MOD1 – DNA ENGINEERING

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Writing Instructor: Neal Lerner
Oral Presentation Instructor: Atissa Banuazizi

Spring 2008

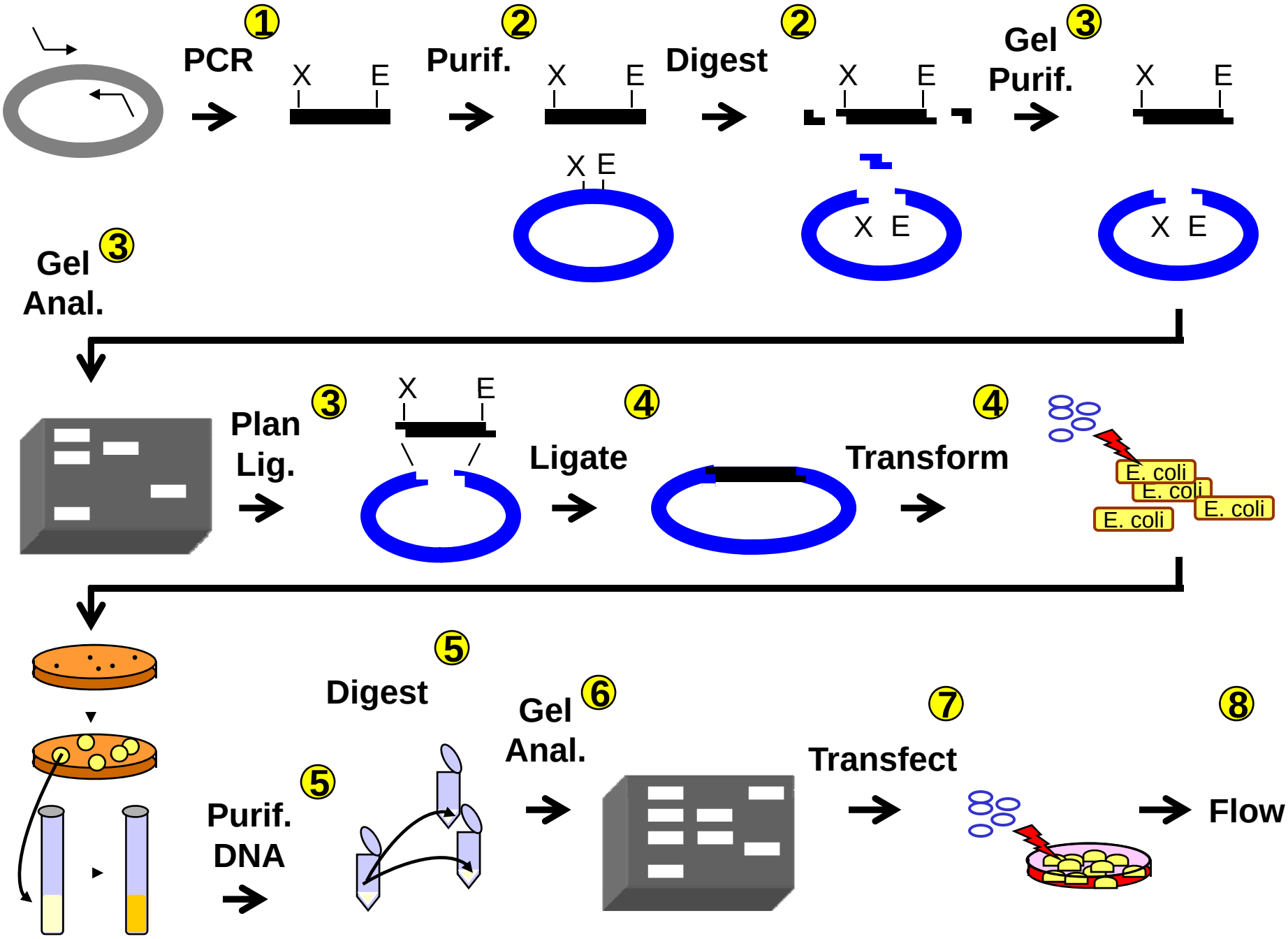
Day 7

Overview of this Module's Experiments

Restriction Analysis – Assuring your DNA is correct

New Cancer Treatments – how your assay might be used

Thoughts about variables that might affect HR frequency


1
PCR
X
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2
Purif.
X
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2
Digest
X
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3
Gel
Purif.
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X E
X E
X E
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Gel
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3
Plan
Lig.
X
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4
Ligate
4
Transform
E. coli
E. coli
E. coli
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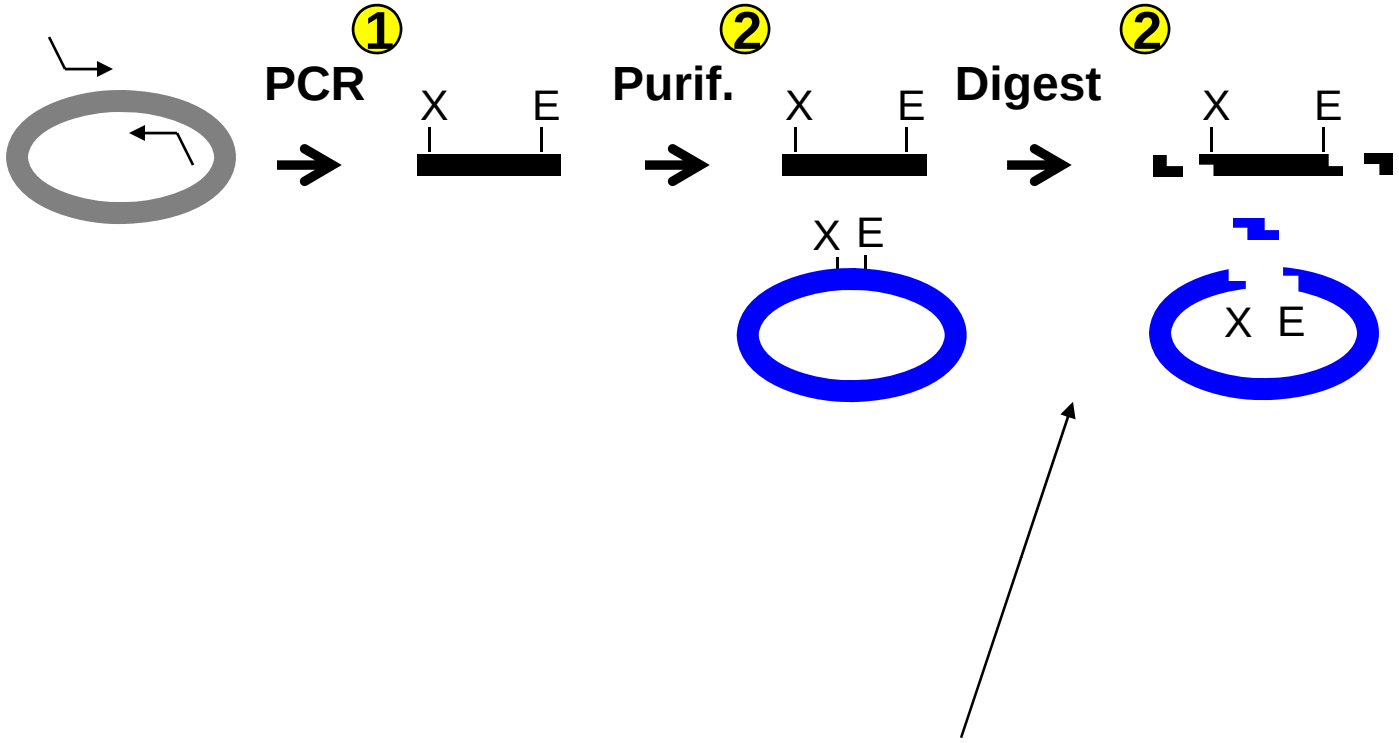
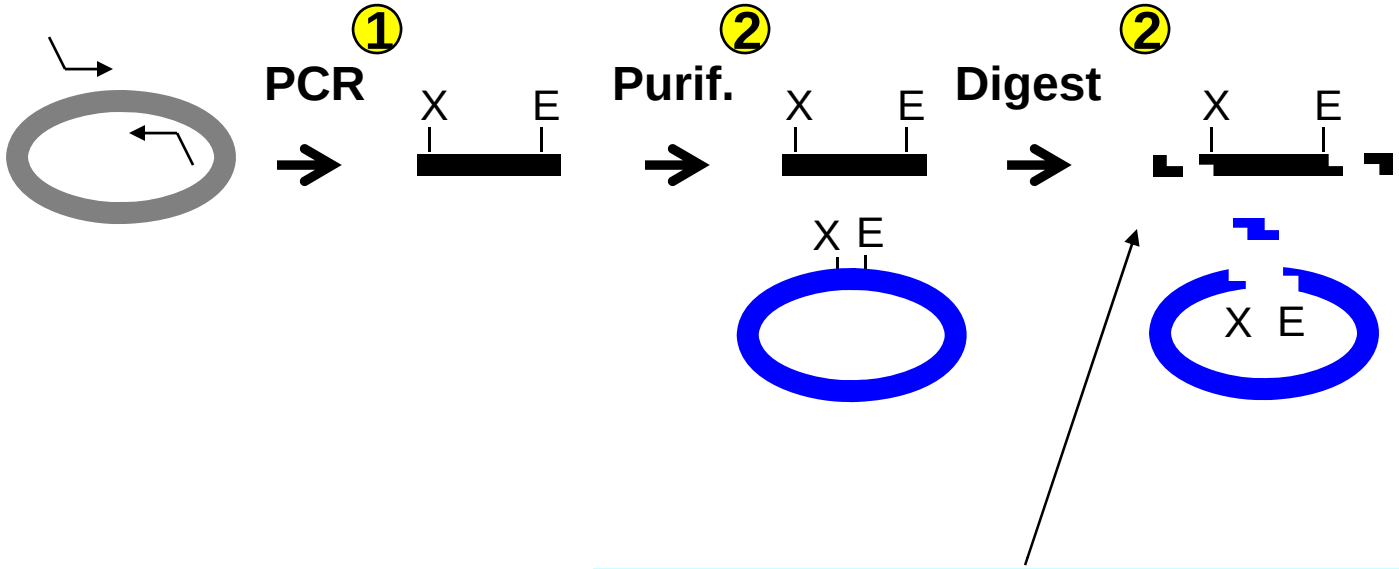
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow

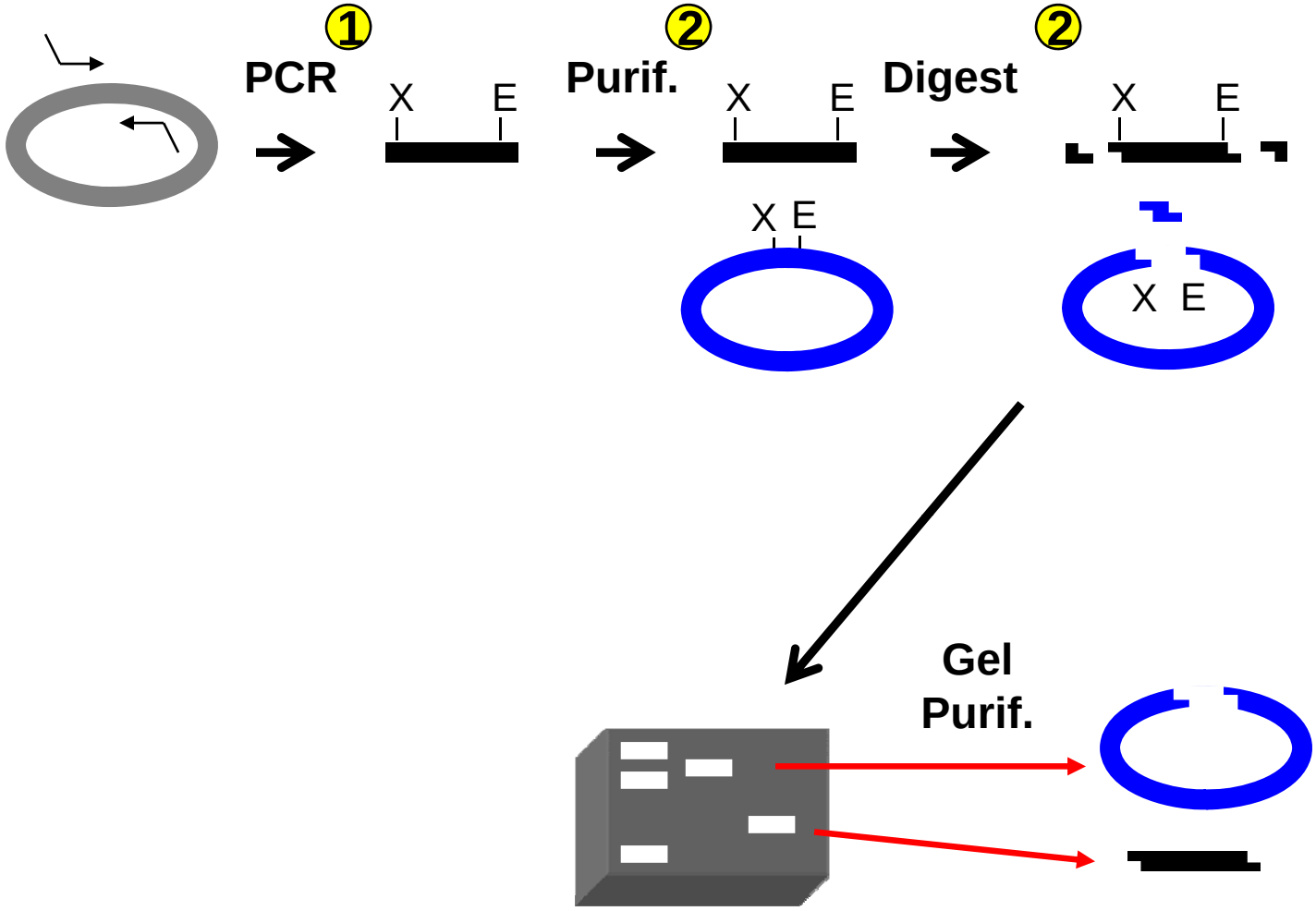


How do you know that your restriction enzymes actually cut the DNA?

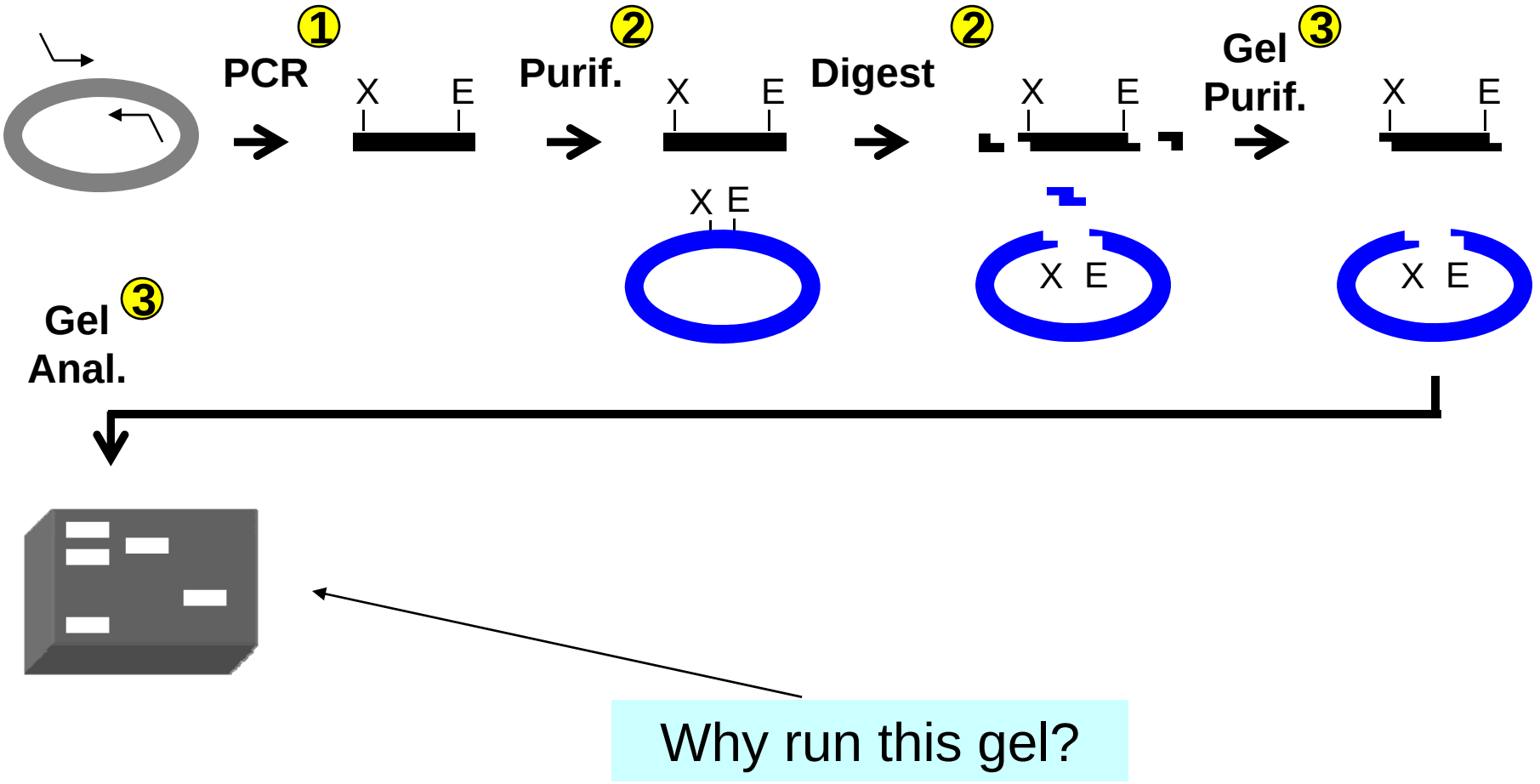


What else is in the reaction with the digested PCR product?

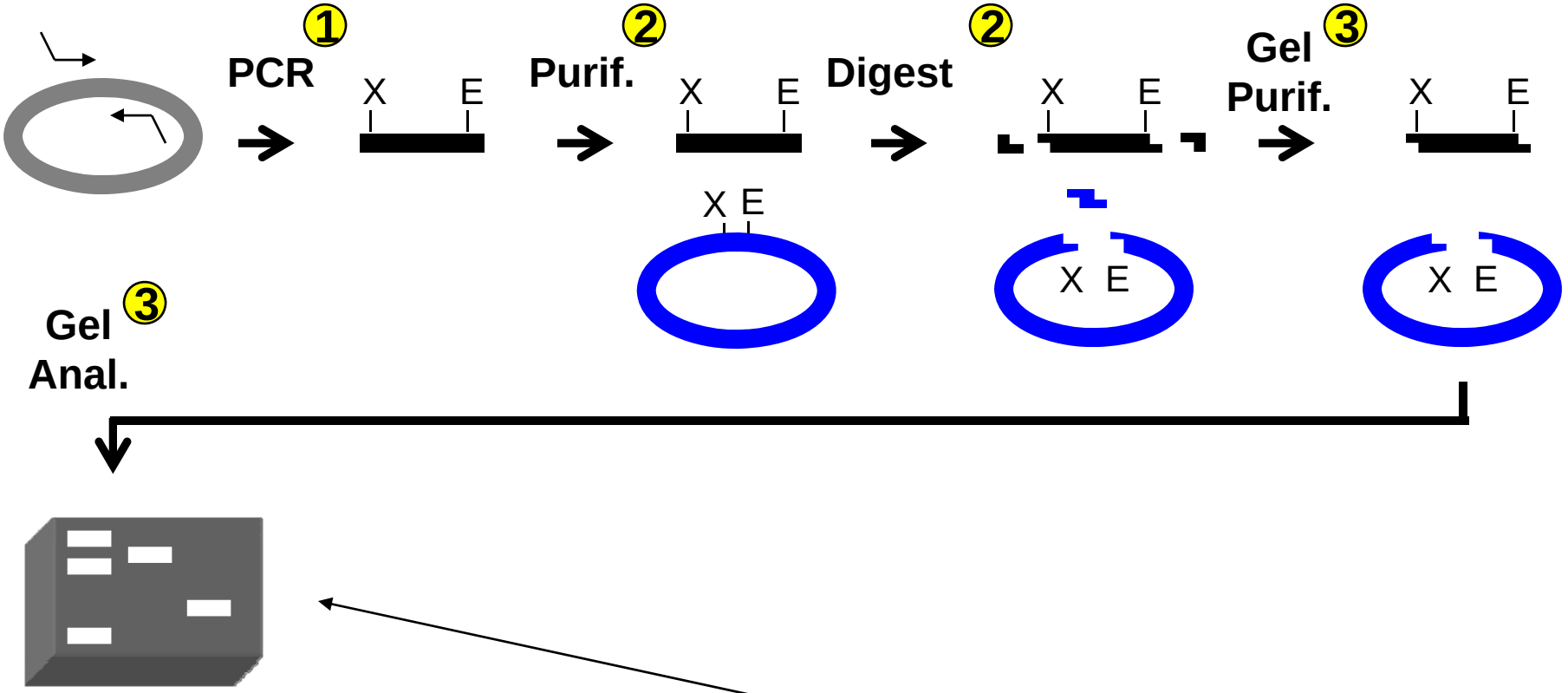
What effect could it have?



Why is it important to excise the DNA from the gel relatively quickly?

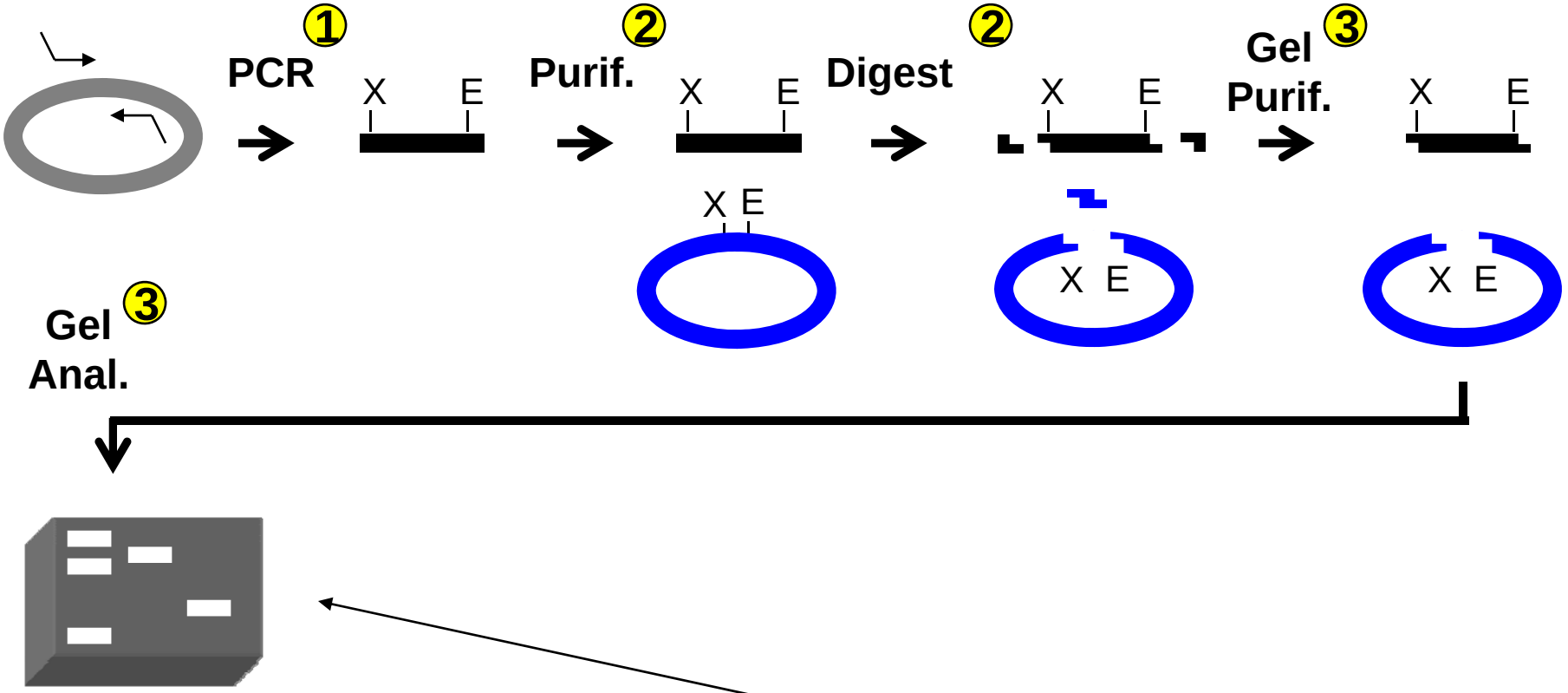


Why run this gel?

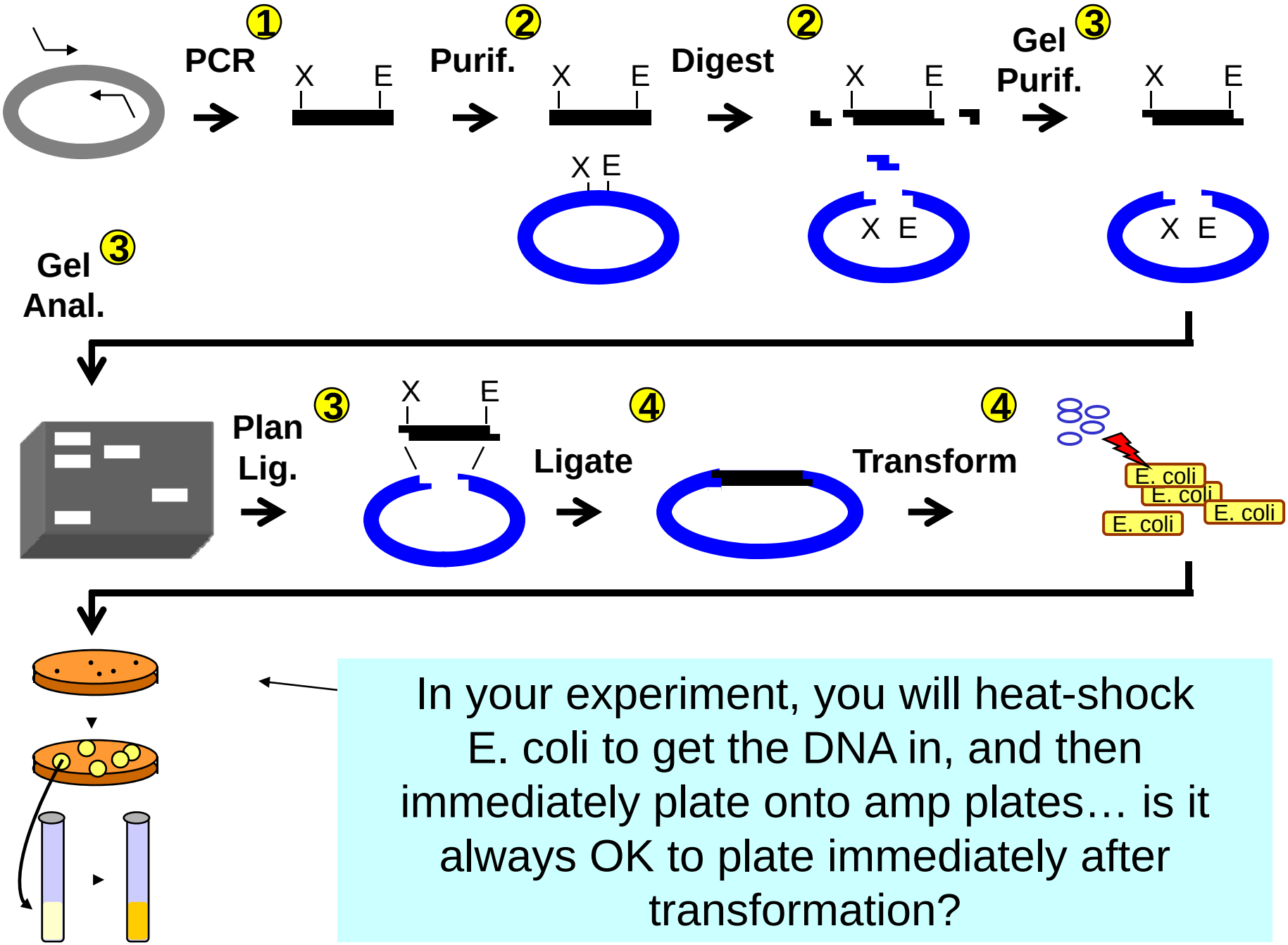


Your objective is a 1:4 vector:insert ratio – Why?

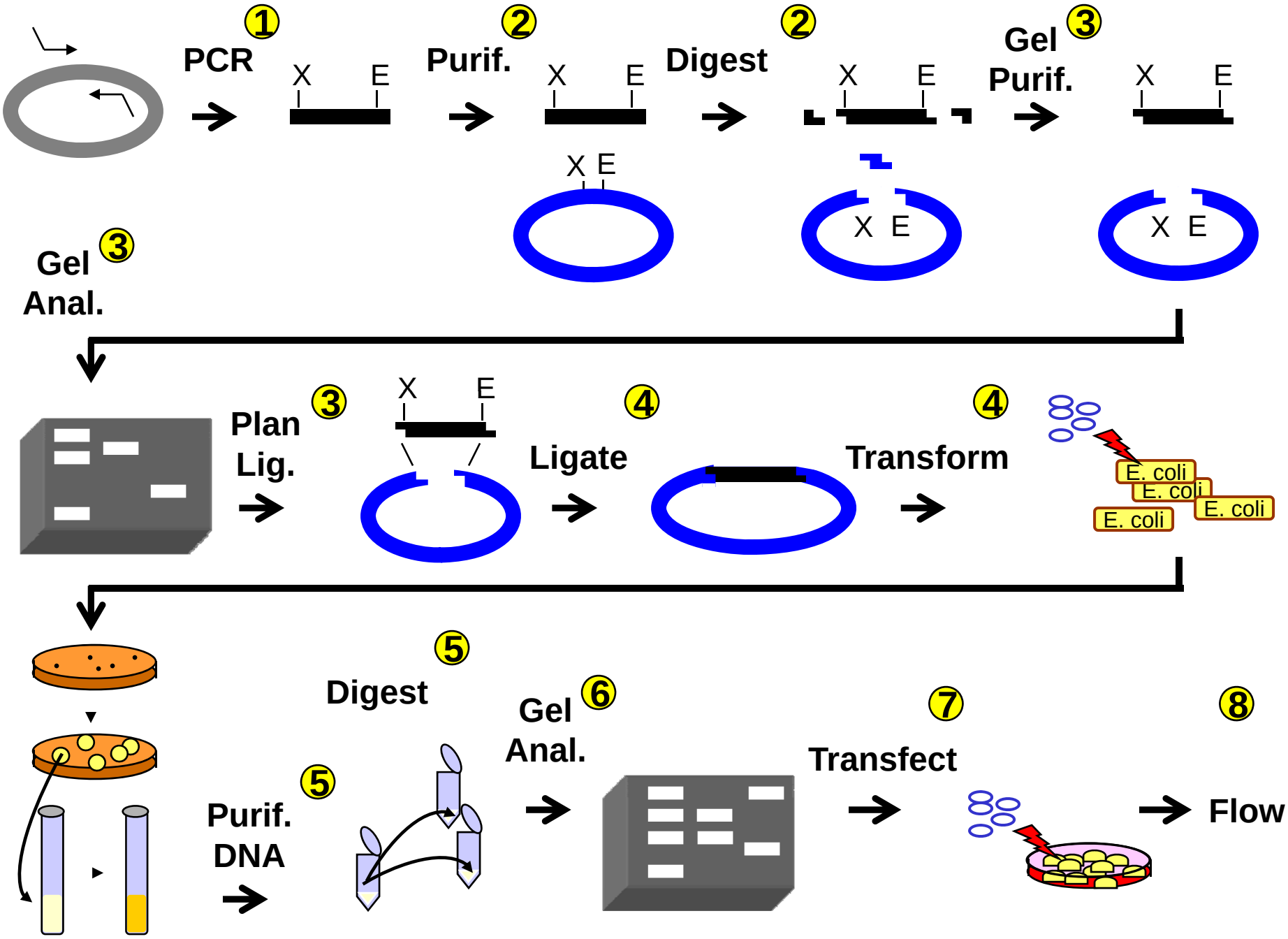
What is it was 1:100?
What if it was 100:1?



How do you figure out how to get a 1:4 molar ratio?



In your experiment, you will heat-shock E. coli to get the DNA in, and then immediately plate onto amp plates… is it always OK to plate immediately after transformation?


1
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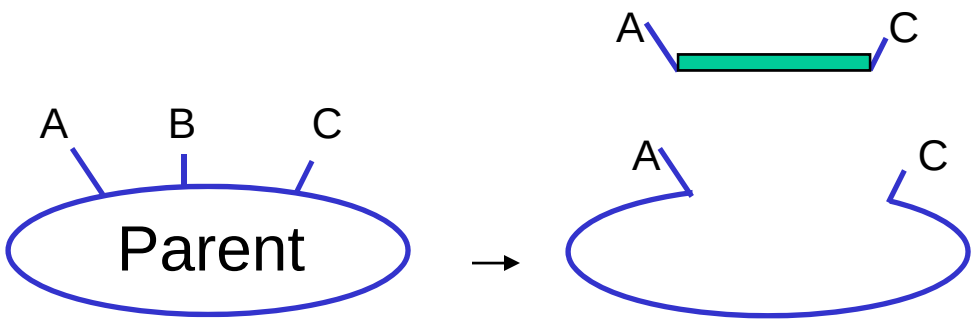
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow

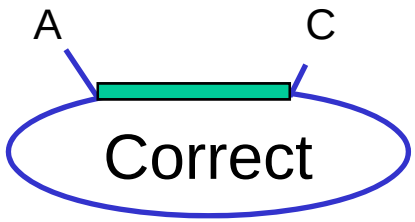
Analysis of Restriction Digestions:

How can you be certain your construct is what you think it is?

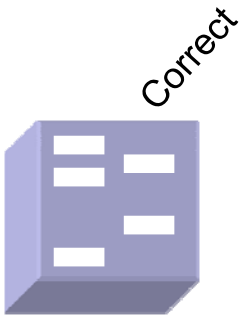
How to test for correct product...



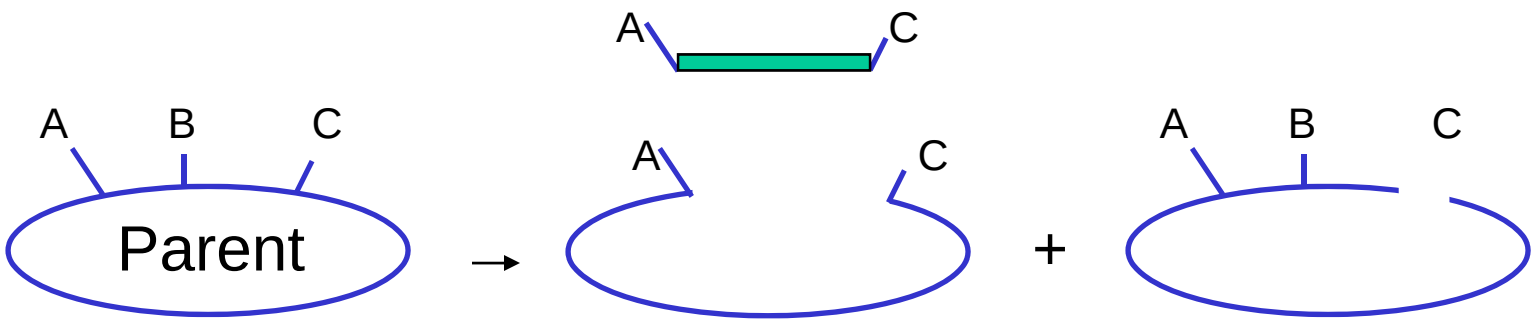
↓ **Ligase**



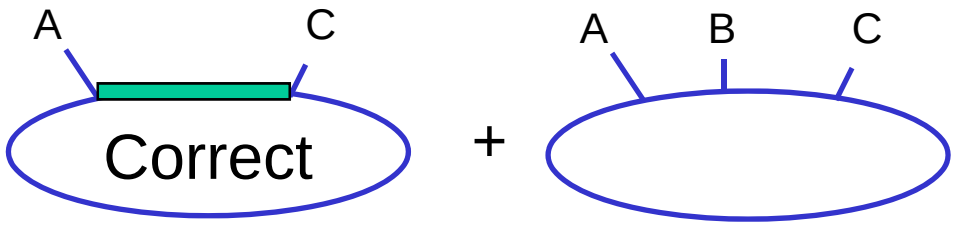
A + C



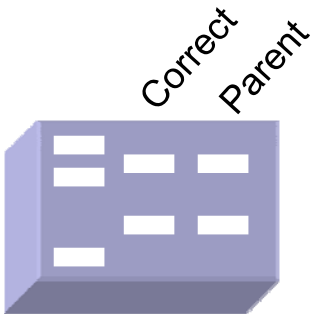
How to test for correct product...



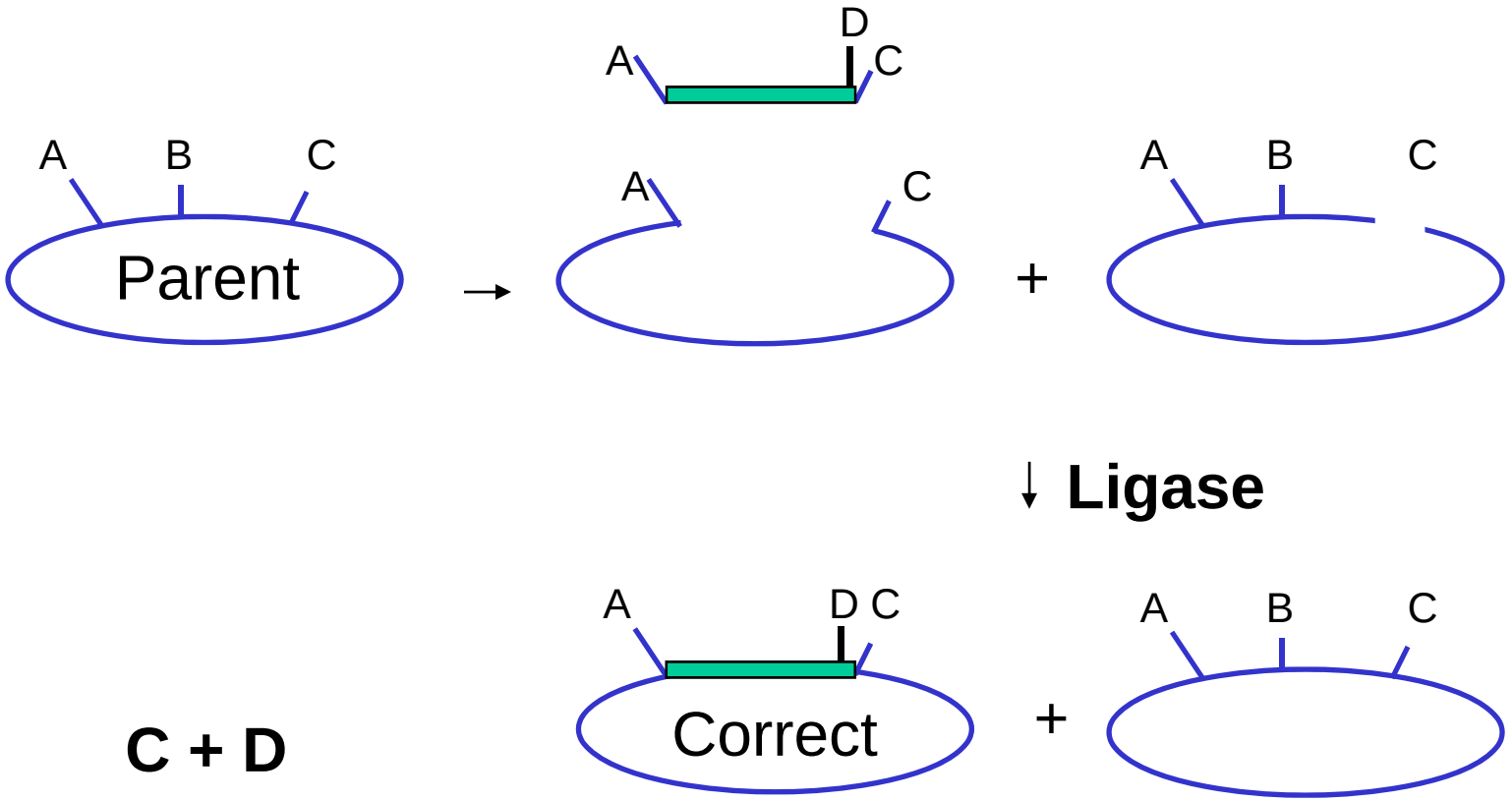
↓ Ligase



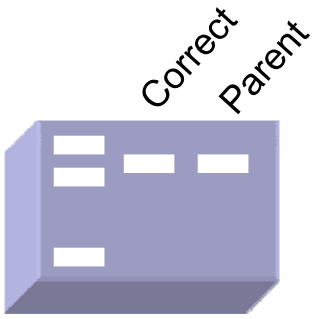
A + C



How to test for correct product...

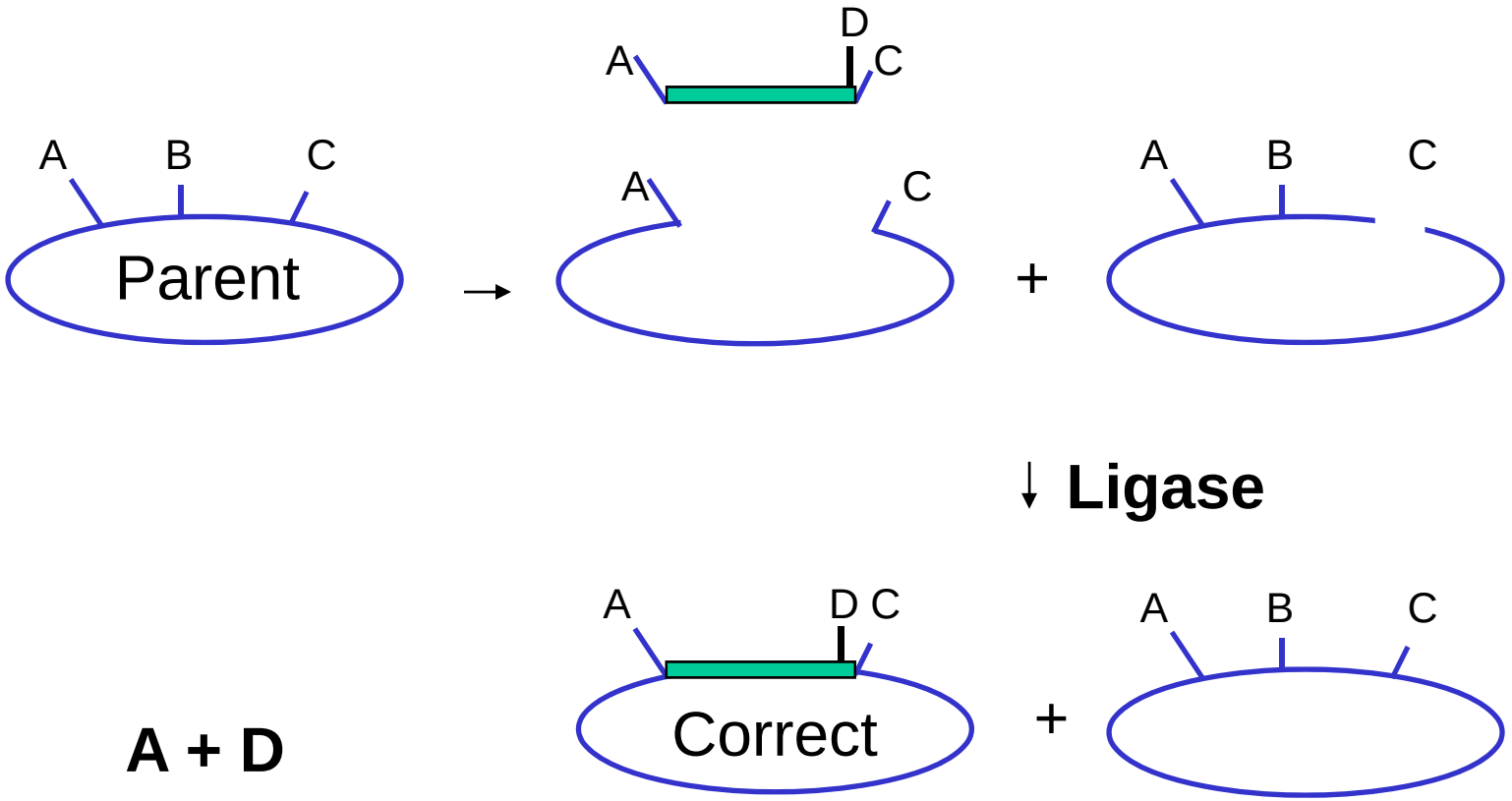


C + D

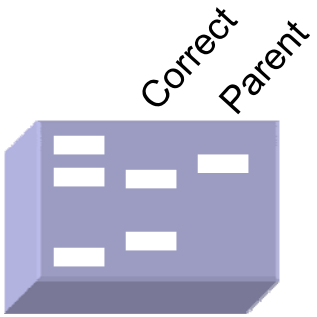


Be sure the expected fragment is easy to see and evaluate (*e.g.*, 0.3 to 2 kb)

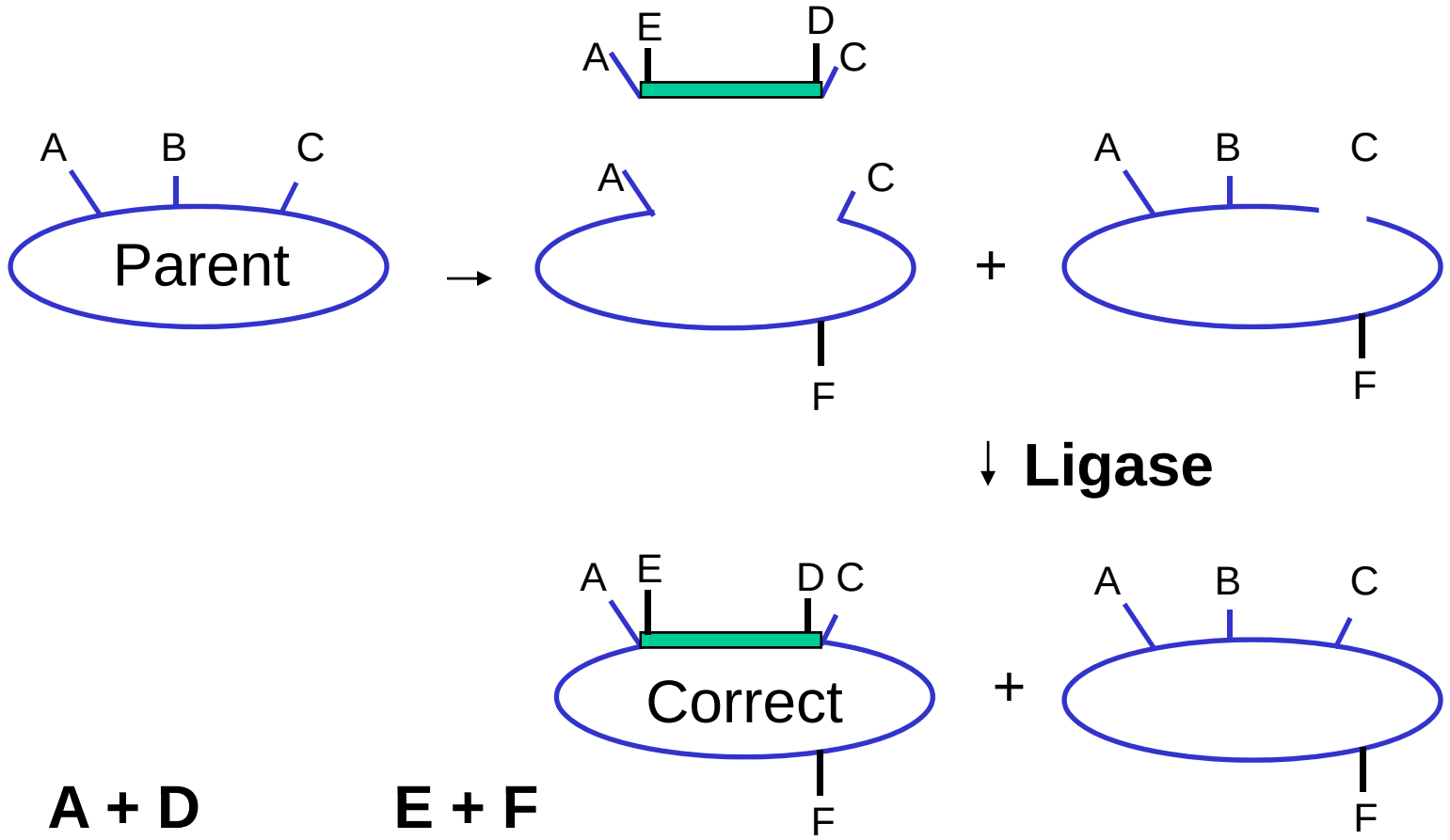
How to test for correct product...



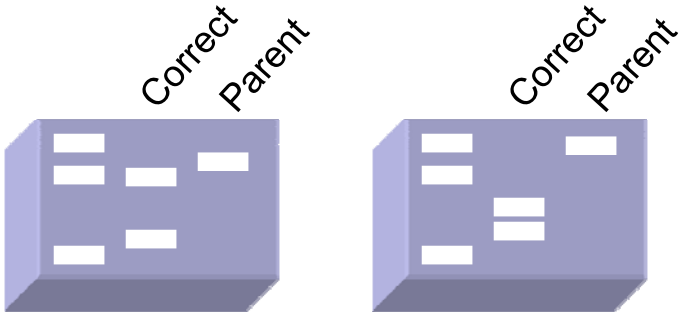
A + D



How to test for correct product...



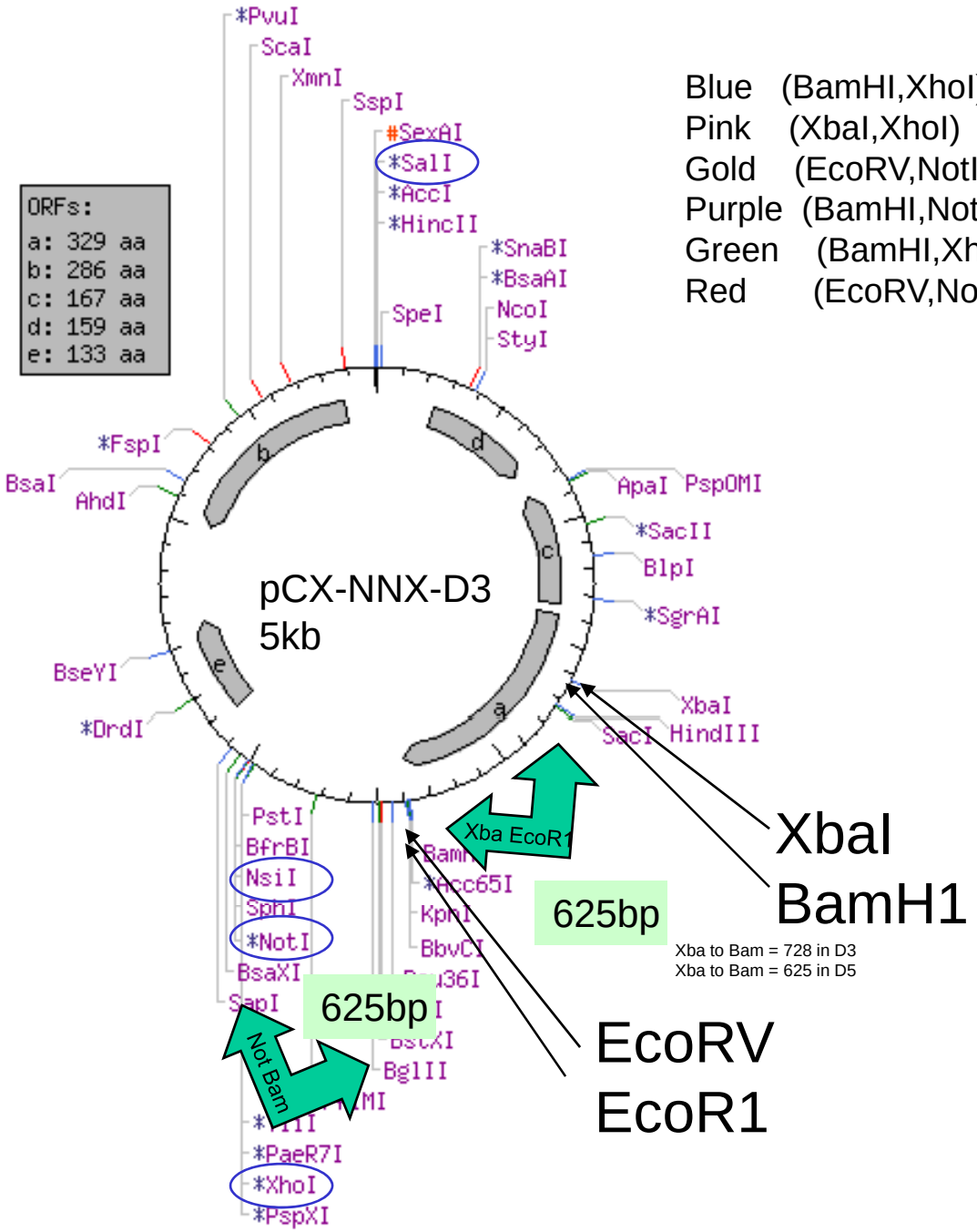
A + D **E + F**



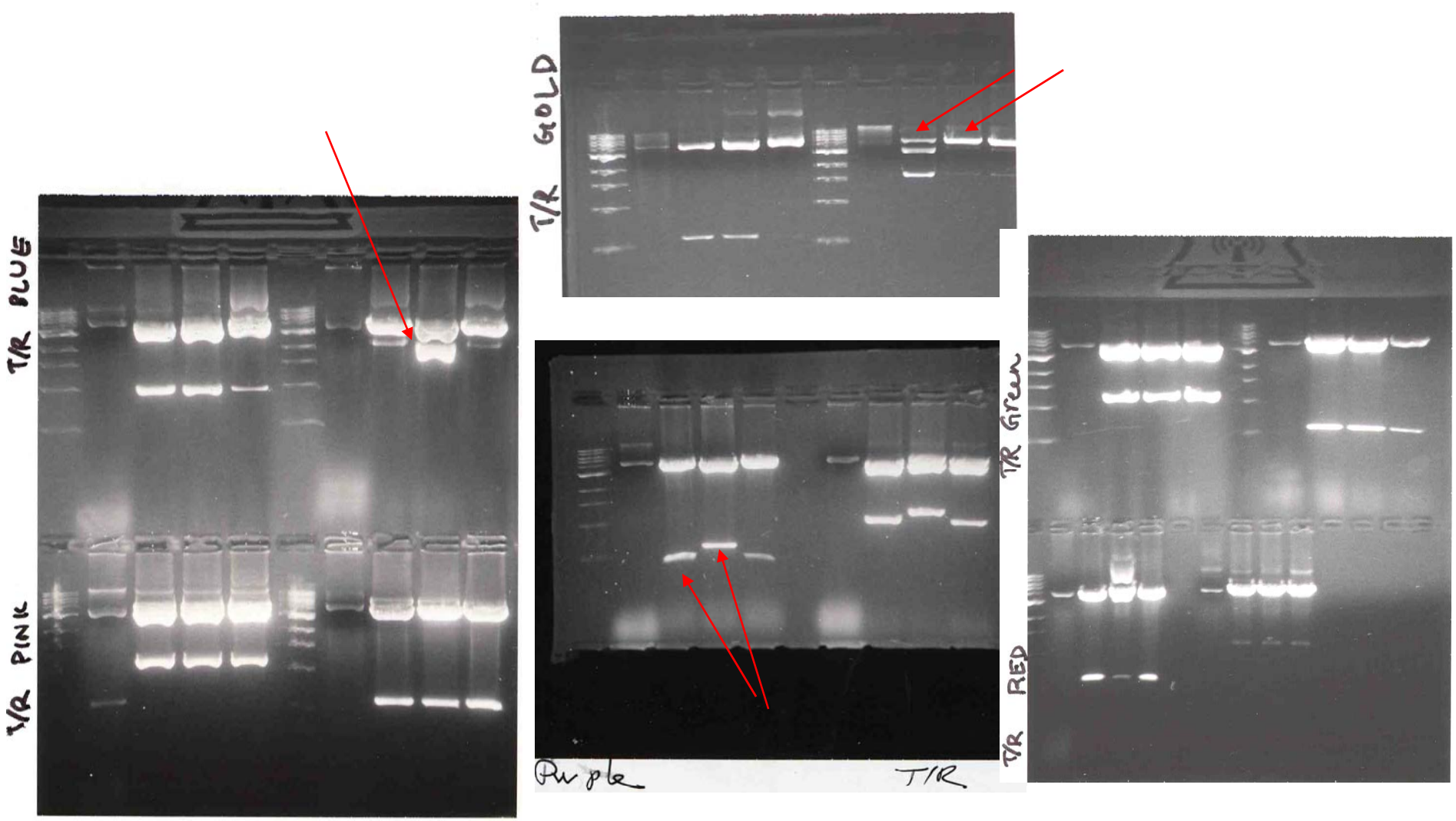
What will E+F find that A+D misses?

Why not use E + D?

Restriction Analysis: Raw Data



Blue (BamHI,XhoI) 1250 (SalI,EcoRV) 2.3 + 2.7
Pink (XbaI,XhoI) 1250 (EcoRV,NotI) 625
Gold (EcoRV,NotI) 625 (BamHI,SalI) 1623
Purple (BamHI,NotI) 1250 (EcoRV,NsiI) 625
Green (BamHI,XhoI) 1250 (EcoRV,NotI) 625
Red (EcoRV,NotI) 625 (BamHI,XhoI) 1200



Blue (BamHI,XhoI) ~1200 (SalI,EcoRV) 2.3 + 2.7
Pink (XbaI,XhoI) 1250 (EcoRV,NotI) 625
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Green (BamHI,XhoI) 1250 (EcoRV,NotI) 625
Red (EcoRV,NotI) 625 (BamHI,XhoI) 1200

Cutting Edge Approaches to Cancer Chemotherapy:

How an HR assay might be used

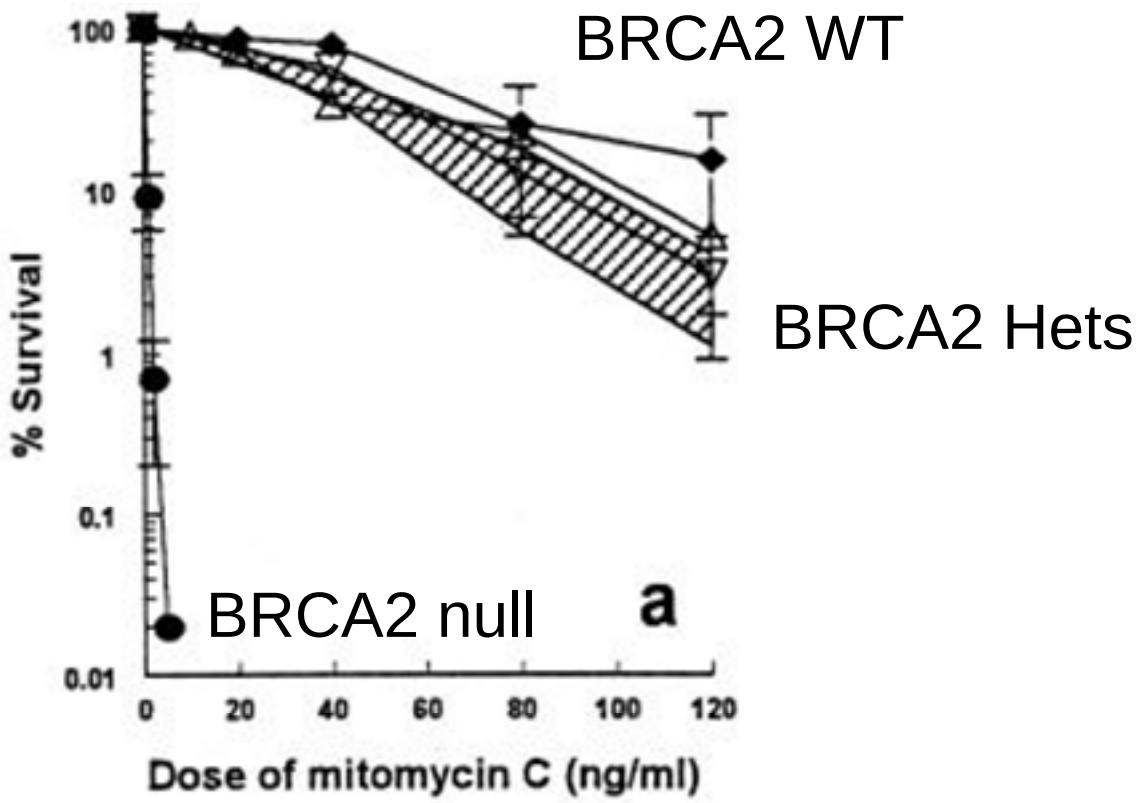
Cancer Treatment today: Why researchers care about Homologous Recombination

Limitations of today's treatments

What we know we want to do

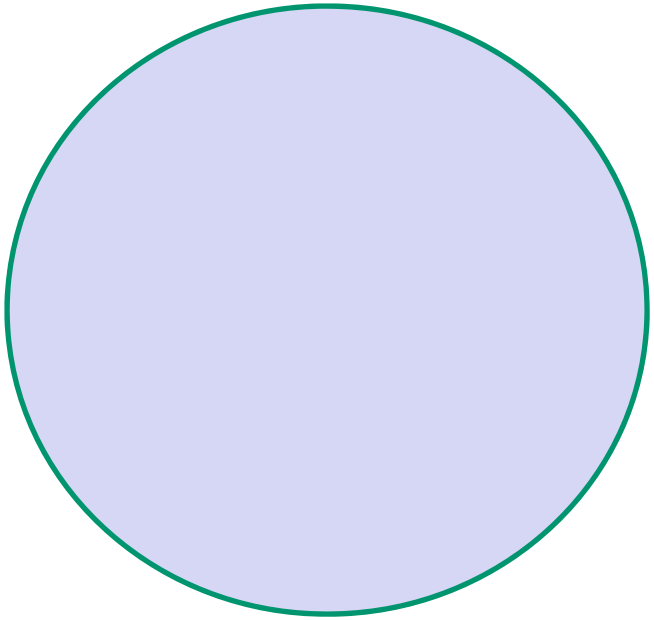
Combinatorial Therapy

-two different drugs at the same time
-a drug plus an siRNA to knock down expression

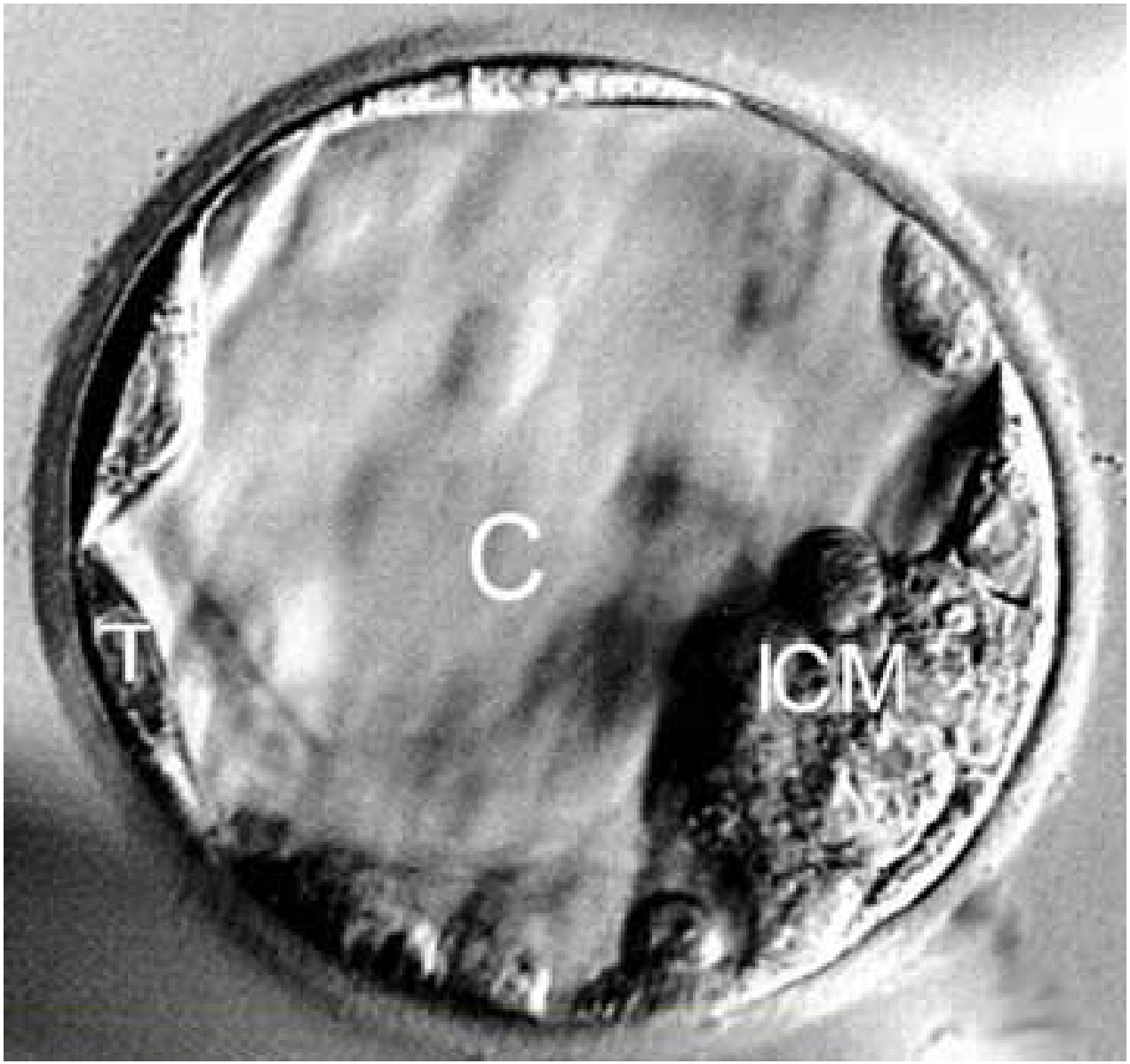


Ashworth Lab; Mol Cell Biol. 2002 January; 22(2): 669–679

What might affect the frequency of recombination between two plasmids?



What kind of cells will you be using in your experiment?


C
T
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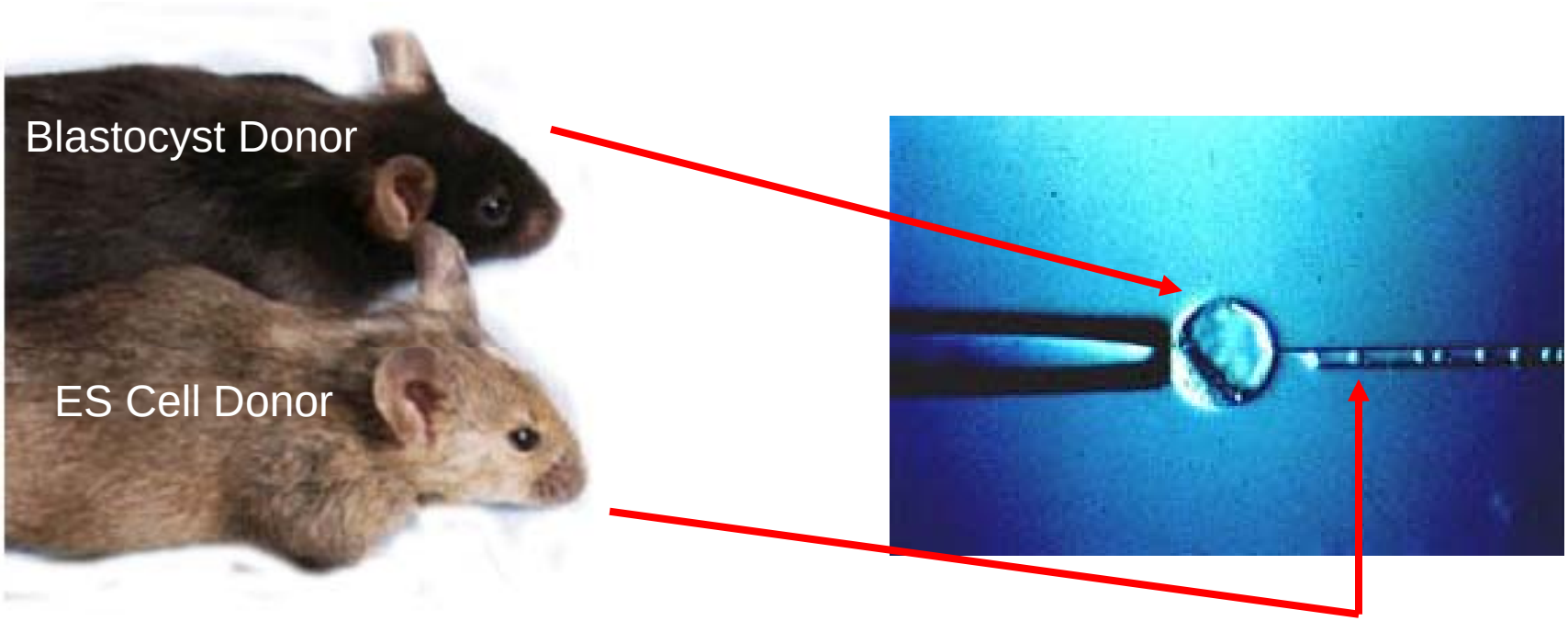
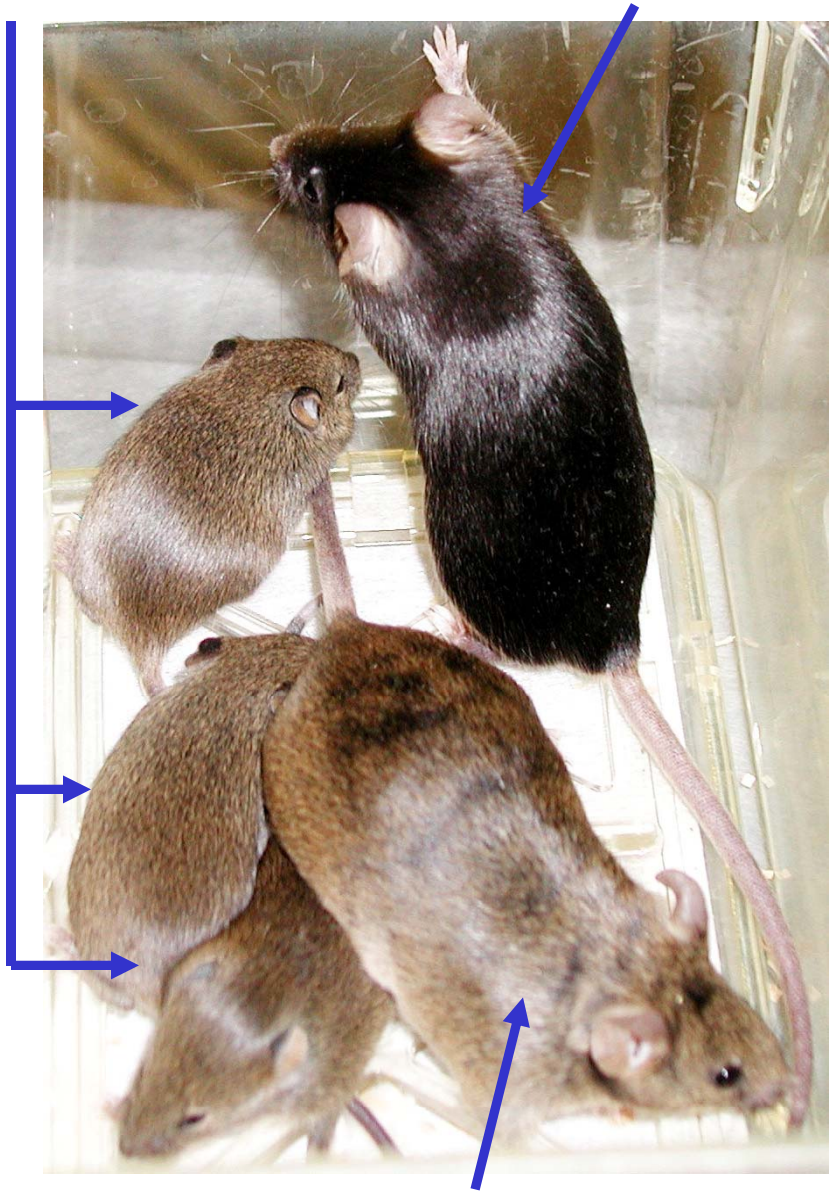

Blastocyst Donor
ES Cell Donor

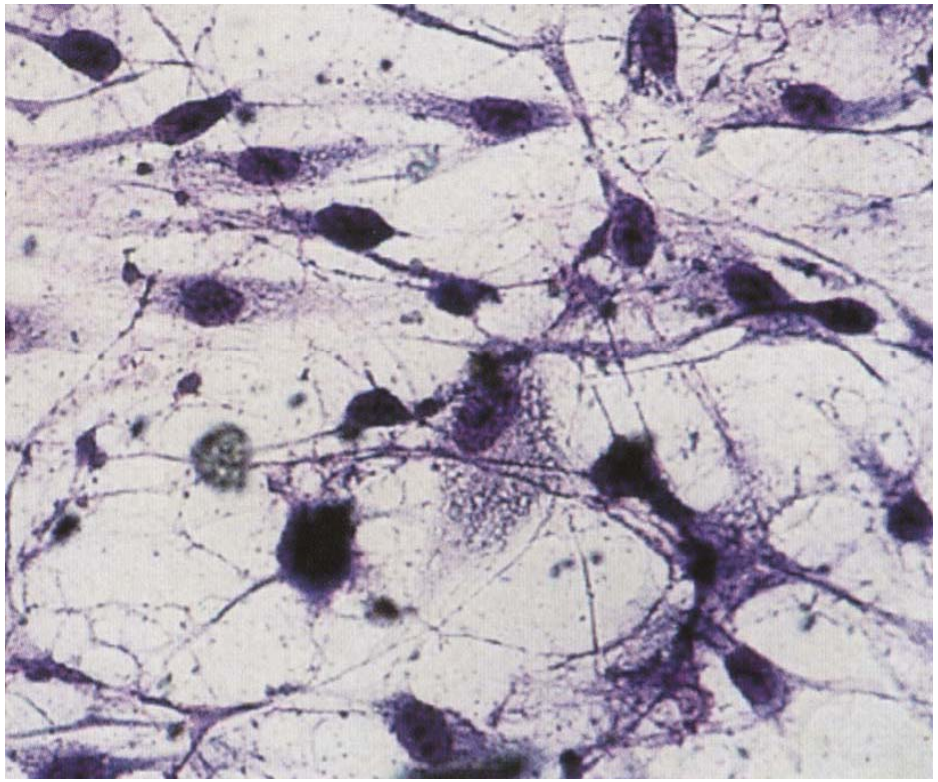
Germline Offspring

C57Bl Male

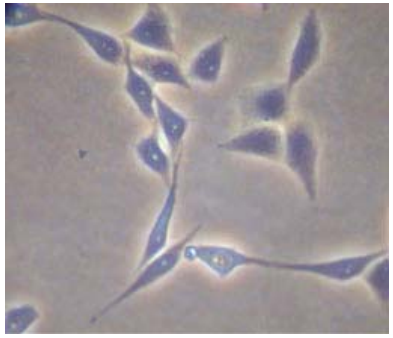


Germline Chimeric Female

Where do mammalian cells come from?

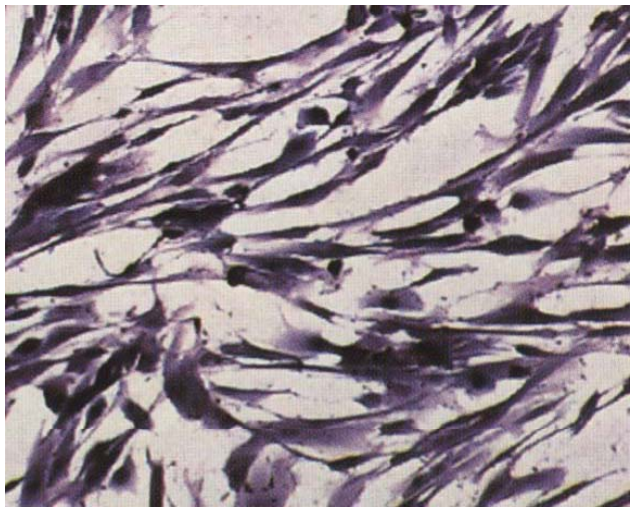


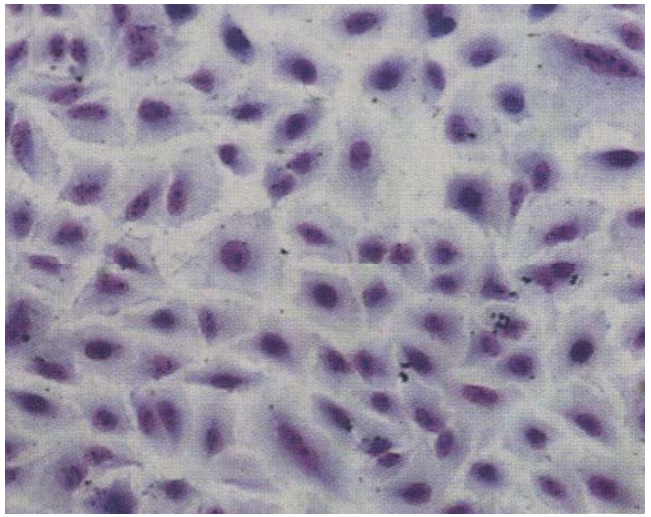
Glial cells



Hela cells

Normal lung fibroblasts





Lung cancer cells

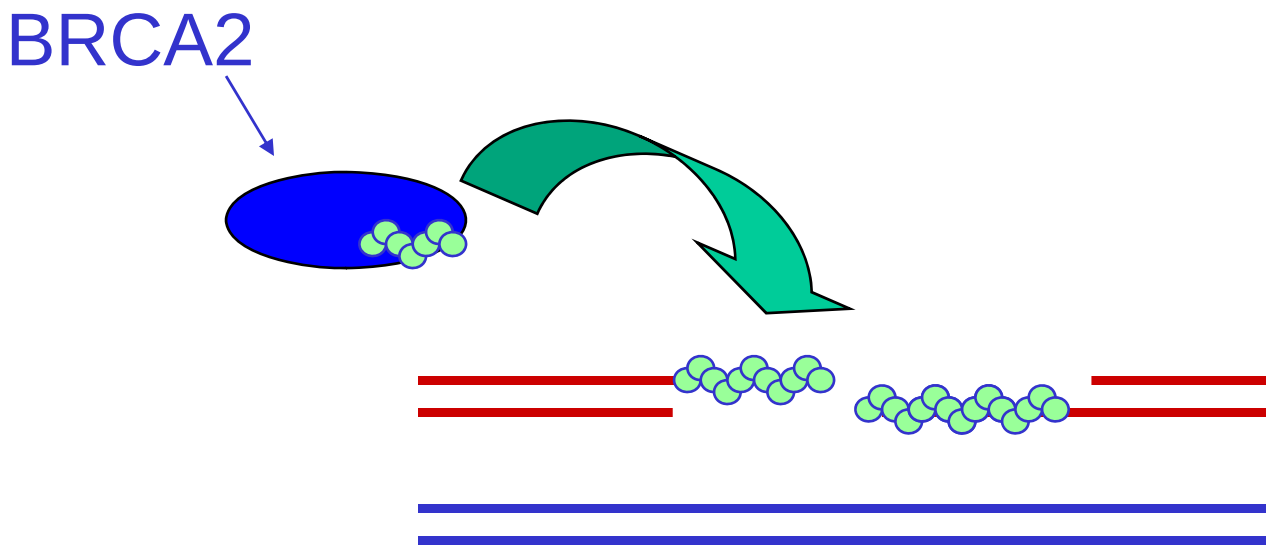
Terminology you need to know:

Primary
Transformed
Undifferentiated
Cell Line (vs ‘strain’)
Isogenic
Genomic Stability
Mixed populations

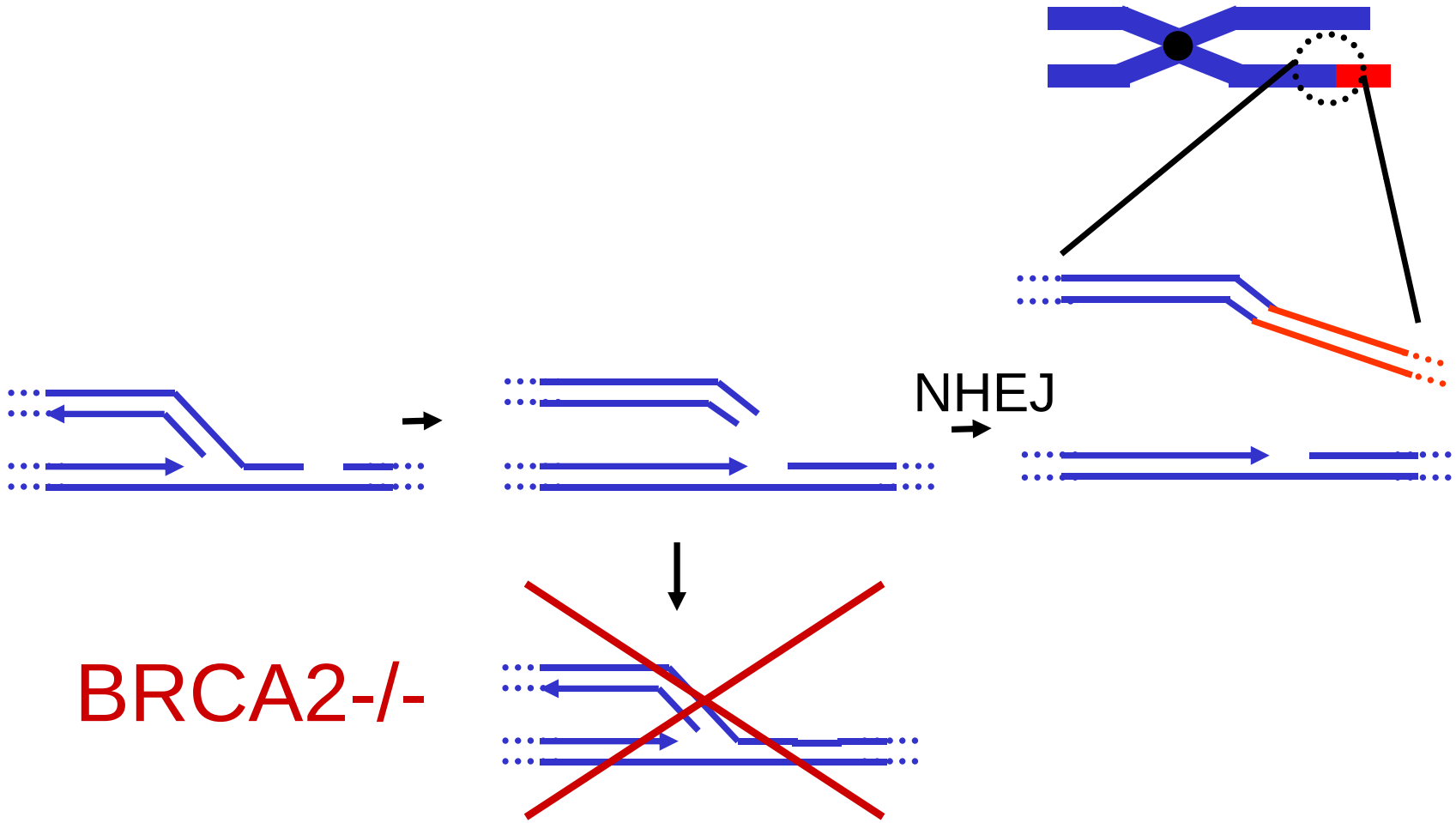
What might affect the frequency of recombination between two plasmids?

Roles of Homologous Recombination:

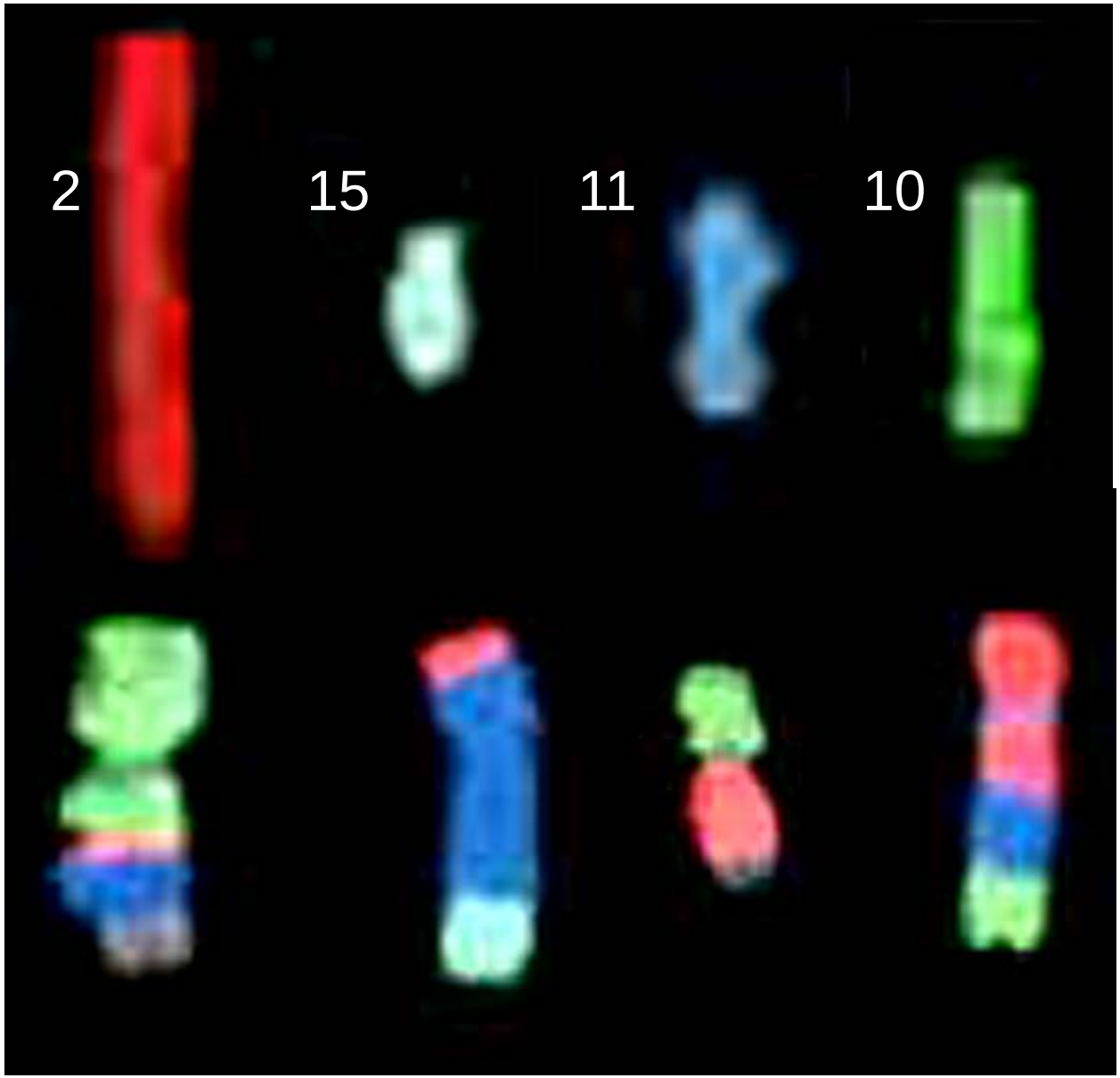
Preventing Cancer & Affecting Treatment



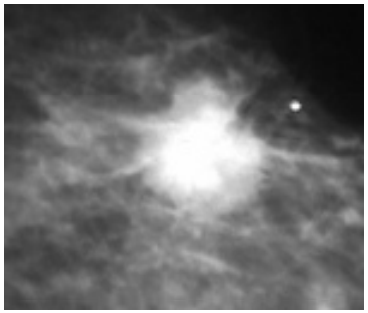
BRCA2 Loads Rad51


NHEJ
BRCA2-/-

Normal Human Chromosomes



BRCA2 -/- Chromosomes

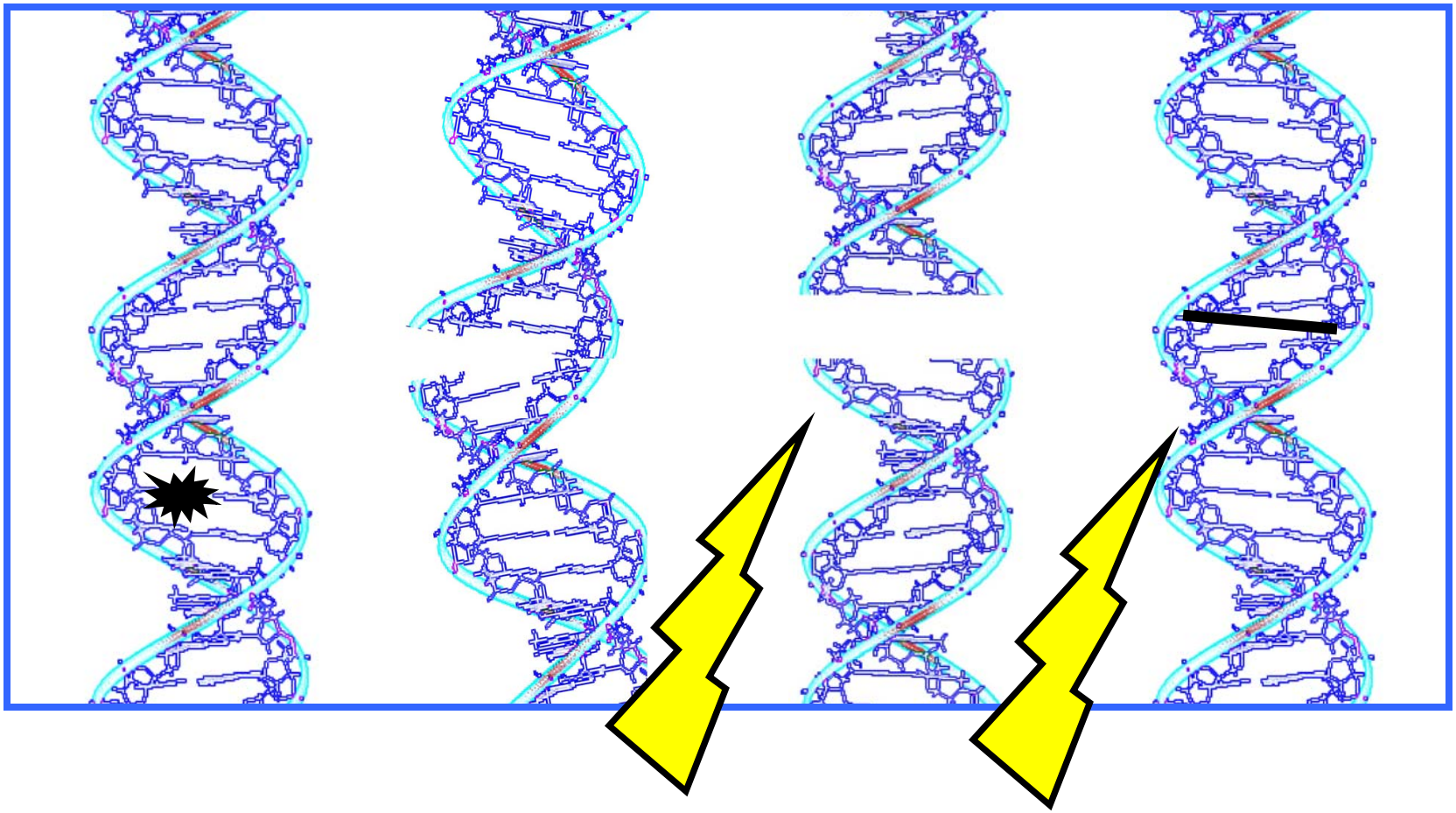


www.rctradiology.com

Raw data from Grigorova *et al*., Cytogen. and Gen. Res. 104:333-340 (2004)

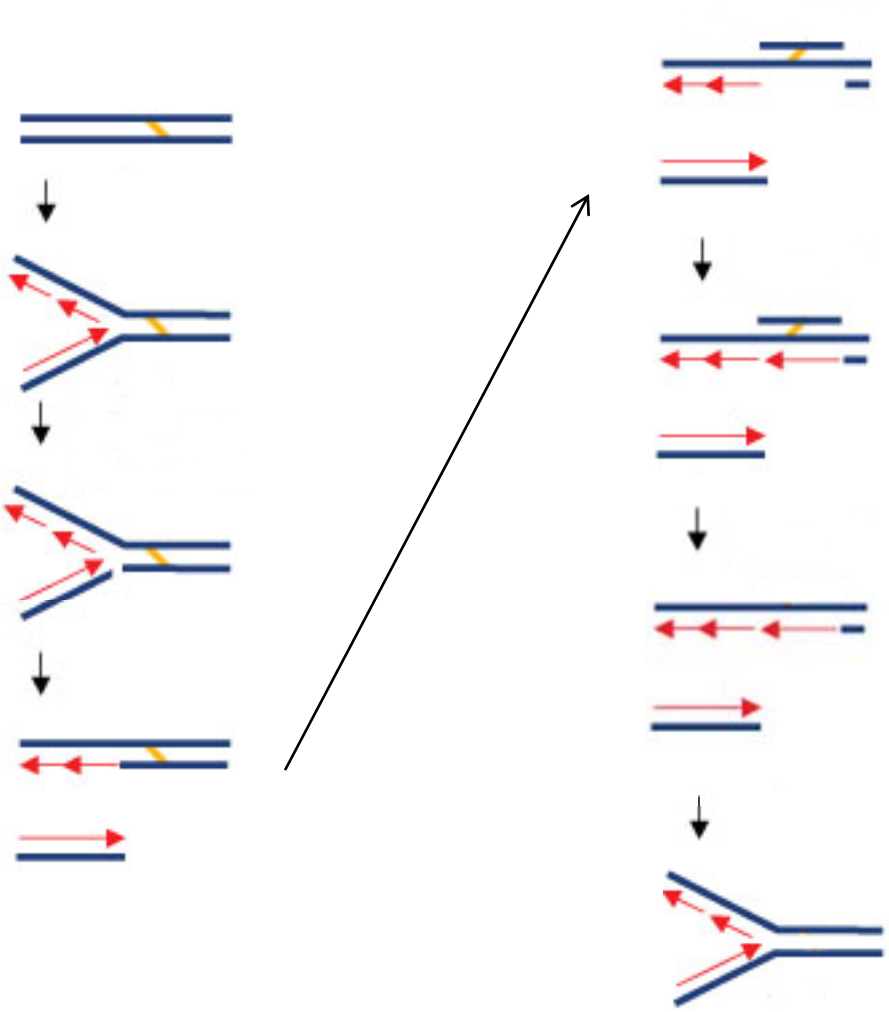
Homologous Recombination

Toxicity

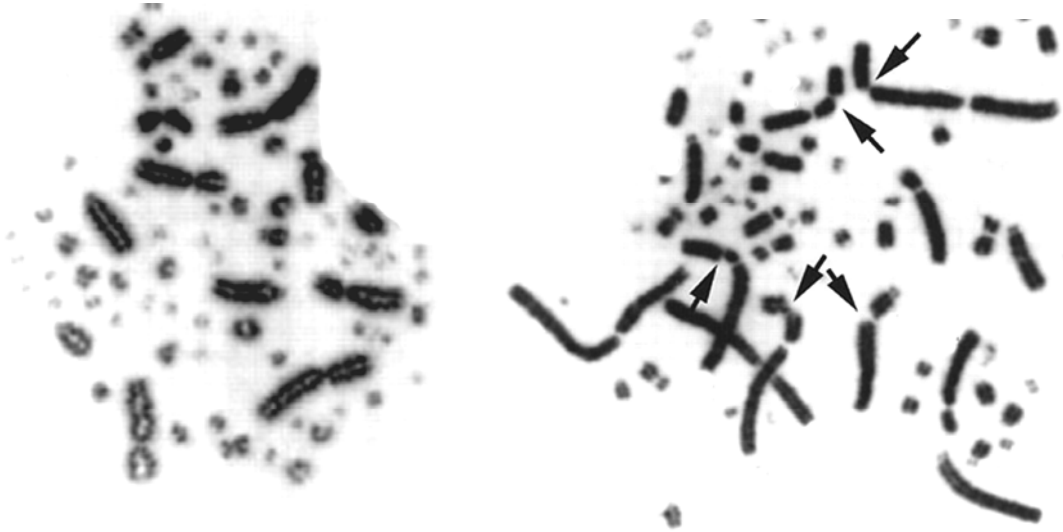


Radiation & Chemotherapy

Replication Fork Animation
By Tet Matsuguchi

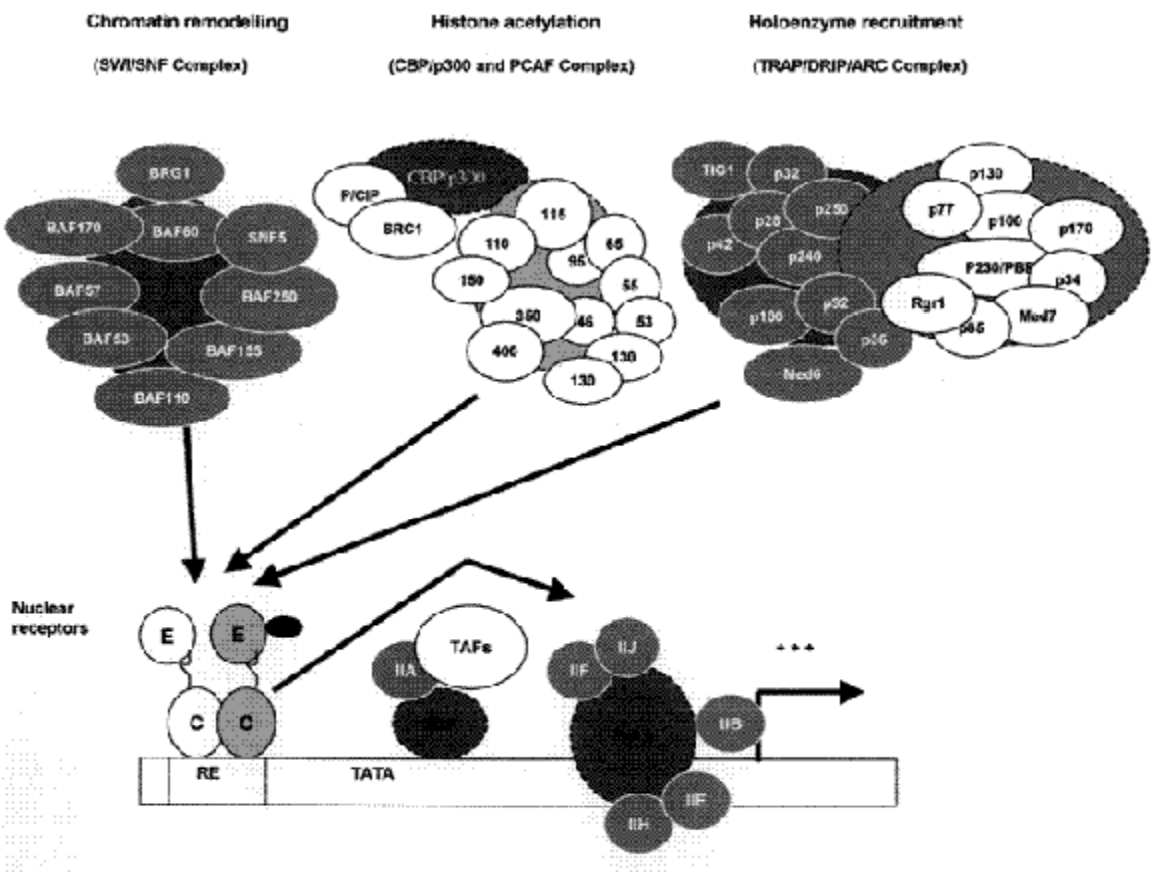


Why you owe Your Life to Homologous Recombination…

Normal  Rad51-/-

Turn Off Homologous Recombination
→ Chromosomes Fall Apart

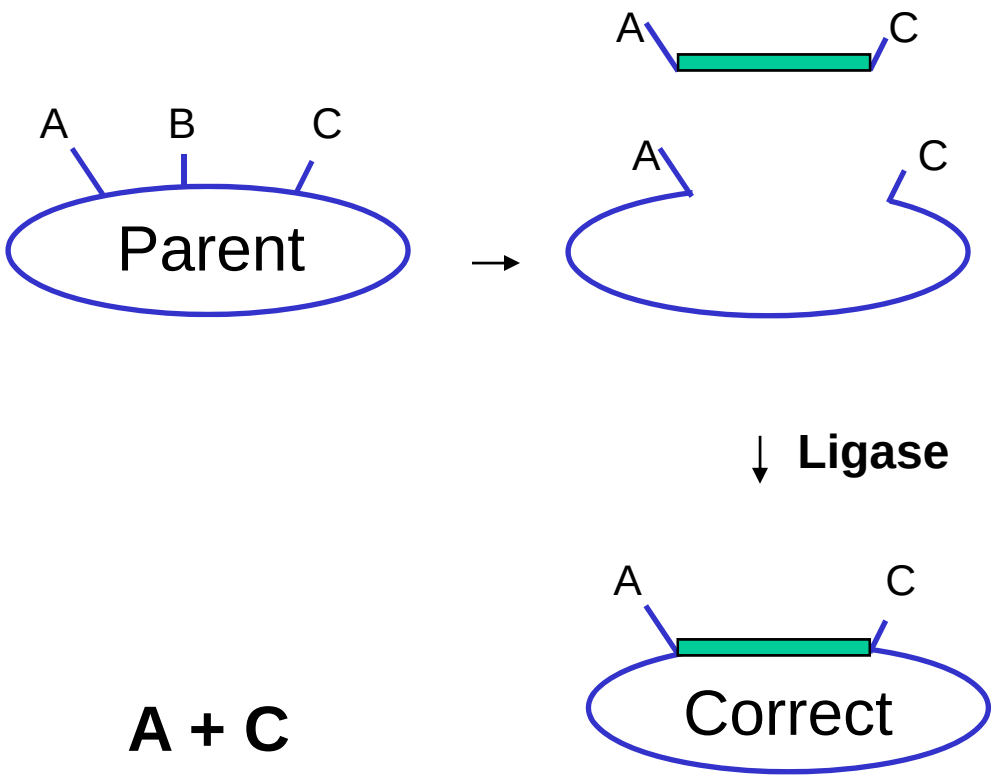
Sonada *et al*., *EMBO J.* **17,** 598–608 (1998).



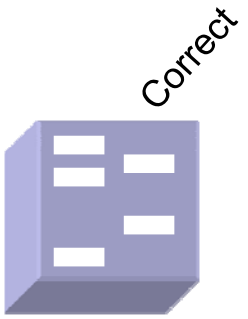
http://www.scielo.cl/fbpe/img/bres/v35n2/img21-02.gif

How can you test to make sure your vector is correct?

How to test for correct product...



A + C



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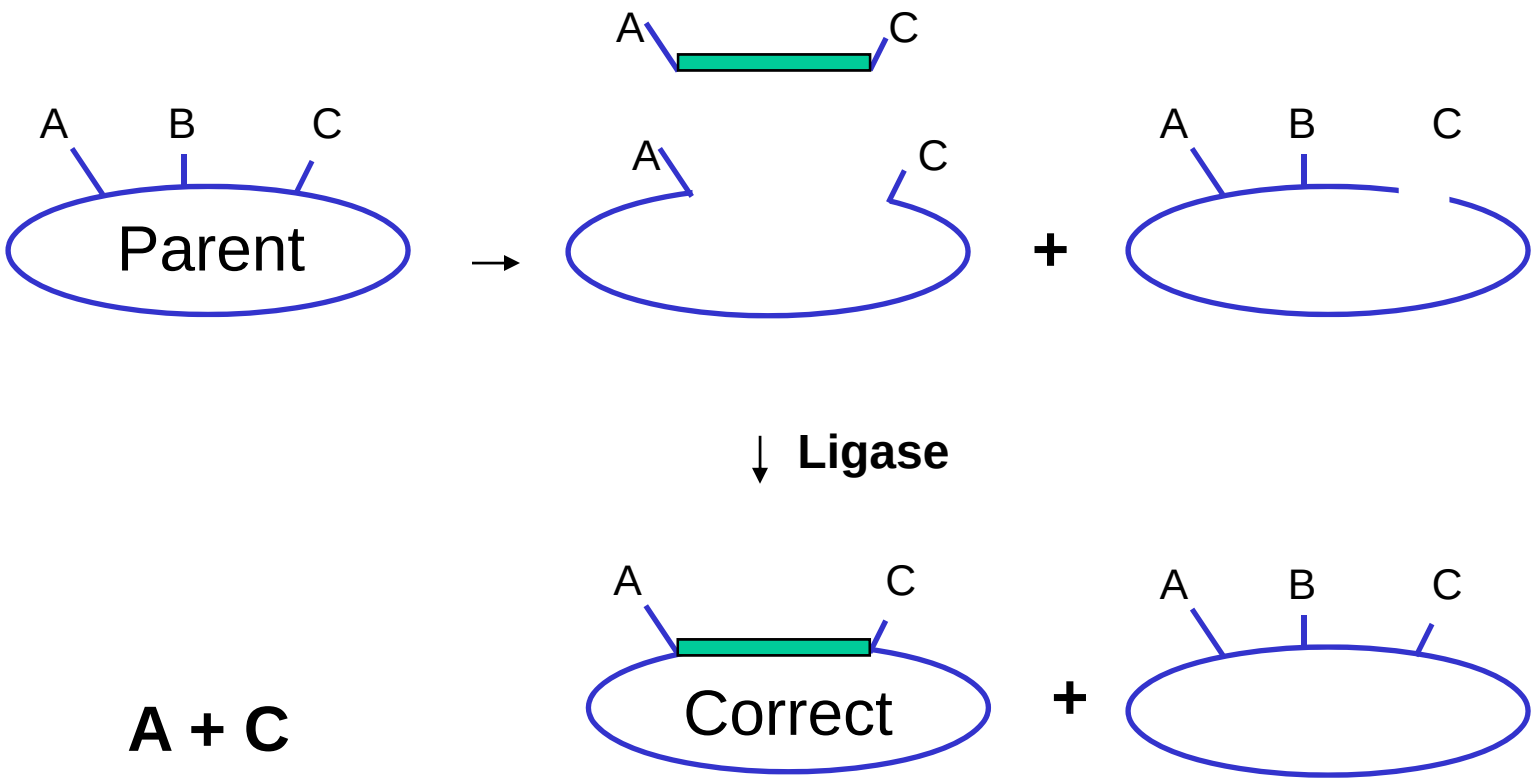

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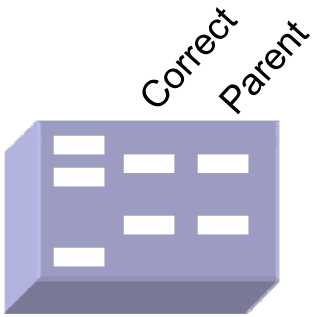

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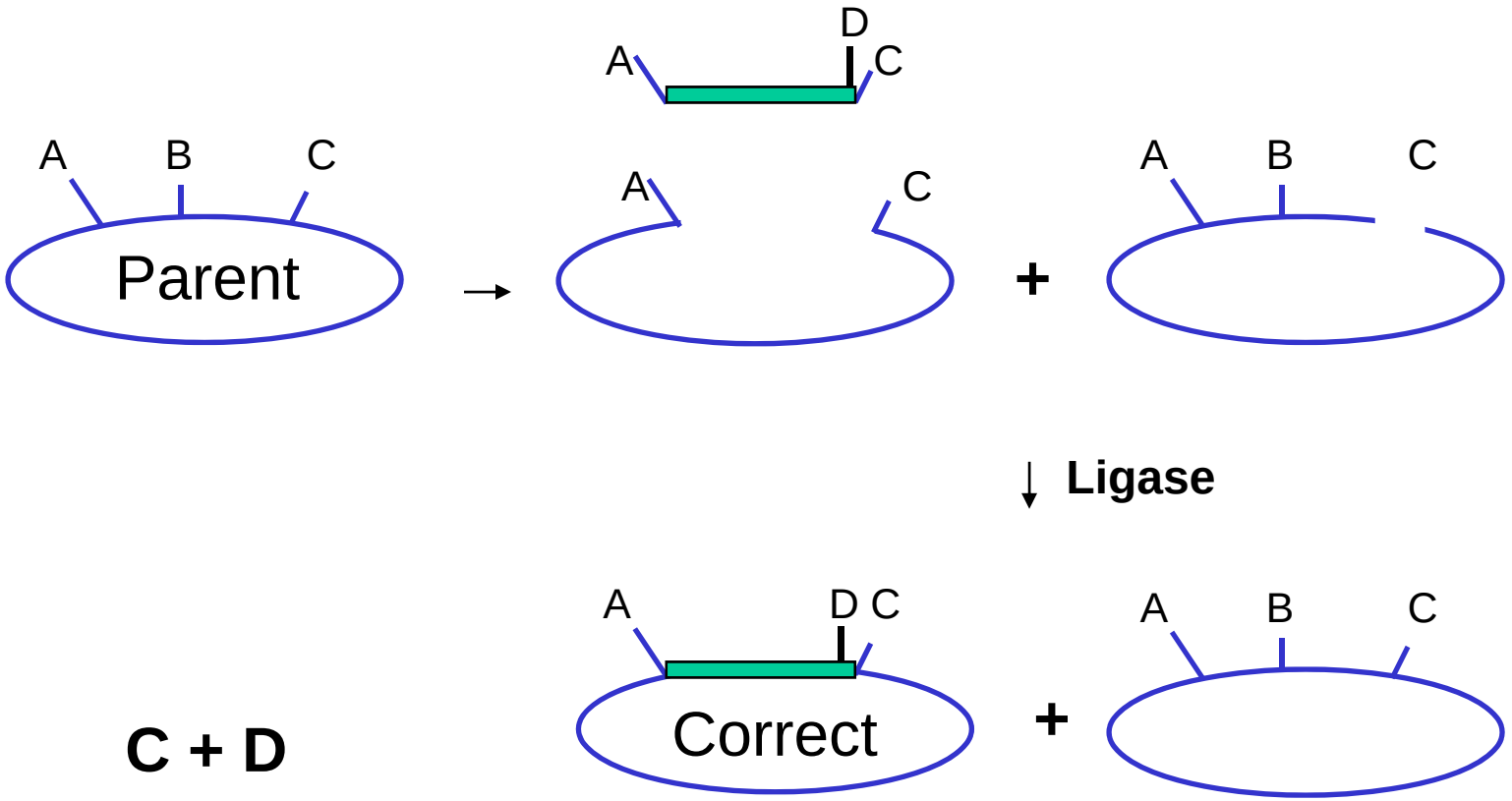
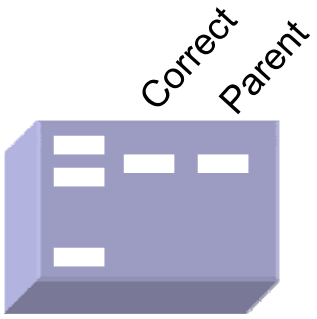
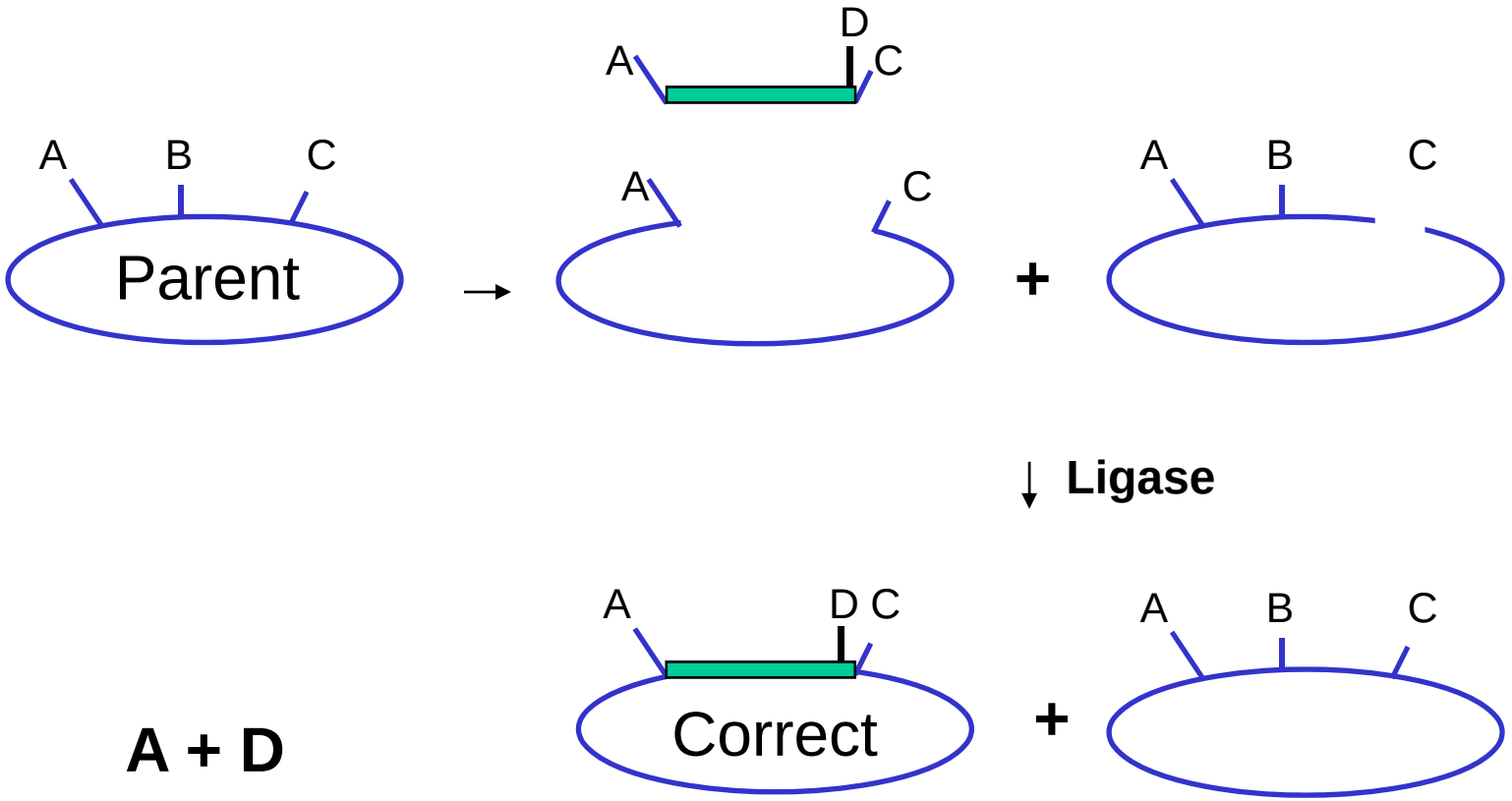


C + D

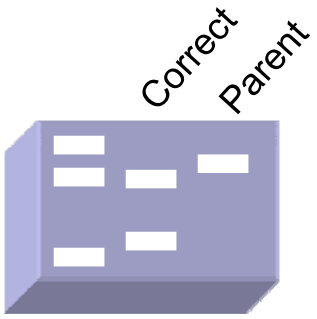


Be sure the expected fragment is easy to see and evaluate (*e.g.*, 0.3 to 2 kb)

How to test for correct product...



A + D



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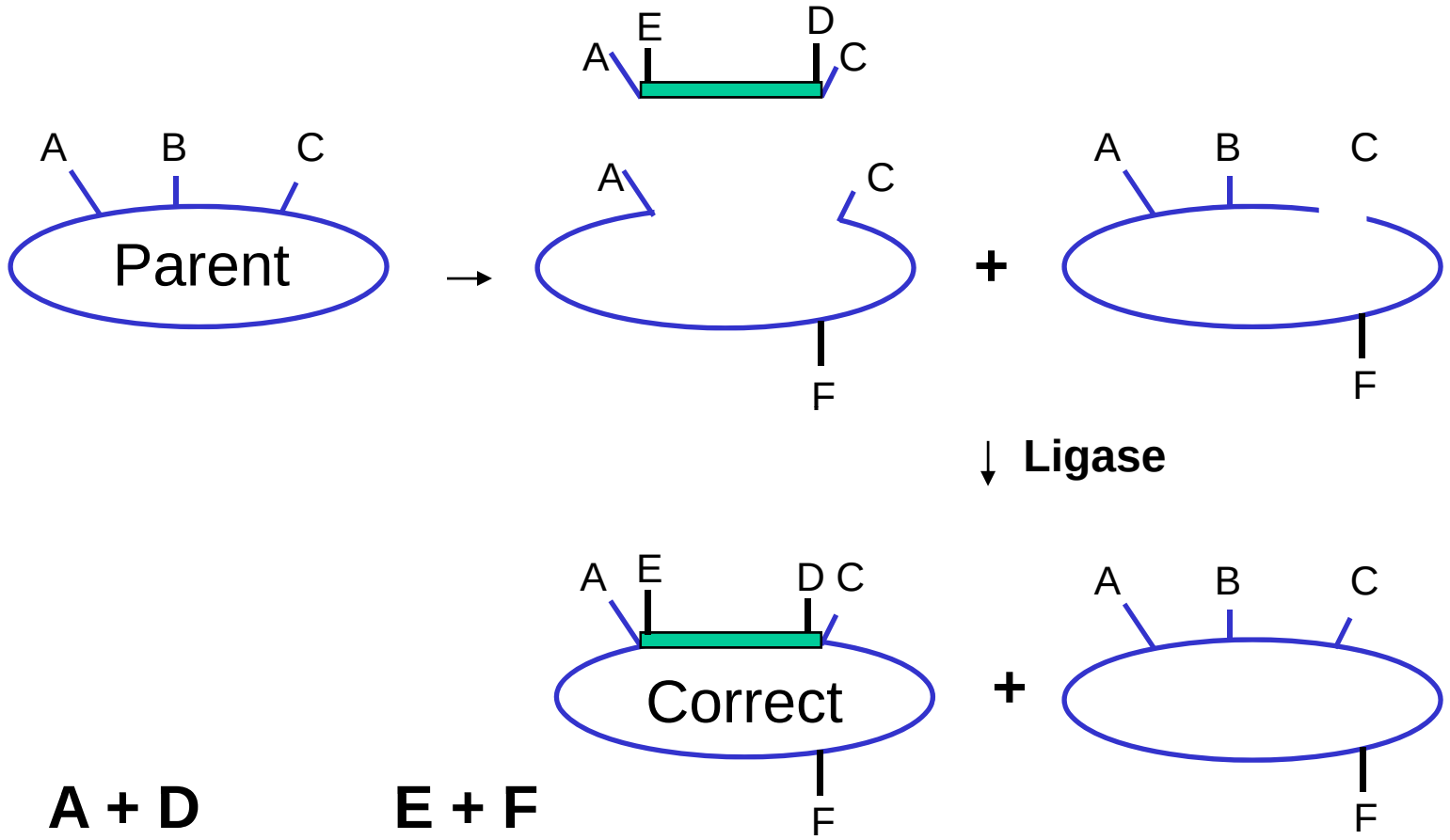
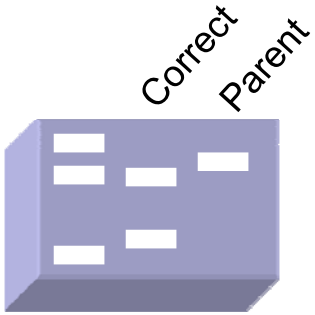
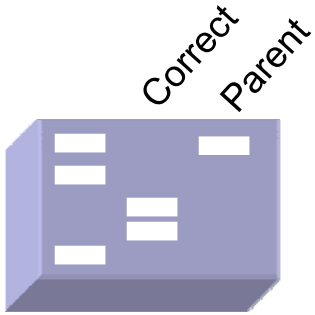


A + D



E + F



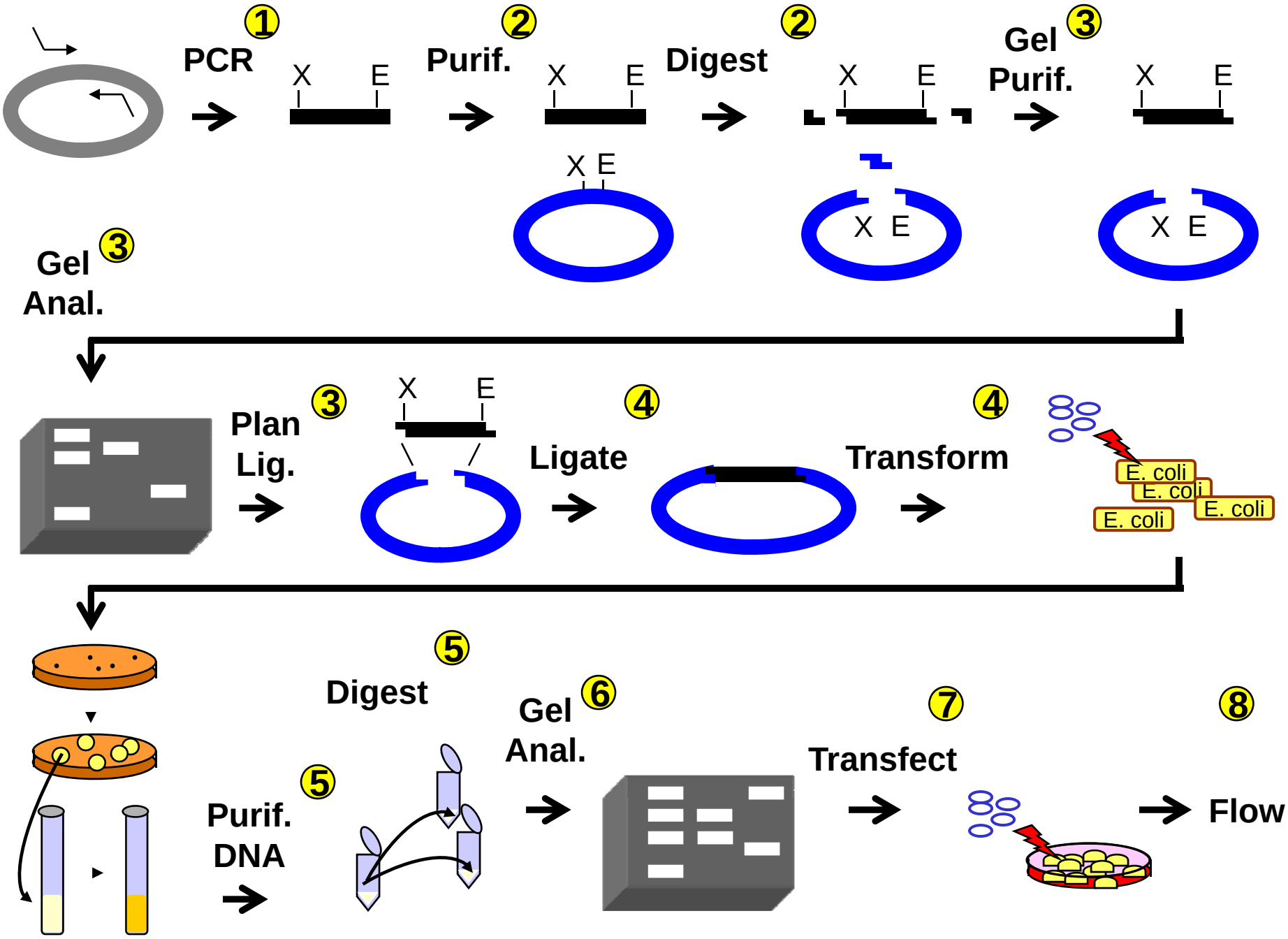
What will E+F find that A+D misses?

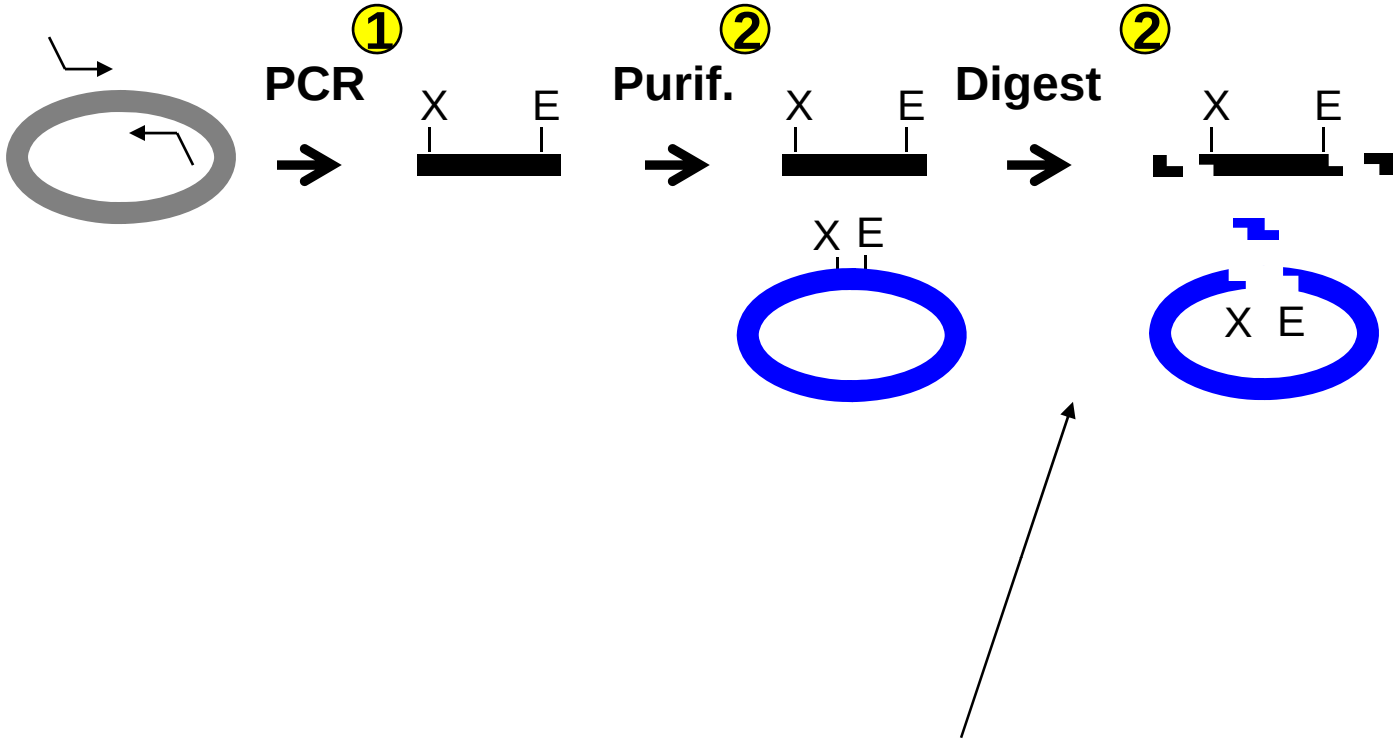
Why not use E + D?

MOD 3 – GENETIC ENGINEERING

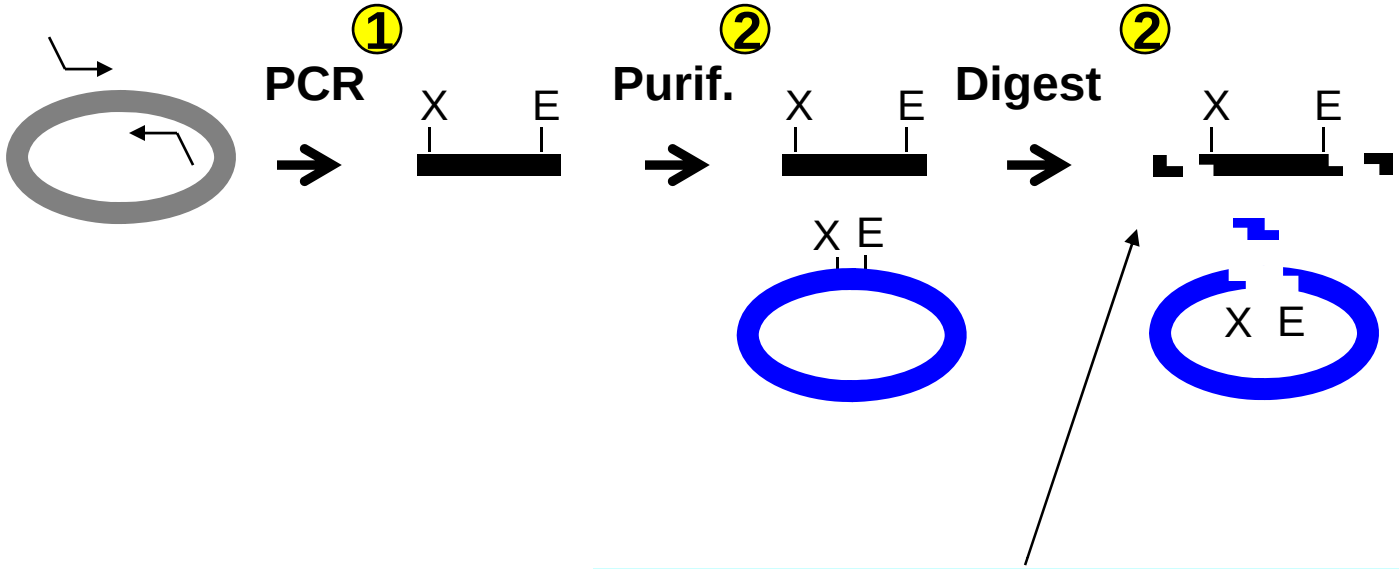
Day 4

1) Roadmap Review
2) Restriction Enzymes
3) Ligase
4) Tips on getting your experiments to work
5) Restriction Mapping


1
PCR
X
E
2
Purif.
X
E
2
Digest
X
E
3
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
3
Plan
Lig.
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow

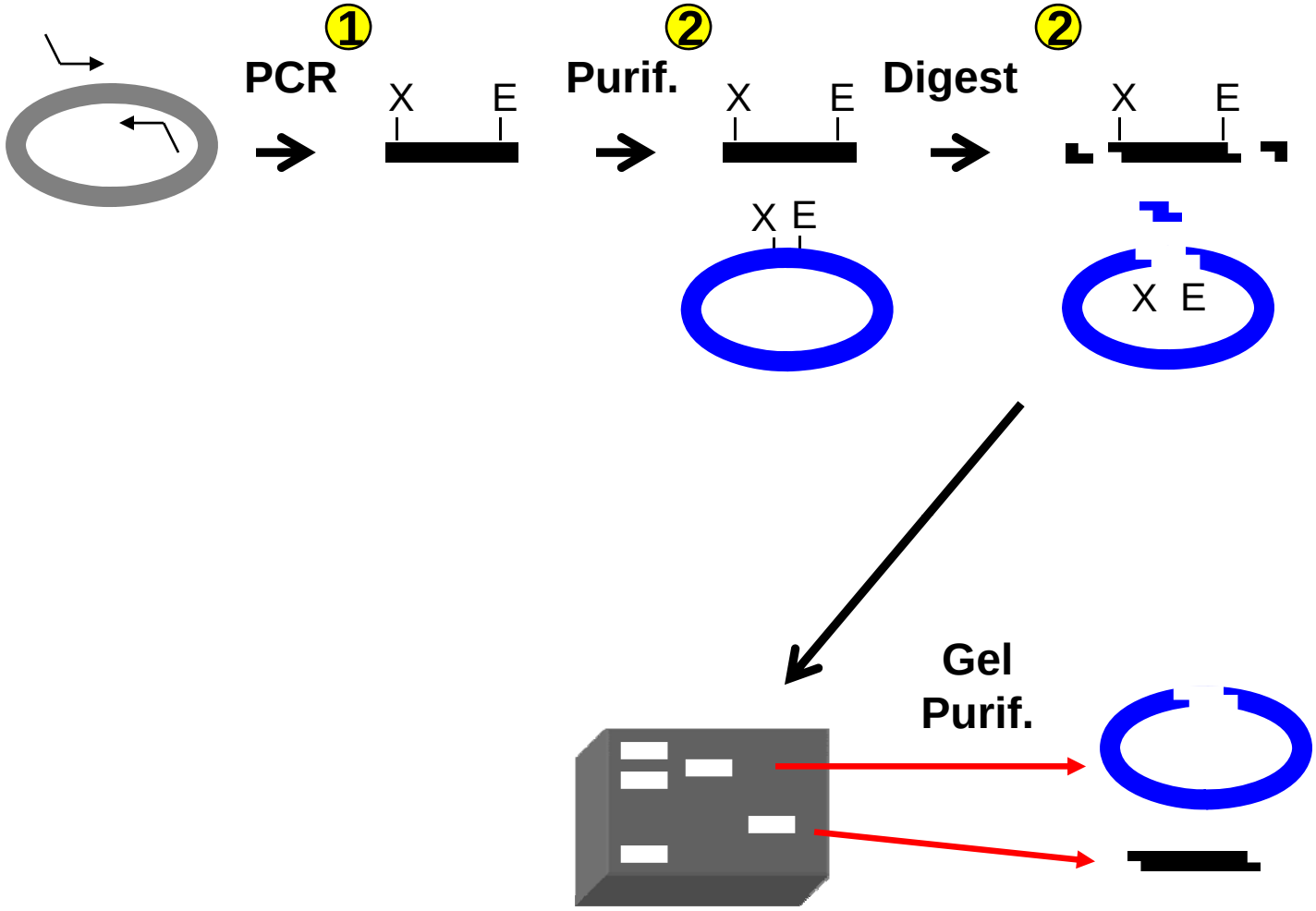


How do you know that your restriction enzymes actually cut the DNA?

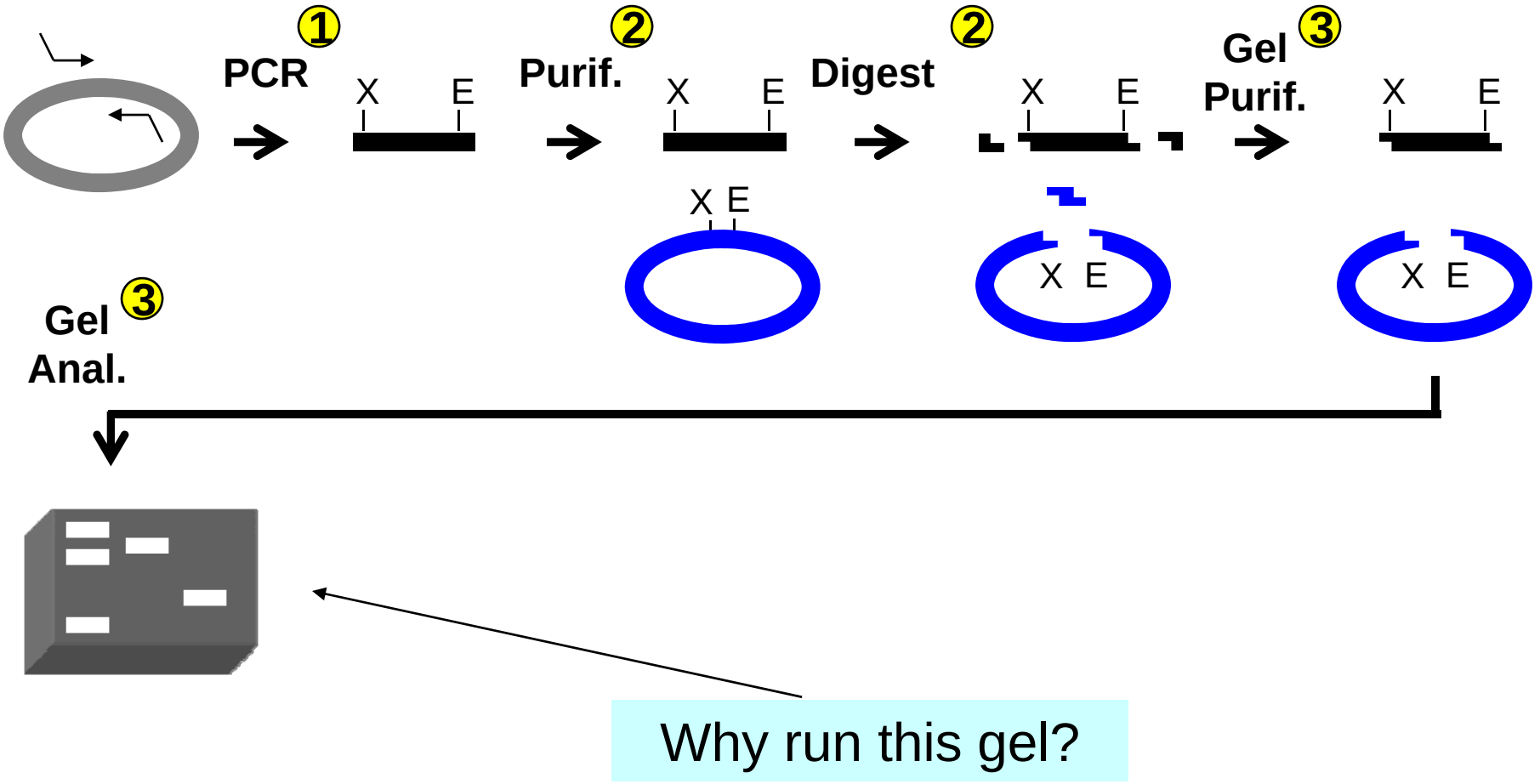


What else is in the reaction with the digested PCR product?

What effect could it have?

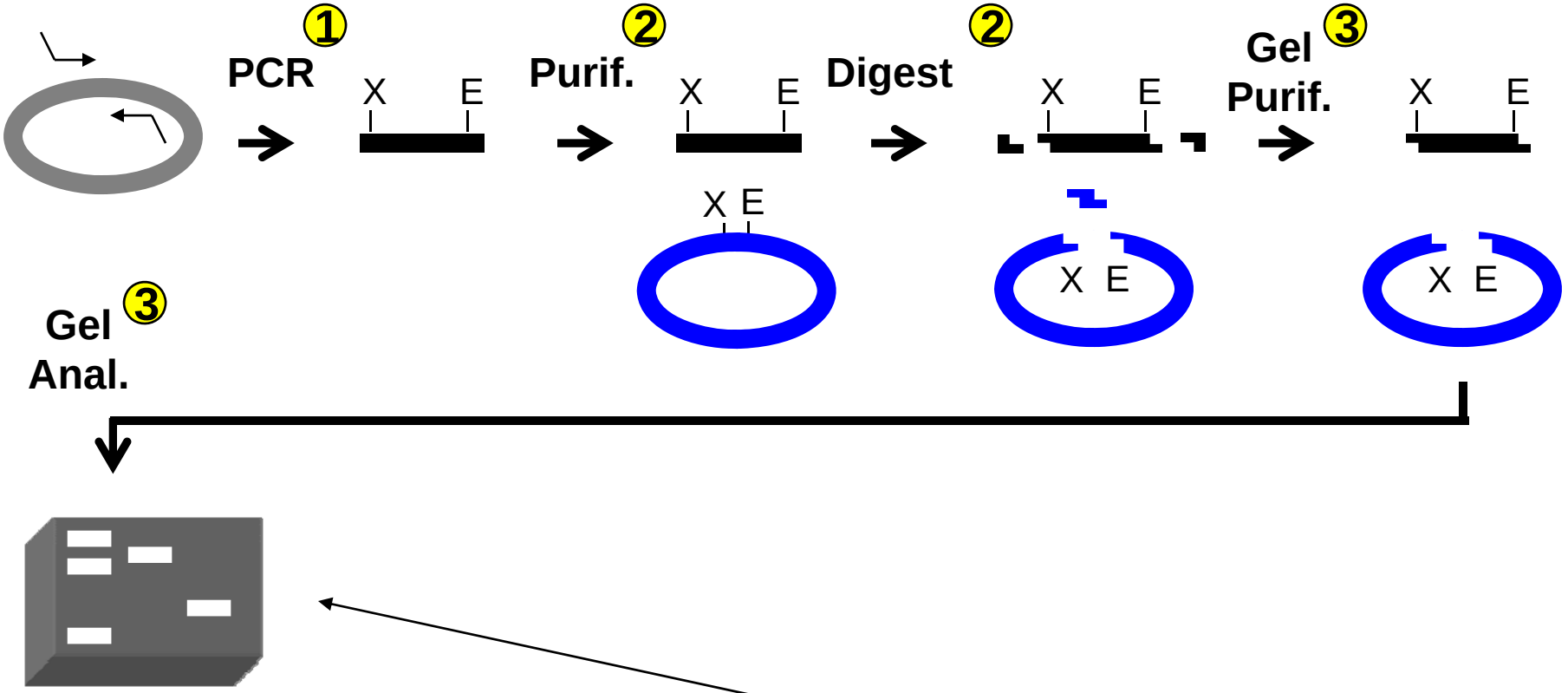


Why is it important to excise the DNA from the gel relatively quickly?



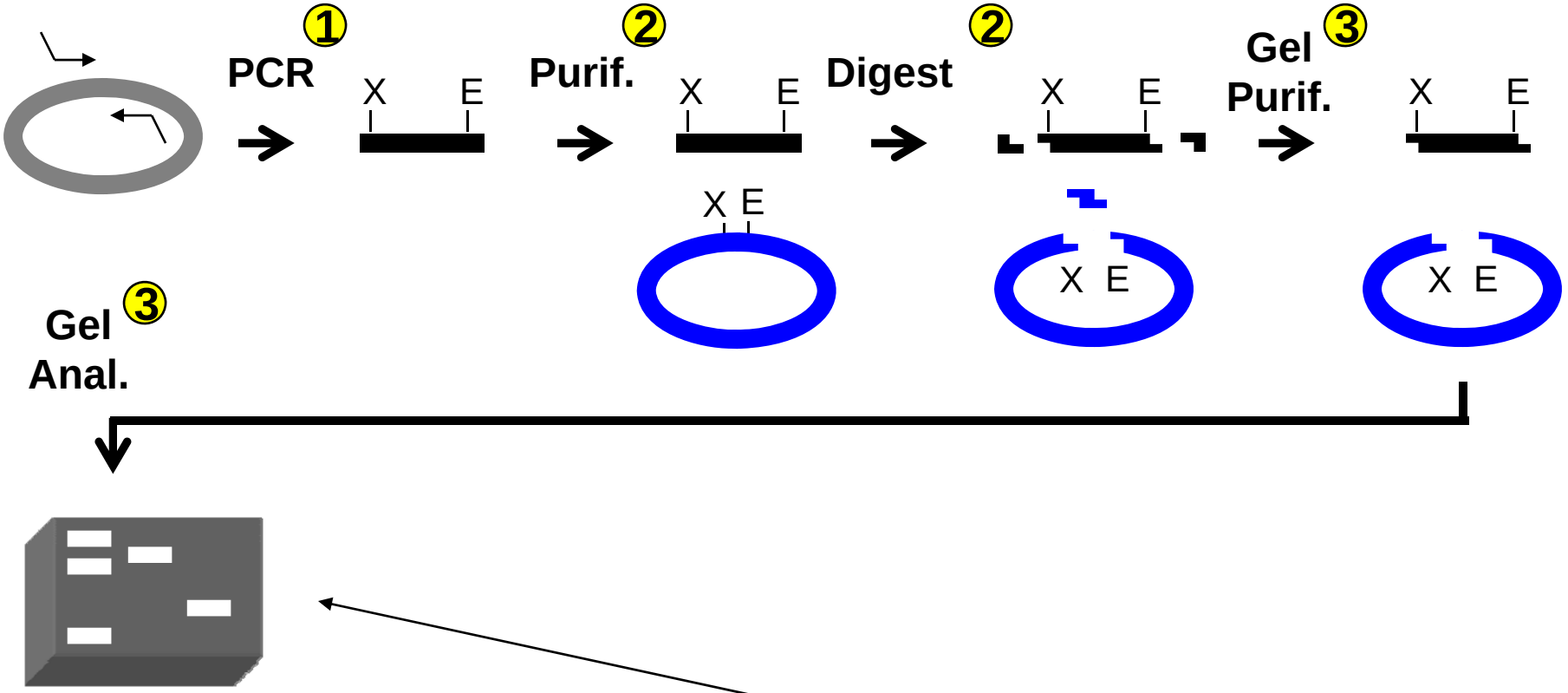
Why run this gel?

Ran out of time here.. Will cover some in during lab time and rest during lecture.



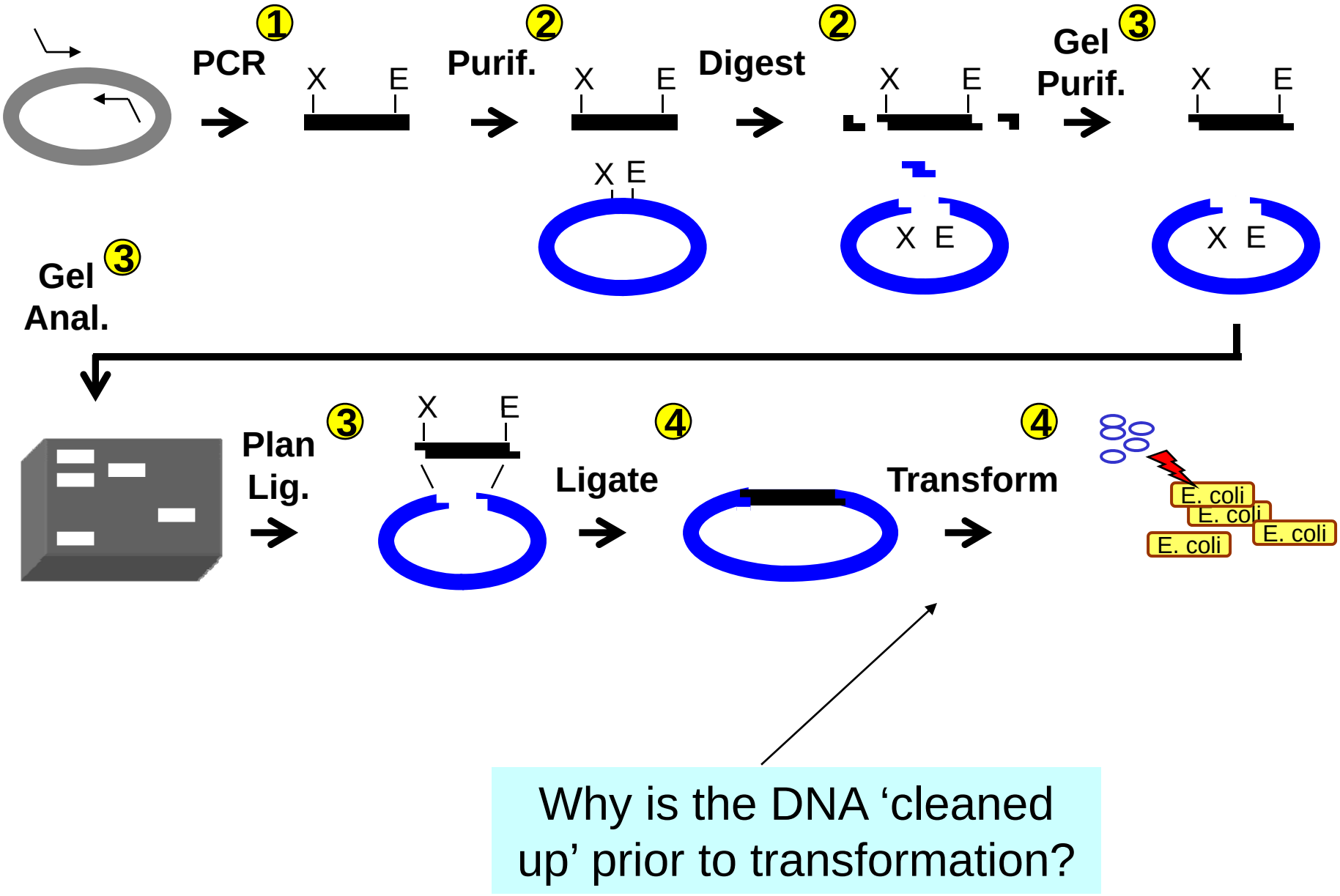
Your objective is a 1:4 vector:insert ratio – Why?

What is it was 1:100?
What if it was 100:1?

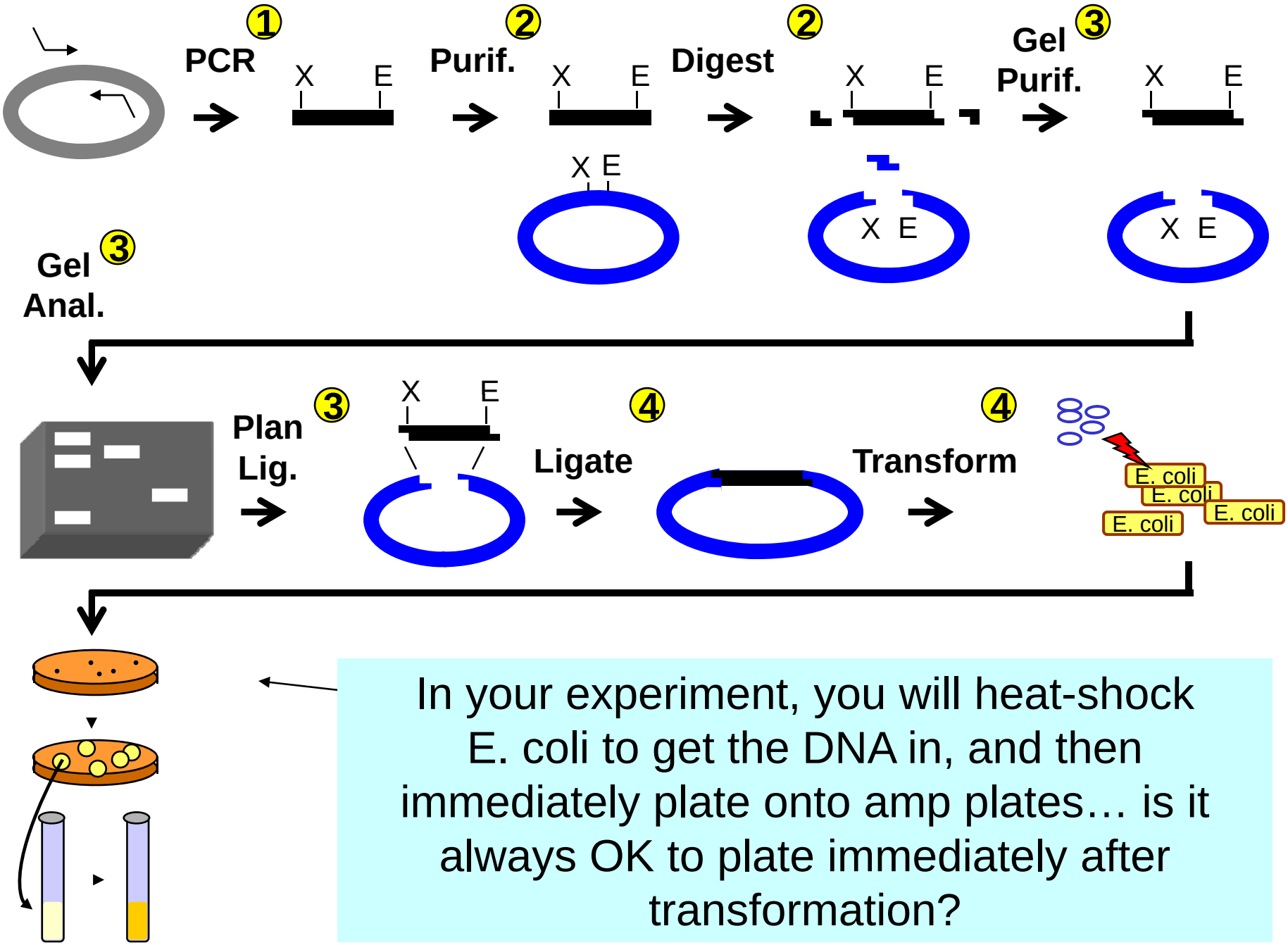


How do you figure out how to get a 1:4 molar ratio?

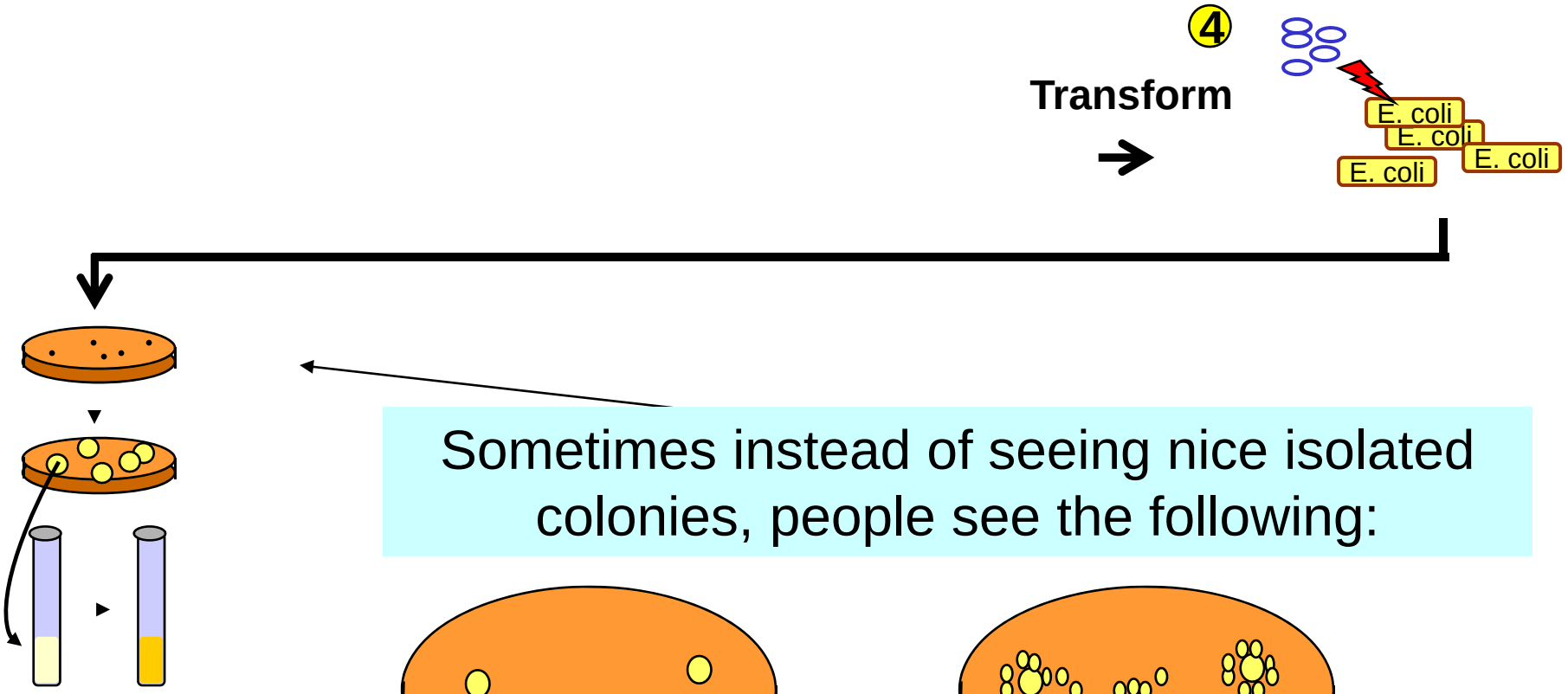
The rest should go into the next lecture.



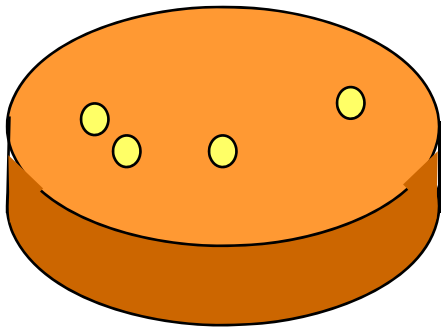
Why is the DNA ‘cleaned up’ prior to transformation?



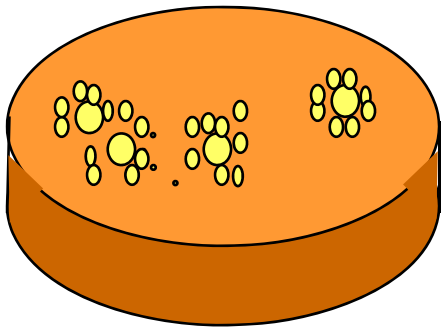
In your experiment, you will heat-shock E. coli to get the DNA in, and then immediately plate onto amp plates… is it always OK to plate immediately after transformation?



Sometimes instead of seeing nice isolated colonies, people see the following:

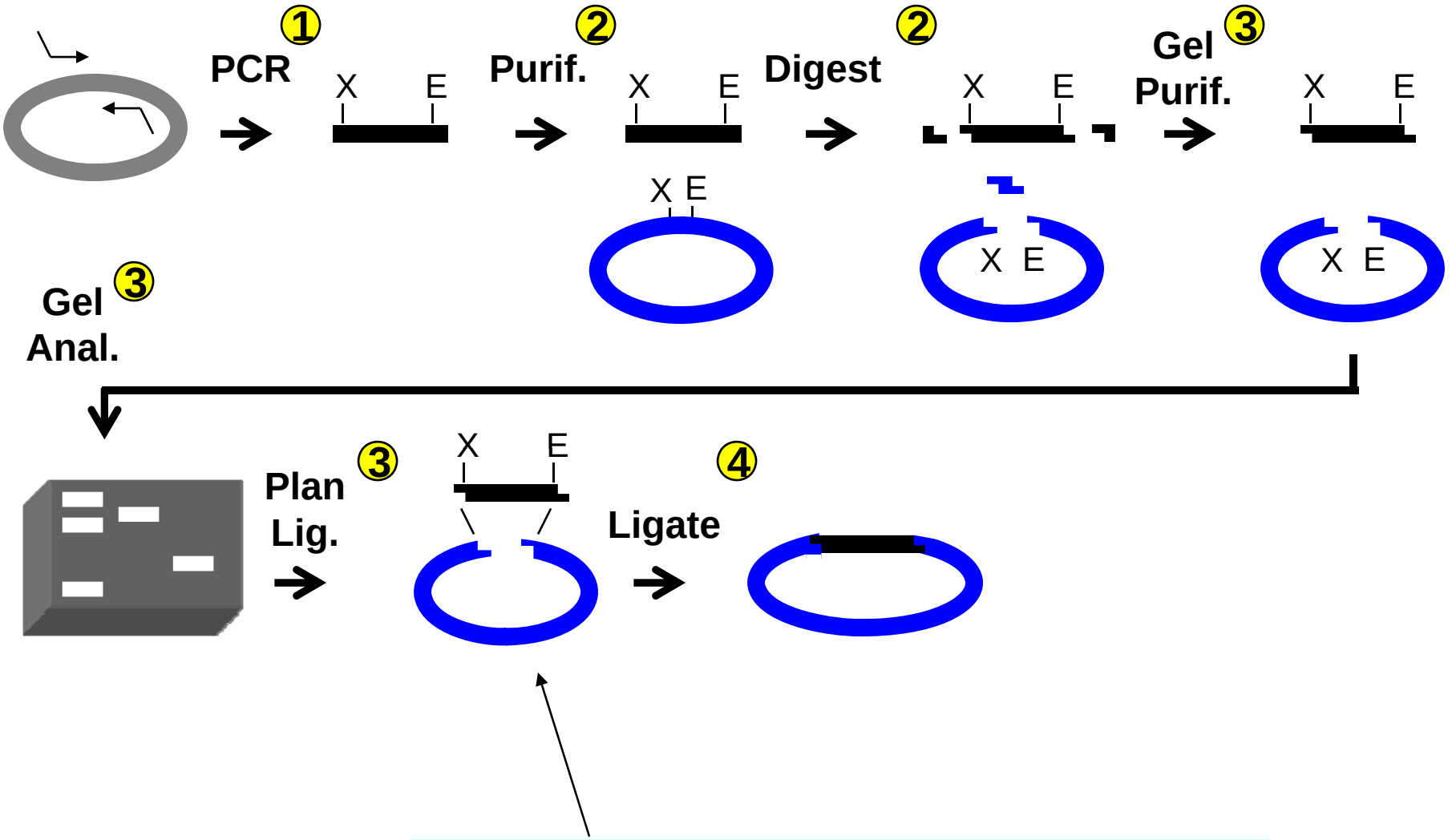


What you hope to see….

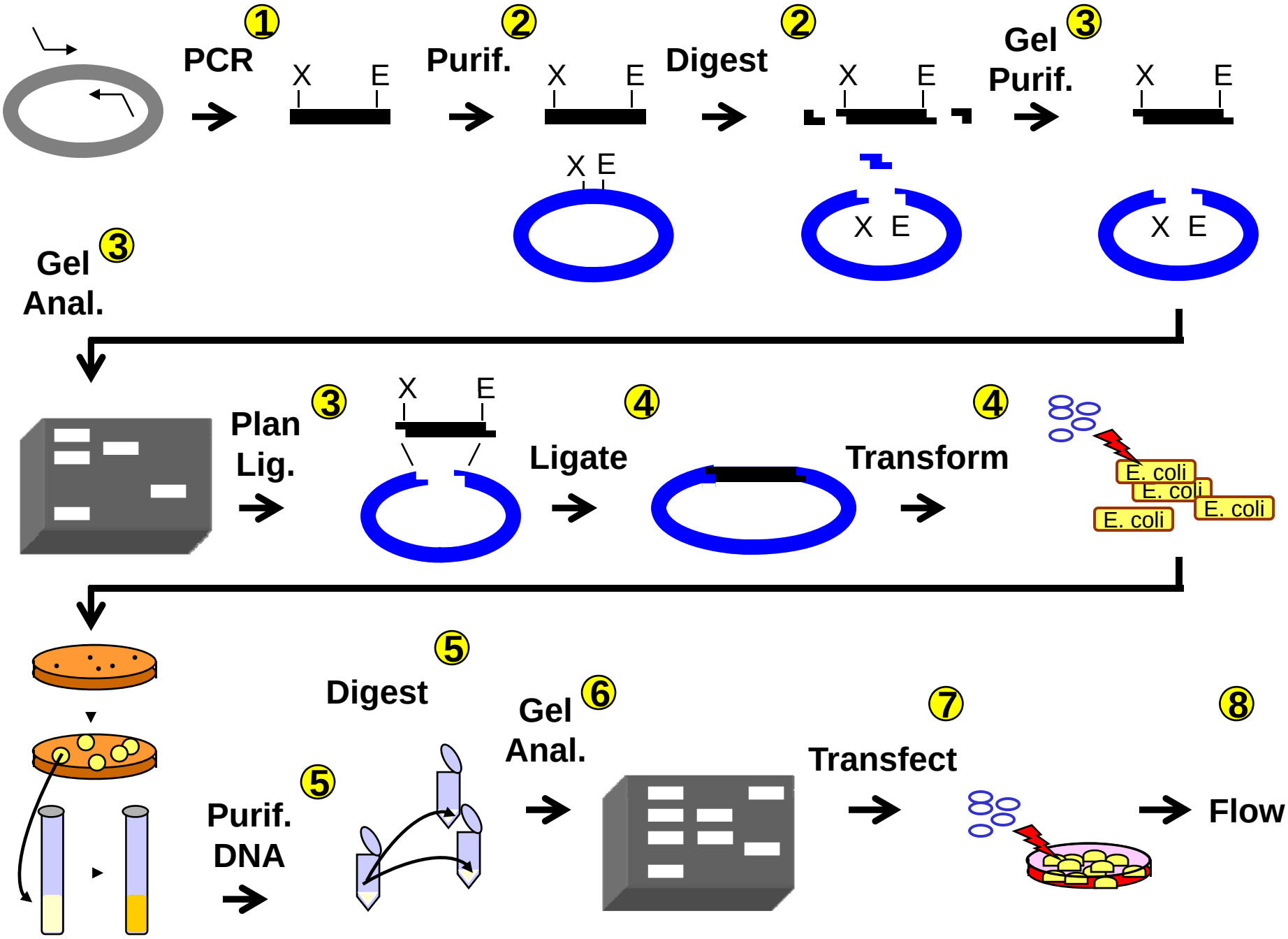


What you sometimes see…

Why? How would you avoid this problem?



Imagine you left your DNA in the fridge for weeks before setting up a 16°C ligation. What might happen?


1
PCR
X
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Purif.
X
E
2
Digest
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E
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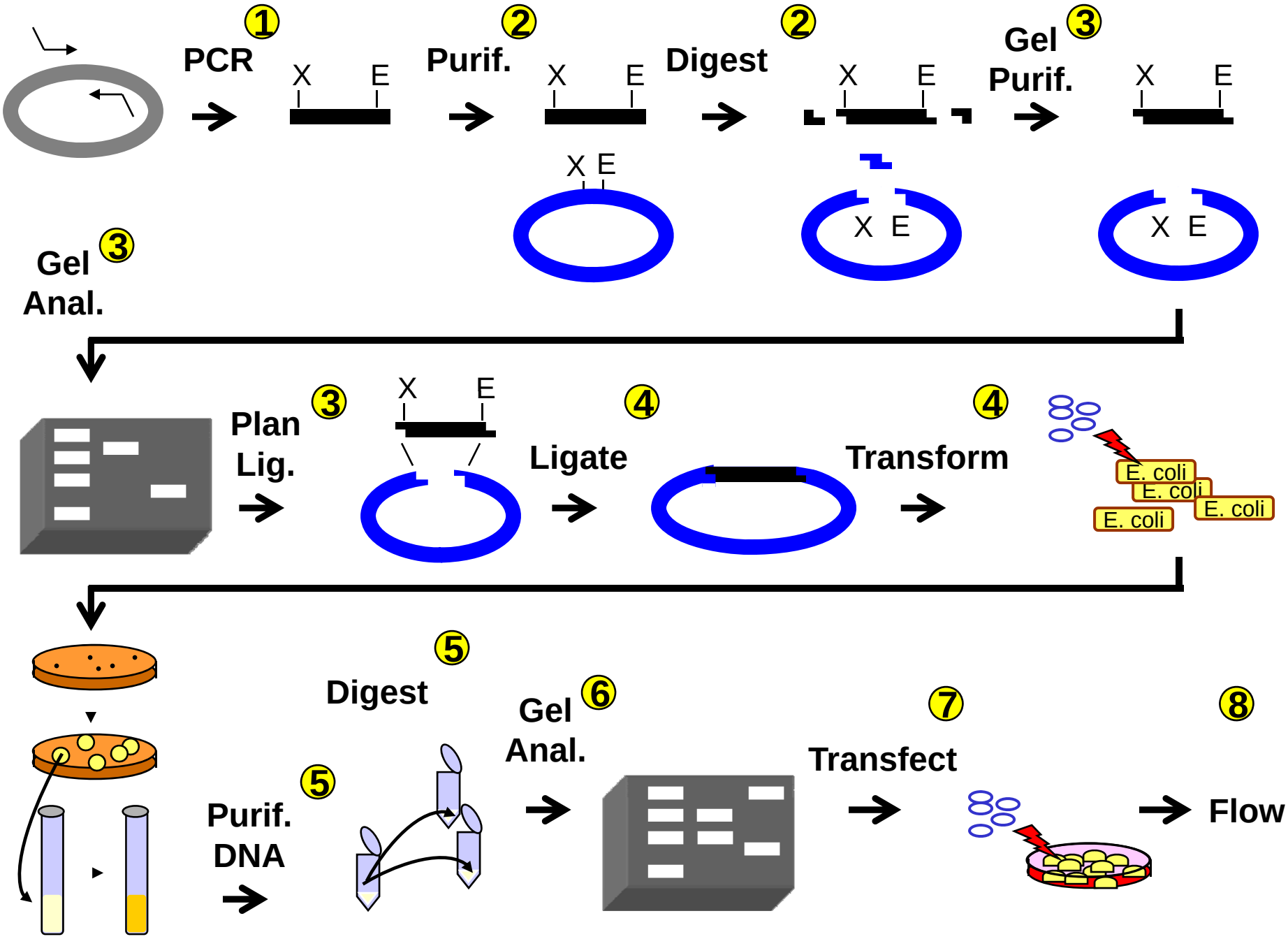
Gel
Purif.
X
E
X E
X E
X E
3
Gel
Anal.
3
Plan
Lig.
X
E
4
Ligate
4
Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
5
Purif.
DNA
6
Gel
Anal.
7
Transfect
8
Flow


1
PCR
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2
Digest
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E
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X
E
X E
X E
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Transform
E. coli
E. coli
E. coli
E. coli
5
Digest
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DNA
6
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Anal.
7
Transfect
8
Flow

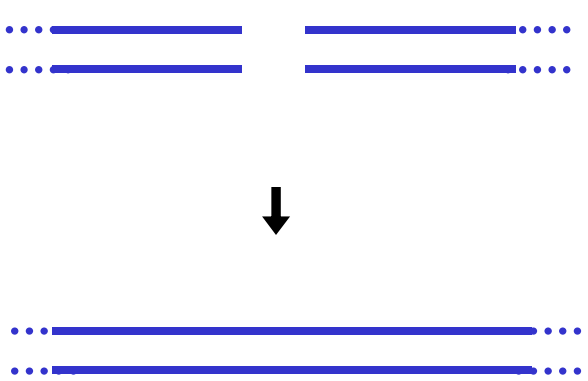
What you will know by the end of this module:

- PCR – Primer design
- Plasmids – Purification & Restriction Analysis
- Mammalian Cell Culture & Transfection
- Basic Statistics
- Flow Cytometry
- Basic Vector Design Know-How

Meta-Level Goal: Avoid assumptions about what is in your test tube and what will happen when you set up reactions.

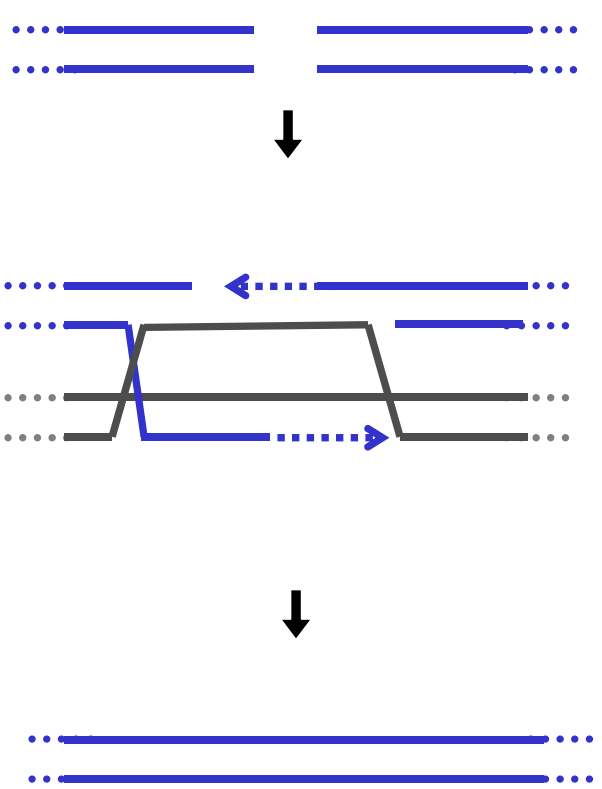
Non-Homologous End-Joining

Double Strand Break

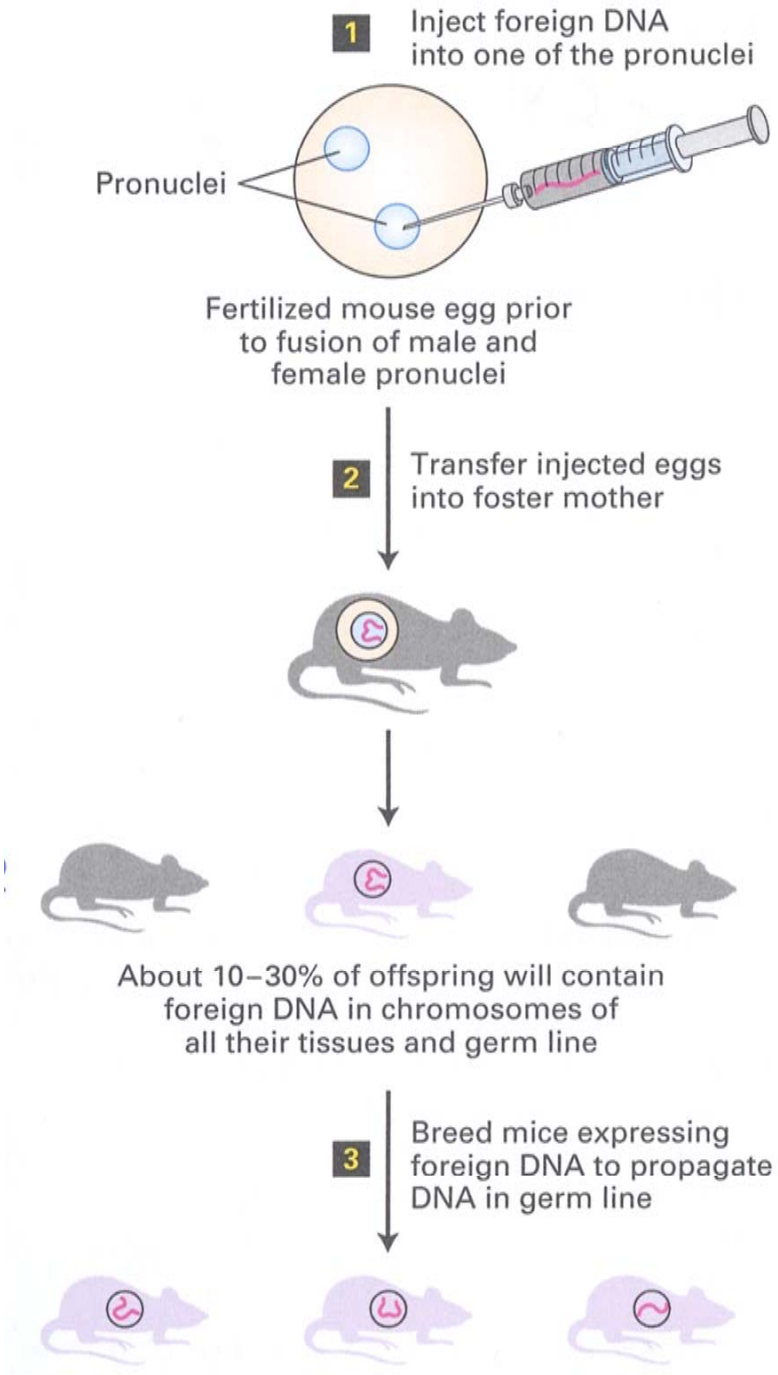


Homology-Directed Repair

Double Strand Break



Why do cells have two different ways to repair a DSB??


1
Inject foreign DNA into one of the pronuclei
Pronuclei
Fertilized mouse egg prior to fusion of male and female pronuclei
2
Transfer injected eggs into foster mother
About 10–30% of offspring will contain foreign DNA in chromosomes of all their tissues and germ line
3
Breed mice expressing foreign DNA to propagate DNA in germ line