

7.013 Quiz 2 ANSWERS

Question	Value	Score
1	14	
2	16	
3	16	
4	24	
5	17	
6	13	
	100	

Question 1

a) What role does the acrosome have in fertilization? Circle all that apply. 3 pts

- i) It responds to egg's chemoattractants.
- ii) It has enzymes that digest the zona pellucida.**
- iii) It has enzymes that digest the nuclear membrane.
- iv) It is the part of the egg that prevents sperm from binding.
- v) None of the above.

b) If calcium is injected into an unfertilized sea urchin egg, what would happen? Circle one. 3 points

- i) Capacitation would occur.
- ii) A slow block to polyspermy would be induced.**
- iii) The egg will become fertilized without sperm.
- iv) The egg will become female.
- v) The sperm will undergo an acrosomal reaction.

c) If we now mix sea urchin sperm with these eggs (in b), will fertilization occur? Circle one. 3 points

- i) No, capacitation has already occurred.
- ii) No, the sperm can no longer attach to the egg.**
- iii) Yes, the egg is now in "ready state".
- iv) No, the egg is already fertilized.
- v) None of the above.

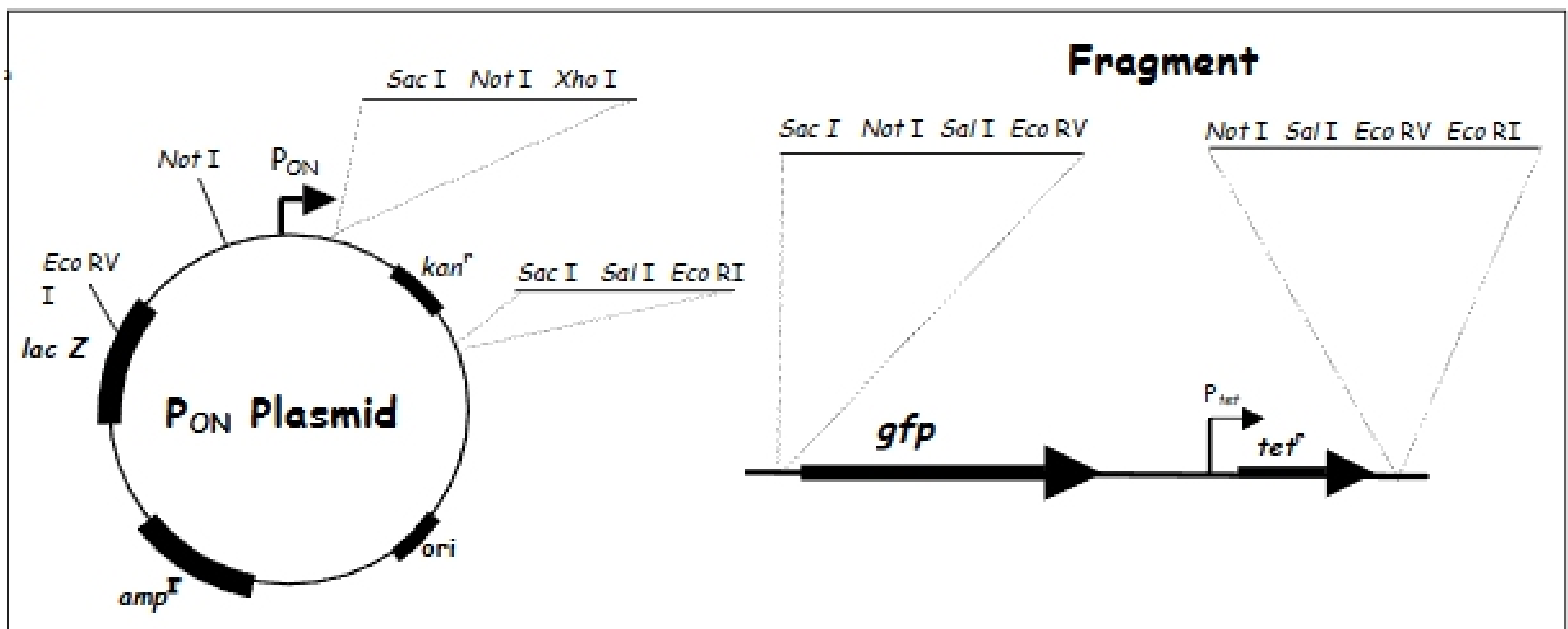
d) Could a human sperm fertilize a hamster egg? Explain your answer briefly. 5 points

No, the egg is protected by a thick zona pellucida that can be penetrated only if the ZP3 ligands within can be bound by the sperm with the appropriate receptor. These interactions are species specific. This triggers the acrosomal reaction which spills out hydrolytic enzymes to make a passage of the sperm through the Zona Pellucida.

Question 2

You wish to make your pet glow in the dark so your first step is to clone *gfp* gene, (encoding green fluorescent protein), downstream of the "ON" promoter which is always active, expressing downstream genes. You have a vector, the P_{ON} plasmid, and you've isolated a DNA fragment containing a promoter-less *gfp* gene as well as the *tet^r* gene conferring resistance to tetracycline.

The vector carries the *lacZ* gene encoding β -Galactosidase which converts the substrate, X-gal, into a blue colored product. It also carries two antibiotic resistance genes: *kan^r* and *amp^r* which allow for growth on kanamycin and ampicillin, respectively. The *lacZ*, *kan^r*, and *amp^r* genes have their own promoters (not shown). Assume all restriction sites are unique unless shown otherwise. Assume that **only** similarly cut DNA ends can ligate with each other. You may use more than one enzyme for your cloning. Assume all digestions are complete (no partial digestions).



a) In order to clone *gfp* in the correct orientation downstream of P_{ON} ...

i) what enzyme(s) would you cut the vector with? 6 points per side (Eco R I-EcoRI -3 pts) (Sac I-Sac I-3 points)

- Eco RI**
- Eco RV*
- Not I*
- Sac I**
- Sal I*
- Xho I*

ii) what enzyme(s) would cut the fragment with?

- Eco RI**
- Eco RV*
- Not I*
- Sac I**
- Sal I*
- Xho I*

b) To select for the desired construct with insert you should plate the ligation on medium with...Circle all that apply. 3 points

- Ampicillin
- Chloramphenicol
- Kanamycin
- Tetracycline**
- X-Gal

c) Bacteria transformed with original vector would grow on medium with...Circle all that apply. 4 points

- Ampicillin**
- Chloramphenicol
- Kanamycin**
- Tetracycline
- X-Gal

d) A fellow student cloned the *gfp* insert into the vector using *Not I*. However he found that there was no expression of *gfp*. Why? 3 points

The promoter P_{ON} would be removed.