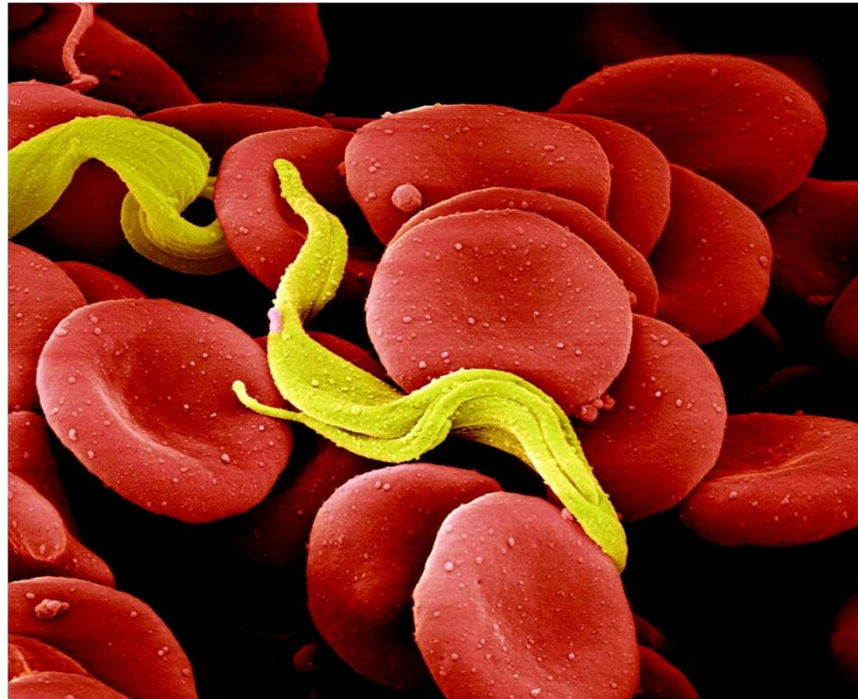
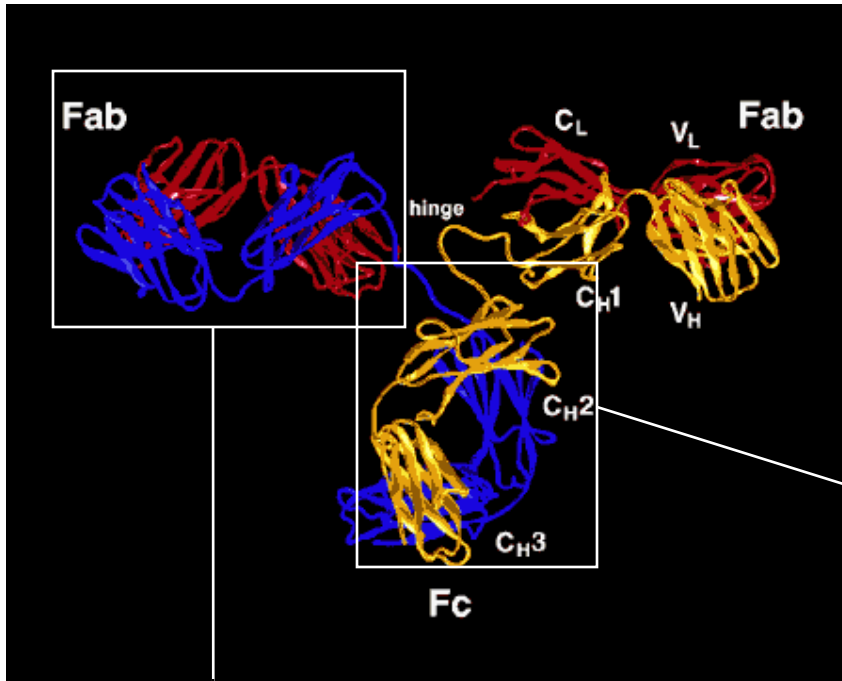


What can parasites teach us about cancers of the immune system



In my lab we study antibody diversification...

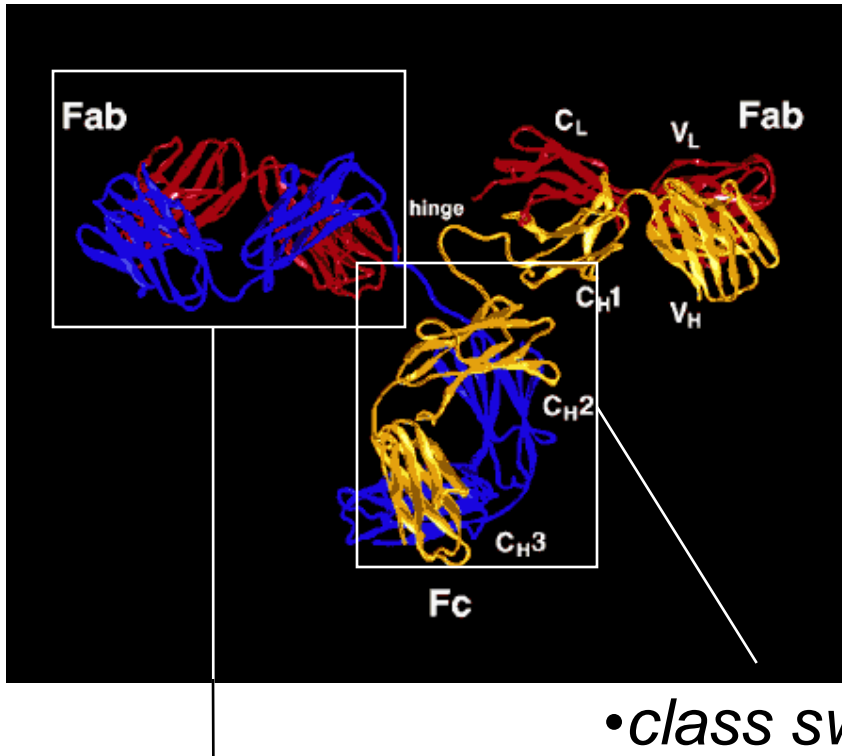


- F_{ab} - Fragment Antigen Binding
- F_c - Fragment Crystallizable

- C - constant region: recruits factors that eliminate pathogens

- V - variable region: binds specific antigens

In my lab we study antibody diversification...



• Diversity is generated by three types of DNA "transactions":

- V(D)J recombination
- Somatic hypermutation/gene conversion
- class switch recombination

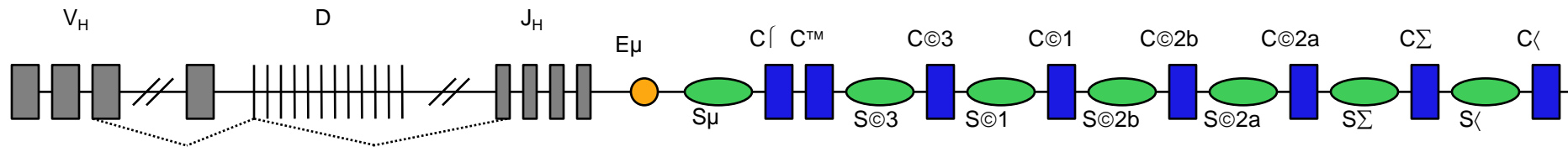
• *class switch recombination*

- *V(D)J recombination*
- *Somatic mutation/Gene conversion*

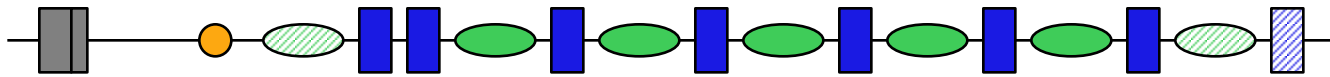
Antibody Diversification

Variable region

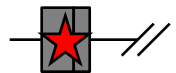
Constant region



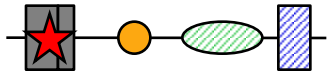
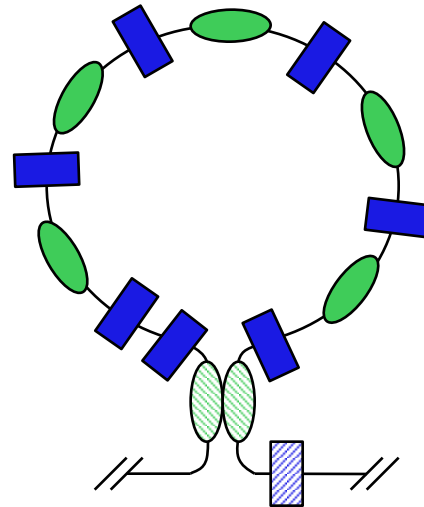
V(D)J recombination



AID Somatic hypermutation



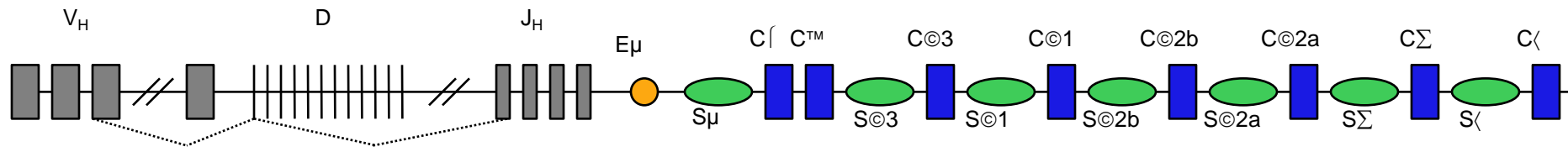
AID Class Switch Recombination



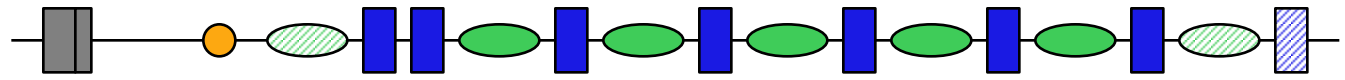
Antibody Diversification

Variable region

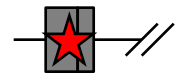
Constant region



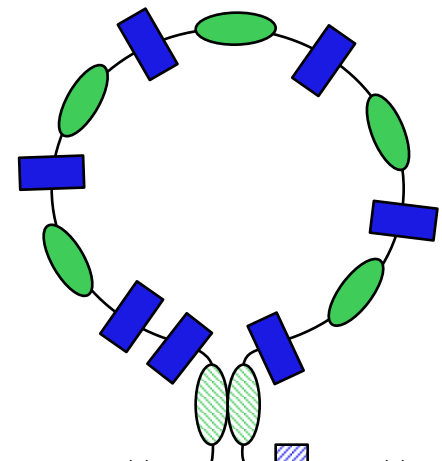
V(D)J recombination



AID Somatic hypermutation



AID Class Switch Recombination



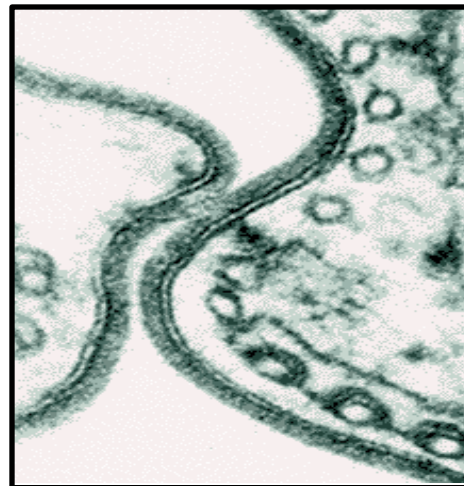
•95% of lymphomas are B cell cancers

•Most of those are errors in diversification reactions

- Why would a molecular immunologist interested in antibody diversification study trypanosomes?

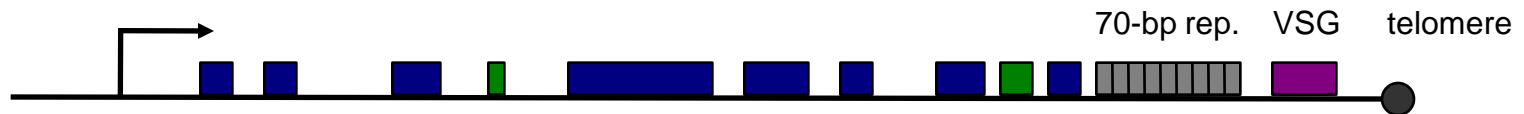
Trypanosoma brucei

- Extracellular parasites, fully exposed to host immune system
- Cells sheathed in dense variant surface glycoprotein (VSG) coat ($\sim 5 \times 10^6$ homodimers) to protect against complement-mediated lysis



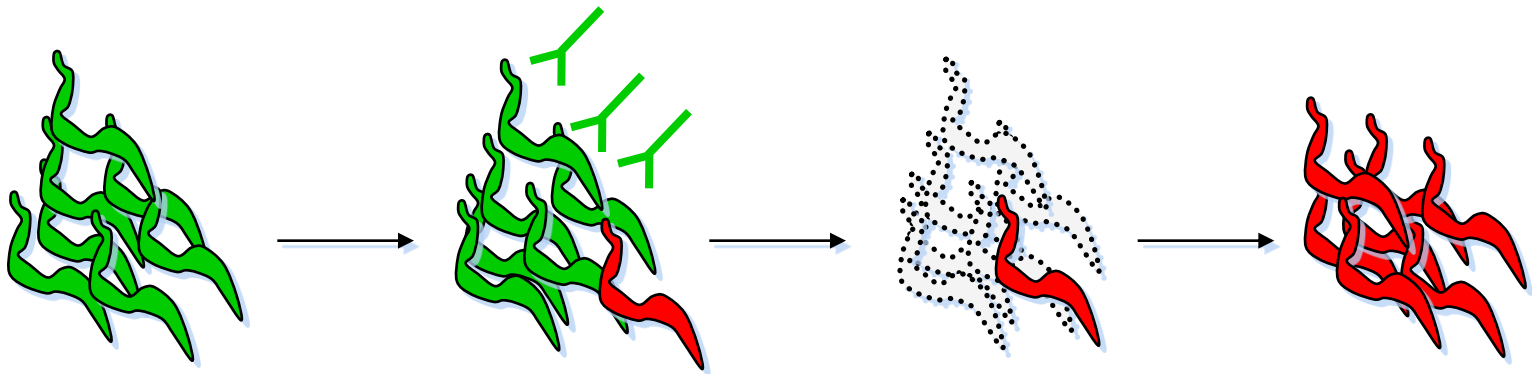
Trypanosoma brucei

- ~1,000 VSG genes and pseudogenes
- Single VSG is expressed from 1 of ~15 telomeric expression sites (ES)
- ESs are transcribed as polycystronic transcription units by PolI



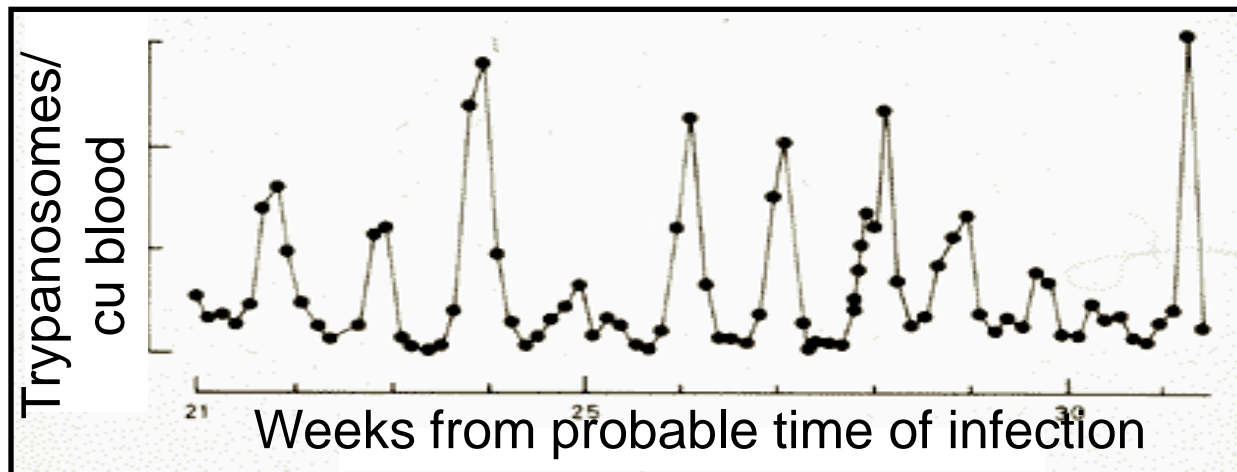
Trypanosoma brucei

- Infected host eventually mounts Ab response to predominantly expressed VSG
- However, continuous switching results in chronic infection



Trypanosoma brucei

- Infected host eventually mounts Ab response to predominantly expressed VSG
- However, continuous switching results in chronic infection

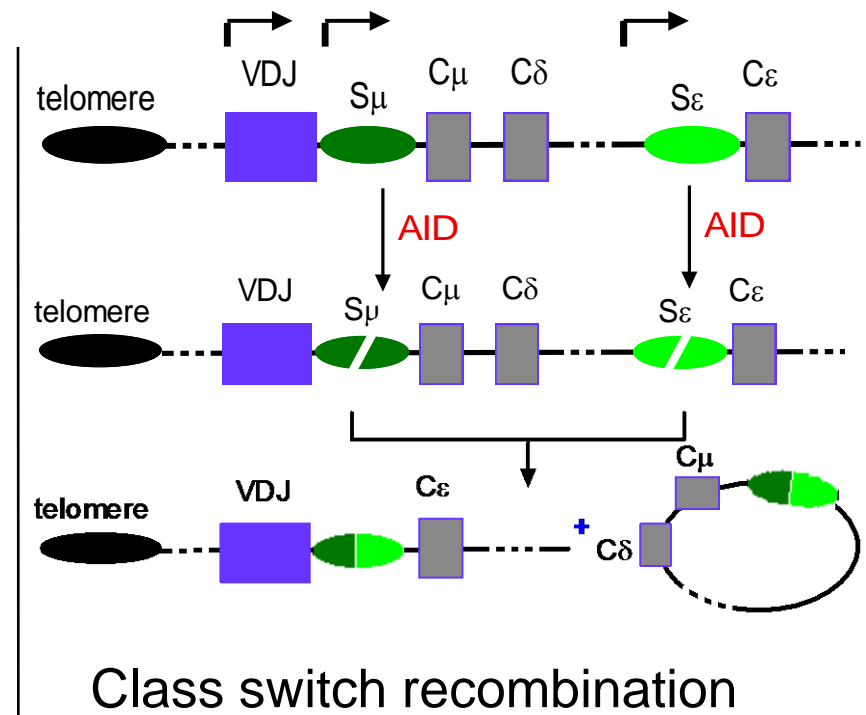


Trypanosoma brucei

- Why would a molecular immunologist interested in antibody diversification study trypanosomes?
 - Surface receptor diversity is not unique to B cells of the immune system
 - Important similarities between *T.brucei* coat switching and class switch recombination in the antibody locus (requirements for transcription, repeats; G>A mutations also present at VSG)

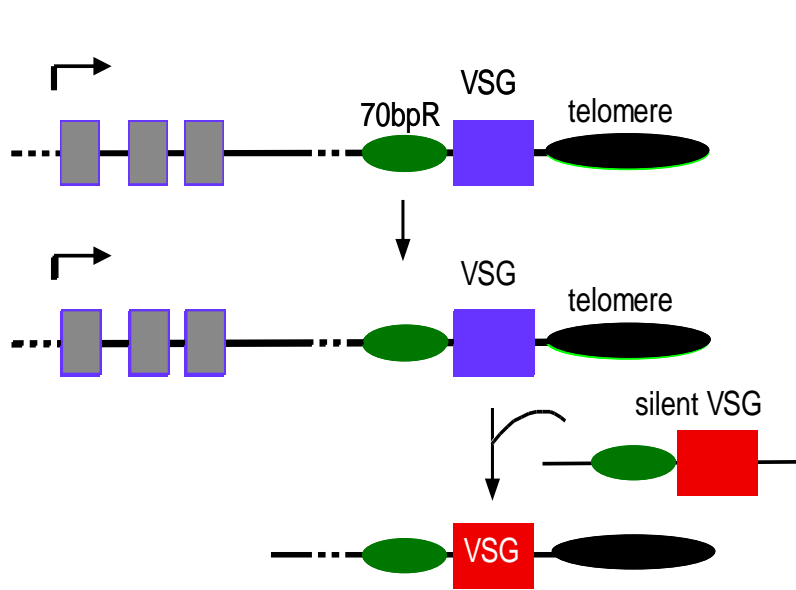
Trypanosoma brucei

- Why would a molecular immunologist interested in antibody diversification study trypanosomes?

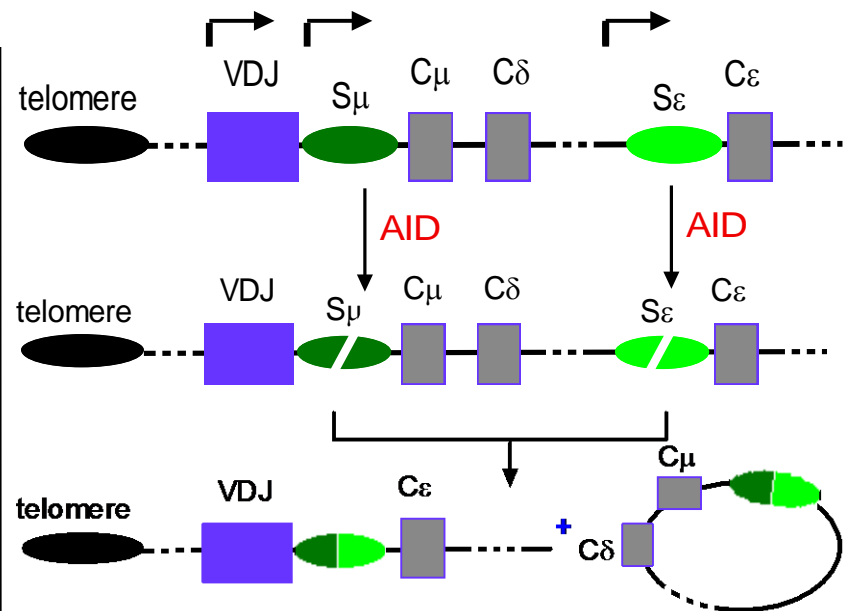


Trypanosoma brucei

- Both require transcription, the presence of repeats
- Some VSGs carry evidence of deamination activity



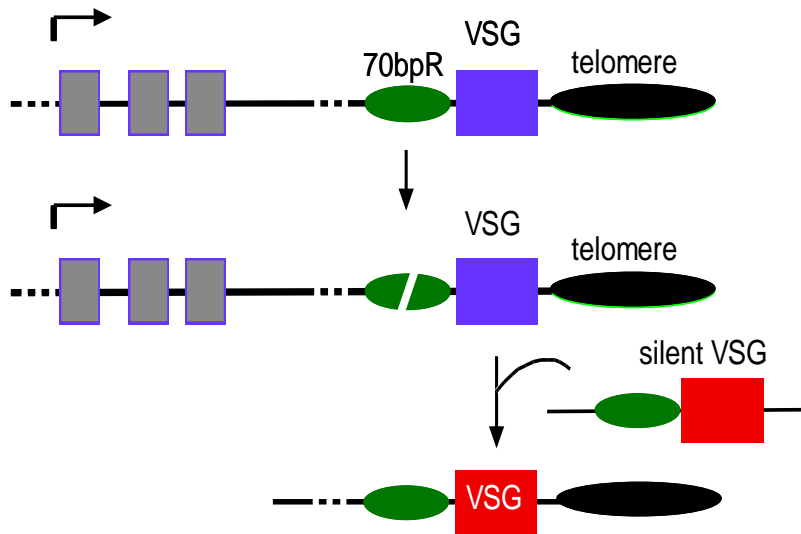
Antigenic variation (VSG switch)



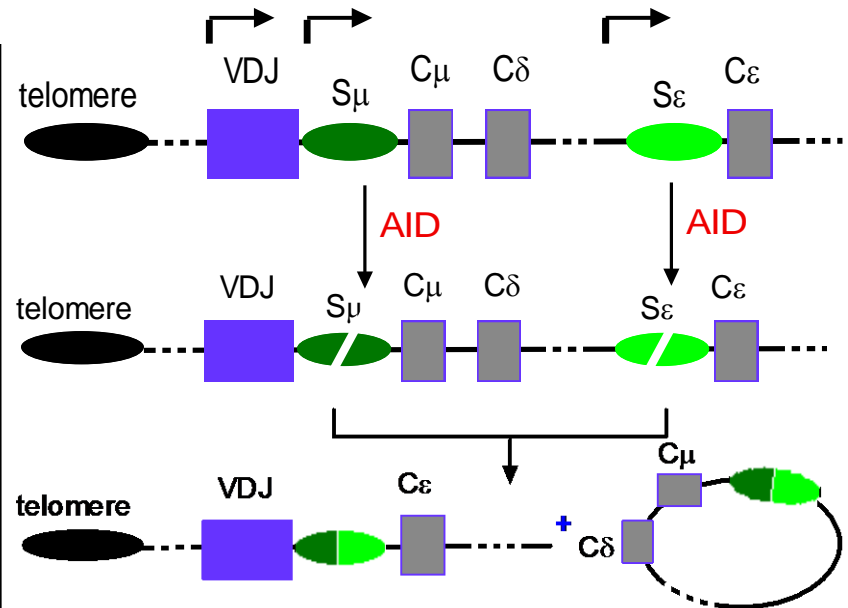
Class switch recombination

Trypanosoma brucei

- A DNA DSB intermediate for VSG switching
(Boothroyd et al, Nature 2009)

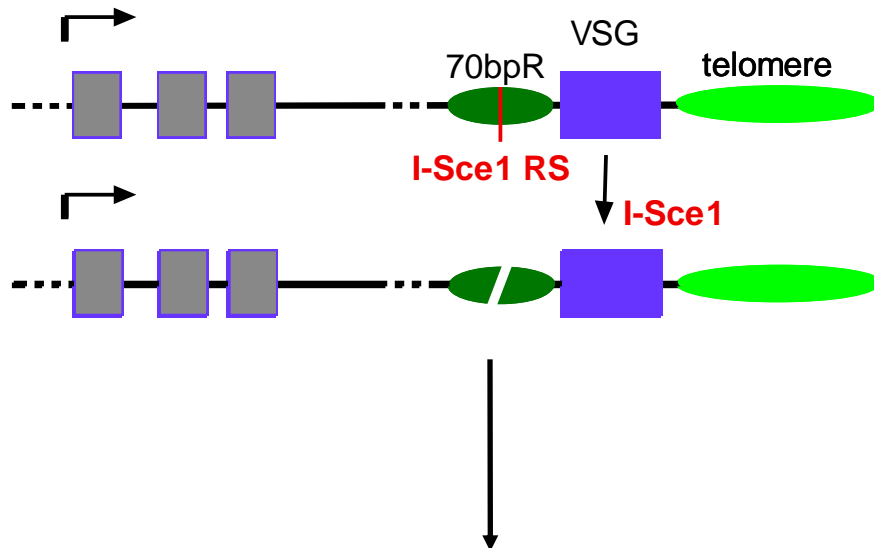


Antigenic variation (VSG switch)



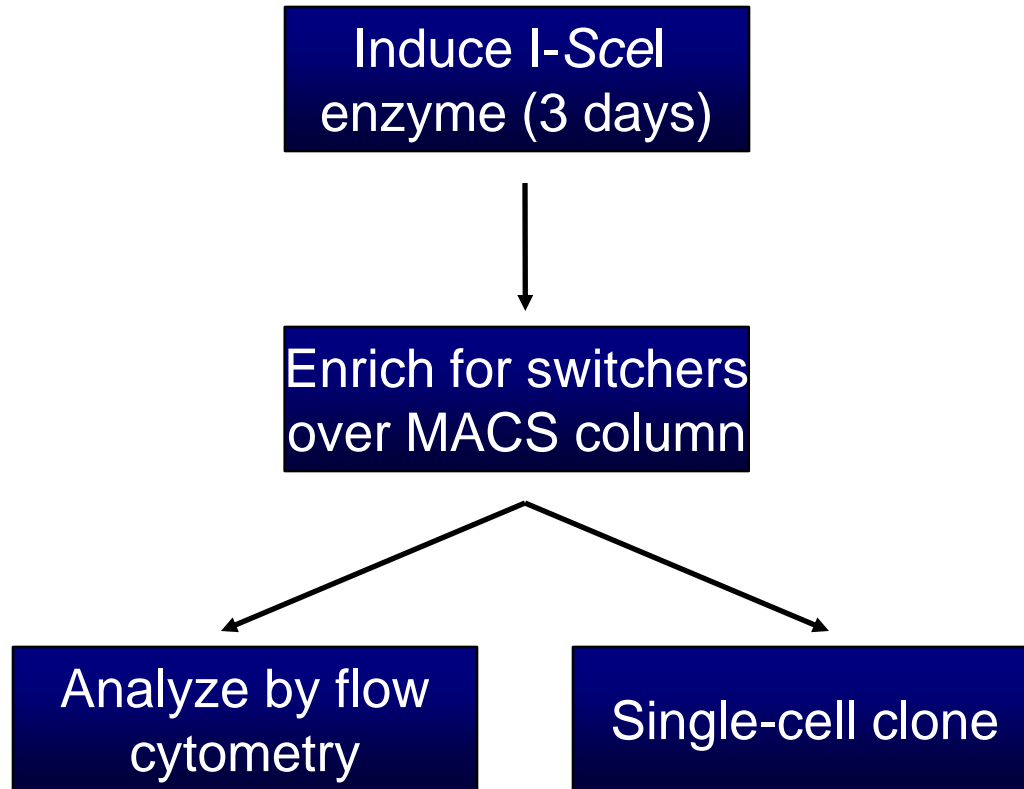
Class switch recombination

- A DNA DSB intermediate for VSG switching?

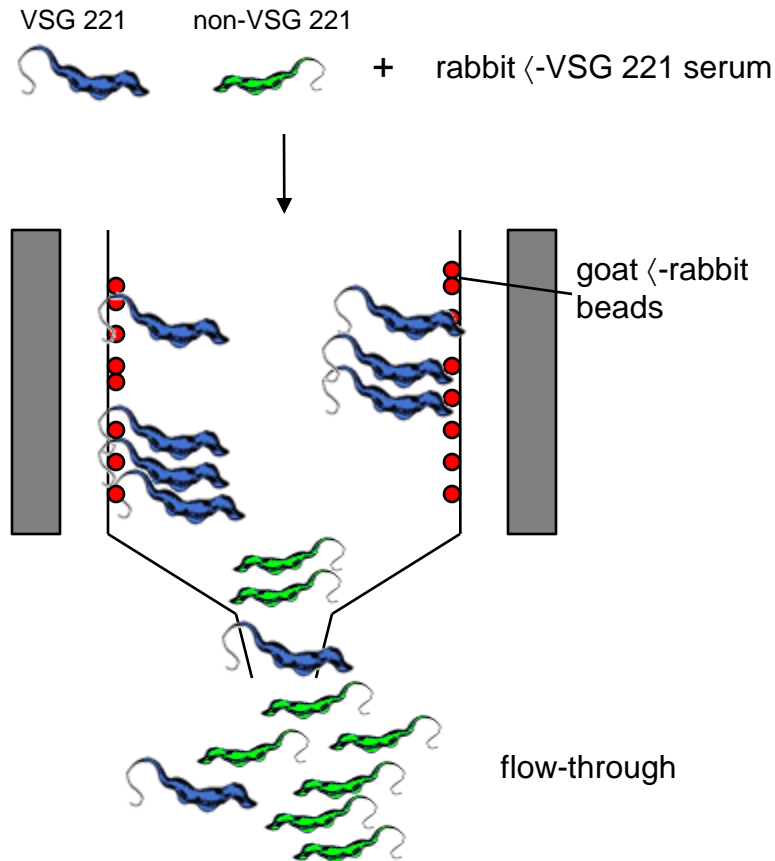


- *Rates of VSG switch? (0.5×10^{-5} in 427; 0.5×10^{-2} in WT)
- *Diversity of switchers (WT: highly diverse but hierarchical)
- *Types of DNA repair involved (duplicative gene conversion)

Will a DSB affect the rate of VSG switching?

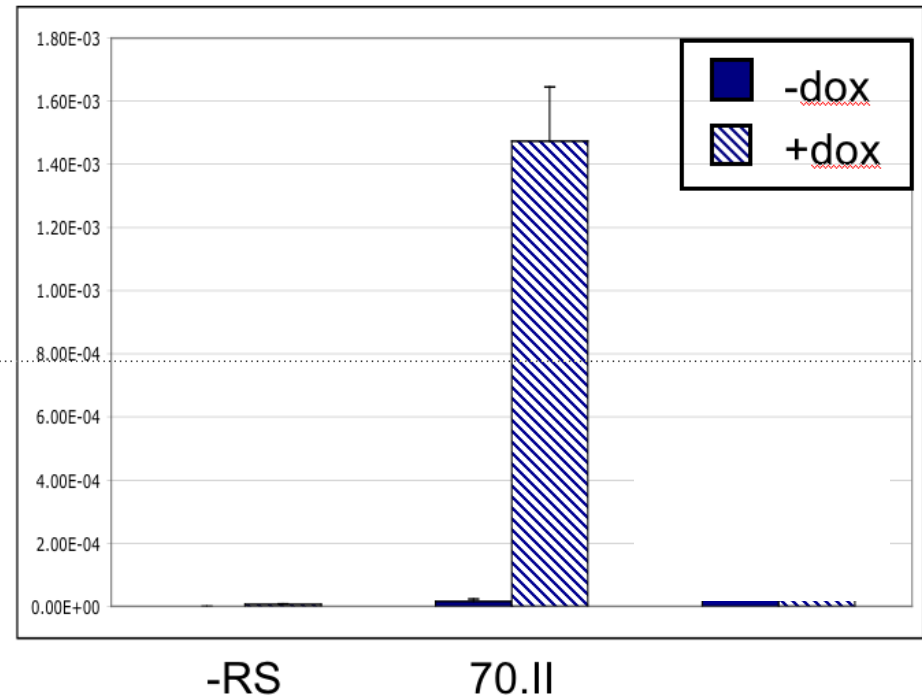
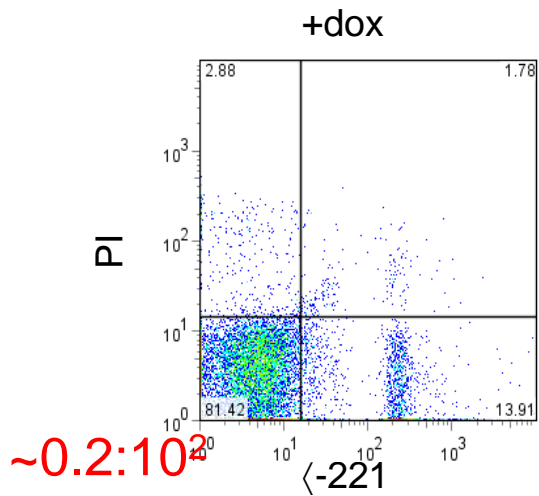
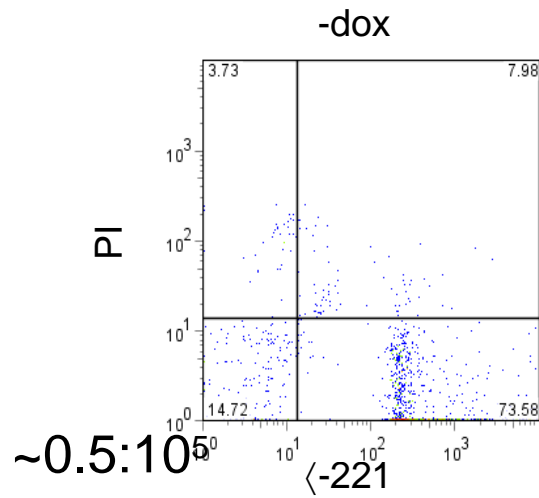


Using MACS to enrich for switchers

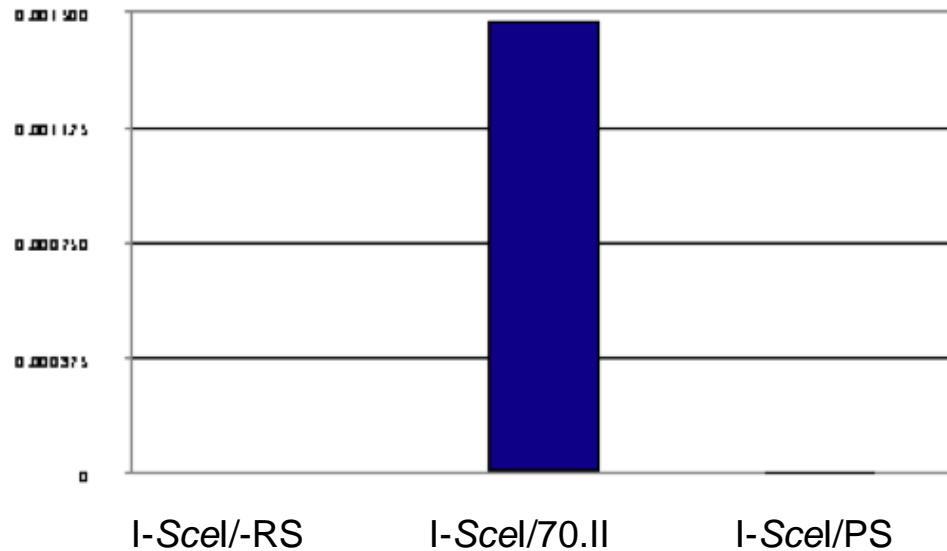
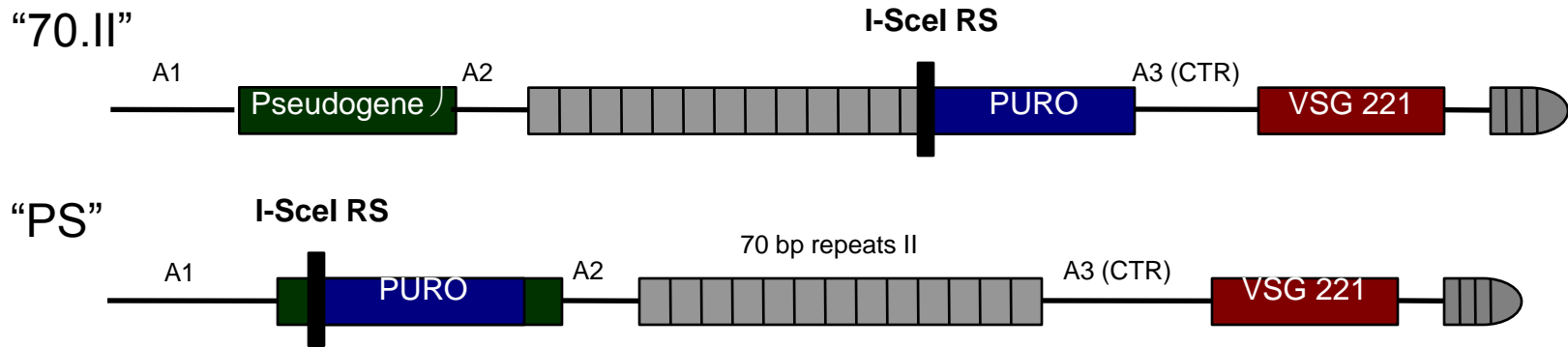


- incubate induced cells w/ serum, then goat ζ -rabbit beads
- run through MACS column
- collect flow-through
- label w/ Alexa 488-conjugated ζ -VSG 221 Ab
- flow cytometry

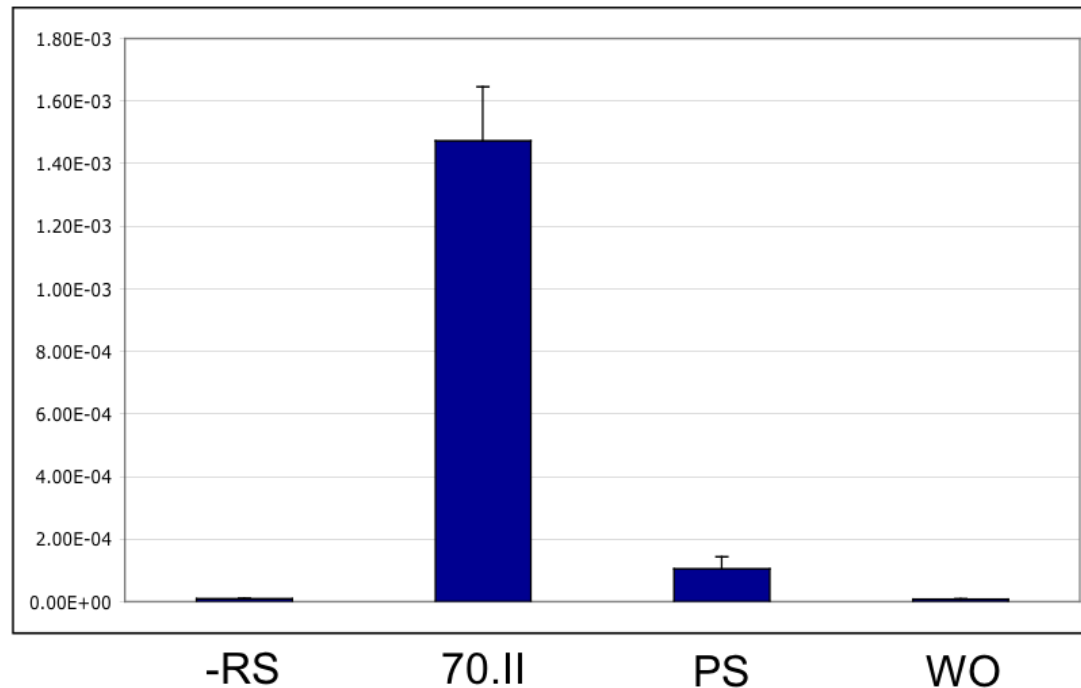
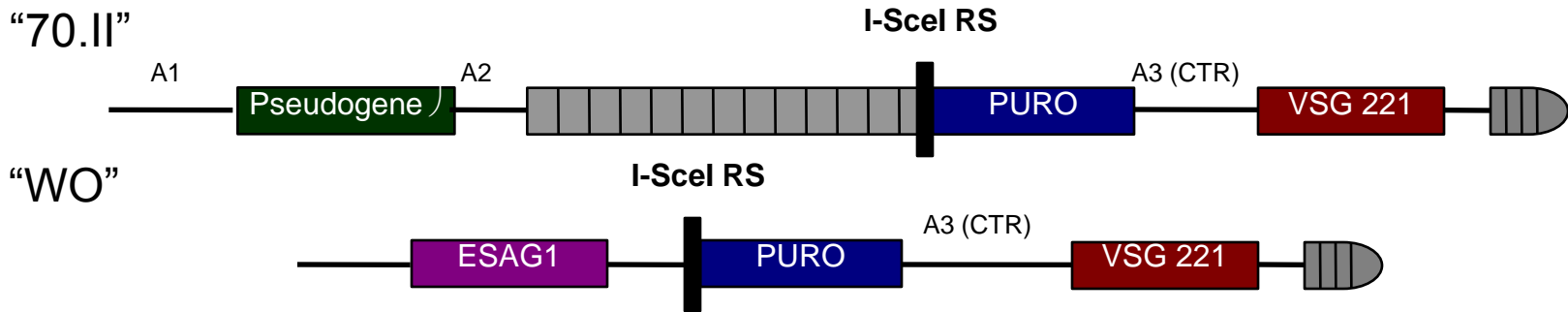
An exogenous DSB increases the rate of VSG switching by 500-fold



A DSB elsewhere in the ES does not increase switching



A DSB in the absence of the 70-bp repeats does not increase switching

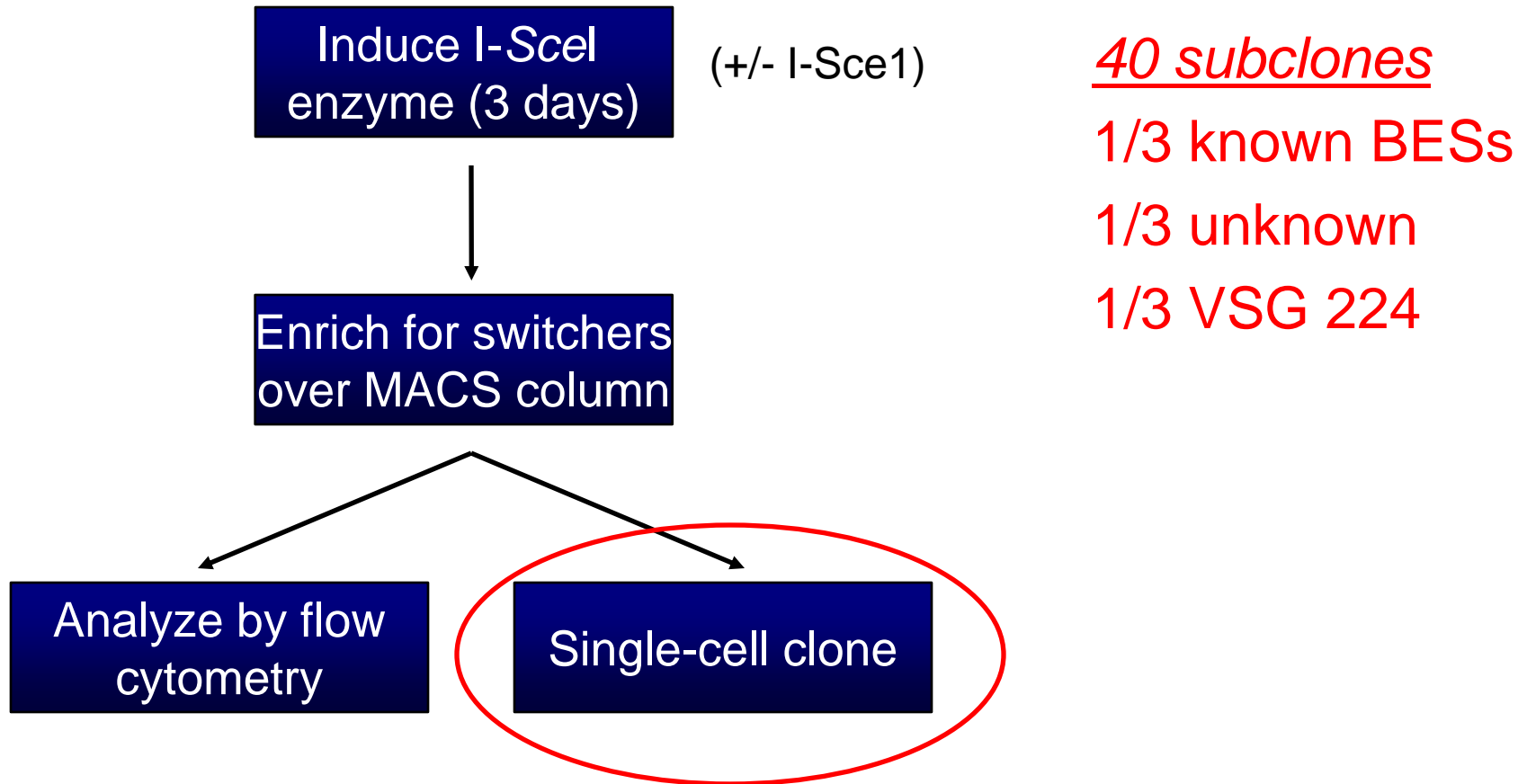


*A **single** DNA DSB increases the rate of VSG switching by 500-fold over background (approaching levels seen in wildtype infections)

*The DSB must be in close proximity to the repeats

*The rate of breakage (0.5% as estimated by Southern) matches the rate of repair (VSG switch)

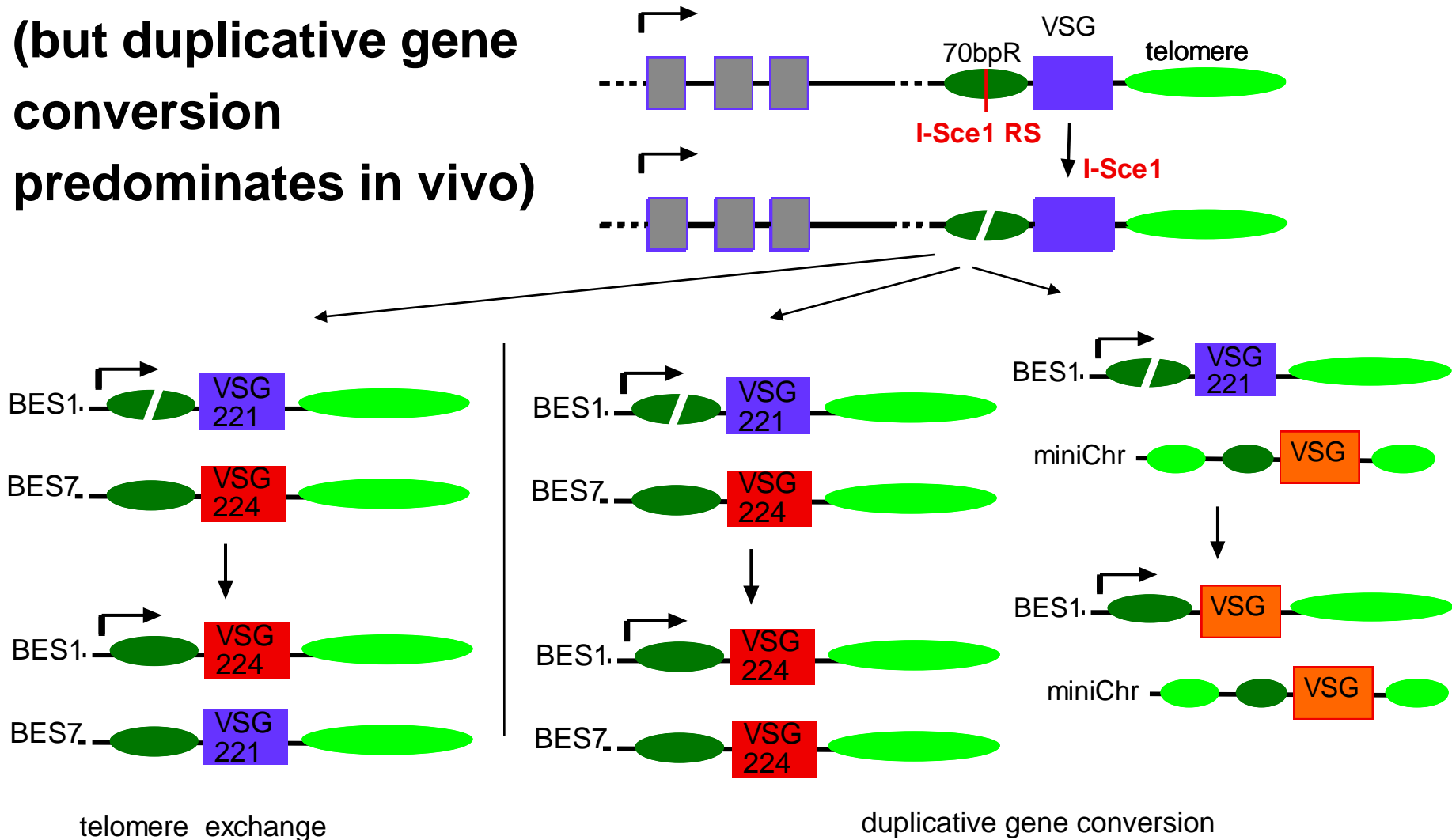
What do the switched clones look like? (how is the DSB repaired)



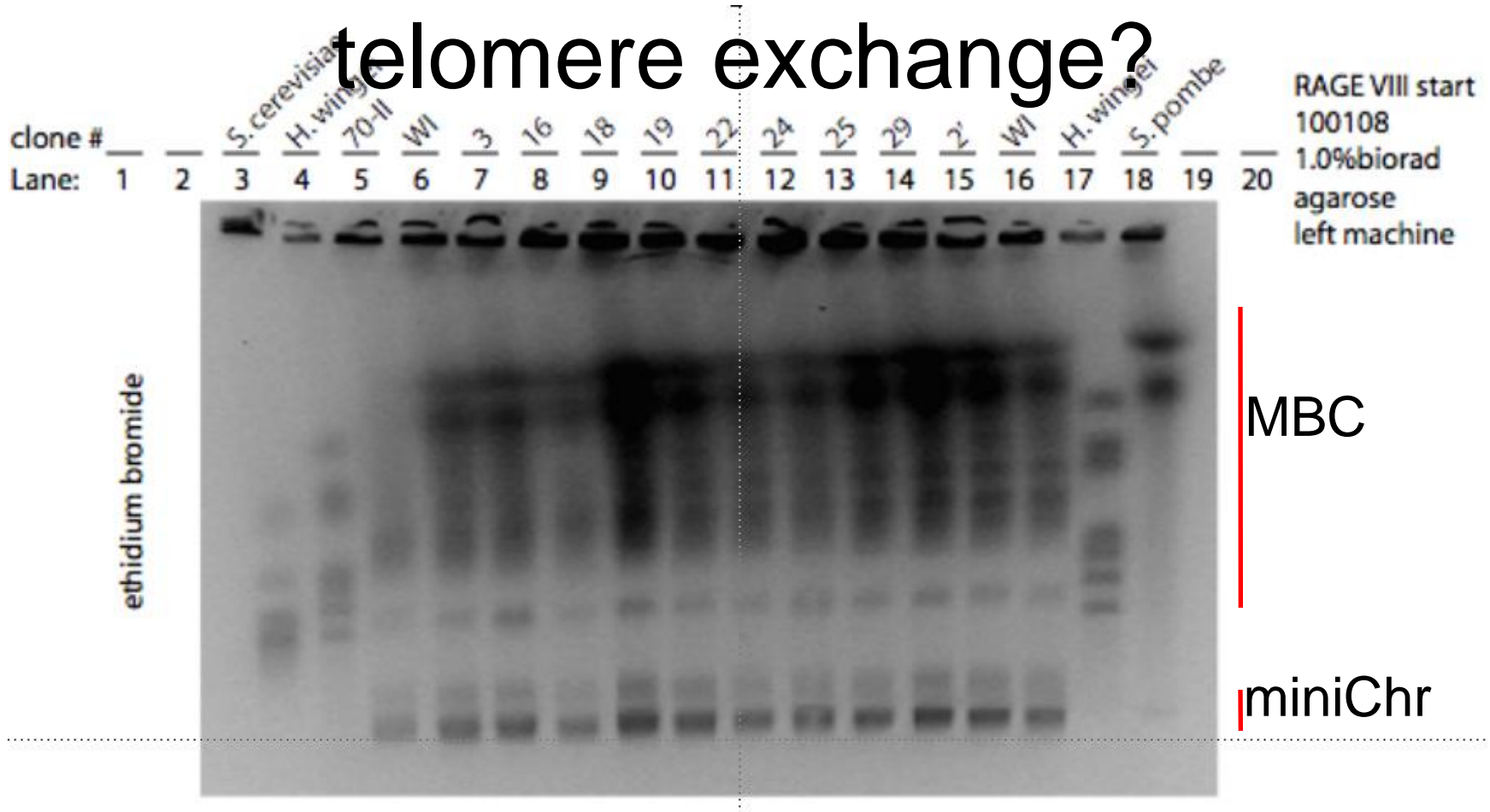
VSG switching: types of DNA recombination products

number of different mechanisms

(but duplicative gene conversion predominates in vivo)

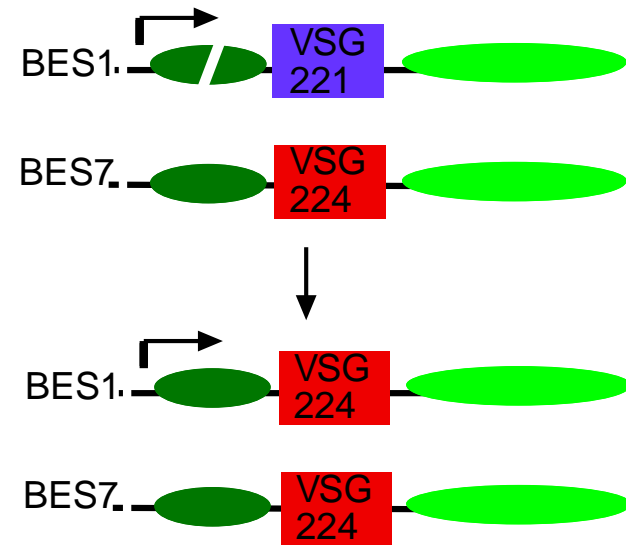
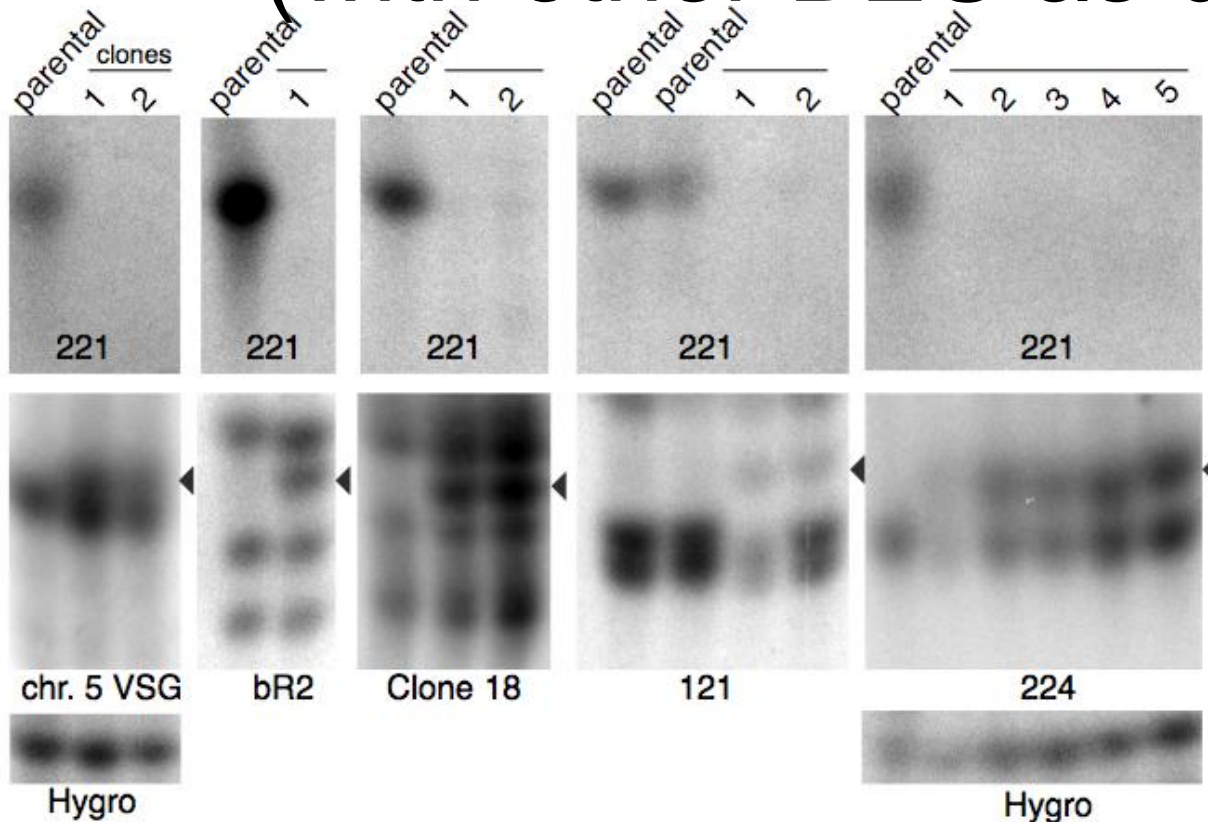


Duplicative gene conversion or telomere exchange?

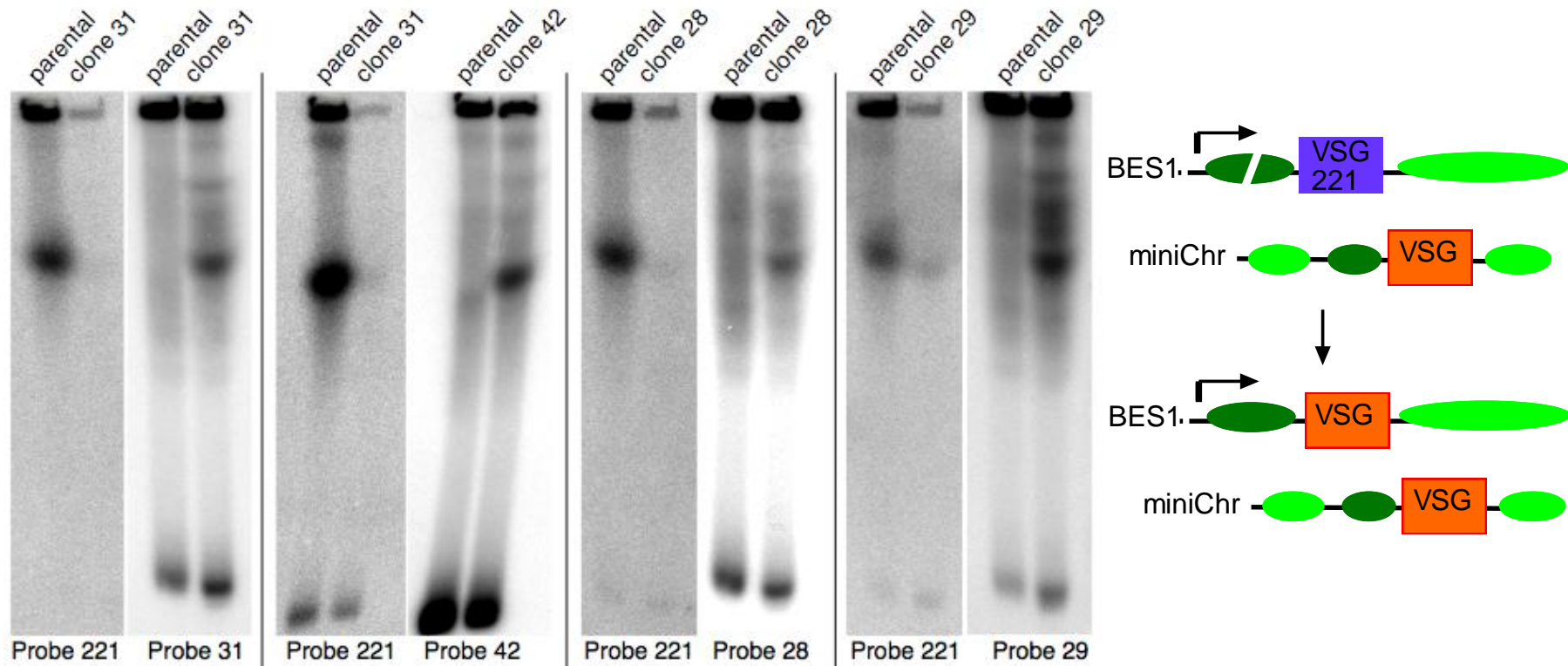


*Rotating agarose gel electrophoresis (similar to pulse field)

Duplicative gene conversion (with other BES as donors)



Duplicative gene conversion (with other minichr as donors)



A DNA DSB mediates VSG switching in *T. brucei*

- *A **single** DNA DSB increases the rate of VSG switching by 500-fold over background (approaching levels seen in wildtype infections)
- *The DSB must be in close proximity to the repeats
- *The rate of breakage (0.5% as estimated by Southern) matches the rate of repair (0.2% VSG switch)
- *Switched clones are very diverse (similar to wt)
Switched clones are the products of DNA repair by duplicative gene conversion with many different donors (similar to wt); repair by *BIR*
- **location, or endonuclease?*

- What can parasites teach us about cancers of the immune system
 - A great model system to study how a single DNA break can generate chromosomal translocations
 - a native model system to study DNA repair by break-induced replication (*BIR*)
 - and lots of other things (ALT maintenance etc)

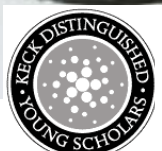
The Parasites

- Catharine Boothroyd
- Galadriel Hovel-Miner
- Tanya Leonova



The Hosts

- Brad Rosenberg
- Claire Hamilton
- Rebecca Delker
- Eric Fritz
- Alex Strikoudis



THE ROCKEFELLER UNIVERSITY

Science for the benefit of humanity