Supplemental Material

CBE—Life Sciences Education

Pavan et al.

Table S1. Multiple Regression Analysis in terms of Raw Scores

	(1) Bloom's Levels 1-2	(2) Bloom's Level 3	(3) Bloom's Level 4	(4) Bloom's Level 5	(5) Bloom's Level 6	(6) Paper Quiz Score	(7) Final Grade	(8) Confidence in Paper Quiz
Returner	3.805*	6.686*	3.195	2.575*	4.139	4.043***	0.401*	0.108
	(0.013)	(0.012)	(0.158)	(0.021)	(0.102)	(0.000)	(0.028)	(0.176)
Method 2 (all fig)	-5.942**	-7.858*	-3.478	0.453	-2.394	-4.343**	-0.364	-0.225*
	(0.006)	(0.036)	(0.279)	(0.774)	(0.505)	(0.003)	(0.158)	(0.047)
Method 1 (some fig)	-1.571	-5.226	-0.187	2.009	4.263	-0.845	-0.245	-0.062
	(0.319)	(0.056)	(0.936)	(0.081)	(0.103)	(0.419)	(0.192)	(0.449)
Method 2 (some fig)	-2.804	-2.245	1.403	1.350	6.214	-0.280	-0.288	-0.263
	(0.345)	(0.662)	(0.750)	(0.533)	(0.207)	(0.887)	(0.416)	(0.089)
Other Method	0.357	0.595	1.948	0.962	-3.261	0.540	-0.068	-0.154*
	(0.808)	(0.815)	(0.372)	(0.370)	(0.182)	(0.580)	(0.697)	(0.045)
Medical Research	-3.031	-4.037	-5.810*	-0.779	-5.434	-3.627**	-0.181	-0.064
	(0.100)	(0.205)	(0.034)	(0.562)	(0.075)	(0.003)	(0.410)	(0.505)
No Research	0.036	1.276	1.544	-0.682	0.606	0.520	0.308	-0.279***
	(0.979)	(0.594)	(0.452)	(0.499)	(0.792)	(0.571)	(0.063)	(0.000)
GPA	15.294***	16.694***	13.194***	8.012***	11.775***	13.517***	3.915***	0.116
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.132)
Confidence	2.268**	4.327**	2.323	0.640	5.693***	2.684***	0.222*	
	(0.007)	(0.003)	(0.061)	(0.293)	(0.000)	(0.000)	(0.026)	
Molecular Biology Lab	-0.265	11.221*	33.470***	-10.941***	22.997***	9.283***	1.070***	-0.290*
	(0.921)	(0.015)	(0.000)	(0.000)	(0.000)	(0.000)	(0.001)	(0.037)
Microbiology Lab	-5.262*	10.440*	6.448	-9.953***	1.219	0.013	-0.067	-0.270*
	(0.037)	(0.017)	(0.085)	(0.000)	(0.770)	(0.994)	(0.824)	(0.040)
Constant	25.404***	-17.996	-37.125***	-9.068*	-25.190*	-6.884	-5.579***	3.567***
	(0.000)	(0.093)	(0.000)	(0.045)	(0.014)	(0.094)	(0.000)	(0.000)
Observations	541	541	541	541	541	541	541	541
R^2	0.313	0.278	0.335	0.290	0.514	0.460	0.533	0.121

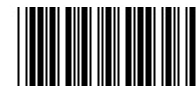
p-values in parentheses $^{*}\ p < 0.05$, $^{**}\ p < 0.01$, $^{***}\ p < 0.001$

Table S1

Multiple regression analysis as in Table 2, except terms are present as raw scores as opposed to standardized beta coefficients.

Coefficients for dummy variables of each specific class were omitted for simplicity.

BIO SCI M116L LEC A (05730)



Paper quiz (Spring Qtr 2013) - LETTER SIZE **5148 (2105)**

Assigned Seat#:_________

Instructions to Instructor:

Do not alter this coversheet in ANY way. Substantial delays and additional fees may apply.

Instructions to Student:

1. Clearly print your Last Name, First Name and the Date
2. Clearly print your Student ID number in the boxes provided. Use large, dark numbers. These numbers are captured automatically during the scanning process.
3. Bubble in each number of your Student ID completely. The bubbles are used only if your written ID number is not captured.
4. Write your Name and Student ID number in the upper right corner of all following pages of your exam.

Last Name, First Name: ____ Date: ____/____/____

STUDENT ID: *For Access UCI student, leave first column blank then enter your 7-digit Student ID number.*

1	O	O	O	O	O	O	O	O	1
2	O	O	O	O	O	O	O	O	2
3	O	O	O	O	O	O	O	O	3
4	O	O	O	O	O	O	O	O	4
5	O	O	O	O	O	O	O	O	5
6	O	O	O	O	O	O	O	O	6
7	O	O	O	O	O	O	O	O	7
8	O	O	O	O	O	O	O	O	8
9	O	O	O	O	O	O	O	O	9
0	O	O	O	O	O	O	O	O	0

--------------------------------- (This space for Instructor/TA use only) ---------------------------------

Graded by: ___________________________________ Total Correct: ________________

Name: ______________________________ Student ID: ________________________

Information questions

Please answer the questions below. This is for informational purposes only, and will not impact your grade.

01. How did you prepare for the quiz?

A. Method 1 (summaries) for all figures
B. Method 1 (summaries) for some figures
C. Method 2 (4 questions) for all figures
D. Method 2 (4 questions) for some figures
E. Other (not Methods 1 or 2)

02. I am very confident that I understand the paper being tested.

A. Strongly agree
B. Agree
C. Neutral
D. Disagree
E. Strongly disagree

03. Outside of lab classes, do you have any additional research experience?

A. Yes, I have done/am doing research in a lab
B. Yes, I have done/am doing research in a clinical/medical setting
C. No

Name: ______________________________ Student ID: ________________________

Paper reading quiz (9 questions, 50 points)

01. Describe the main purpose of this paper briefly, and in your own words. ***[2 points]***

1/2

02. Describe the main findings of this paper briefly, and in your own words. ***[2 points]***

1/2

03. a) What is one method commonly used (before this) to identify CpG methylation in DNA? ***[1 point]***

1/2

b) Normally, we run PCR for ~30 cycles. Why did the authors need to run ~60 cycles of PCR? ***[2 point]***

1/2

c) Normally, the first denaturation step in PCR is 94°C for ~2 mins. Why did the authors need to initially denature at 94°C for 10 mins? ***[2 points]***

1/2

04. Why did the authors use two forward and one reverse primer in their method? ***[2 points]***

1/2

Score

_____/11

Name: ______________________________ Student ID: ________________________

05. Why did the authors chose to study methylation patterns of CDKN2A/INK4a and COL1A2? ***[2 points]***

1/2

06. Answer the following questions about Figure 1B:

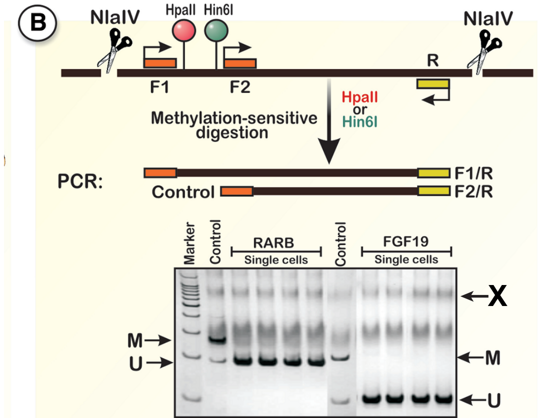


Figure 1.Principle of the RSMA test system for the analysis of DNA methylation patterns in single cells. (B) DNA is fragmented by a restriction enzyme (here NlaIV), that cleaves outside of the target region. Primer mixes contain two forward primers (F1+F2) and one reverse primer (R). The analyzed CpG dinucleotides (here HpaII and Hin6I sites) are located between the two forward primers. If the restriction site is methylated, the restriction enzyme cannot cleave and both PCR products are synthesized, whereas for unmethylated restriction sites only the short PCR product (internal PCR positive control) is produced.

a) Clearly circle the lanes that contain DNA that was orginally methylated. ***[2 points]***

4

b) In this experiment, *Hpa*II was used to digest the DNA. I repeat this experiment, but instead of using *Hpa*II, I use *Msp*I, which is a methylation-insensitive isoschizomer of *Hpa*II. Write down which bands I would expect to see in all the lanes (M, U, both, neither, or a mix). Explain your answer. ***[4 points]***

3

c) What is the best hypothesis for why we see the band labelled “X” in all the lanes? ***[4 points]***

5

Score

_____/12

Name: ______________________________ Student ID: ________________________

d) What might be one change you could make to try and eliminate band "X"? ***[2 points]***

5

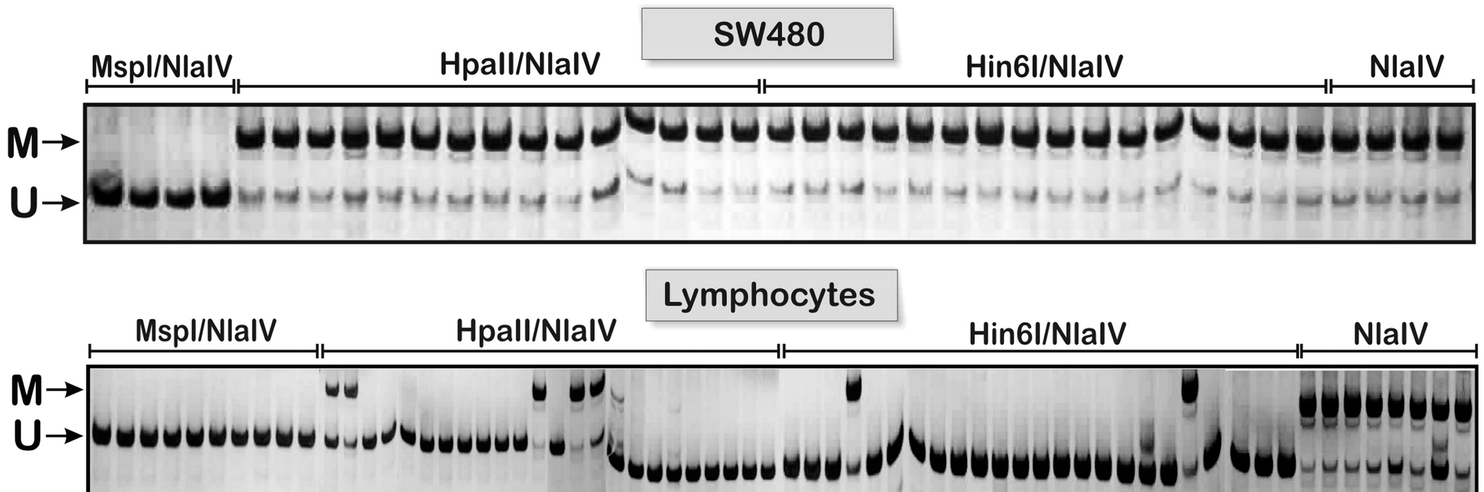


Figure 2. Polyacrylamide gel electrophoresis of products from multiple parallel single-cell PCRs as exemplified for the CDKN2A promoter. Each lane represents a PCR product from a single cell in SW480 cells (top row) and lymphocytes (bottom row). All of the SW480 cells in the presented slide contained only fully methylated HpaII and Hin6I sites, indicated by the presence of two bands, the uncleaved (methylated) long band (191bp, F1/R) and the short control band (157bp, F2/R). In contrast, most of the lymphocytes contained only the short band, indicating that the analyzed CpG dinucleotides were unmethylated. Some cells were treated with MspI/NlaIV as control for the enzymatic reaction and should produce only the short band. In parallel, some cells were treated with NlaIV alone, which serves as positive control for the long PCR product. If the chosen PCR conditions are appropriate, both amplification products should be visible. Typically, the short control band is noticeable weaker compared to the long band, which may be explained by the fact that in later PCR cycles the short band may also serve to a significant extent as primer for the long PCR product. In addition, due to better hybridization efficiency, the longer PCR product may be amplified preferentially. M, methylated; U, unmethylated.

07. Based on Figure 2 from the paper (shown above) answer the following questions:

a) What percentage of Lymphocytes are methylated at the CDKN2A promoter? ***[2 points]***

3

b) Can you conclude that *Hpa*II sites are more methylated than *Hin*6I sites in the CDKN2A promoter of Lymphocytes? Explain your answer. ***[3 points]***

6

c) What was the purpose of including the *Msp*I/*Nla*IV digest in this experiment? ***[2 points]***

1/2

Score

_____/9

Name: ______________________________ Student ID: ________________________

Wed Thurs Fri Morning Afternoon

d) What was the purpose of including the *Nla*IV only digest in this experiment? ***[2 points]***

1/2

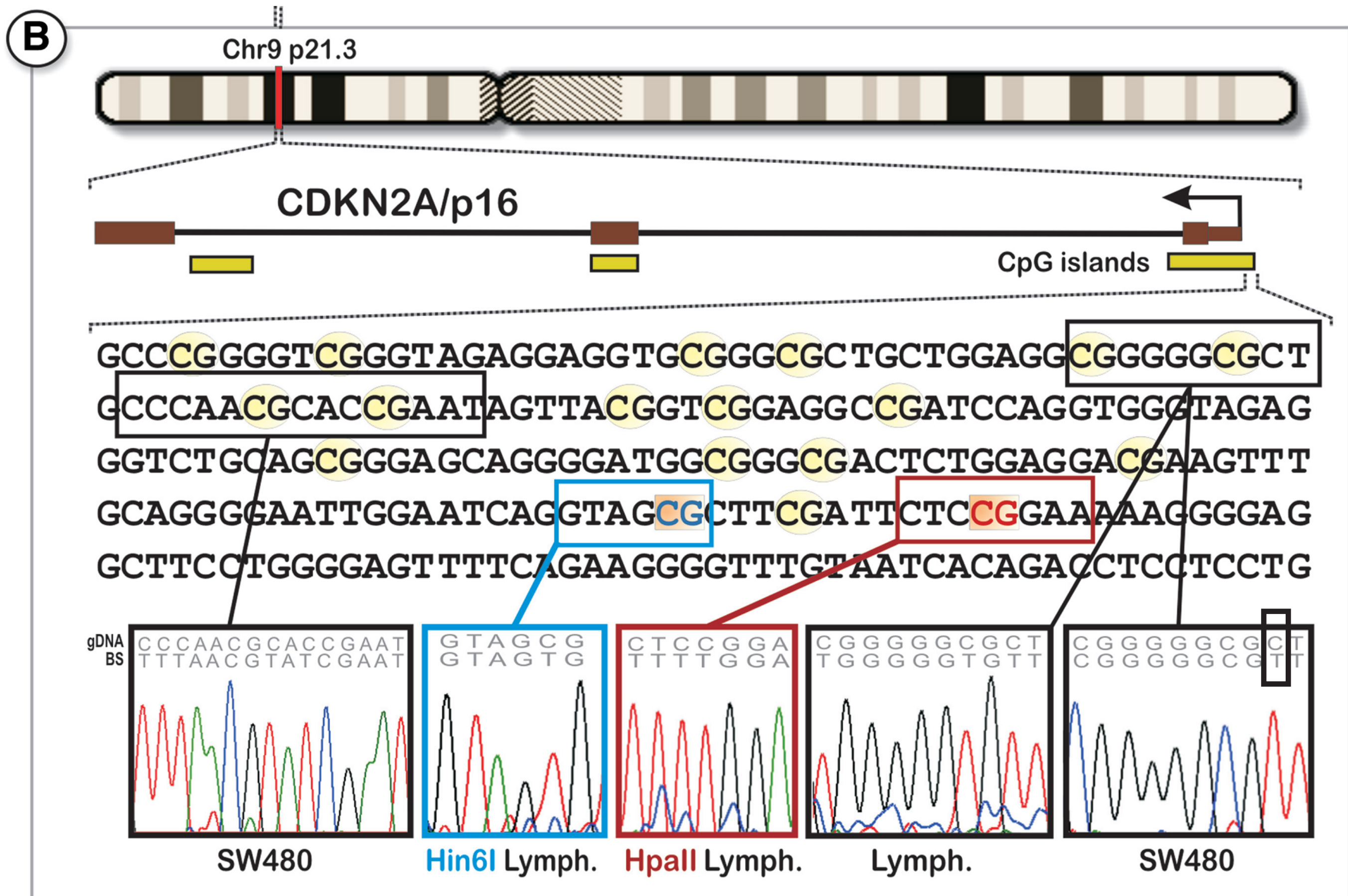


Figure 3. Bisulfite sequencing of larger cell populations to verify the results from single-cell measurements. (B) Structure and analyzed sequence of the CDKN2A gene. Sample sequencing traces from different tissues are shown on the bottom. The bisulfite sequencing results confirm the methylation data derived from single-cell methylation profiling by the RSMA technology.

08. Answer the following questions based on Figure 3B (shown above) from the paper:

a) Clearly circle and label as "A" the example sequence traces that show differences in methylation between Lymphocytes and SW480 cells at the **SAME** CpG island: ***[3 points]***

3

Score

_____/5

Name: ______________________________ Student ID: ________________________

Wed Thurs Fri Morning Afternoon

b) Consider the C residue shown boxed in the SW480 example trace. Why was this C converted to a T upon bisulfite treatment? ***[1 point]***

3

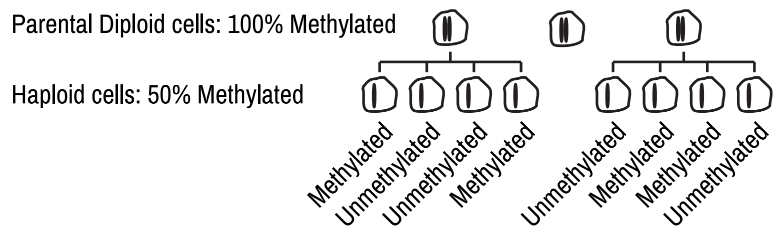
c) Consider the C residue shown boxed in the SW480 example trace. Could this C be acted upon by the same Methyl Transferase that affects the other C residues in this example trace? Why or why not? ***[3 points]***

3

d) We already knew that SW480 cells were hypermethylated at the CDKN2A promoter, compared to Lymphocytes. So, what was the purpose of doing the experiment shown in the figure? ***[3 points]***

1/2

09. You perform RSMA (Restriction-enzye based Single-cell Methylation Assay - the technique described in this paper) on a particular cell line for the *Comp* gene. 100% of single cells from the cell line show methylation at the *Comp* locus. When you generate haploid cells from this cell line, you find that exactly 50% of the haploid cells are methylated! The individual cell that gave rise to the haploid cell line is positive for methylation. You generate a number of different haploid cell lines from distinct individual cells, and in every case, 50% of the haploid cells are methylated at *Comp* , despite the parental cell being methylated (the figure shows a schematic representation of the experiment). There is no differential distribution of any cellular components when either the diploid or haploid cells divide. What is the best hypothesis that explains your observations? ***[6 points]***



5

Score

_____/13

M118L Paper Quiz
May 20, 2013

Name/ID#: ___________________________

TA: _________________________________

How did you prepare for the quiz?

A. Method 1 (4 questions) for all figures **B.** Method 1 (4 questions) for some figures
C. Method 2 (summaries) for all figures **D.** Method 2 (summaries) for some figures
E. Other (not Methods 1 or 2)

I am very confident that I understand the paper being tested.

A. Strongly agree **B.** Agree **C.** Neutral
D. Disagree **E.** Strongly disagree

Outside of lab classes, do you have any additional research experience?

A. Yes, I have done/am doing research in a lab
B. Yes, I have done/am doing research in a clinical/medical setting **C.** No

1. What is the main question the authors are asking? Answer must be one sentence/phrase. (2 pt)

1, 2

2. What is the answer to that question? Be specific. Answer must be one sentence/phrase. (2 pt)

1, 2

3. In regards to this study, what is the key difference between *Bacillus* and *Escherichia*? (1 pt)

1, 2

4. Answer the following questions (a-d) regarding Figure 1B.

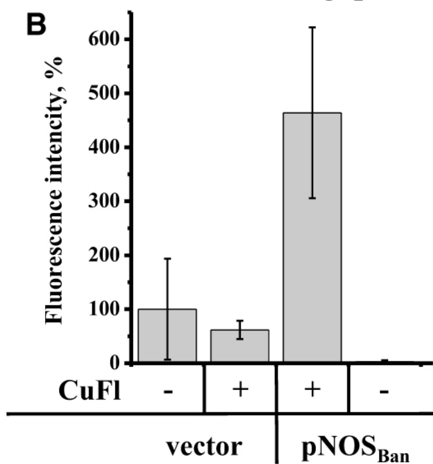


Fig 1. Bacteria-Derived NO Signals inside *C. elegans*. B. Bacterial NO production in *C. elegans*. Representative fluorescent quantification of *C. elegans* treated with a Cu(II)-based NO-detecting probe (CuFL). WT worms were fed E. coli harboring either empty vector or $pNOS_{Ban}$ at 20°C.

a. What is $pNOS_{Ban}$? Be specific. (1 pt)

1, 2

b. This experiment is measuring NO produced by (**BACTERIA/WORMS/BOTH**). (1.5 pt) **1, 2**

c. You would expect worms eating *E. coli* with $pNOS_{Ban}$ in the absence of CuFL to have a (**LONGER/SHORTER/THE SAME**) lifespan as worms eating the same *E. coli* but without CuFL. (1 pt) **3**

d. Explain how you came to this conclusion (in c). (1.5 pt) **3**

5. Answer the following questions (a-c) regarding Figure 2D.

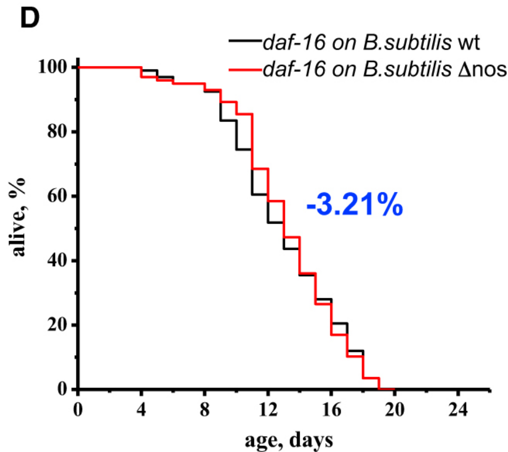


Fig 2. NO-Dependent Life Extension Depends on *daf-16* and *hsf-1*. D. Endogenous or bacterially derived NO does not extend the lifespan of *daf-16* worms. N2 worms were fed either WT or *Δnos B. subtilis* on NGMga agar plates

a. The 50% survival time for these worms is __________ days. (1 pt) 4

b. Explain how the authors came to the conclusion stated in the figure legend. (1.5 pt)

4

c. *DAF-16* is a transcription factor that promotes insulin-like signaling (ILS). If the authors added a chemical inhibitor of ILS to wild type worms that are eating wild type *B. subtilis*, **illustrate the expected results on the graph above**. (1.5 pt) 3

6. Answer the following questions (a-e) regarding Figure 3B.

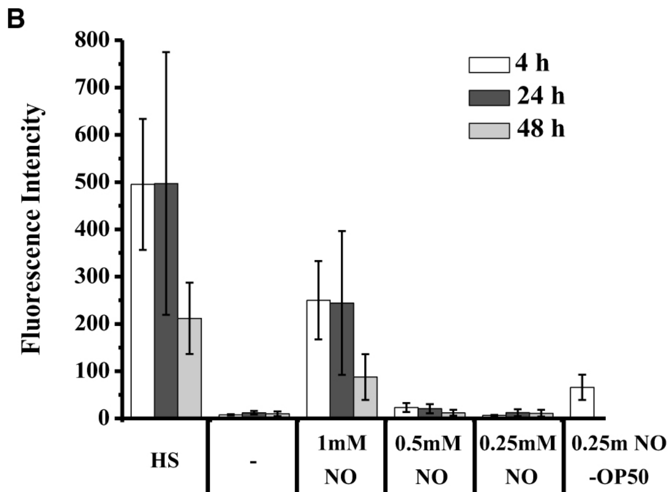


Fig 3. NO Induces the Expression of HSPs in *C. elegans*. B. Fluorescent quantification of TJ375 worms (hsp16::GFP) treated with NO. Twenty worms were randomly picked for quantification 4, 24, and 48 hr after NO exposure. HS: 30 min of HS at 30°C.

a. On a molecular level, describe how these *C. elegans* express GFP? (1 pt)

1, 2

b. What is the **specific** purpose of the HS portion of this figure? (1 pt)

6

c. This figure (**DOES/DOES NOT**) allow us to conclude that NO producing bacteria activate the heat shock response in *C. elegans*. (1 pt) 4

d. TRUE or **FALSE**. The data above **imply** that heating worms will increase lifespan. If true, explain why. If false, rewrite the statement so it is a true statement. (1.5 pt)

4

e. Imagine the worm strain overexpressed chaperone proteins. How might we expect the 1mM NO results to differ? (1.5) 3

7. Answer the following questions (a-d) regarding Figure 4C.

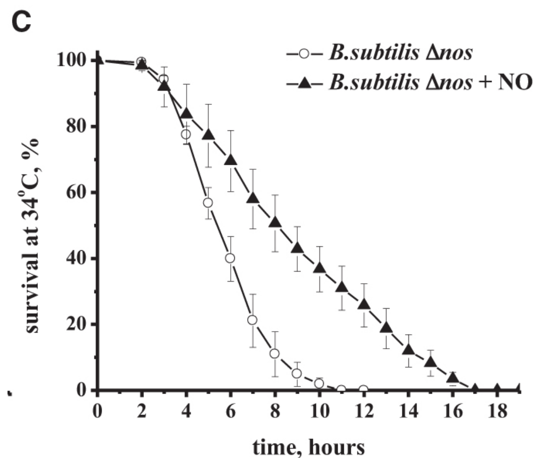


Fig 4. NO Increases *C. elegans* Thermotolerance. C. Exogenous NO complements the lack of bacterial NO in restoring the thermotolerance of worms grown on *Δnos B. subtilis*. Worms were treated twice with 1mM MAHMA NONOate at L4 and A2. At A3, animals were shifted to 34°C and scored every hour for dead worms.

a. What is MAHMA NONOate? (1 pt)

1, 2

b. How does this result support the main question of this paper? (1 pt)

6

c. Imagine *C. elegans* were fed wild type *B. subtilis*. Draw the expected result on the graph and label as **C**. (1.5 pt)

3

d. Imagine *C. elegans* were fed *E. coli* with an empty vector plasmid. Draw the expected result on the graph and label as **D**. (1.5 pt)

3

7. In lab, you conducted the nematophagous experiment. Some students added *B. cereus* to the CMA plates and saw an increased *C. elegans* percent survival compared to control CMA plates lacking the bacteria. Imagine that *B. cereus* lacks an active NOS gene.

You now plan on repeating the experiment adding *B. subtilis* to the CMA plate. Write a hypothesis regarding this proposed experiment. (2 pt)

5

8. Based on the results in this paper, propose a relevant future experiment the authors could conduct. (2 pt)

5

PNAS

Carcinogenic bacterial pathogen *Helicobacter pylori* triggers DNA double-strand breaks and a DNA damage response in its host cells

Isabella M. Toller[a,1], Kai J. Neelsen[a,1], Martin Steger[a], Mara L. Hartung[a], Michael O. Hottiger[b], Manuel Stucki[b], Behnam Kalali[c], Markus Gerhard[c], Alessandro A. Sartori[a], Massimo Lopes[a,2], and Anne Müller[a,2]

[a]Institute of Molecular Cancer Research and [b]Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich, 8057 Zürich, Switzerland; and [c]Department of Medicine, Technical University Munich, 81675 Munich, Germany

Edited by Jeffrey W. Roberts, Cornell University, Ithaca, NY, and approved July 28, 2011 (received for review January 24, 2011)

Class Survey

1. How long did you spend on this paper (including the paper assignment)

A. More than 2 hours
B. 2 hours
C. 1 hour
D. Less than 1 hour
E. No time

Class Survey

2. I have a very strong understanding of this paper.
   A. Strongly disagree
   B. Disagree
   C. Neutral
   D. Agree
   E. Strongly agree

What is the main question this paper is asking?

A. Why is *H. pylori* a cause of gastric cancer?
B. How does *H. pylori* affect chromosome stability?
C. How does the DNA repair machinery fix double stranded breaks?

Figure 1

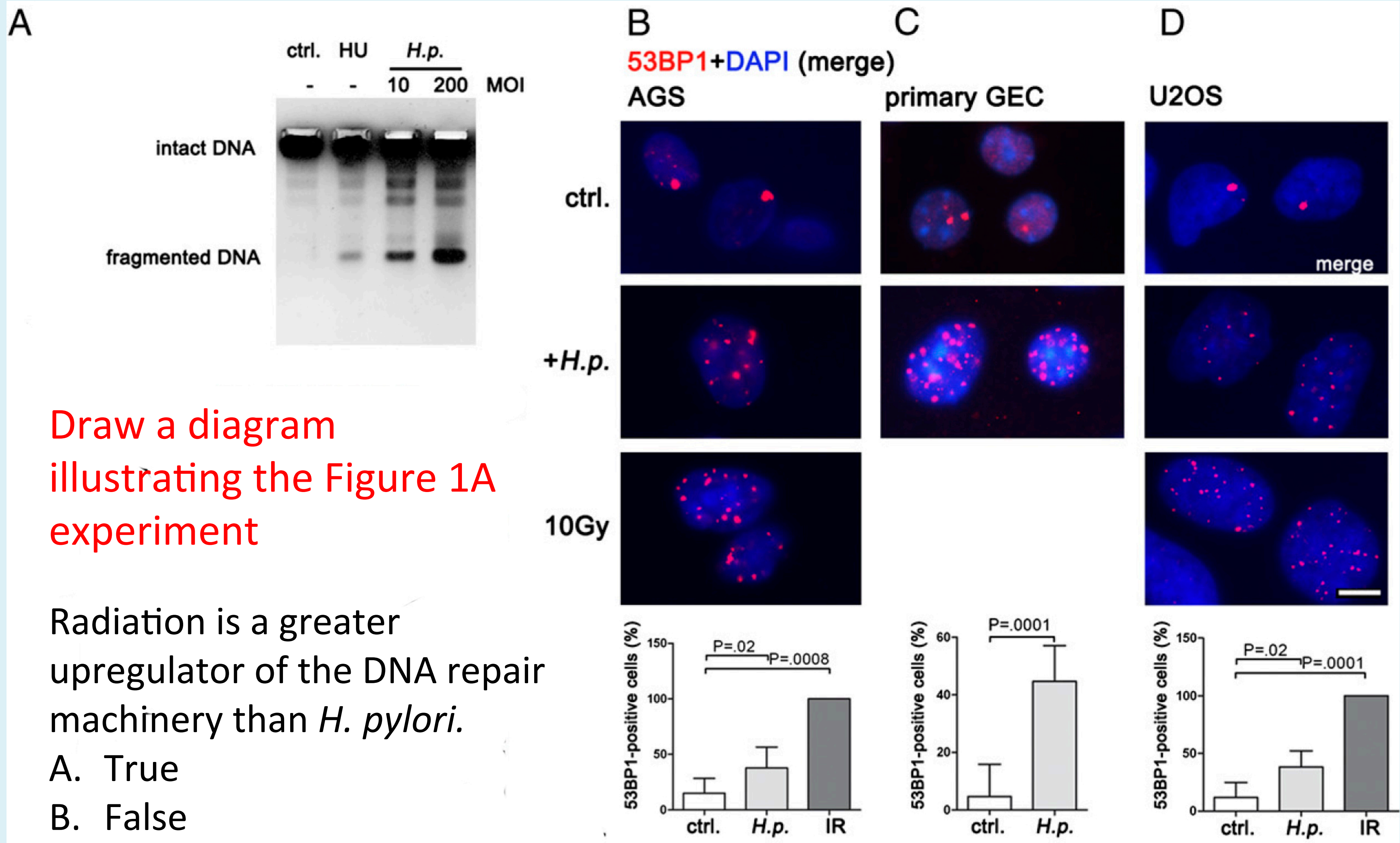
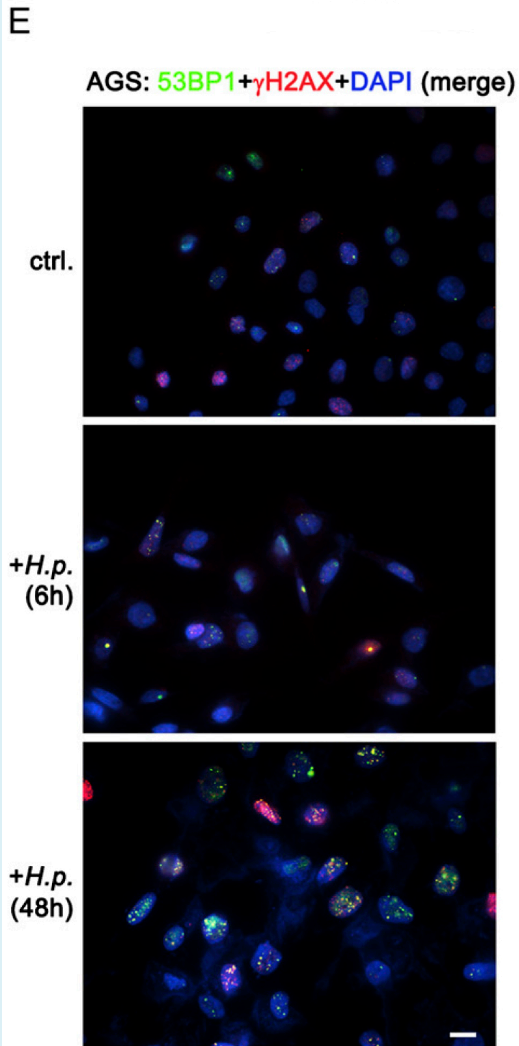
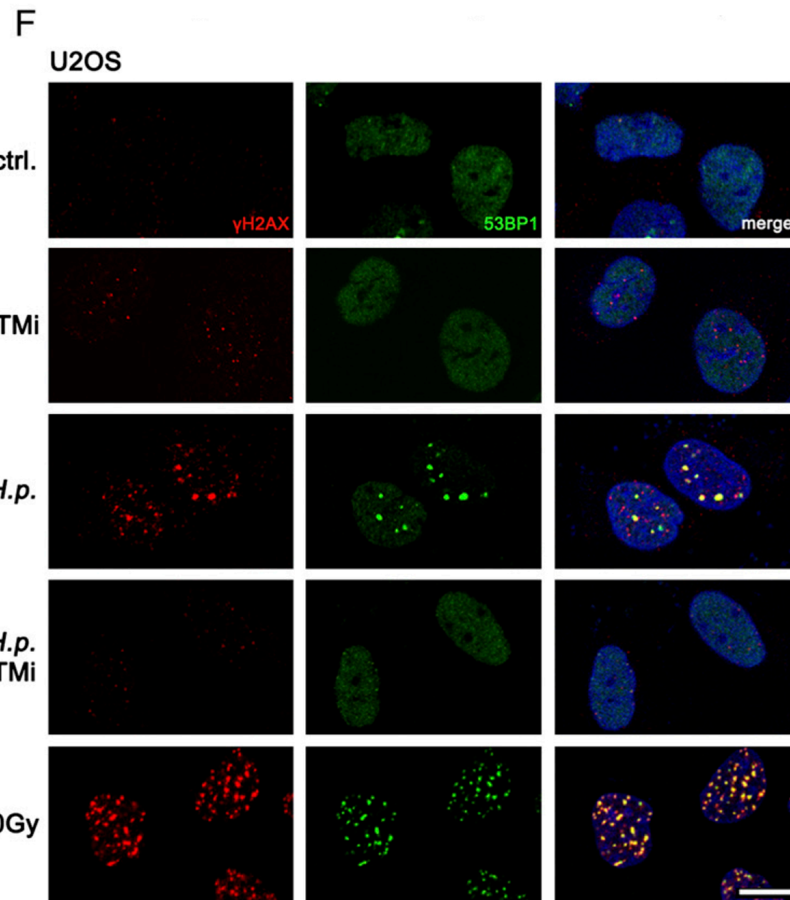


Figure 1



Imagine the H pylori induced repair pathway was not ATM dependent. What would Fig 1F look like?



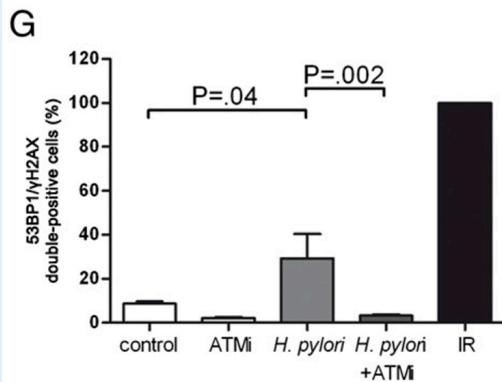


Figure 2

Multiple breaks in a chromosome are only observed when cells are infected with *H. pylori.*

A. True
B. False

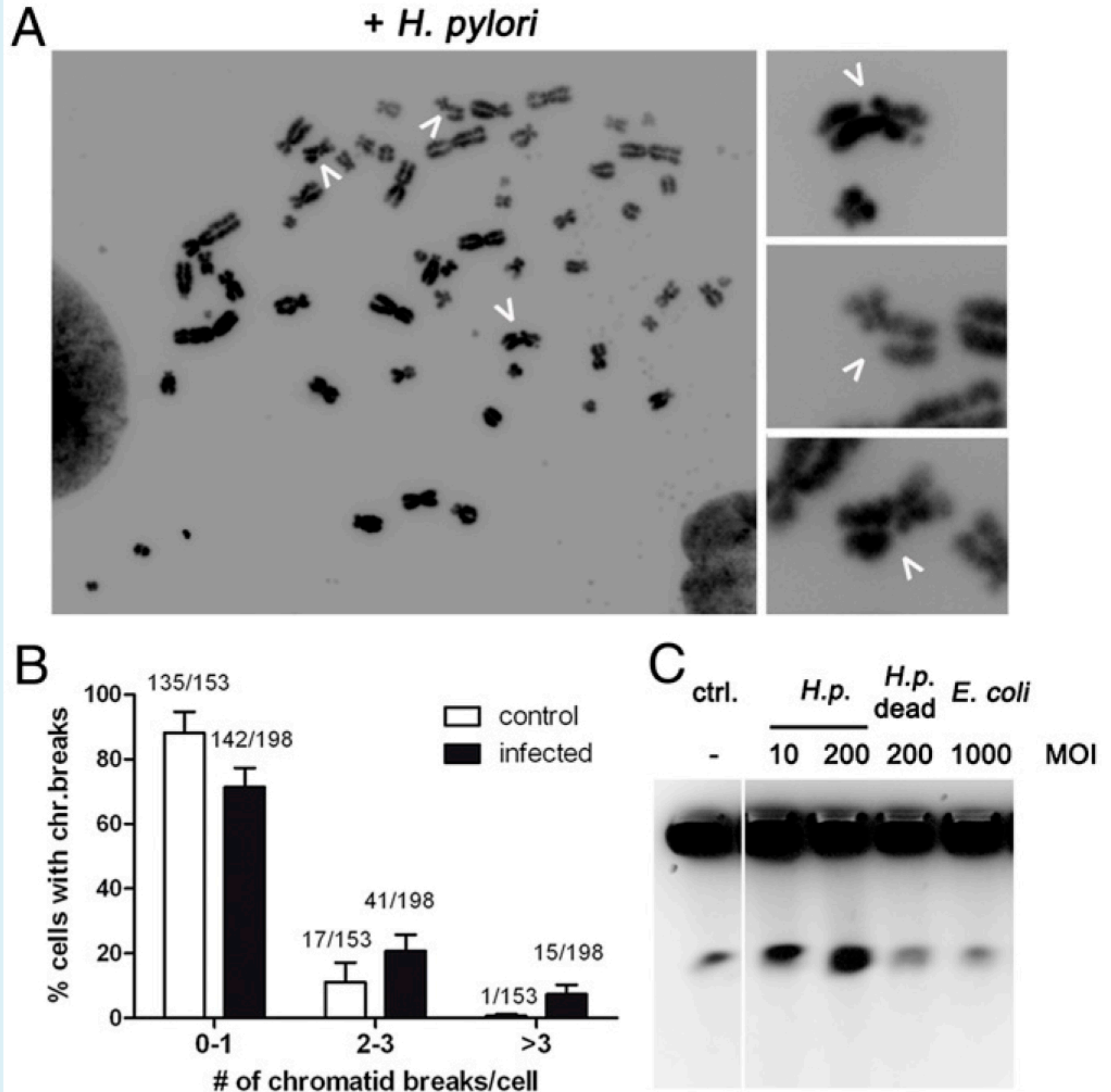


Figure 2

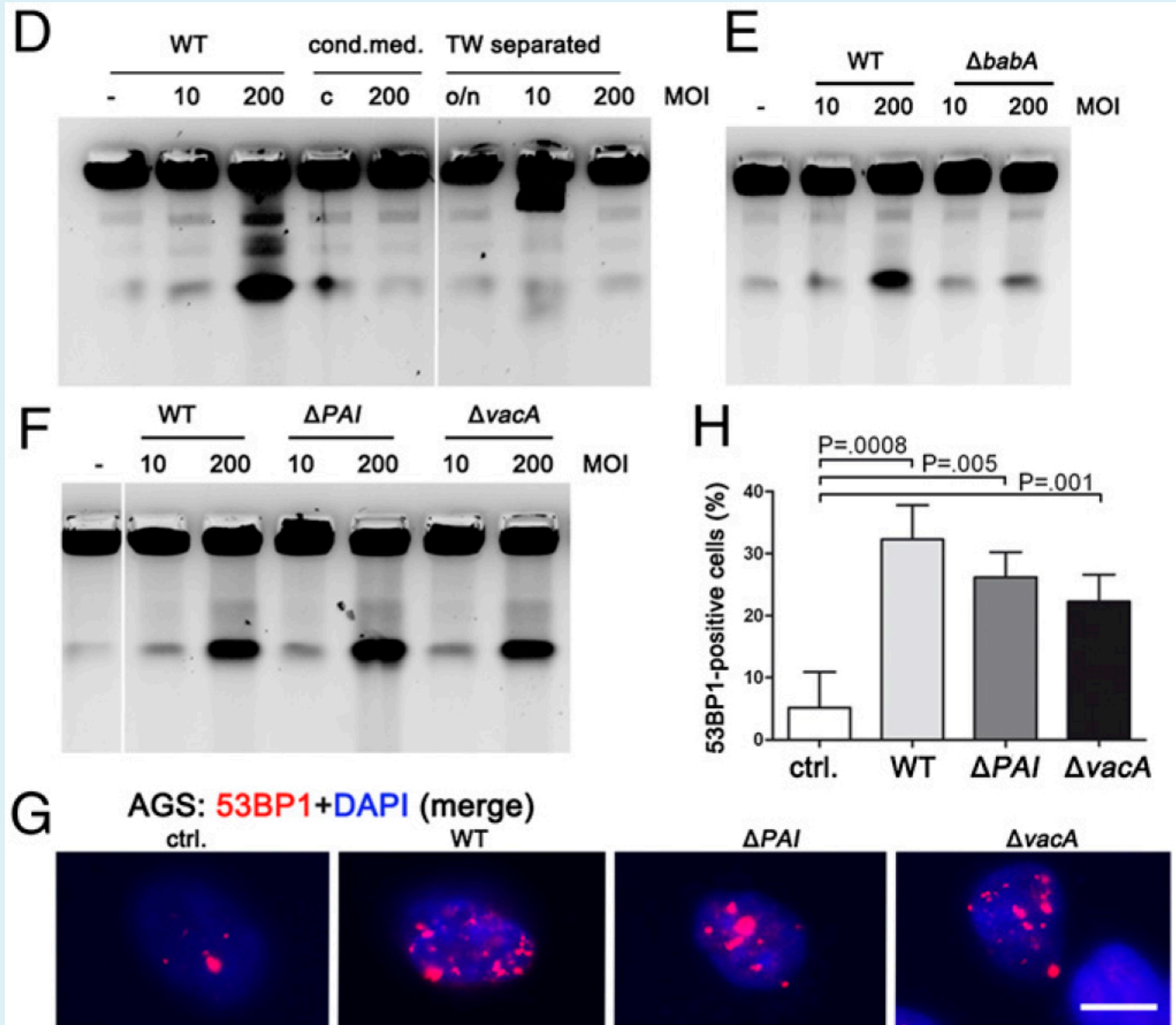
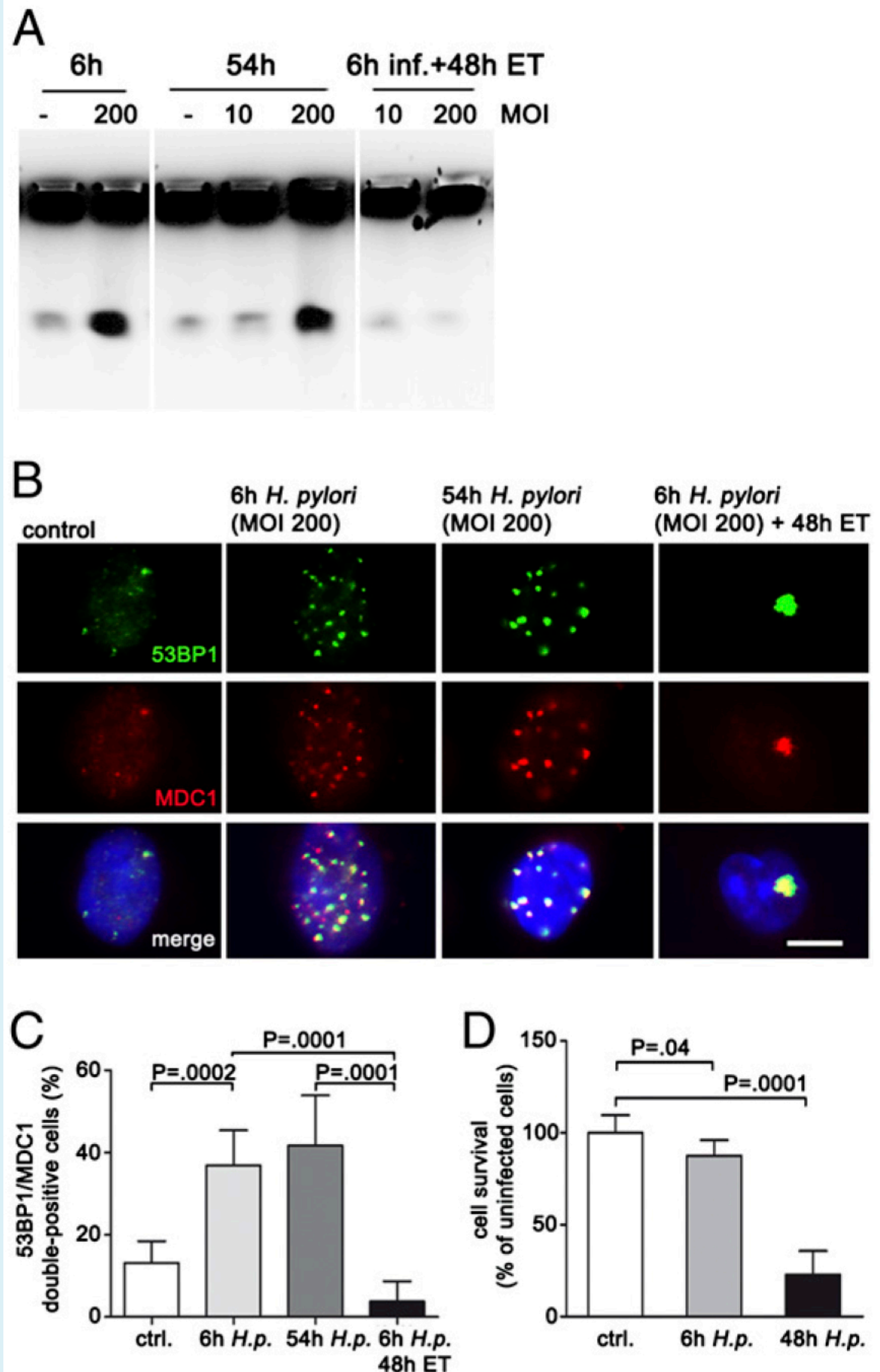


Figure 4

What question are these sets of tests trying to answer?

A. Is *H. pylori* responsible for double stranded break creation?
B. Is the DNA repair machinery assembled following *H. pylori* infection?
C. Are the consequences of *H. pylori* infection reversible?

Does a 6 hour H pylori treatment result in decreased cell survival?



Future Directions

Paper reading assignment 2

Paper was:

A. Interesting

B. Interesting but hard to read

C. Boring

D. Boring and hard to read

Paper reading assignment 2

Assignment 1 VS 2:

A. Assignment 1 was better

B. Assignment 2 was better

C. Both were useless

D. Both were good

Paper reading assignment 2

Main purpose of paper?

A. Investigate mechanism of VSG switching

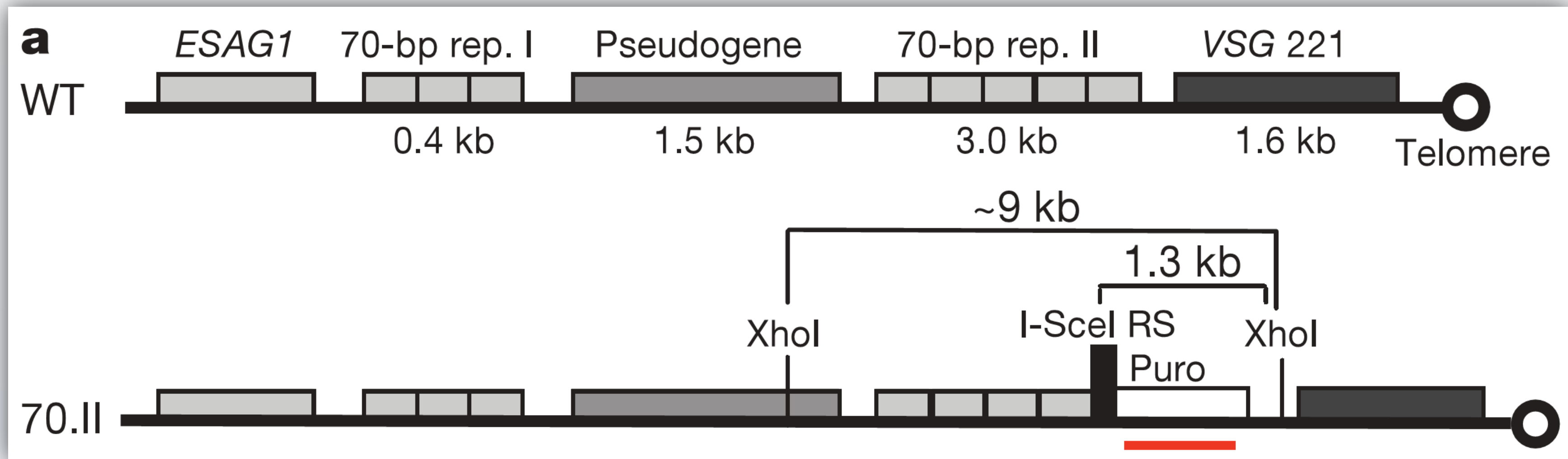
B. Mutating VSG 221 locus

C. Switching VSG expression

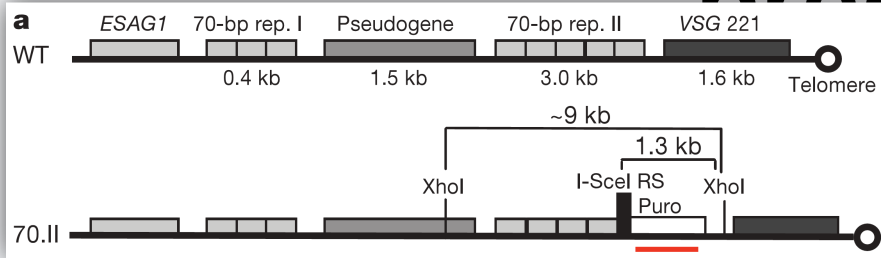
D. Increasing VSG switching

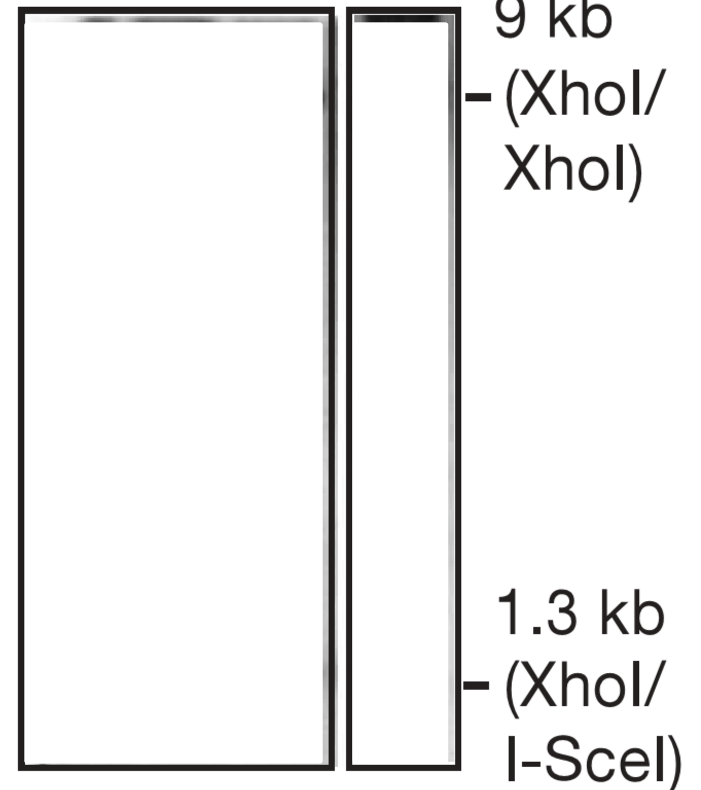
E. Mutating 70bp repeat region

1: The VSG 221 region



1: I-Sce site is inserted where predicted





Paper reading assignment 2

Main hypothesis of paper?

A. I-Sce1 triggers VSG switching

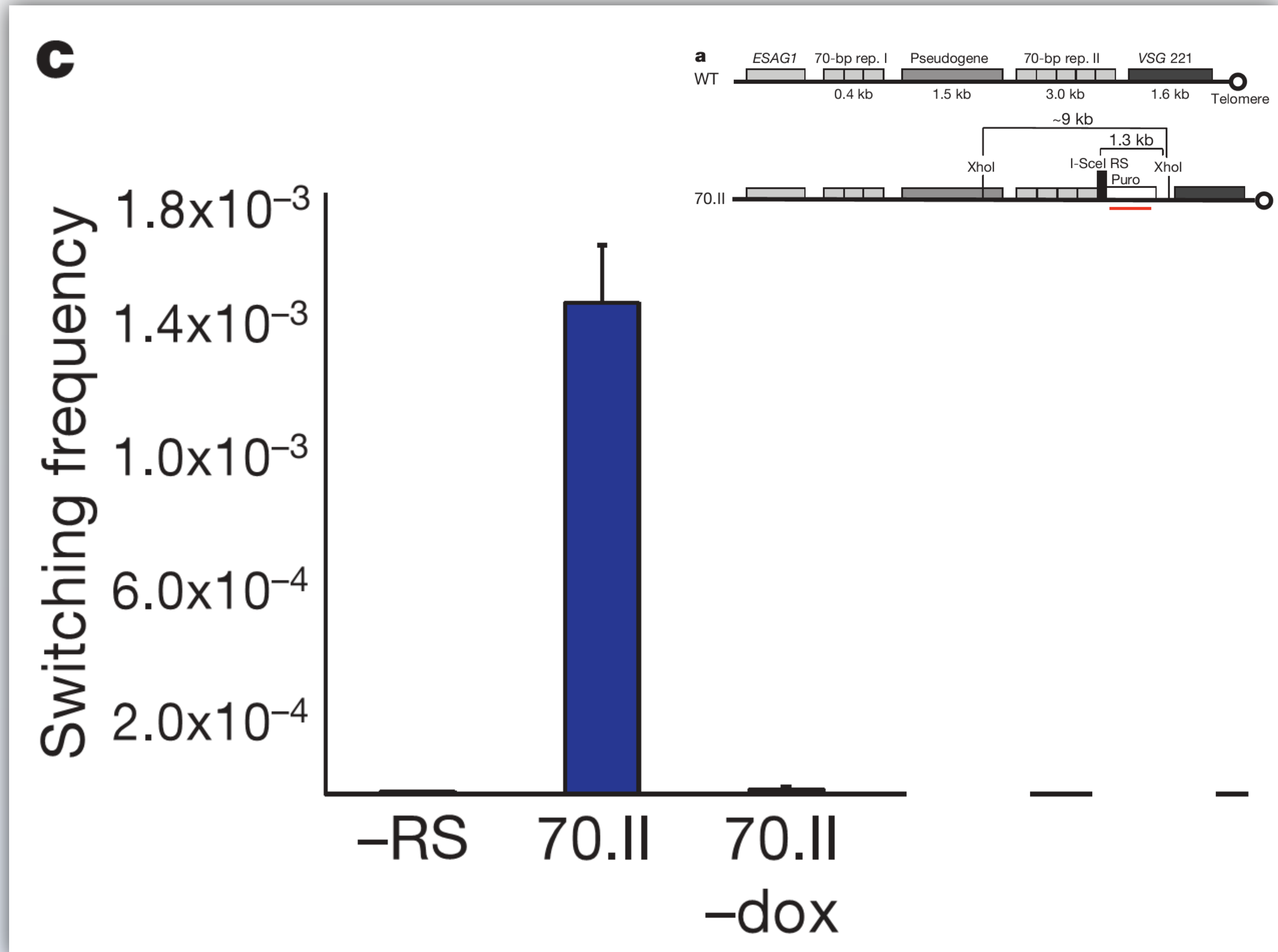
B. 70 bp repeats required for VSG switching

C. DSBs trigger VSG switching

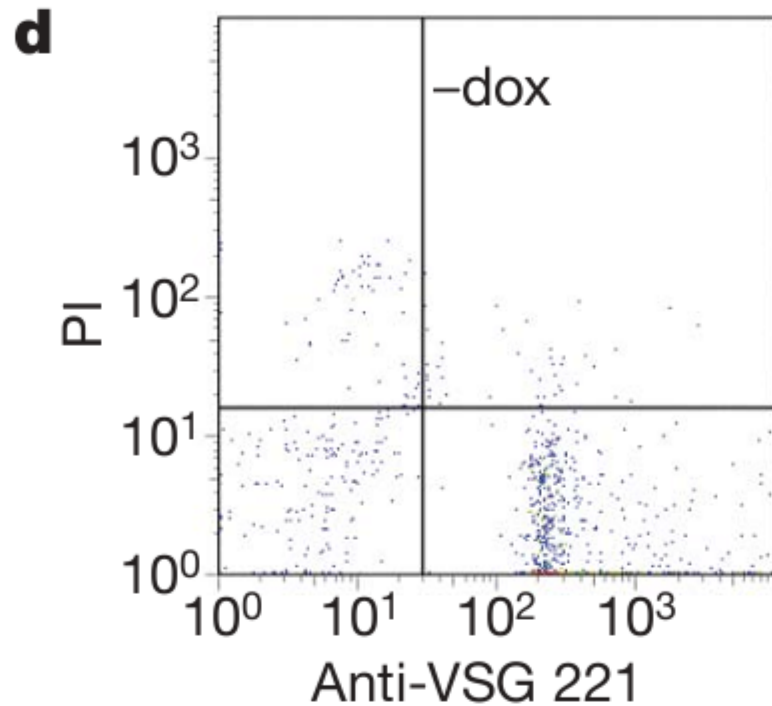
D. Mutations in VSG221 trigger VSG switching

E. Dox triggers VSG switching

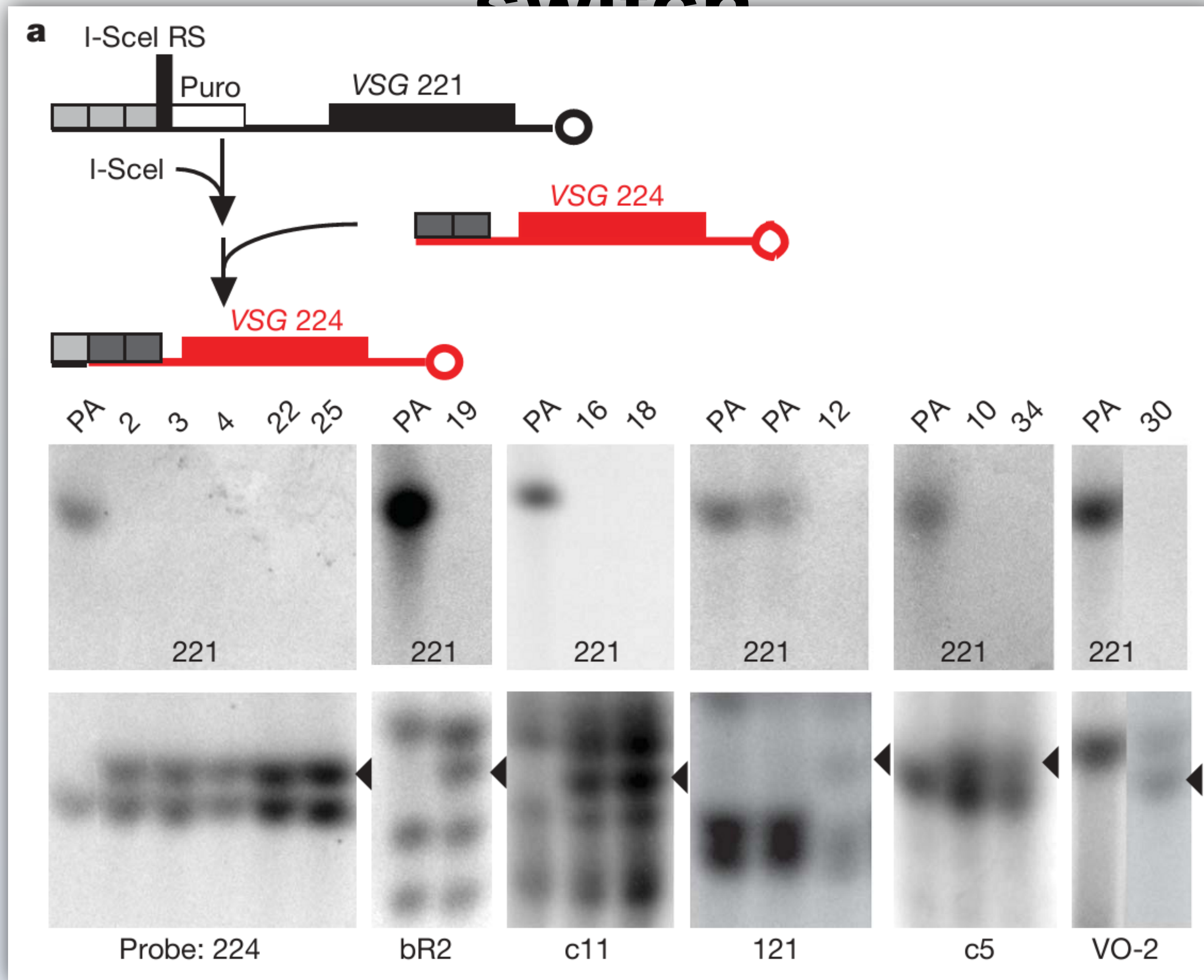
1: DSBs trigger VSG switching



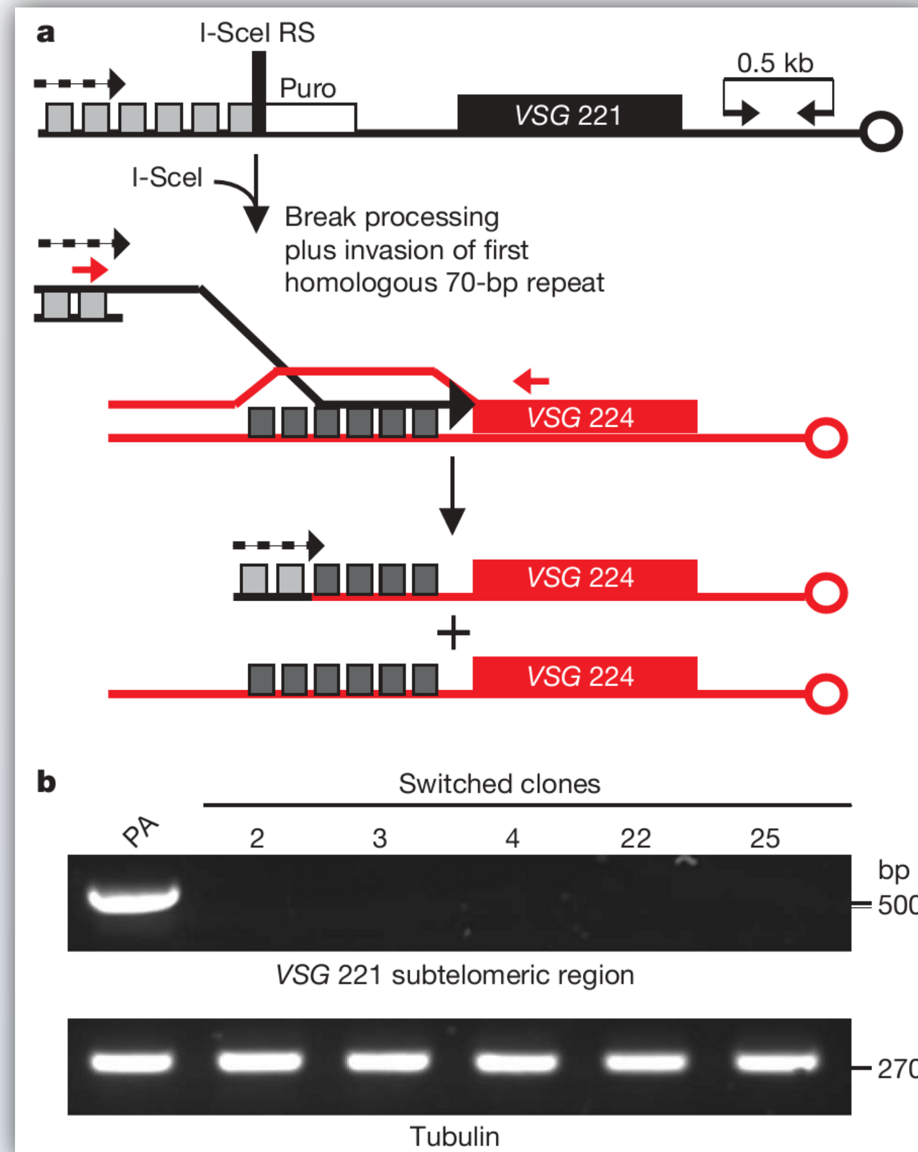
1. Confirmation: DSBs trigger switching



2. DSB trigger random duplicative switch



3. Telomeric region lost after switch



DSB repair importance

How did they show that DSB repair caused switching?

A. Create DSB breaks!

B. Sequence switched region.

C. Mutations in DSB repair = No switching

D. PCR of telomeres

Last question

What is am important question left to answer?

A. How does I-Sce1 create DSBs?

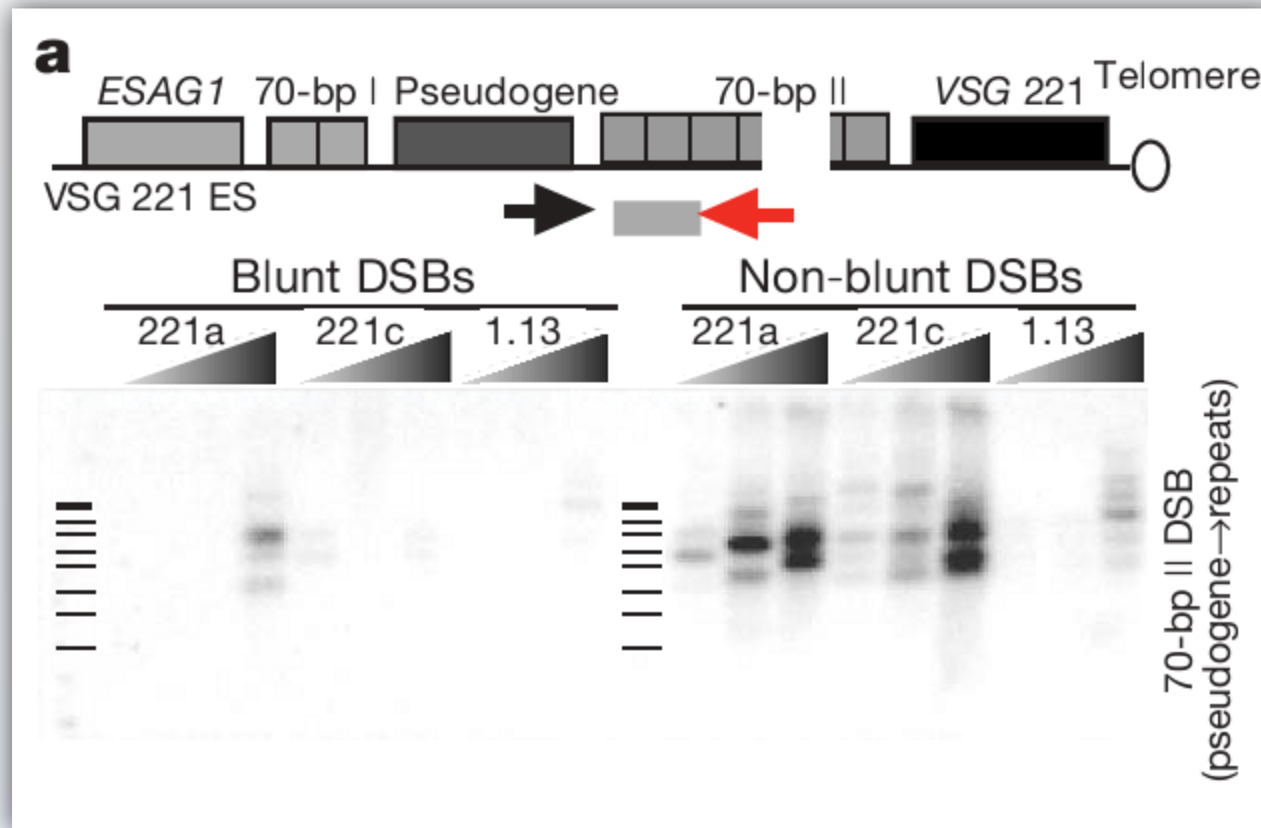
B. How does DSB repair occur?

C. Artificial = *In vivo*?

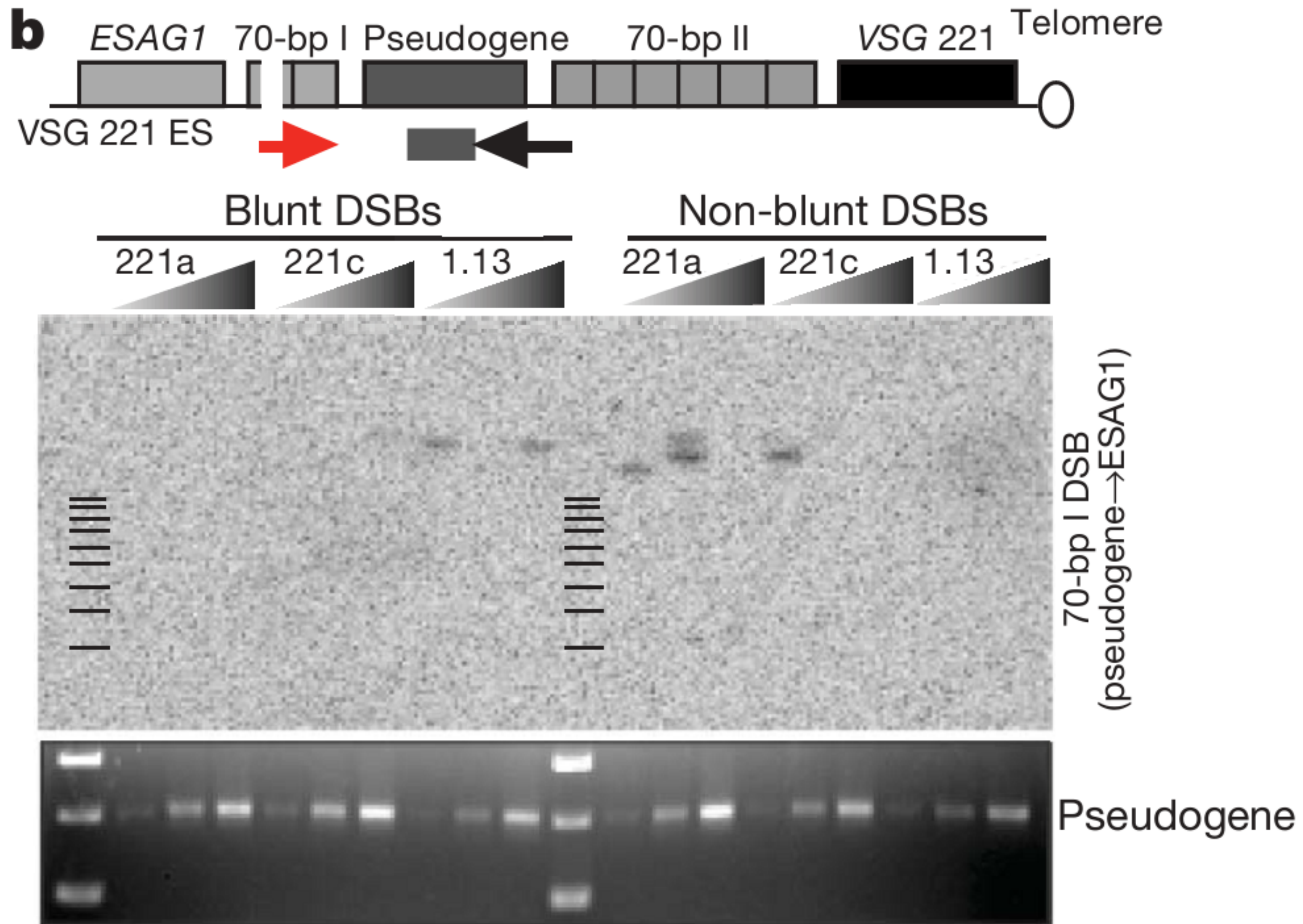
D. Frequency of DSB?

E. Bigger DSB = More frequent switching?

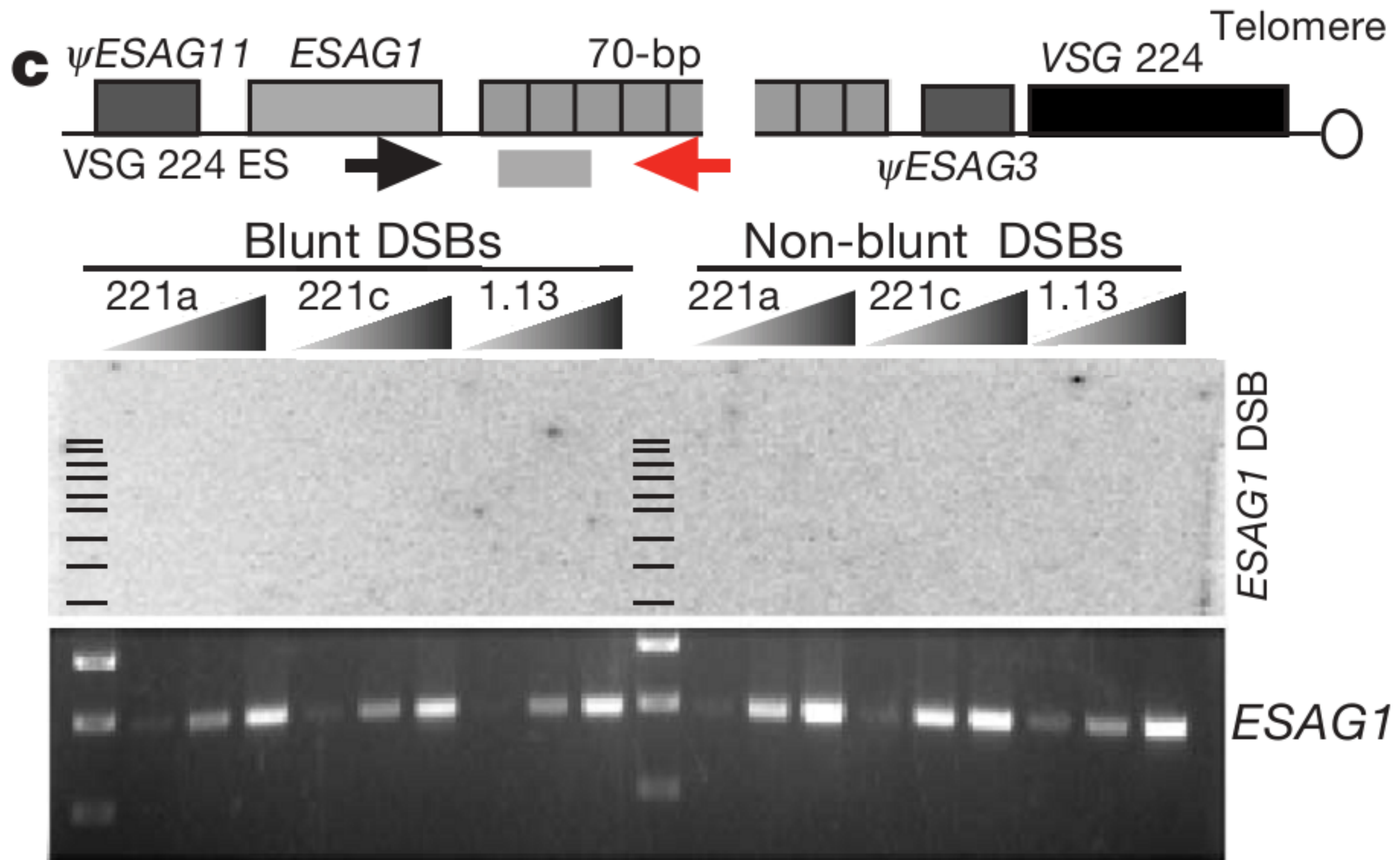
In vitro = In vivo?



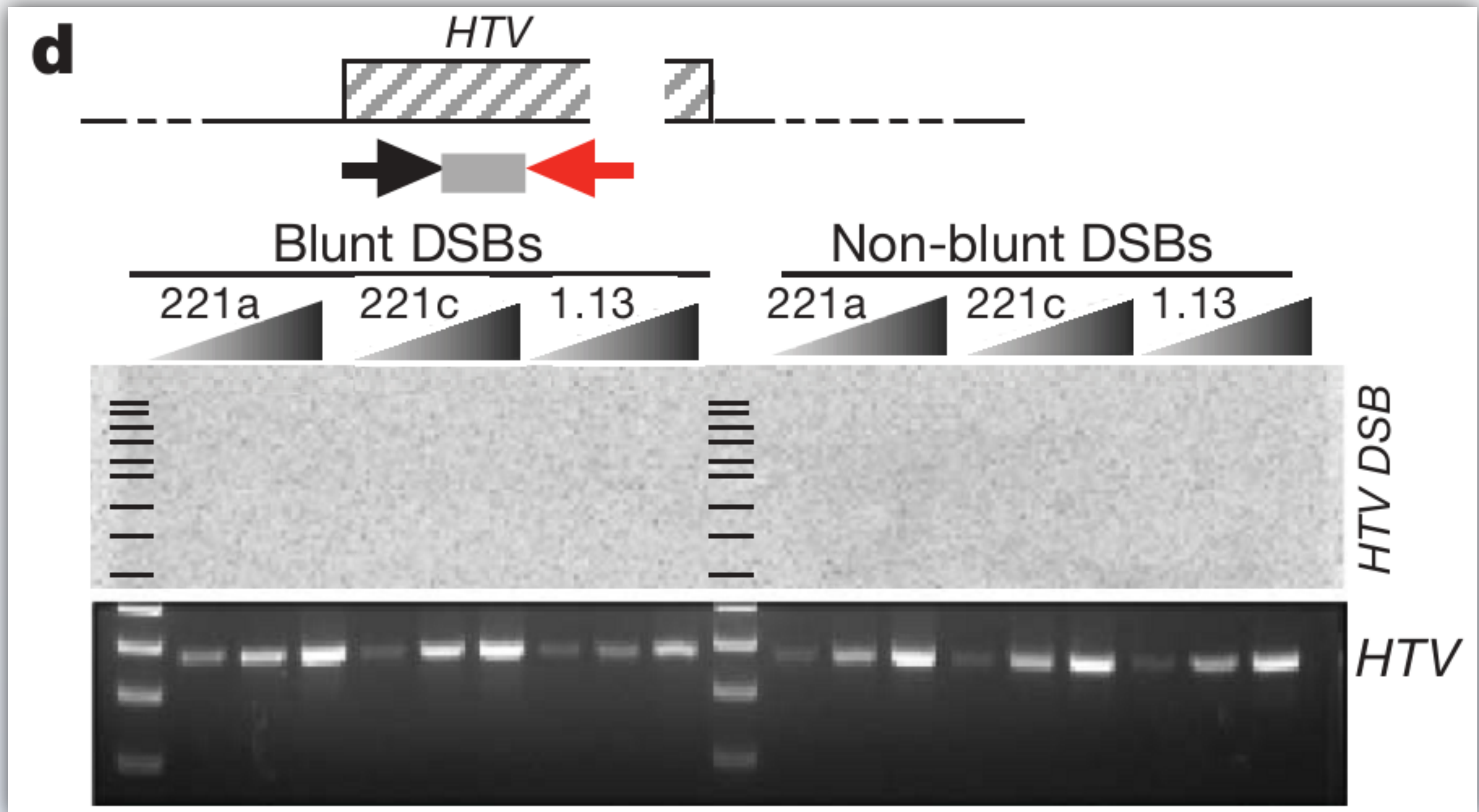
4. In vitro = In vivo?



4. In vitro = In vivo?



Determining our fragment size



Paper 2

How do VSG antigens switch?

DSBs trigger?

Yes! In 70bp repeats, in lab

Switch by DSB repair

In lab = In wild!