

M2D2: Site-directed Mutagenesis

3/15/13



- First module 2 quiz on Wednesday, FNT also due
- Primer design summaries are also due Wednesday -- see wiki for all the information that you need
 - expectations vs. observations? propose reasons why
 - individual assignment, no revision
 - submit to 20109.submit@gmail.com

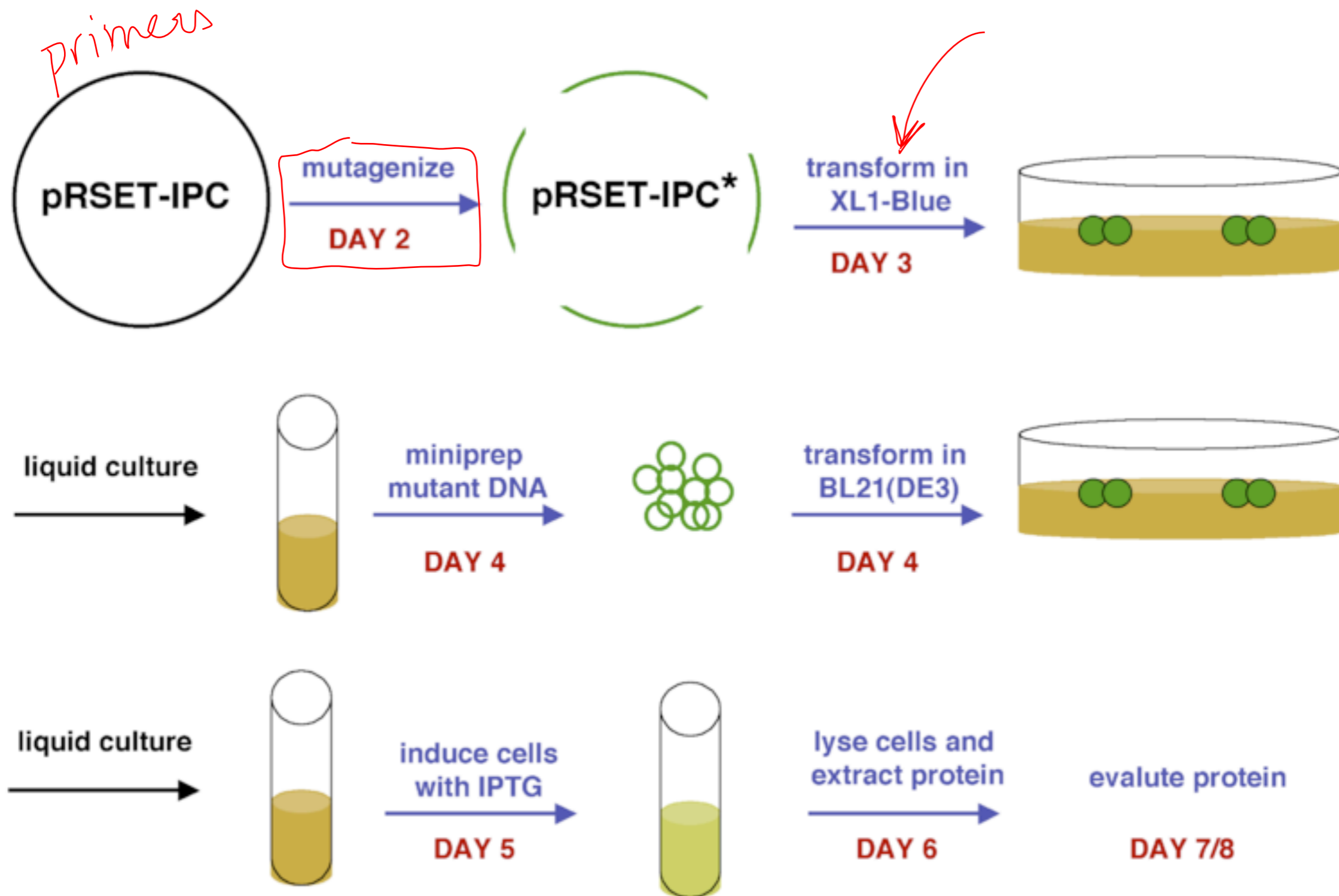
- Do something that makes you happy this weekend:

1 - blue
2 - yellow
3 - green
4 - purple
5 - platinum
6 - orange
7 - pink
8 - red



Shannon's
happy shamrock shake

Protein Engineering -- Experimental Overview

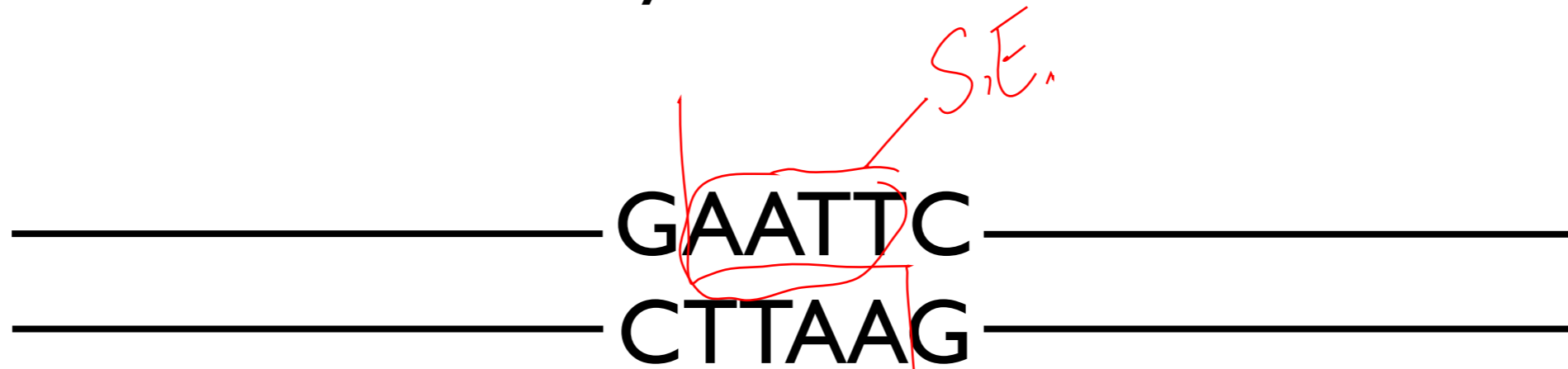


Protein Engineering -- Rational Design



Review: Restriction Enzymes

EcoRI



Purpose of RE: 109
 — sequencing
 — cloning
 — screen

Bacteria — protection against viral DNA

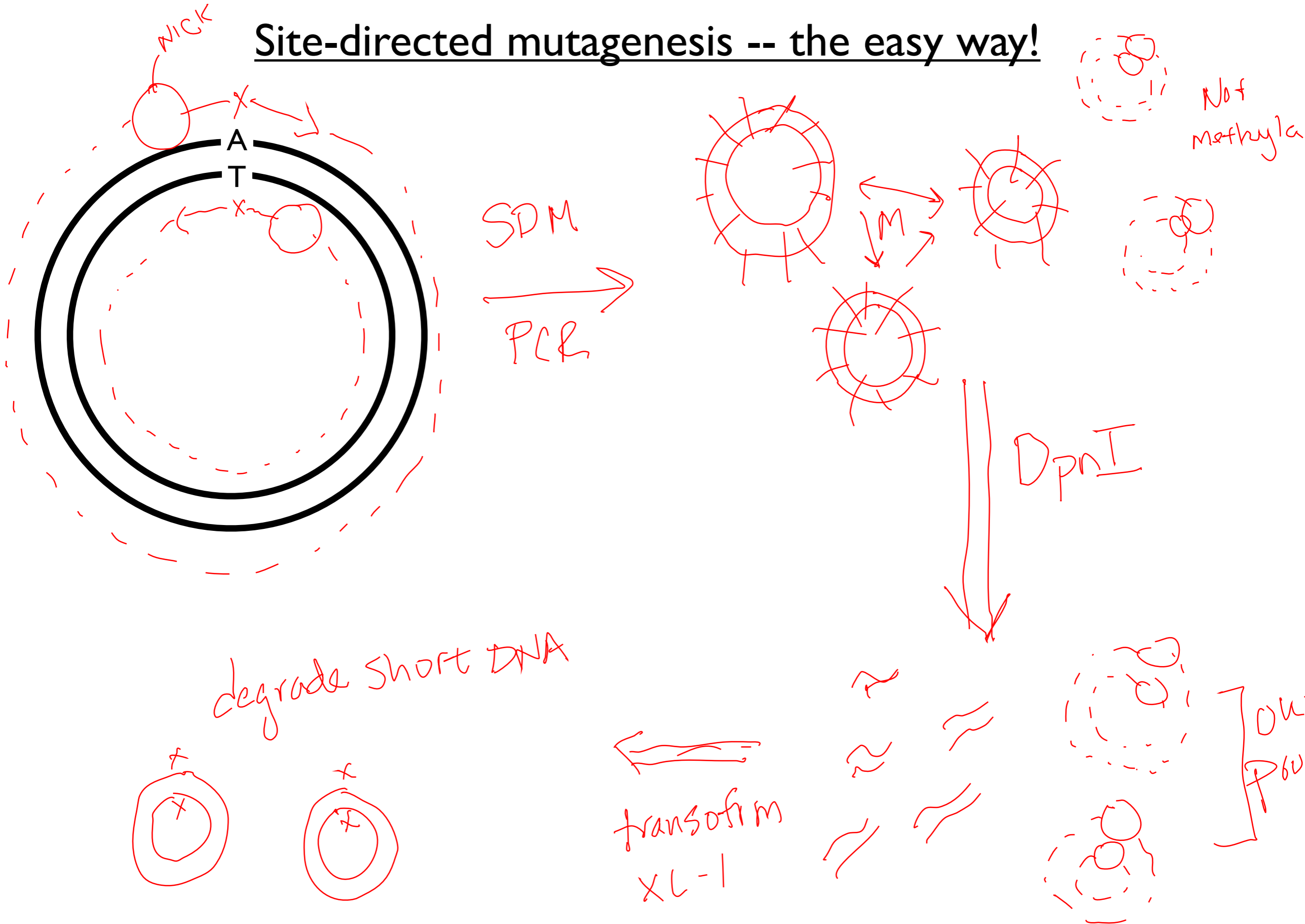
Most restriction sites are palindromic -- but not all

Some enzymes recognize modified DNA: methylation

Some enzymes are promiscuous:



Site-directed mutagenesis -- the easy way!



Today in lab:

1. Resuspend primers
 - Pay attention to [] and volume
2. Set-up SDM PCR reaction: Be careful of contamination potential
3. During incubation:
 - Zeinab JC talk (~2pm)!
 - Reading time (~45 min) for Nagai et al.
4. Paper discussion
5. Start DpnI restriction enzyme digest

One website that might be useful to you:

<http://micro.magnet.fsu.edu/primer/lightandcolor/fluoroexcitation.html>