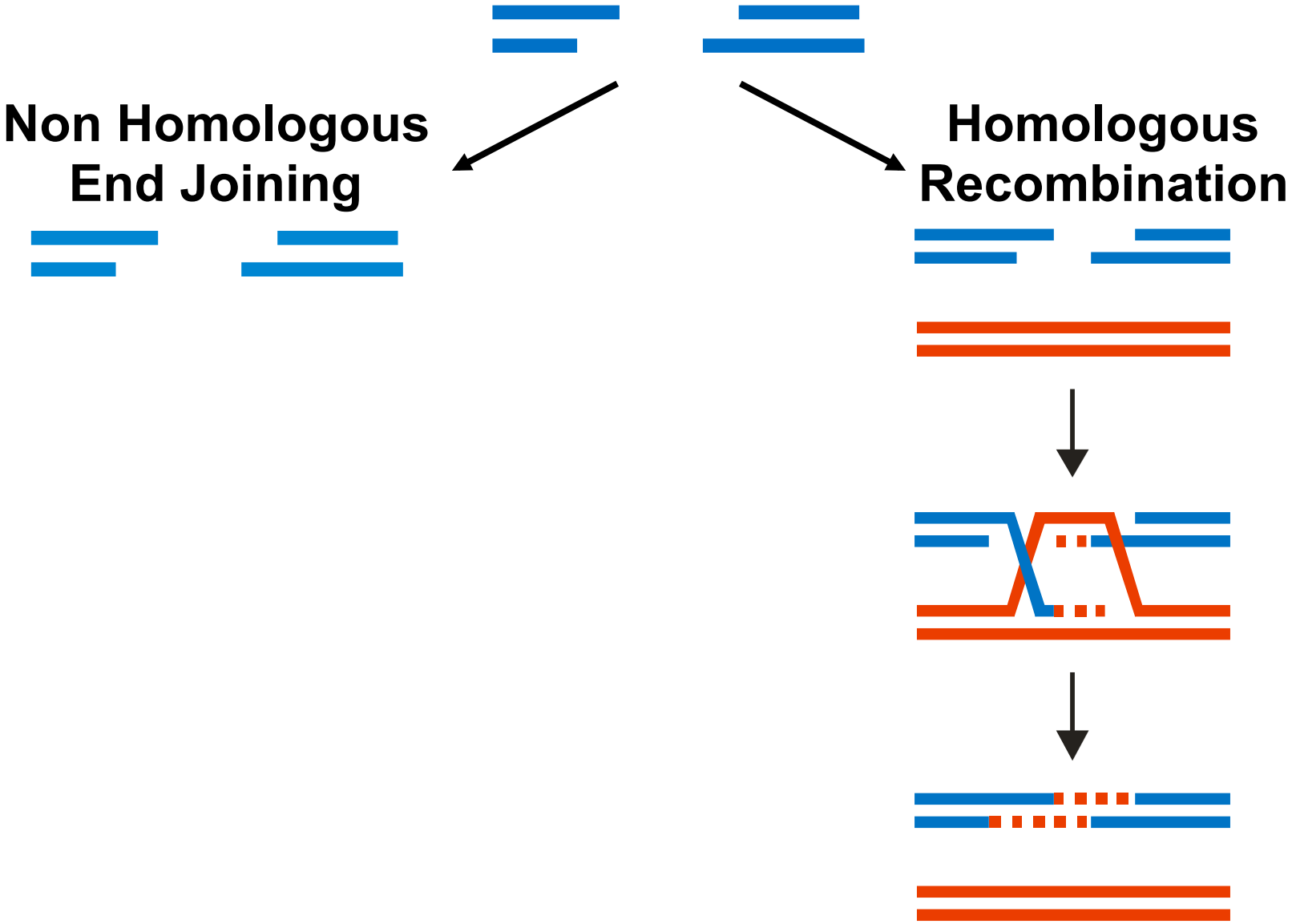
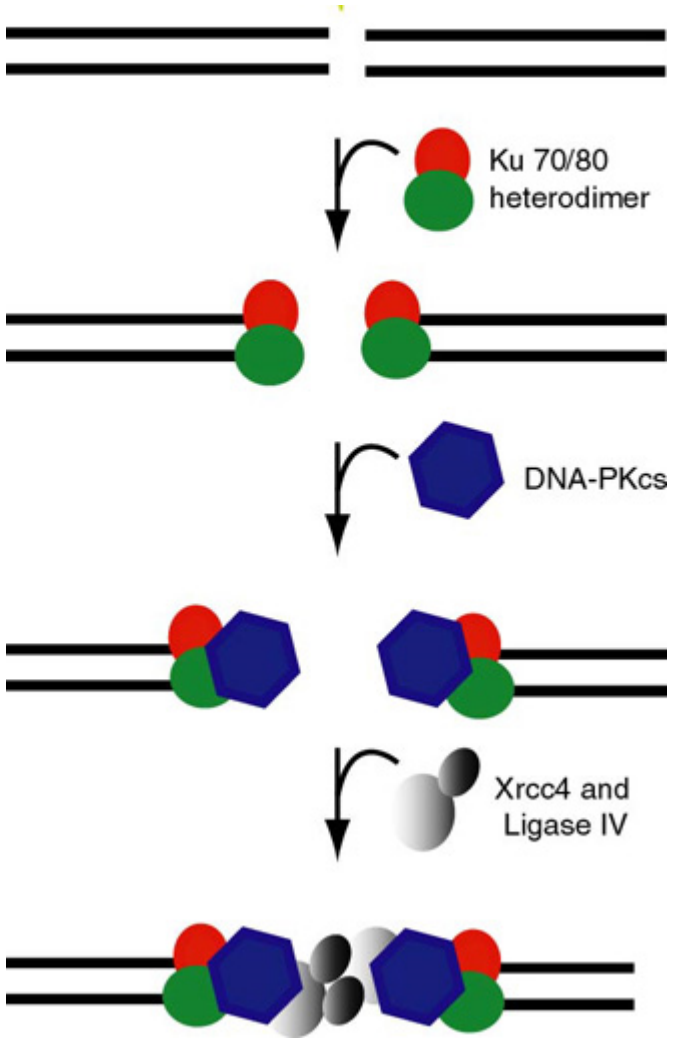
DNA double-strand break repair



Non-Homologous End Joining (NHEJ)

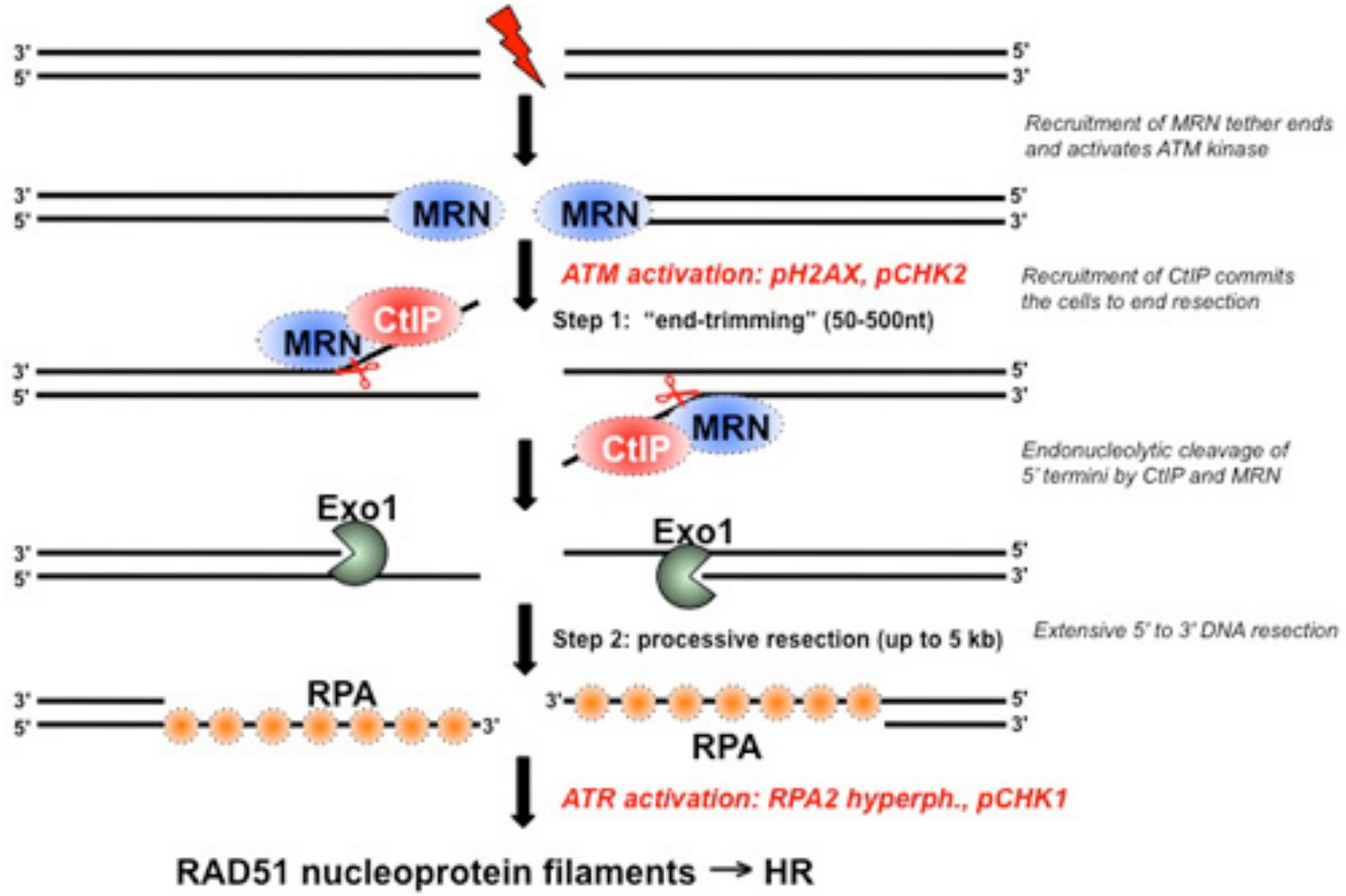


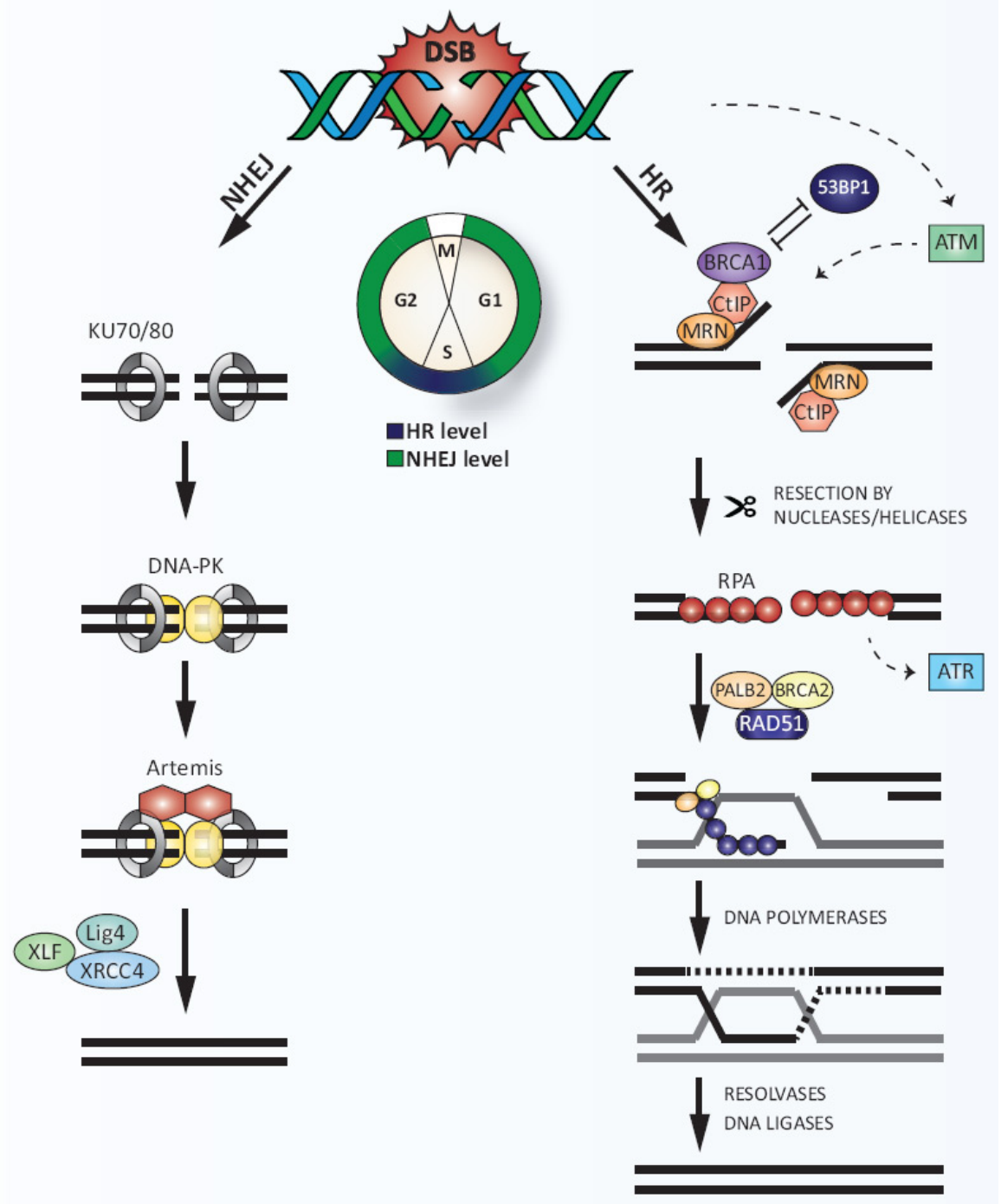
Ku70
Ku80

DNA-PKcs

Xrcc4
Ligase IV

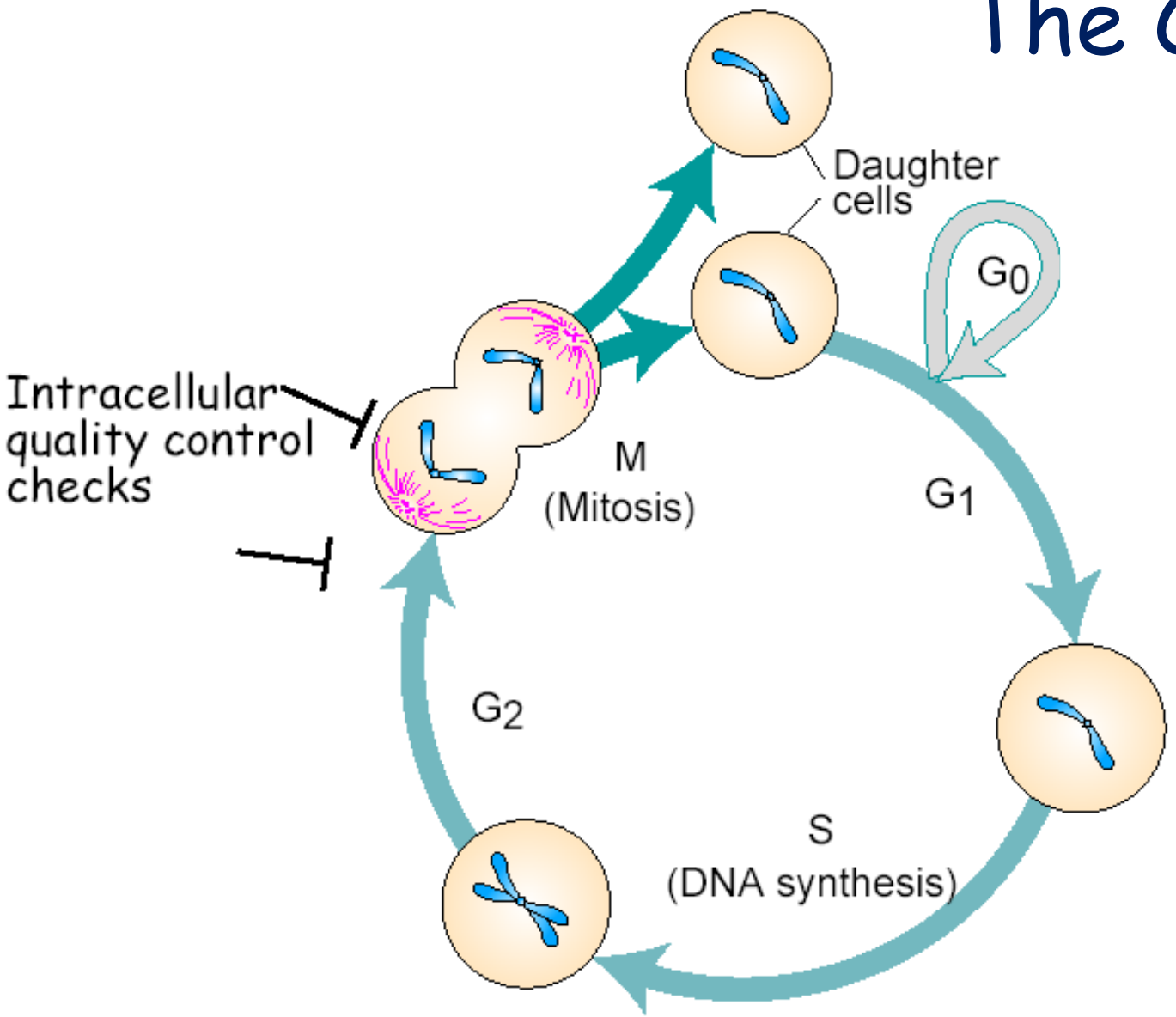
The MRN complex (Mre11/Rad50/Nbs1) competes with Ku for binding DSBs





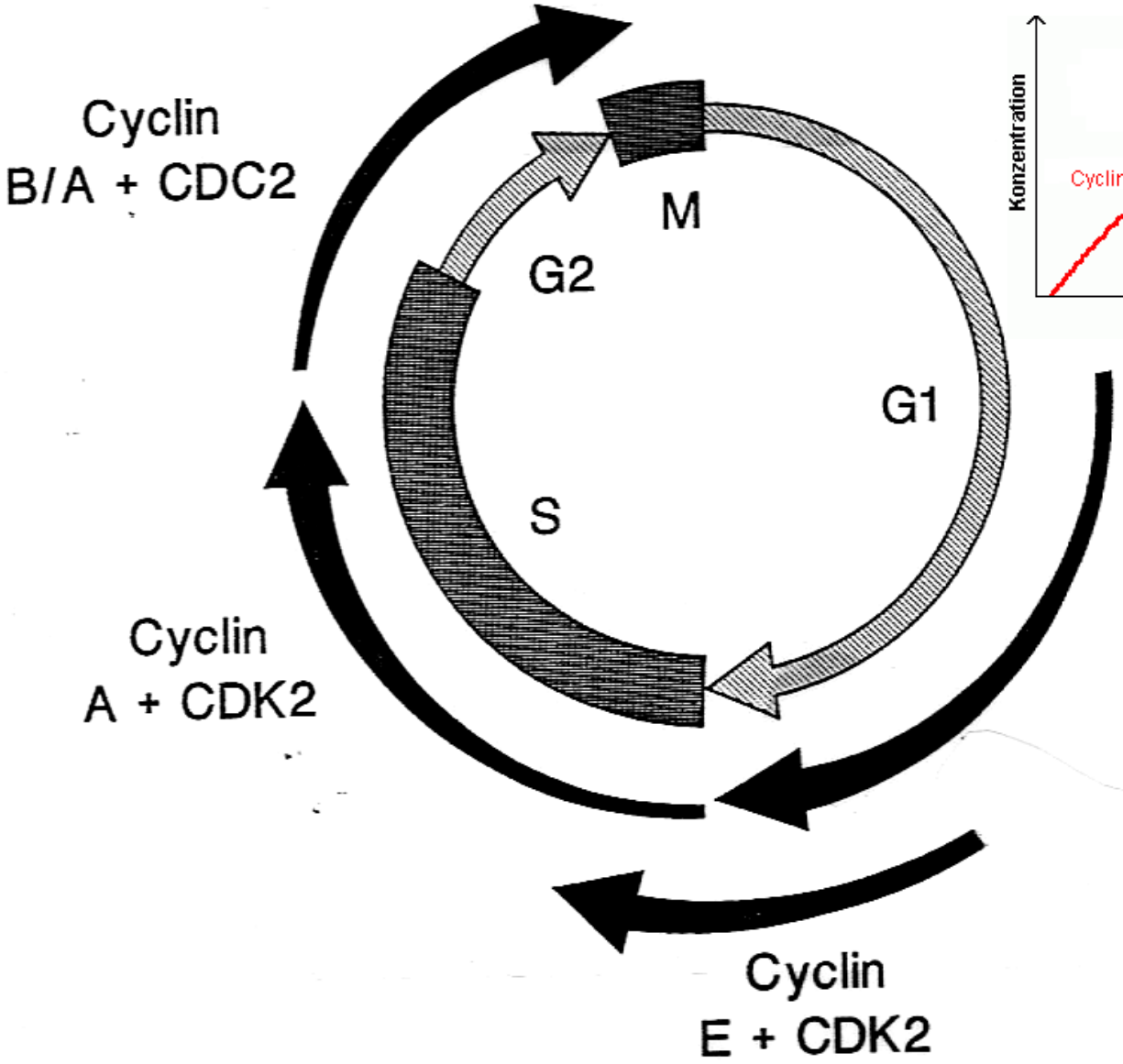
How does the cell decide which pathway to use?

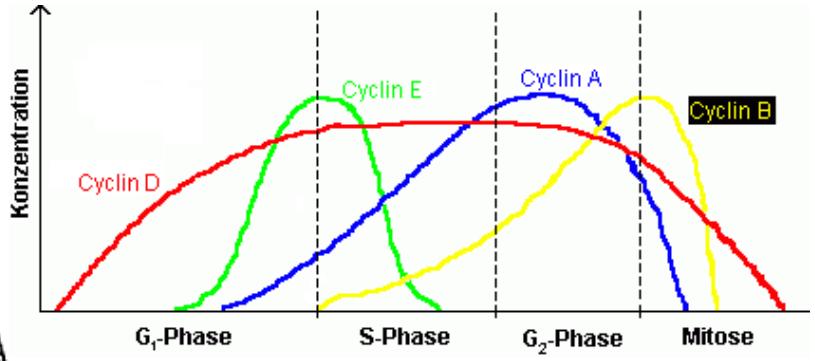
The Cell Cycle

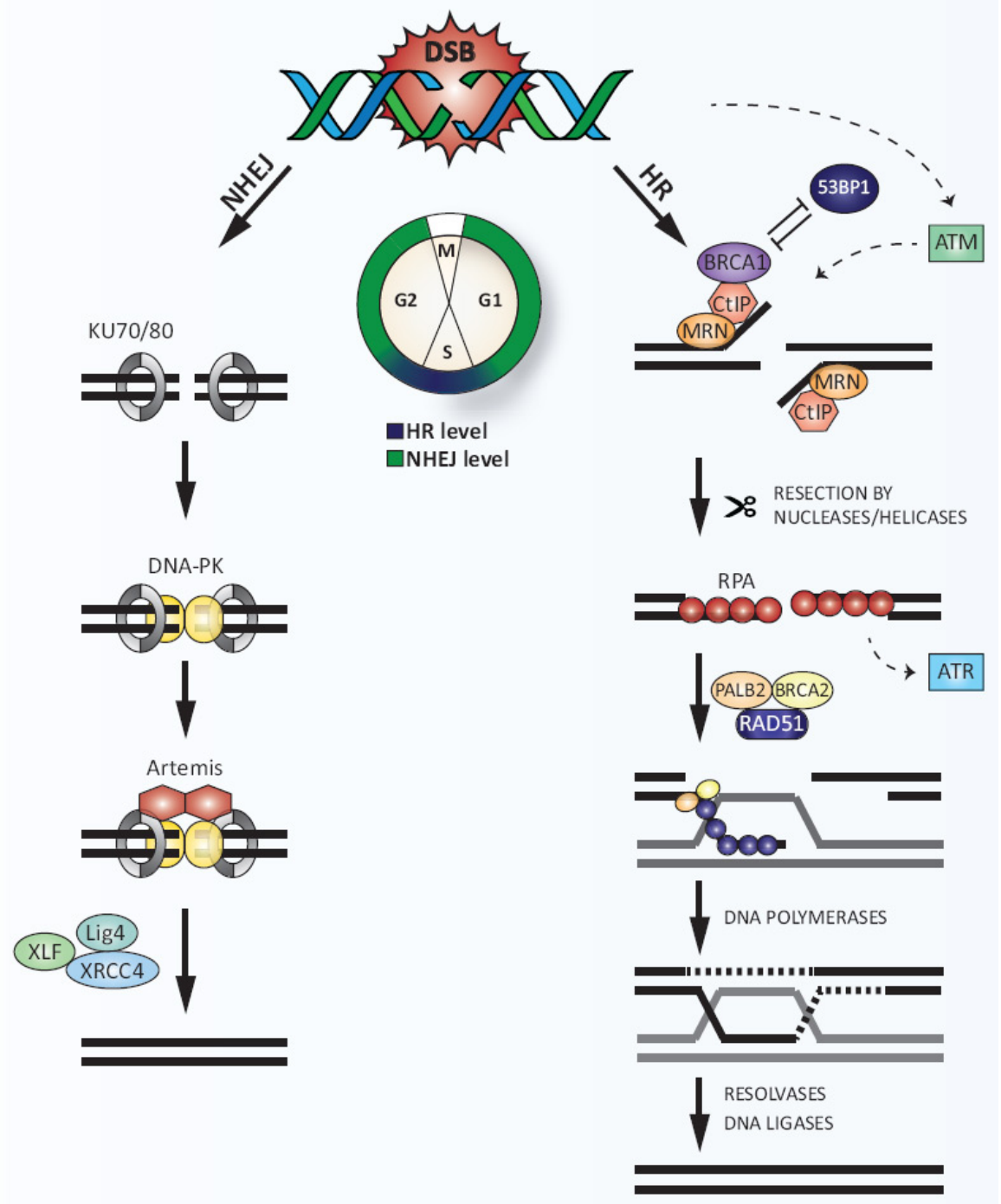


Duplication of chromosomes
DNA Replication

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







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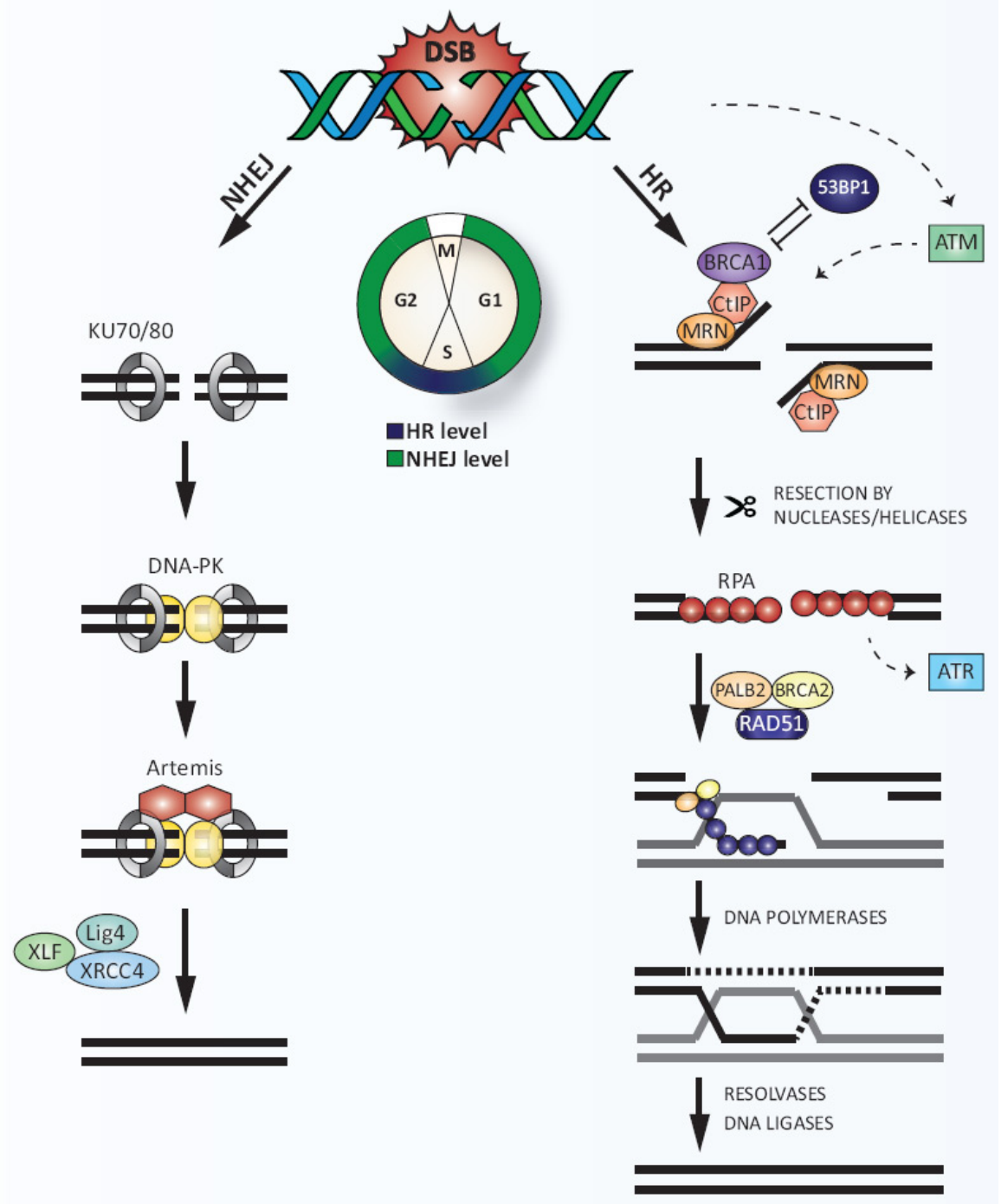
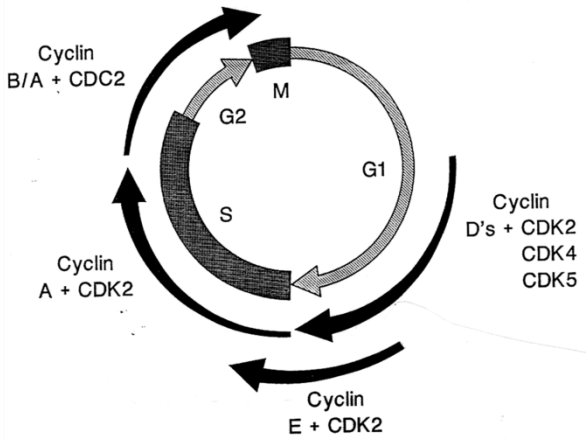
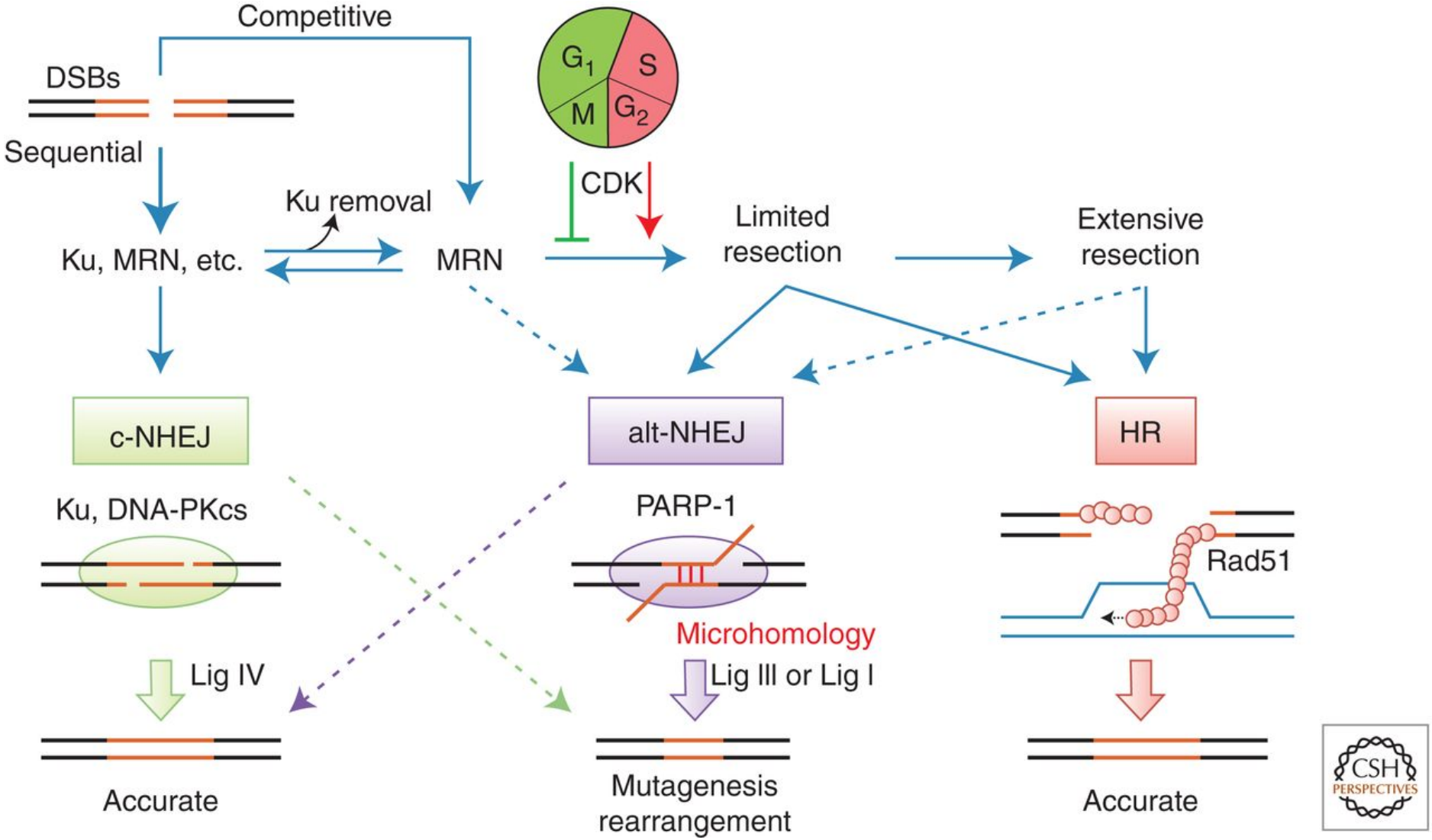


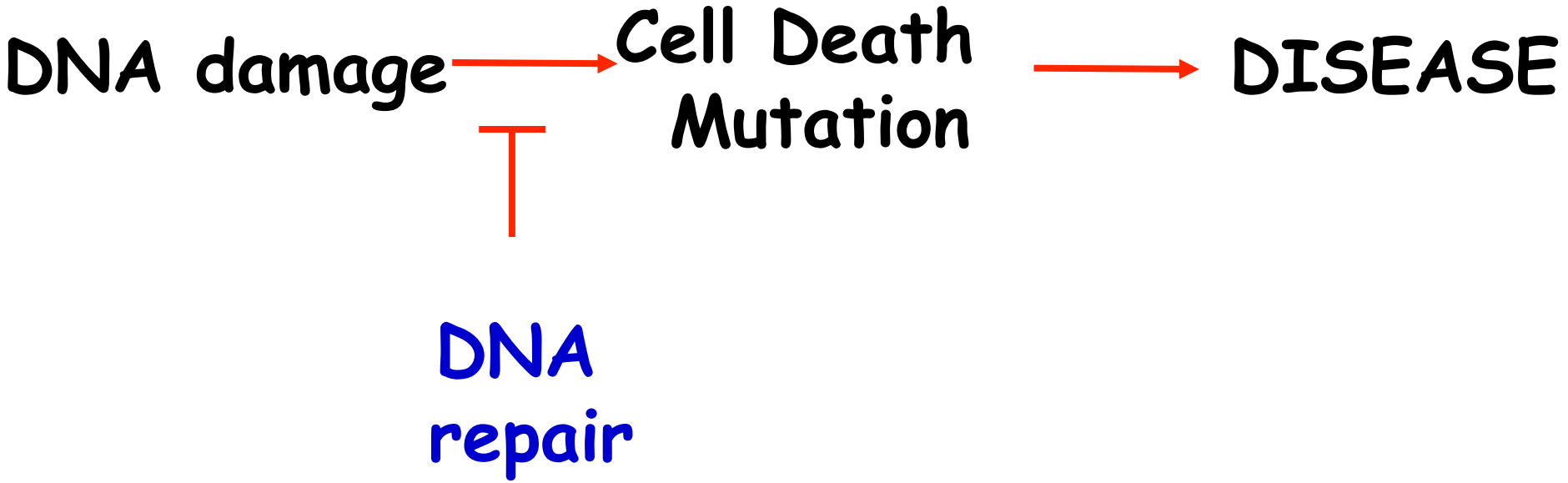
CyclinA-CDK2 targets the CtIP/BRCA1 complex

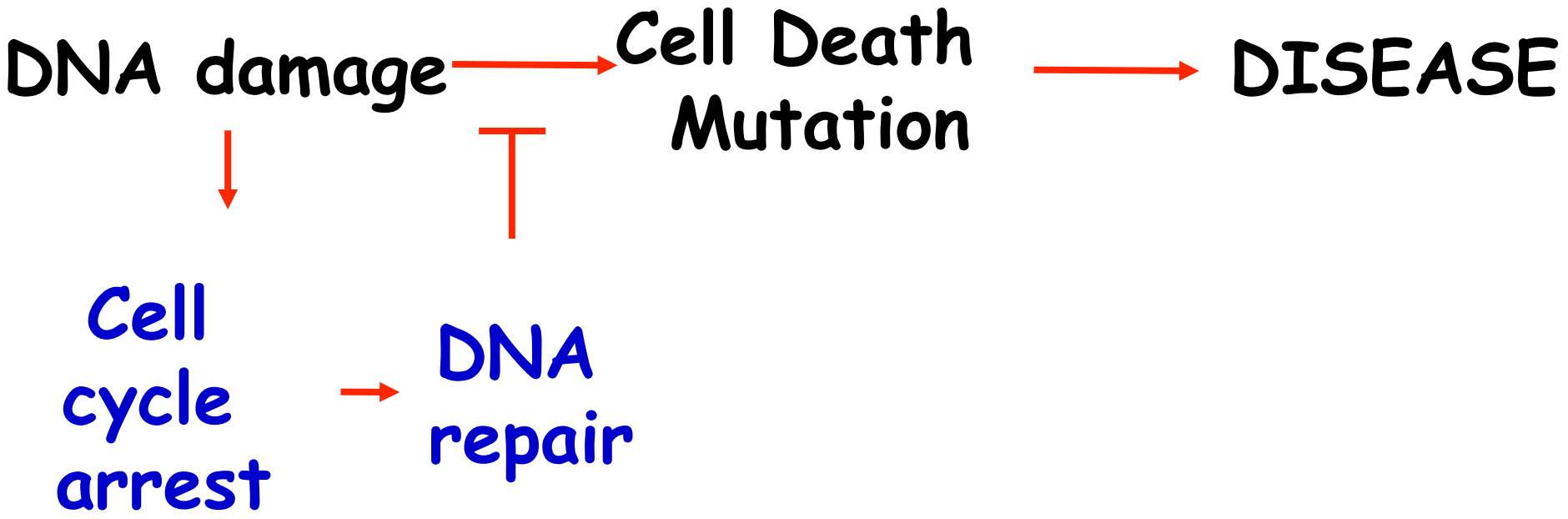


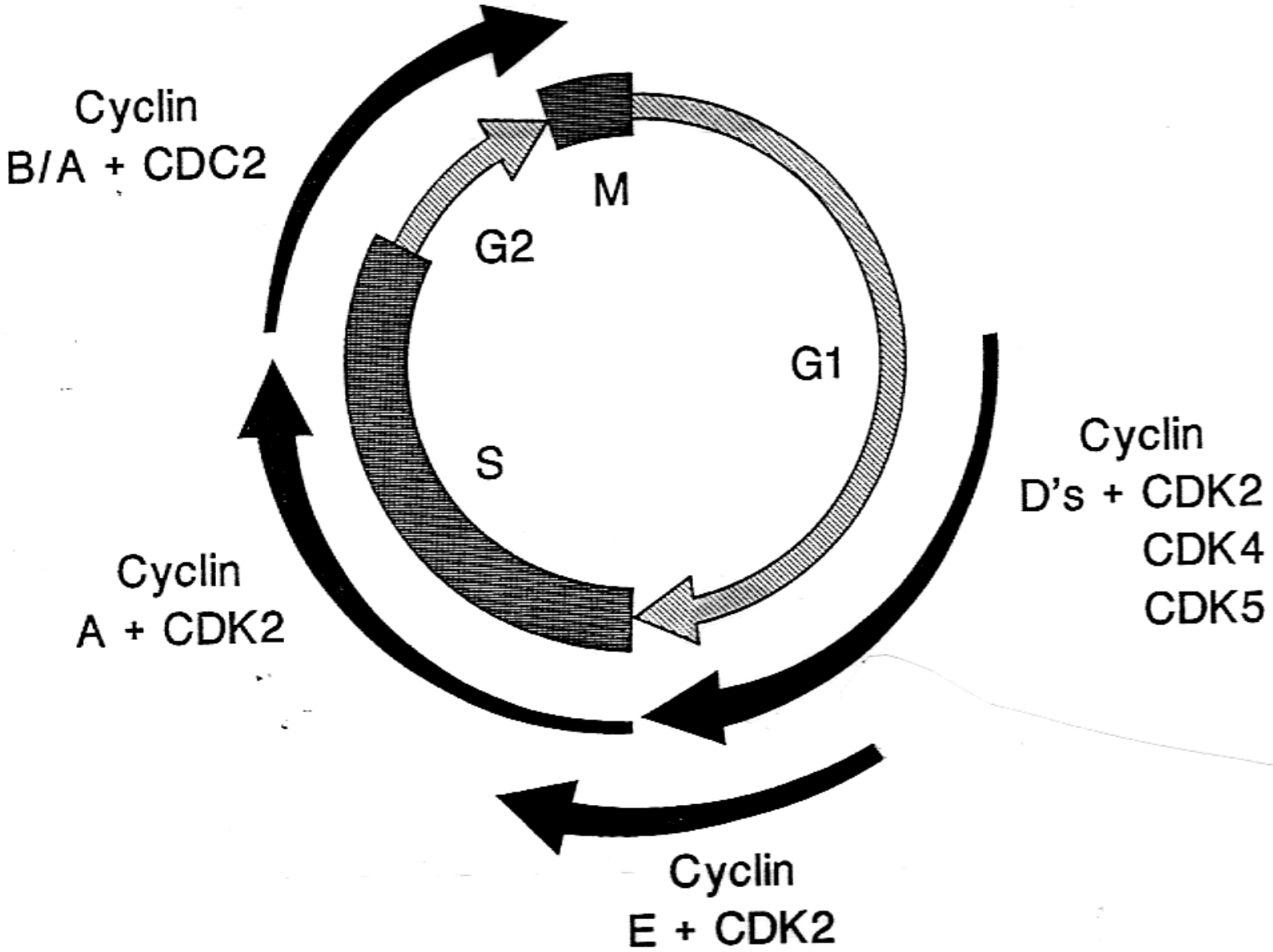
Disposition of DSBs between repair pathways.



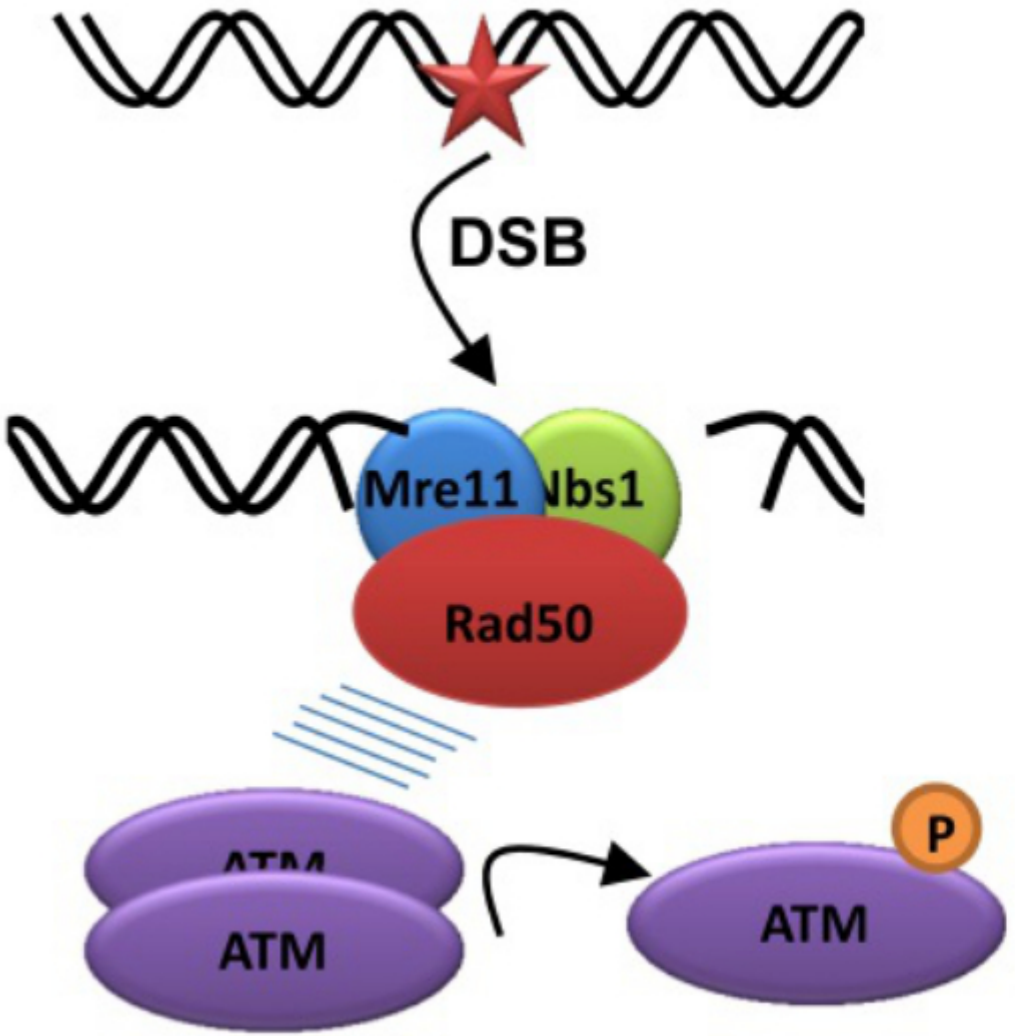
Chiruvella K K et al. Cold Spring Harb Perspect Biol 2013;5:a012757

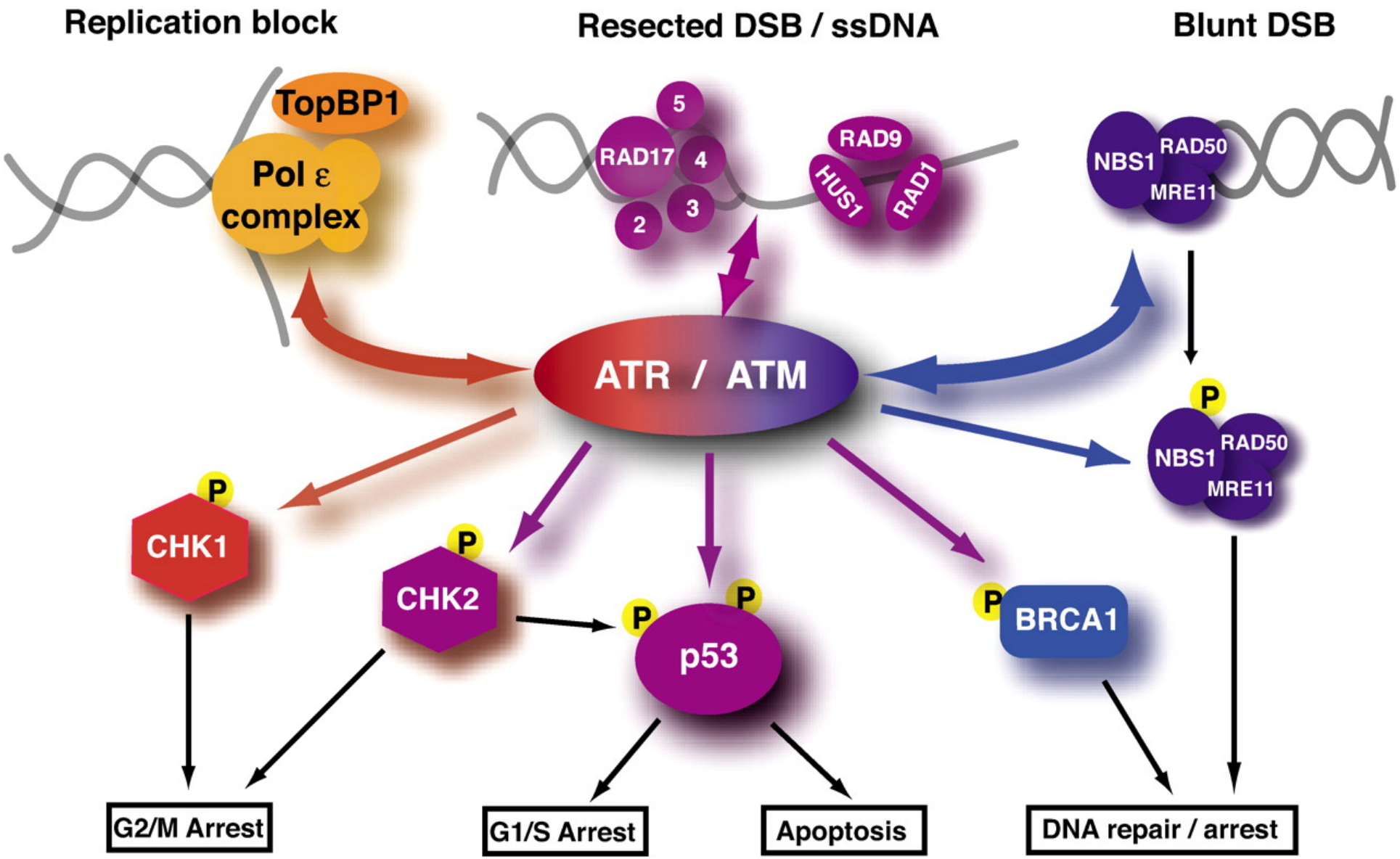

DNA damage
Cell Death
Mutation
DISEASE
DNA
repair


DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair

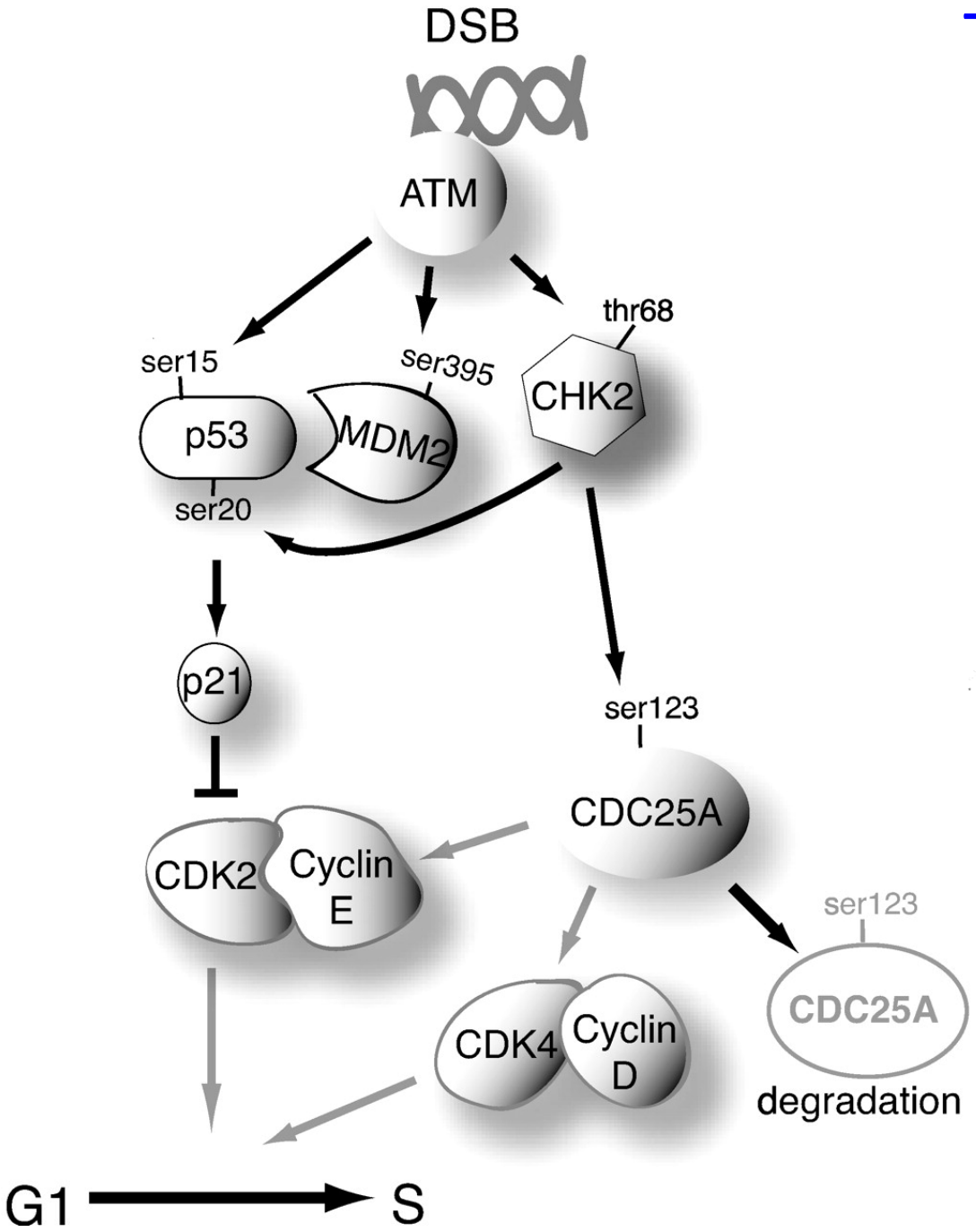

Cyclin
B/A + CDC2
M
G2
G1
S
Cyclin
D's + CDK2
CDK4
CDK5
Cyclin
A + CDK2
Cyclin
E + CDK2

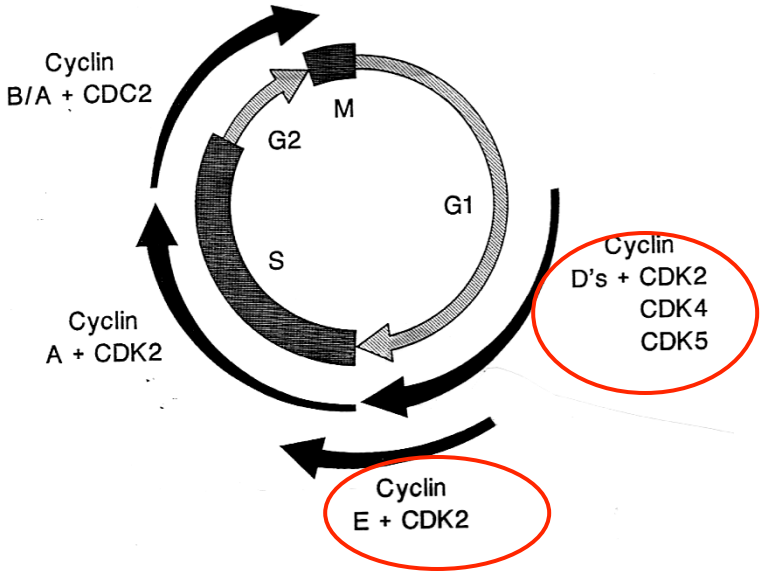
Signaling at DSBs – ATM kinase activated




Replication block
Resected DSB / ssDNA
Blunt DSB
TopBP1
Pol ε complex
5
RAD17
4
3
2
RAD9
HUS1
RAD1
NBS1
RAD50
MRE11
ATR / ATM
P
CHK1
CHK2
p53
BRCA1
G2/M Arrest
G1/S Arrest
Apoptosis
DNA repair / arrest

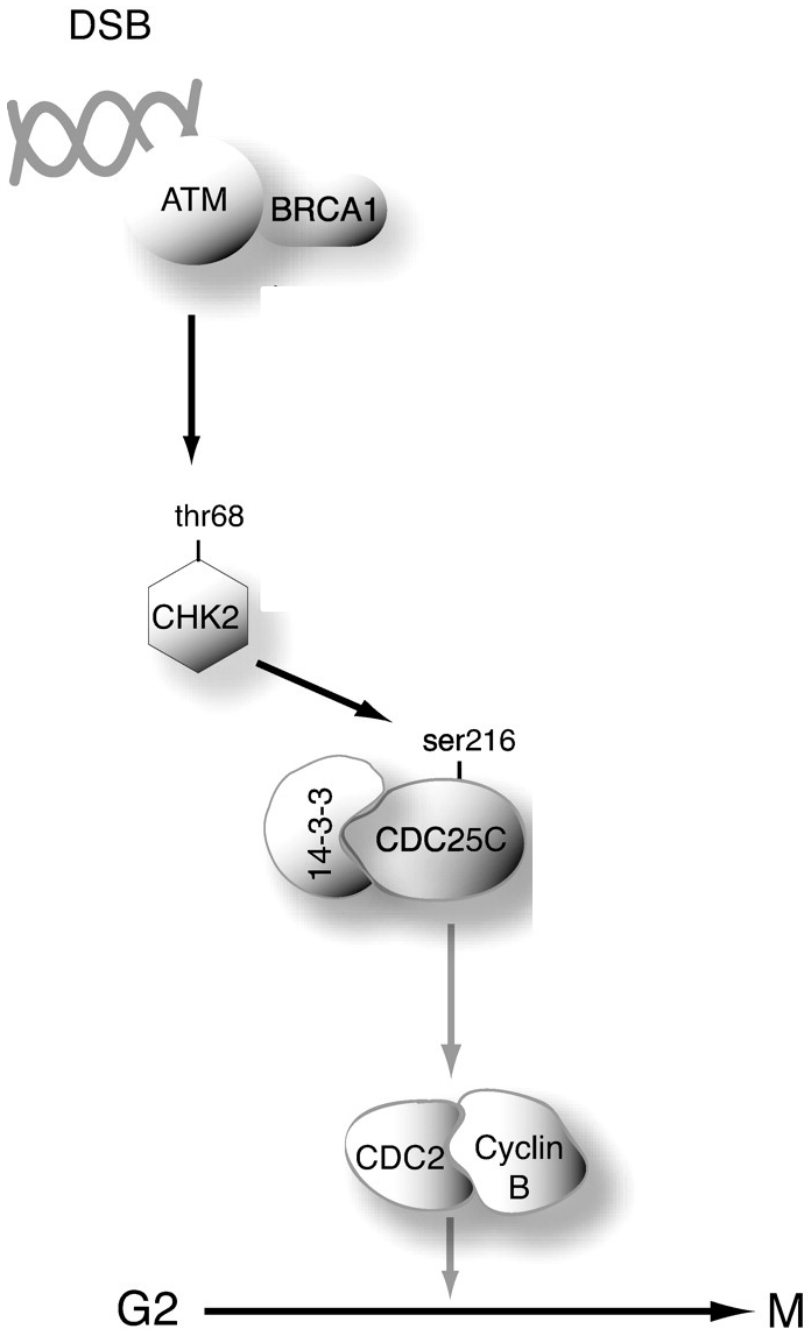
Two routes to activate a G1 arrest

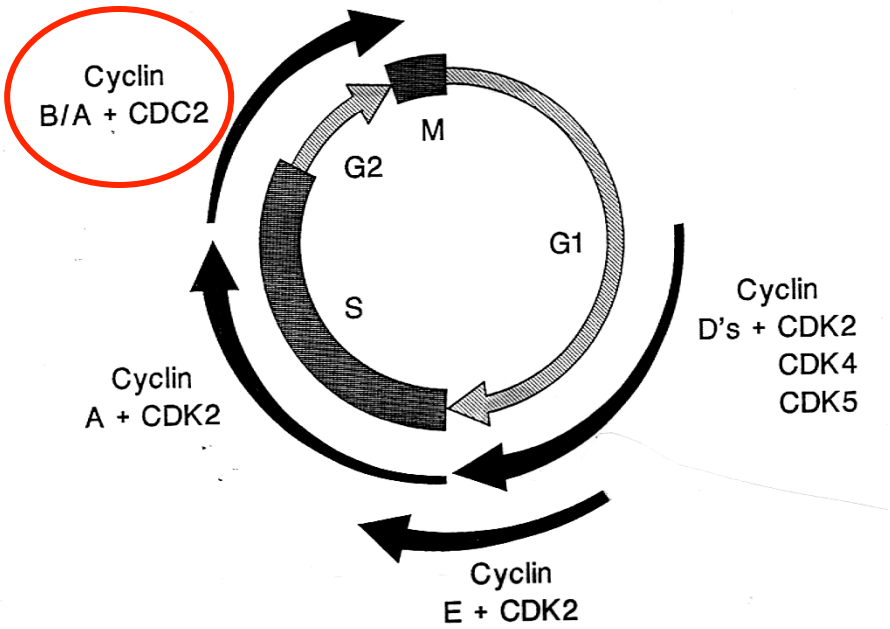




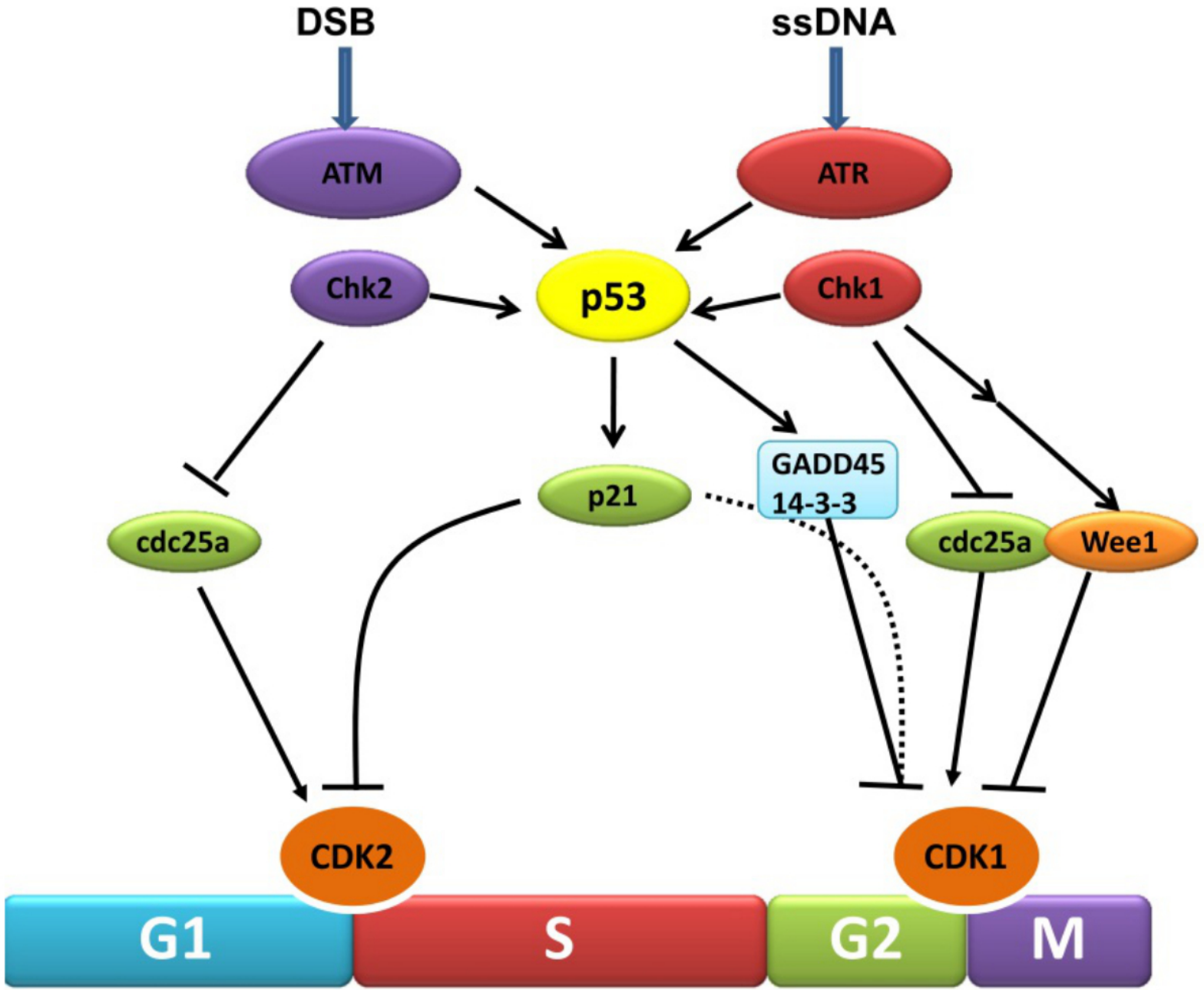
CDC25A is a phosphatase that must act on CDK2 and CDK4 to activate the complexes

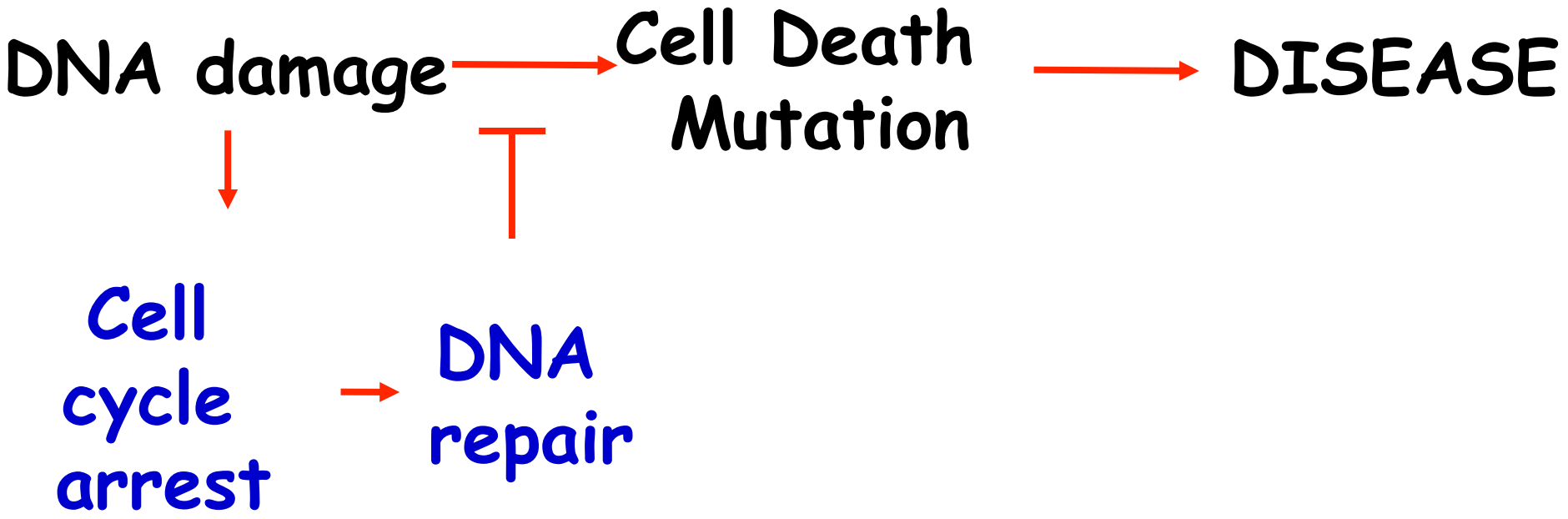
G2/M Arrest





CDC25C phosphatase has different substrate..Cdc2 to target G2/M transition


DSB
ssDNA
ATM
ATR
Chk2
p53
Chk1
p21
GADD45
14-3-3
cdc25a
cdc25a
Wee1
CDK2
CDK1
G1
S
G2
M


DNA damage
Cell Death
Mutation
DISEASE
Cell
cycle
arrest
DNA
repair

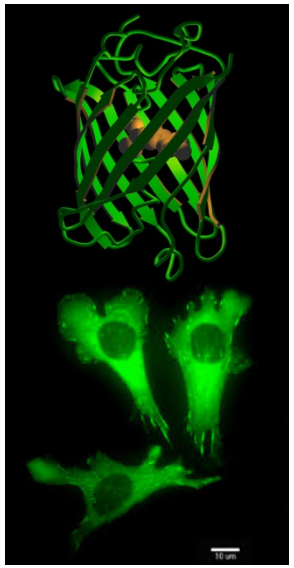
Ataxia Telangiectasia – Cancer Prone

Defective DNA Damage Responses can affect both neurodegeneration and cancer susceptibility

Ataxia Telangiectasia

- 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

Key Experimental Methods for Module 1



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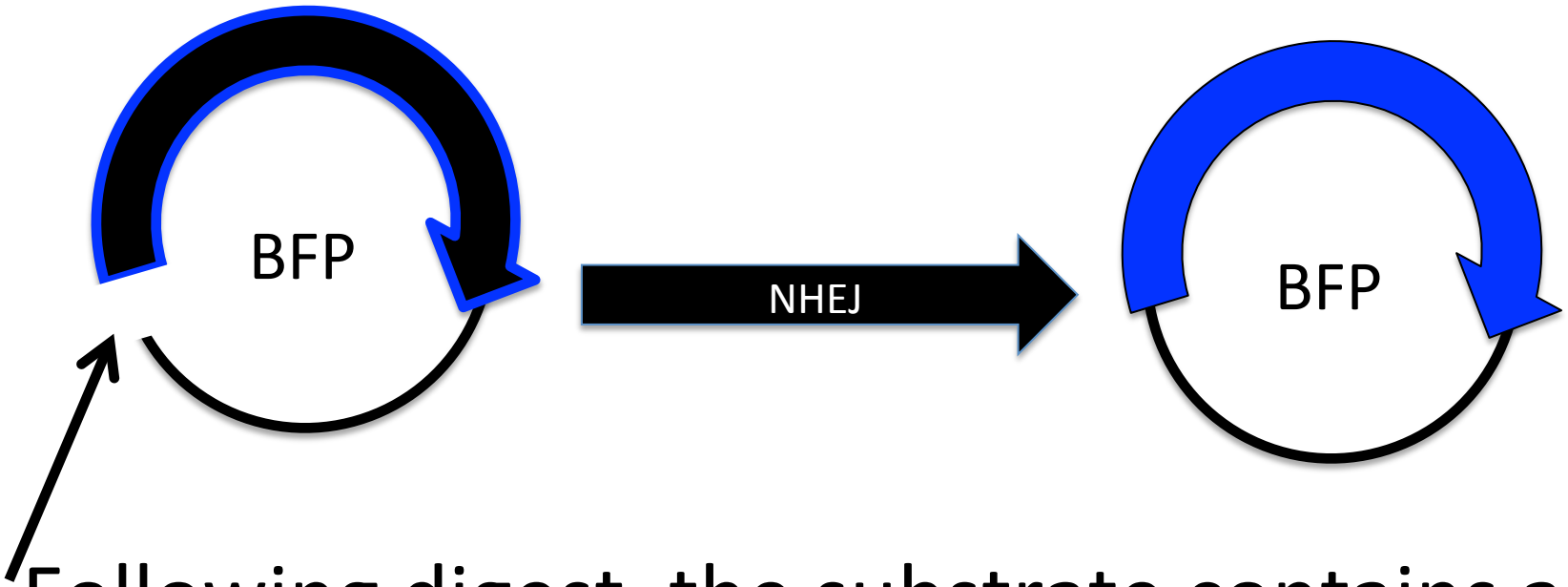
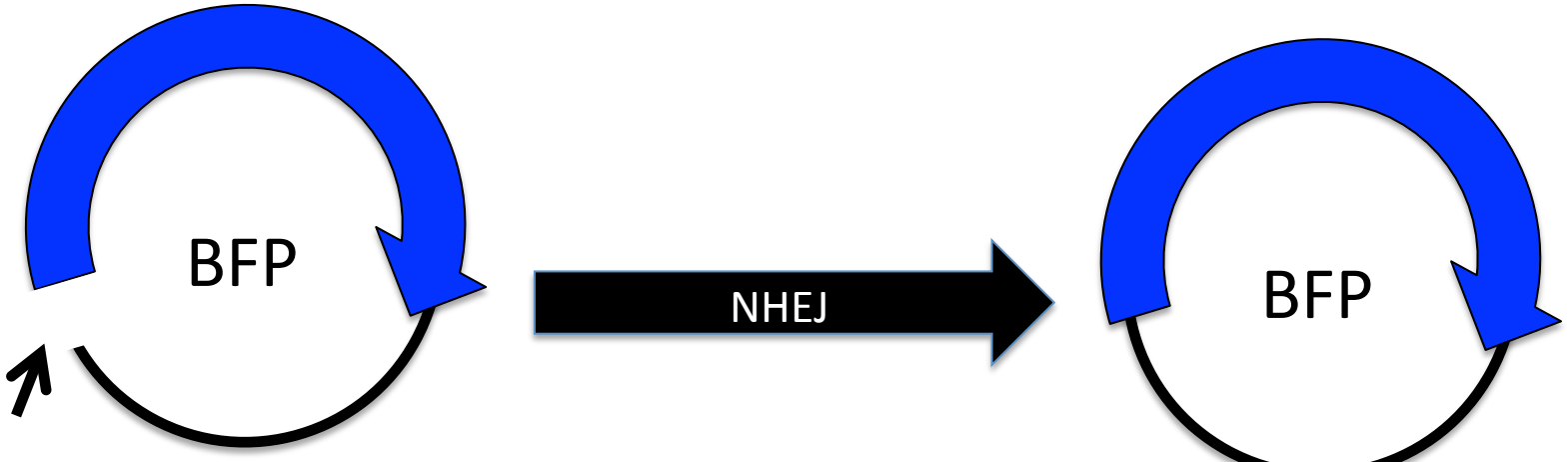
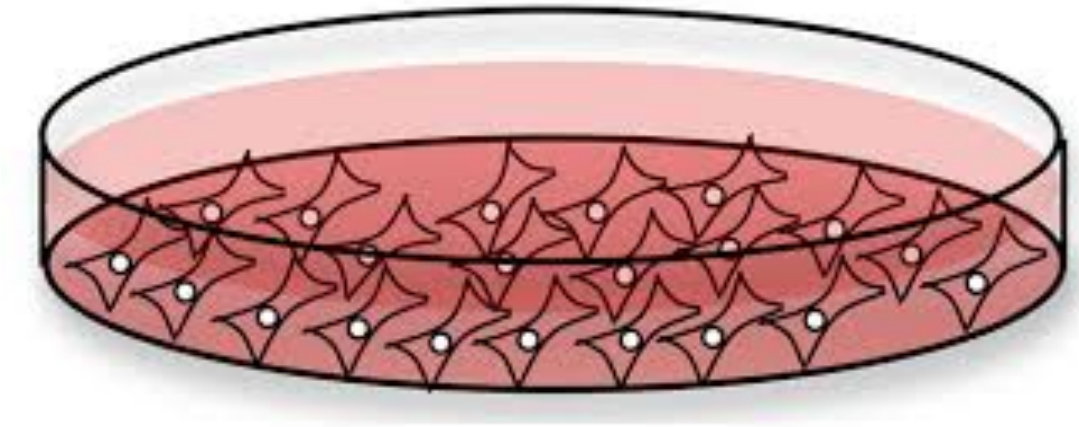
Basis for the fluorescent reporter assay:

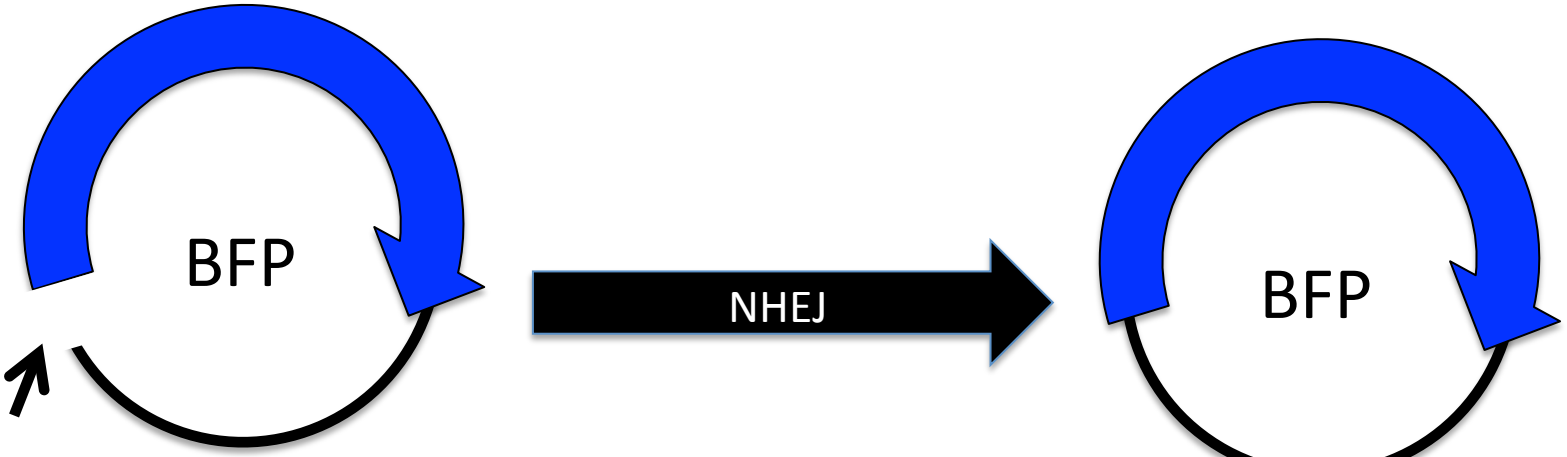


Following digest, the substrate contains a DSB in the 5’ UTR that prevents fluorescent reporter expression



Substrate contains a DNA double strand break





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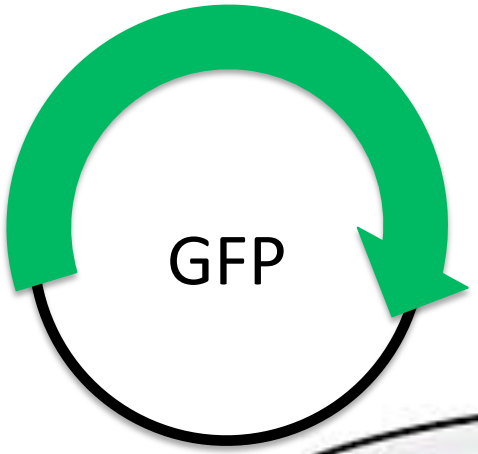
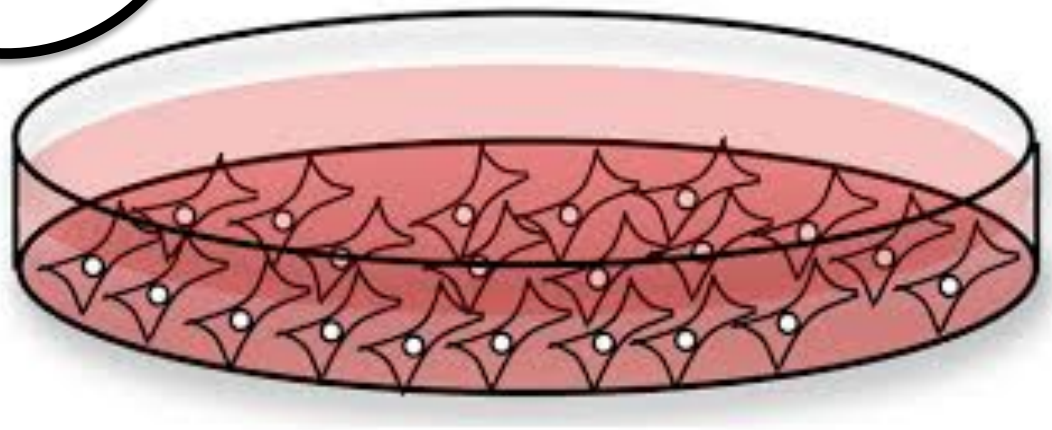
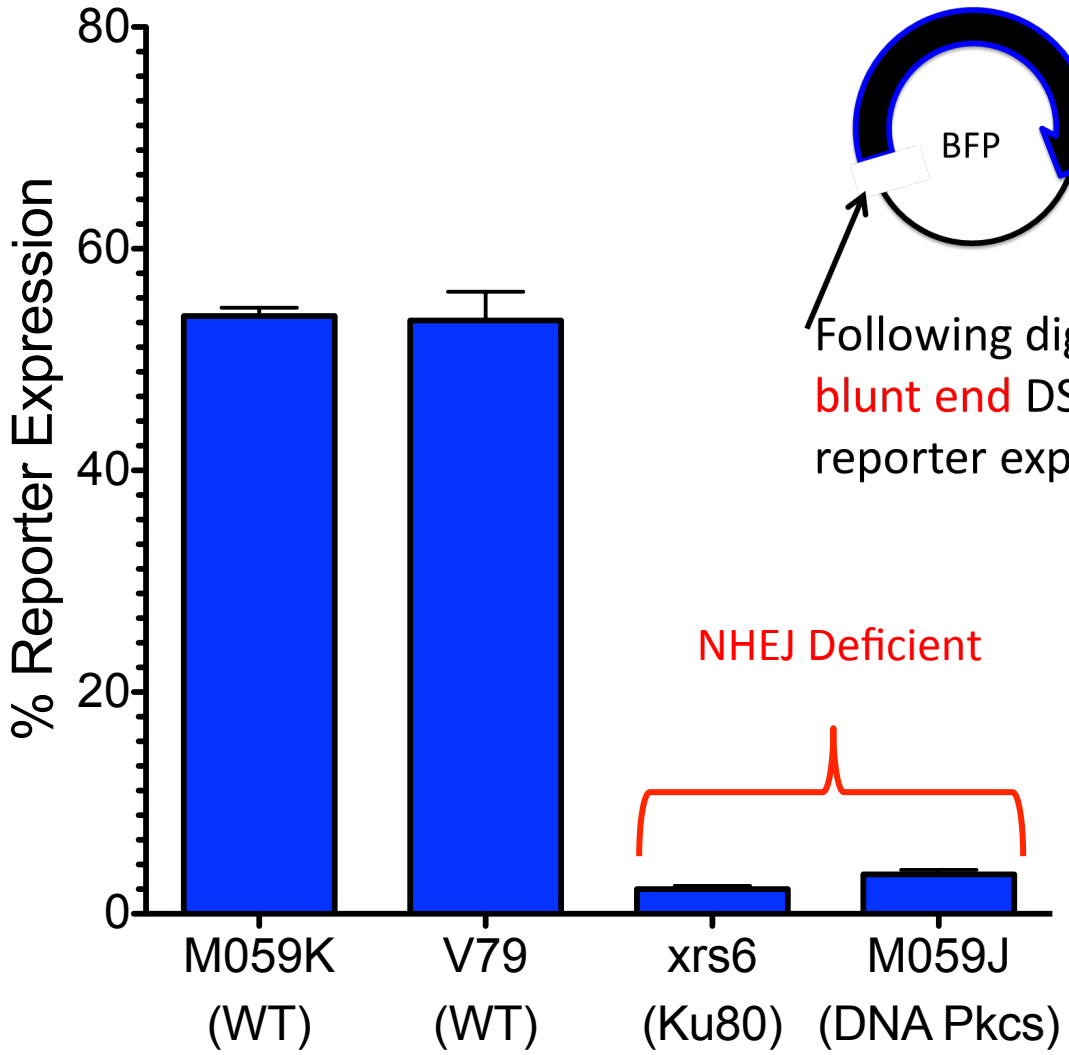
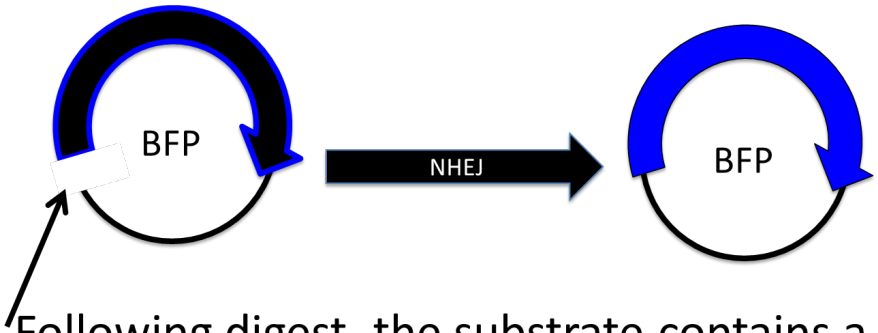




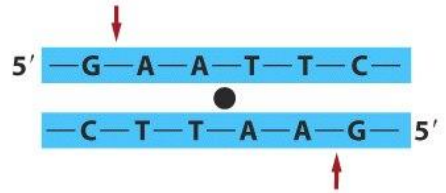
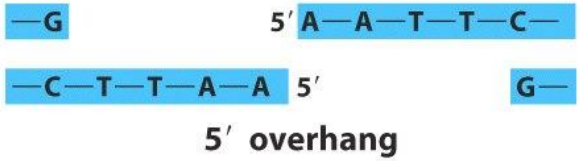
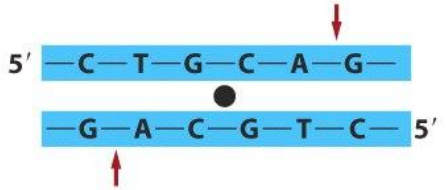
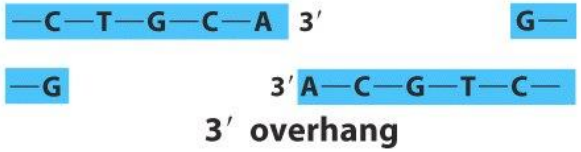
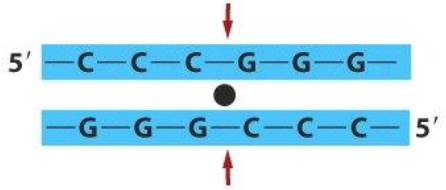
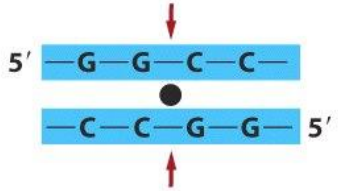
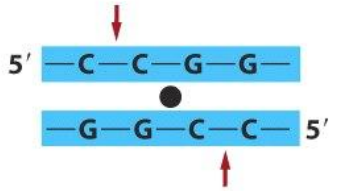
NHEJ HCR in WT and NHEJ defective cells at 18 hours post-transfection:

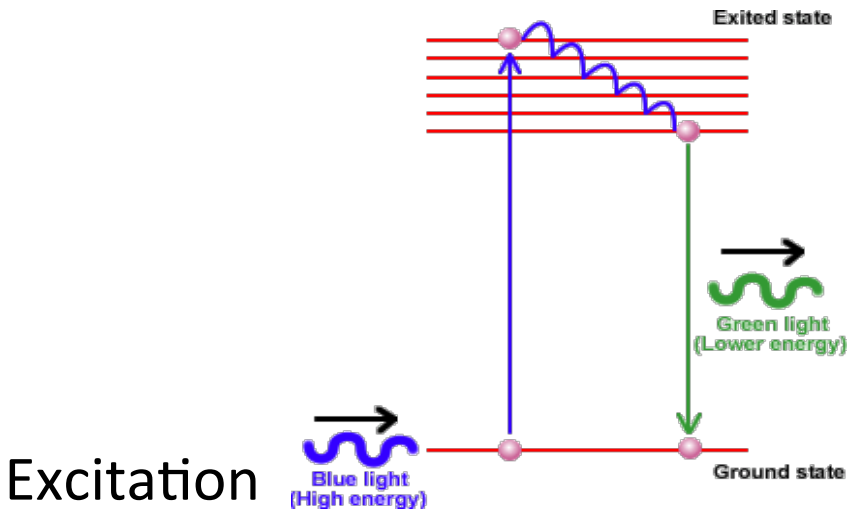




Following digest, the substrate contains a blunt end DSB that prevents fluorescent reporter expression

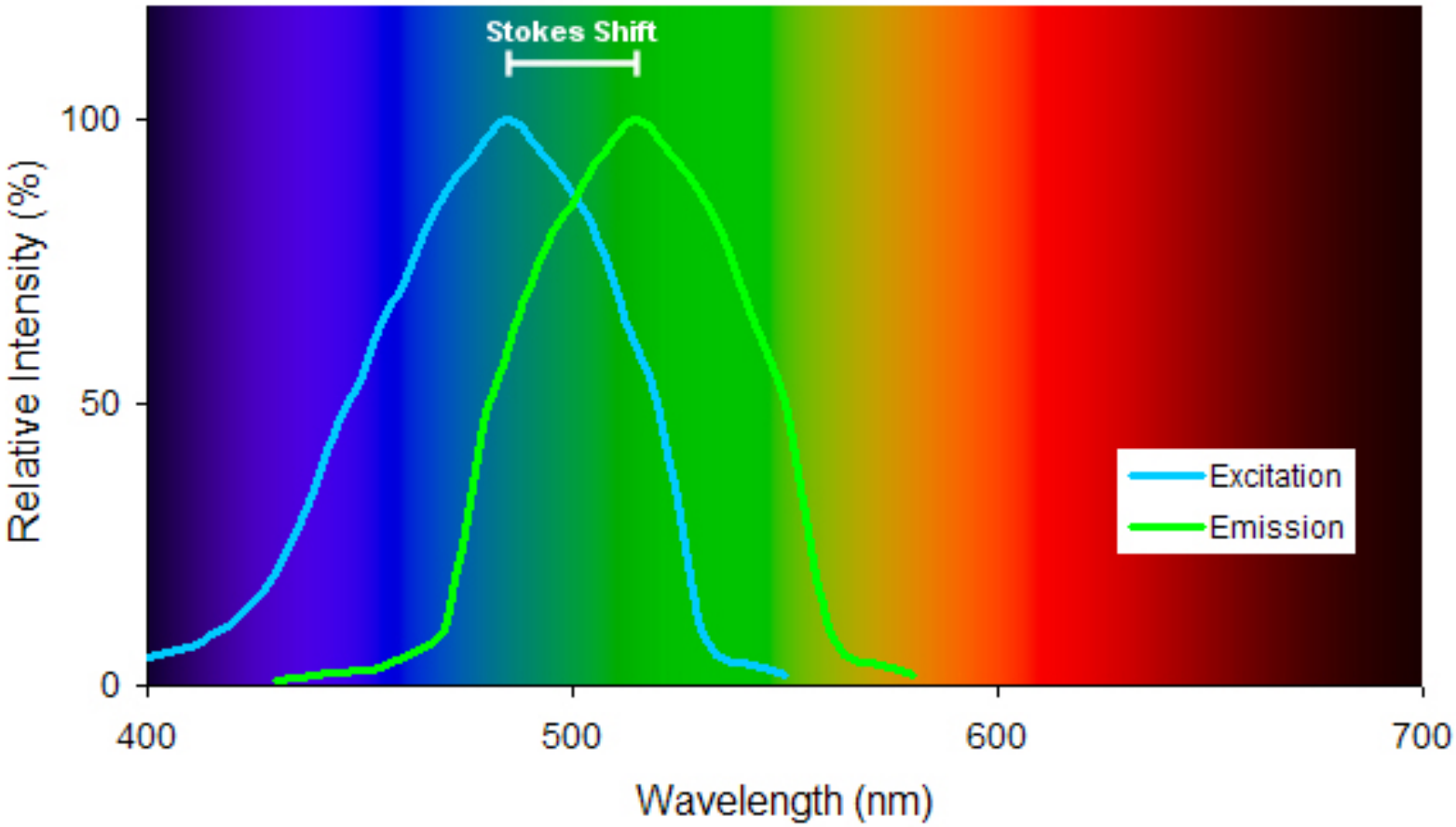
Some restriction enzymes

Enzyme	Source organism	Restriction recognition site in double-stranded DNA	Structure of the cleaved products
(a)			
*Eco*RI	*Escherichia coli*		
*Pst*I	*Providencia stuartii*		
*Sma*I	*Serratia marcescens*		
(b)			
*Hae*III	*Haemophilus aegyptius*		
*Hpa*II	*Haemophilus parainfluenzae*		

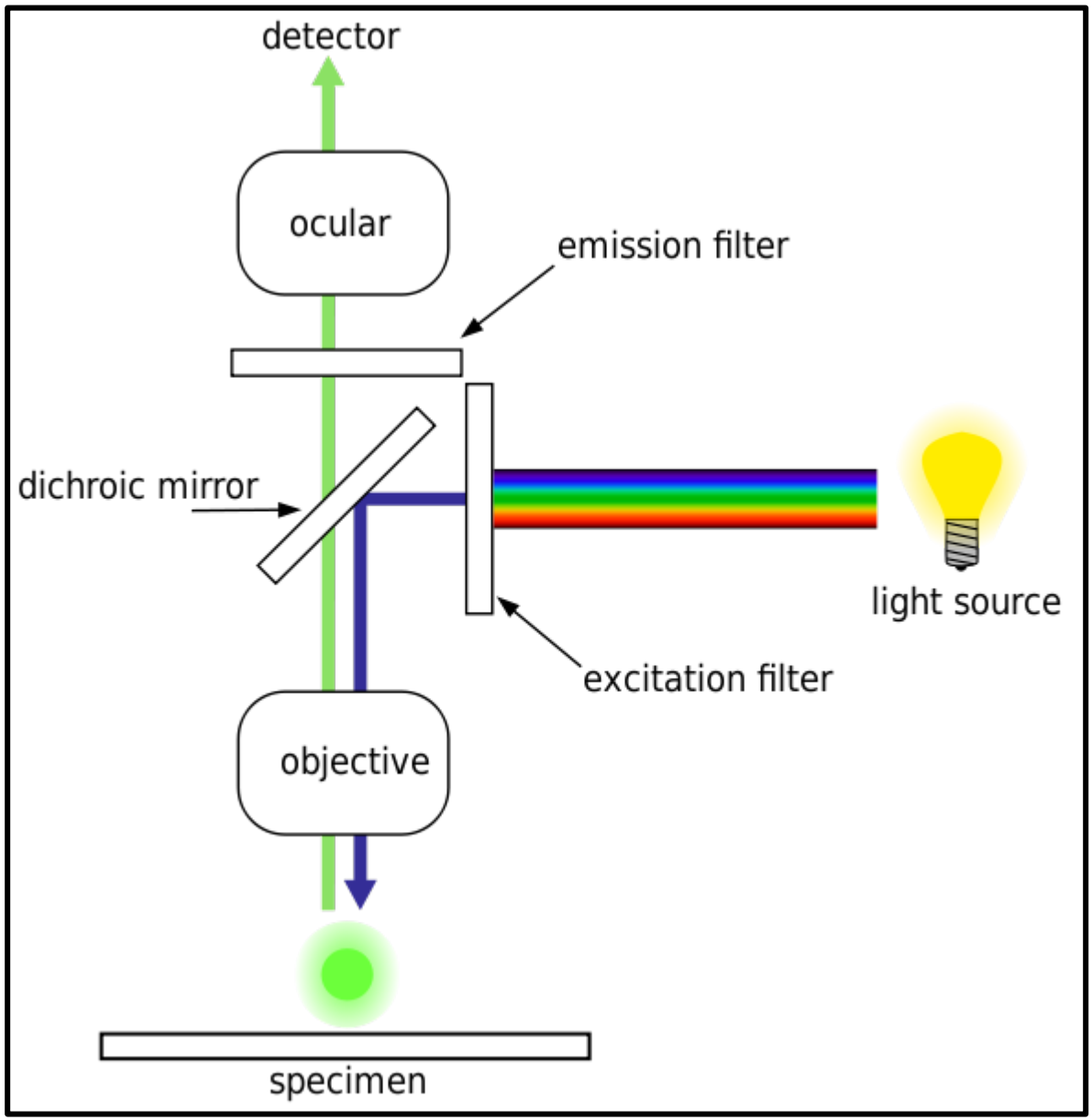


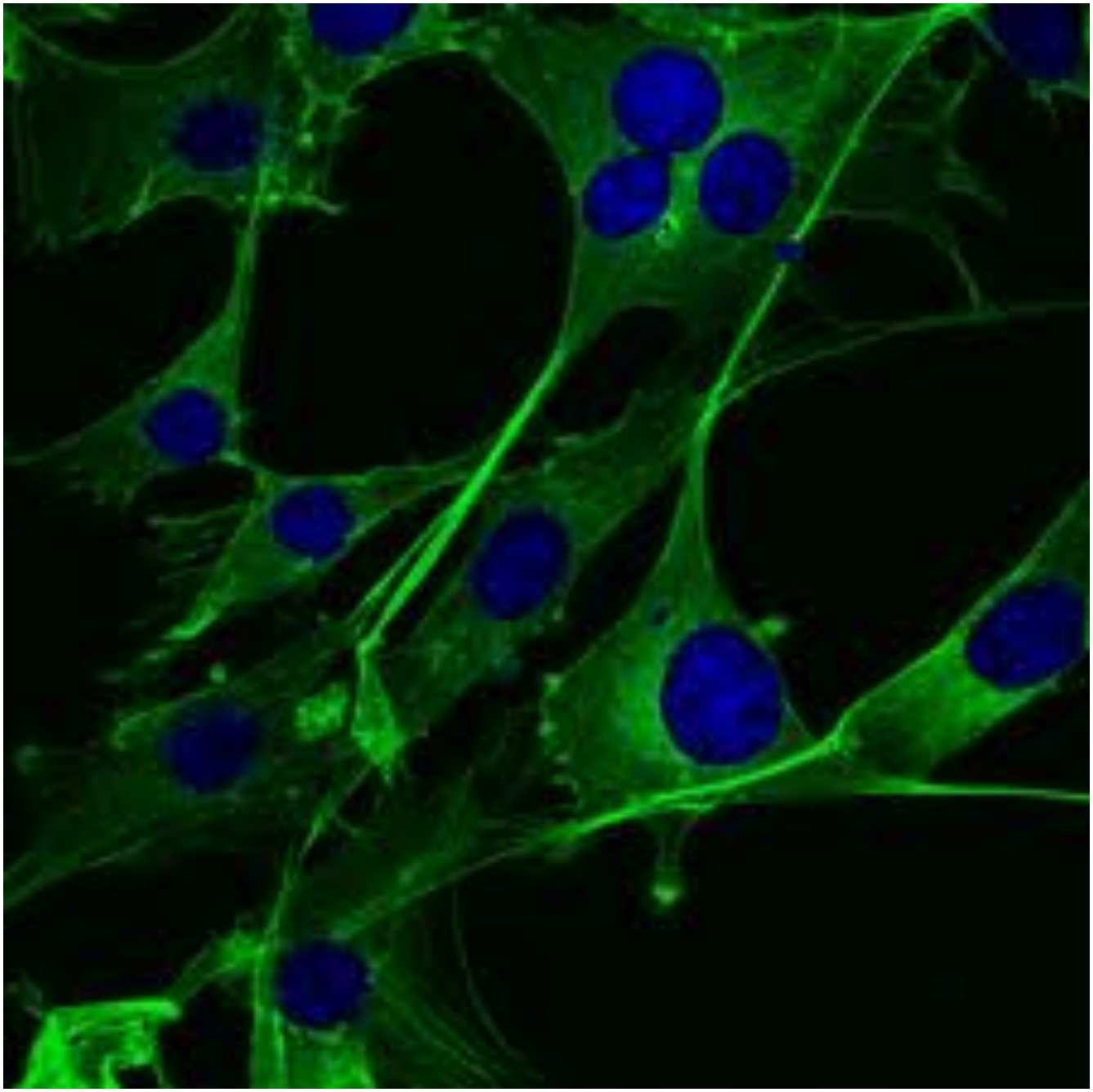
Emission

Excitation



Fluorescence Detection

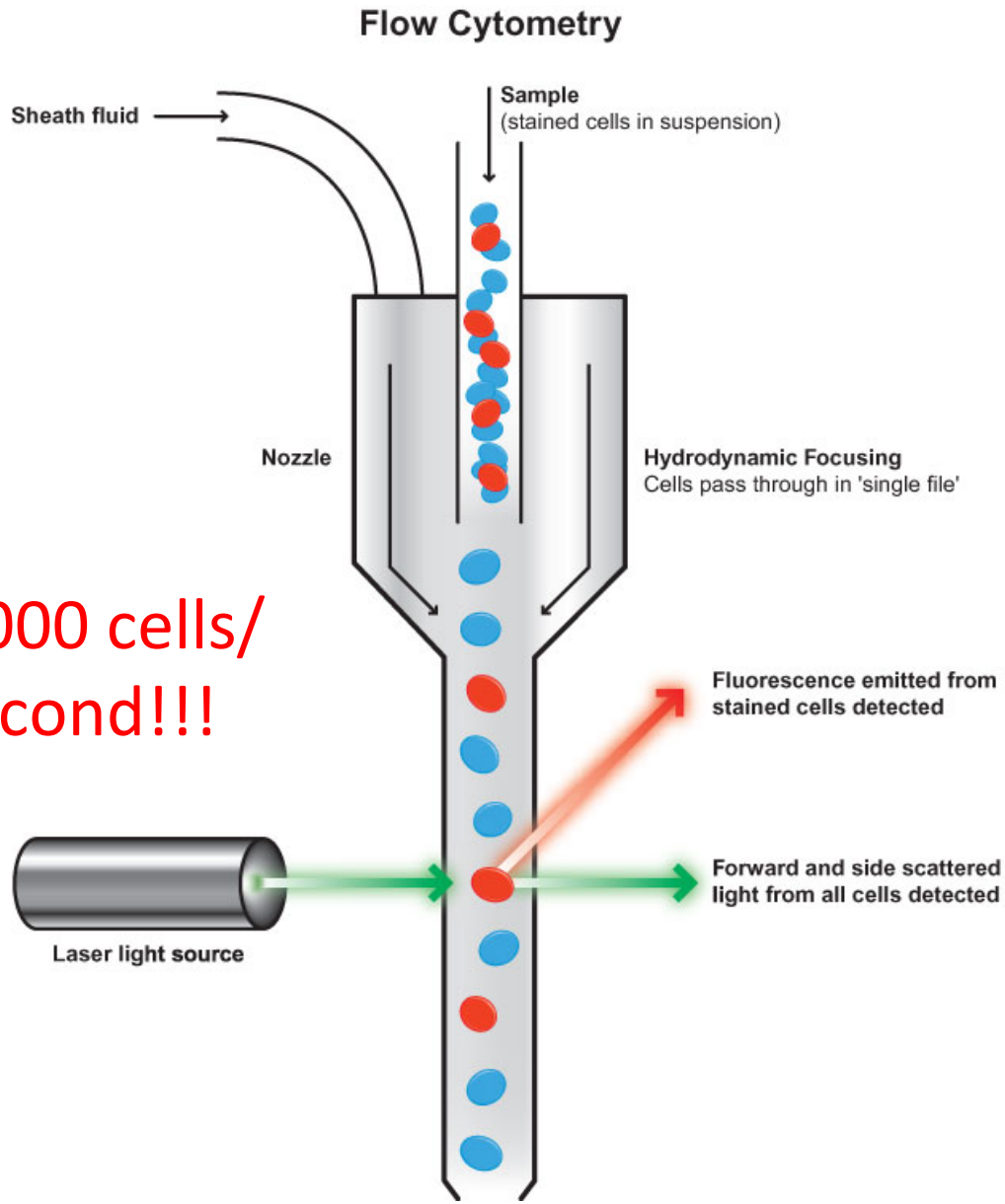




DNA – Blue

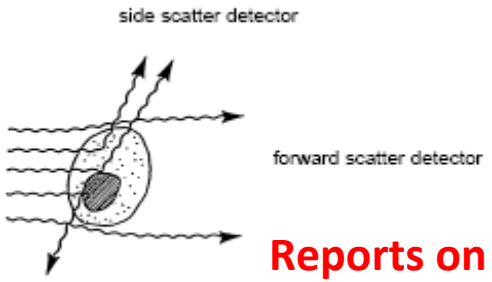
GFP - Green

Flow Cytometry

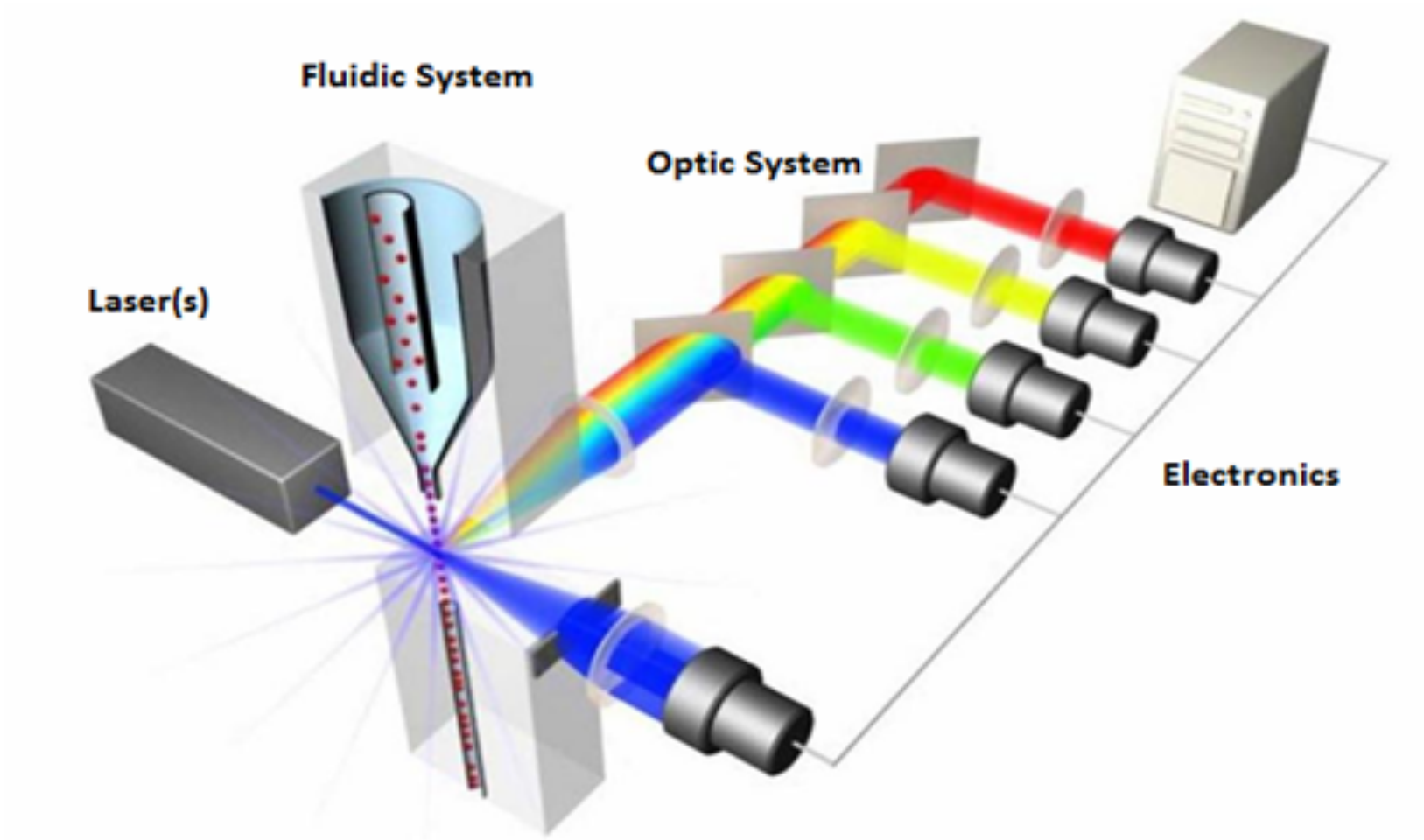


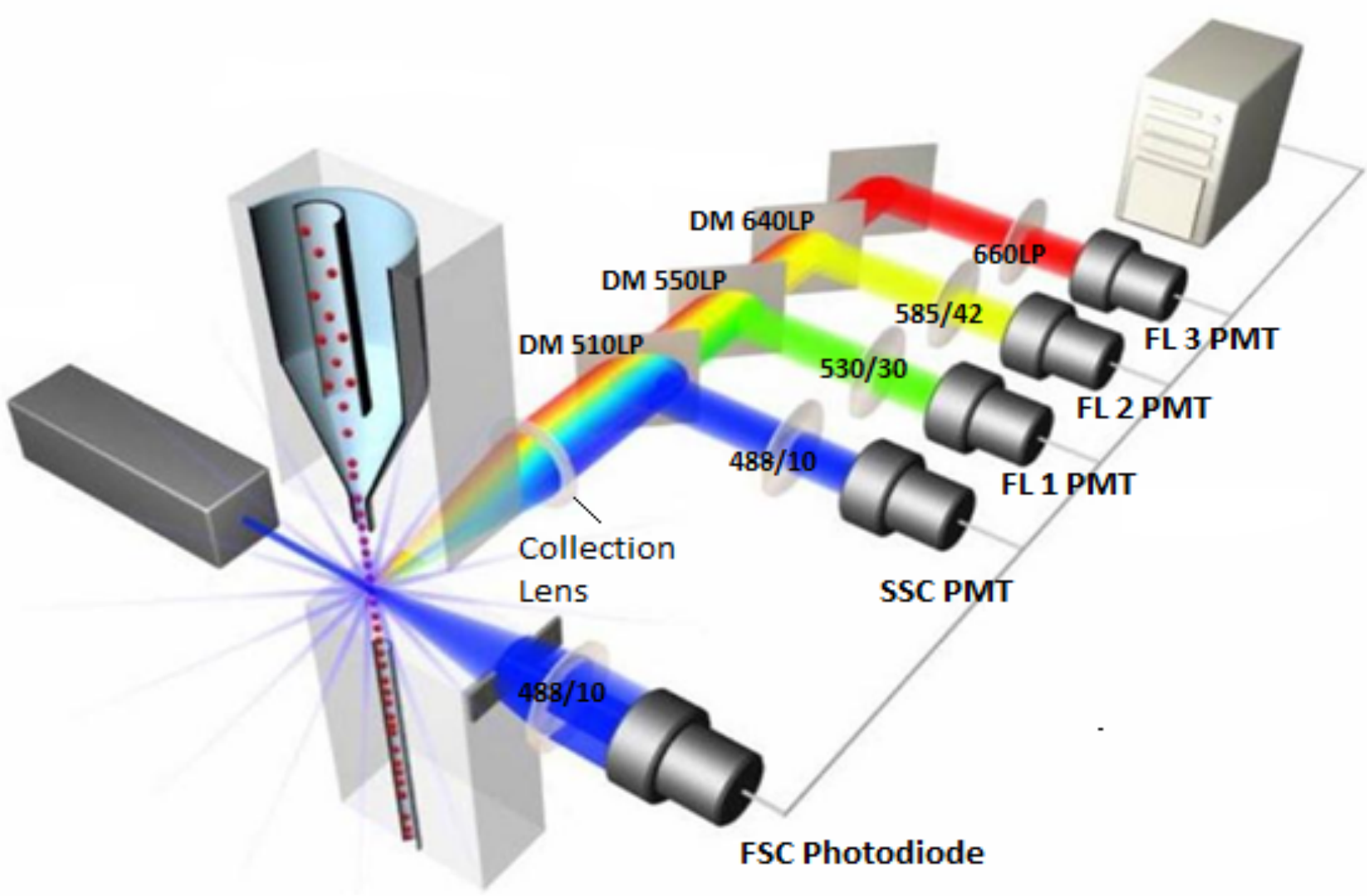
50,000 cells/ second!!!

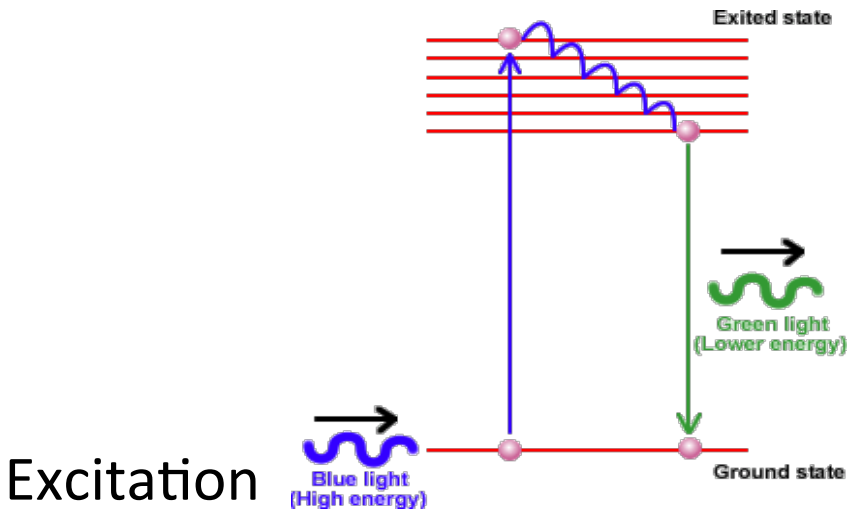
Reports on cell complexity



Reports on cell size

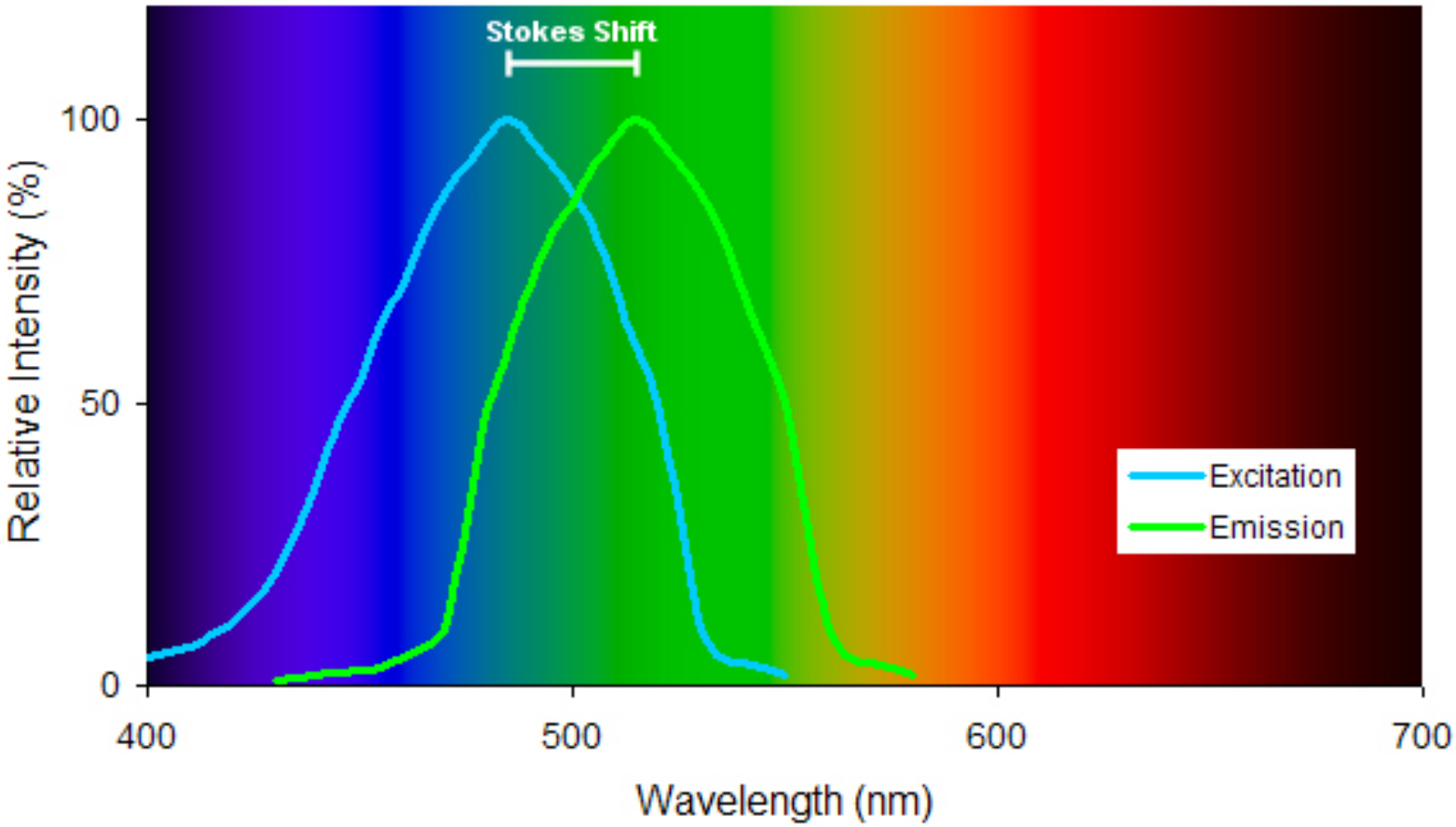

Fluidic System
Optic System
Laser(s)
Electronics


DM 640LP
660LP
DM 550LP
585/42
FL 3 PMT
DM 510LP
530/30
FL 2 PMT
488/10
FL 1 PMT
Collection
Lens
SSC PMT
488/10
FSC Photodiode

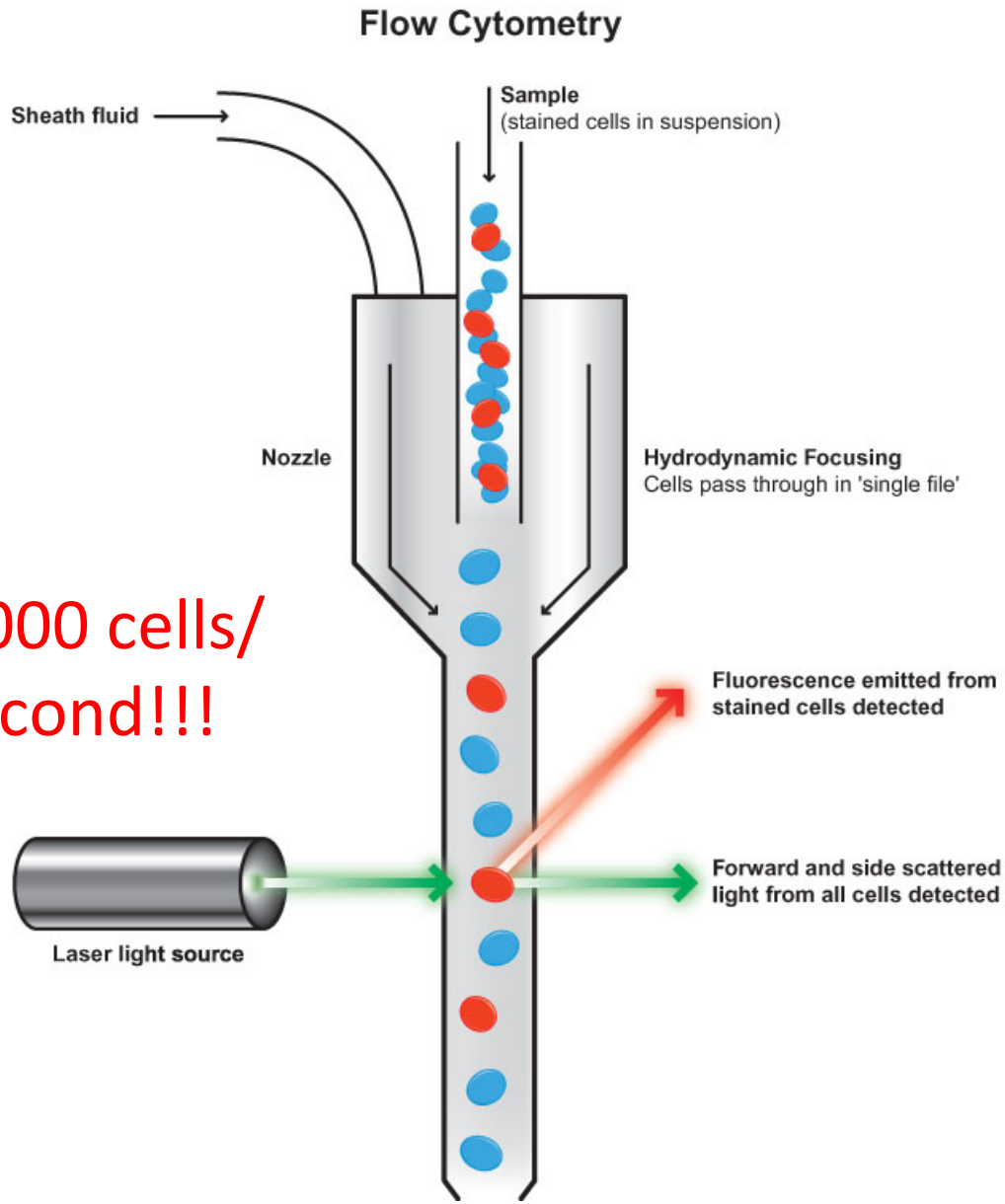


Emission

Excitation

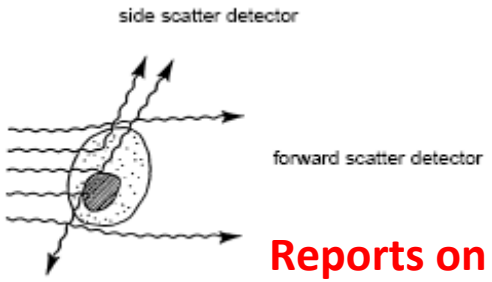


Flow Cytometry



50,000 cells/ second!!!

Reports on cell complexity



Reports on cell size

Flow Cytometry

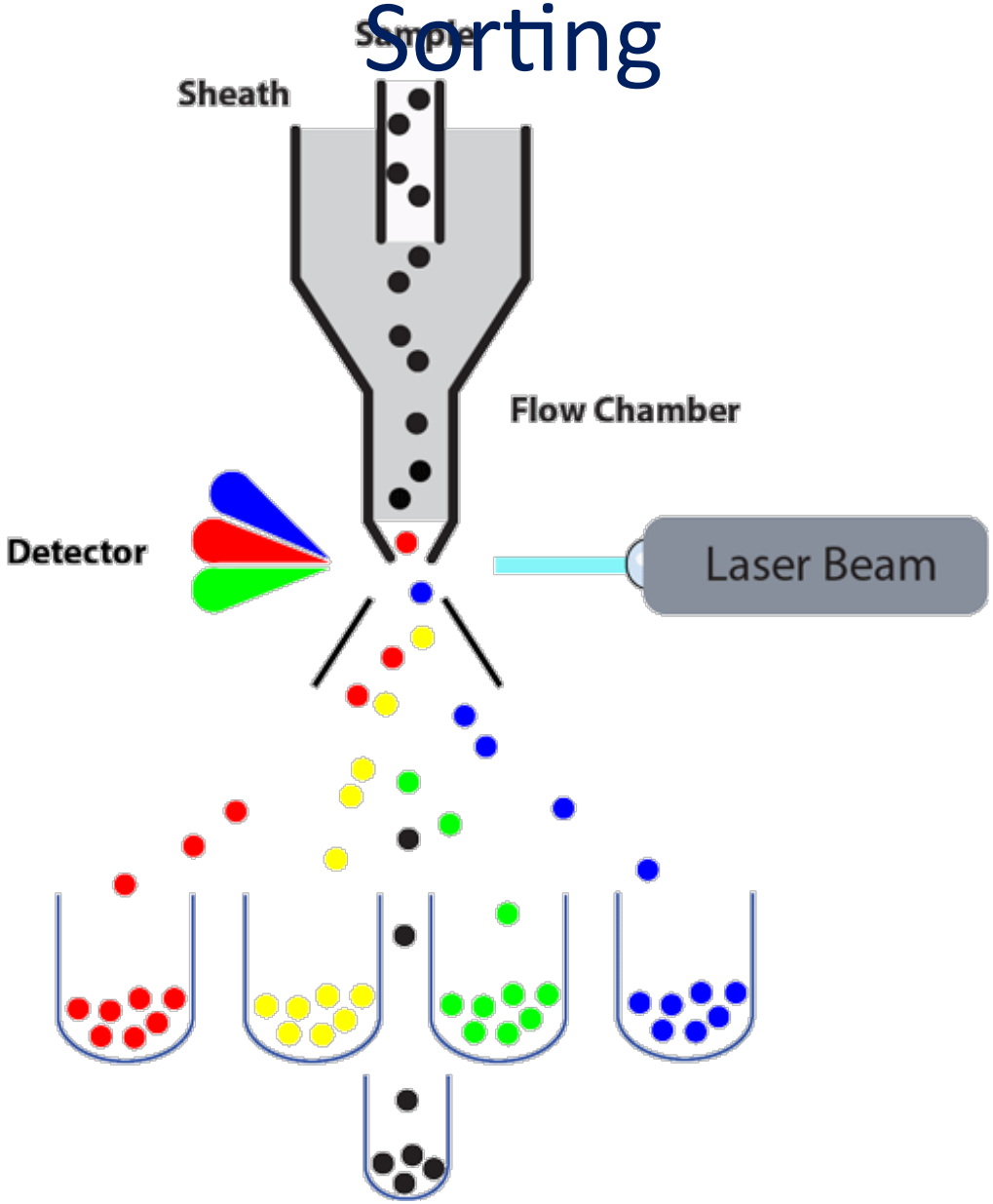
Flow cytometry analyzes cells one by one

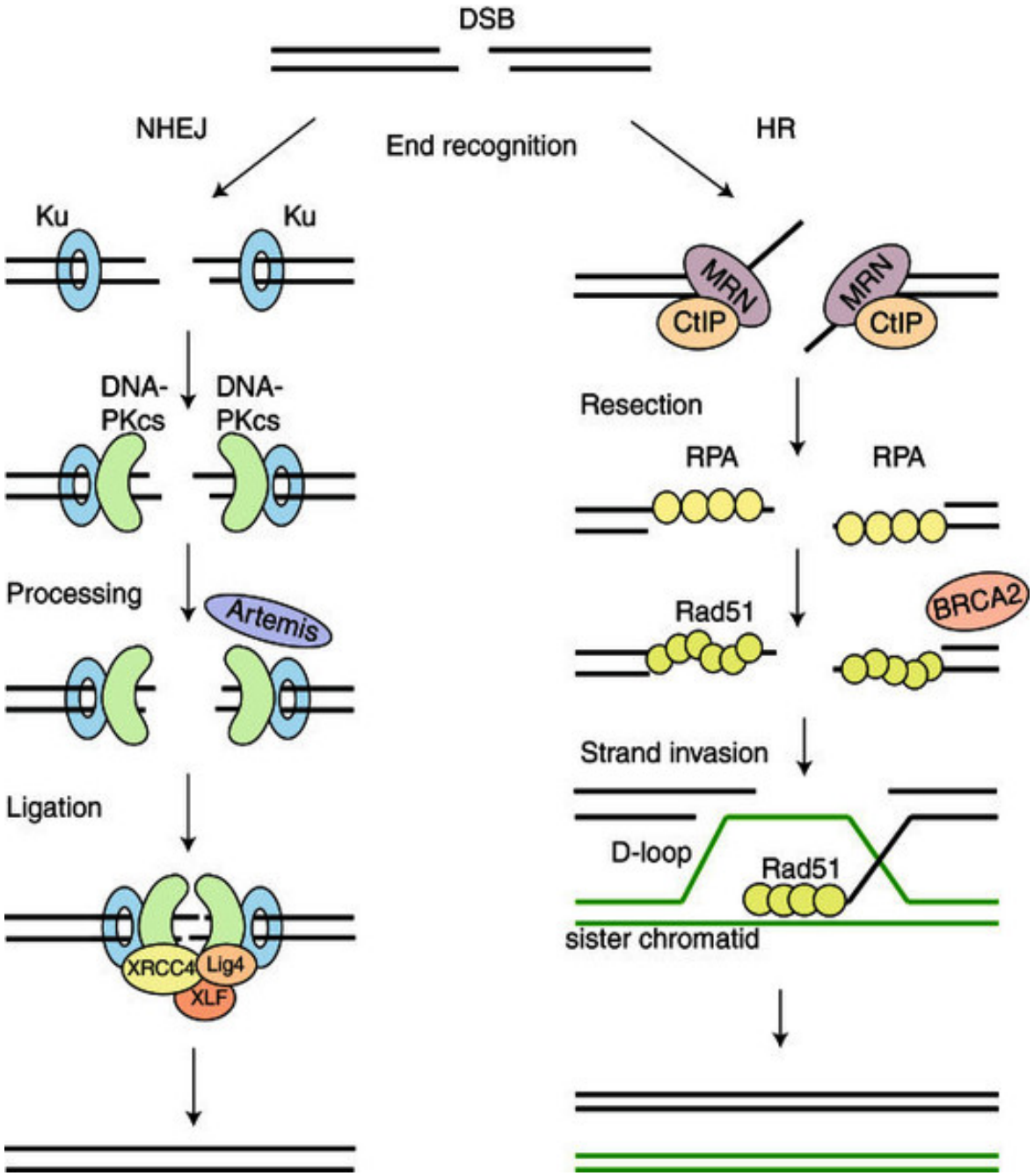
Fluorescence, diffracted and reflected light can be measured for each cell

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





How does the cell decide which pathway to use?