E1. A. ß-ONPG was used to measure the level of expression of the *lac* operon. More specifically, the cleavage of ß-ONPG to produce a yellow compound requires the action of ß-galactosidase. So the assay is indirectly measuring the amount of ß-galactosidase protein. The reason why there was no yellow color in one of the tubes was because the repressor was preventing the expression of the *lac* operon, and this prevents the expression of ß-galactosidase enzyme. Some other methods to measure the expression level of the *lac* operon could include the following:

1. Conduct a Northern blot to measure the amount of mRNA that is produced from the *lac* operon.
2. Conduct a Western blot using antibodies against one of the three proteins encoded by the *lac* operon.
3. Measure the uptake of radiolabeled-lactose into the cells. This would measure the amount of the lactose permease that is expressed from the *lac* operon.

B. The merozygote has two copies of the *lacZ* gene so it makes twice as much ß-galactosidase.

E2. In samples loaded in lanes 1 and 4, we expect the repressor to bind to the operator because there is no lactose present. In the sample loaded into lane 4, the CAP protein could still bind cAMP because there is no glucose. However, there really is no difference between lanes 1 and 4, so it does not look like the CAP can activate transcription when the *lac* repressor is bound. If we compare samples loaded into lanes 2 and 3, the *lac* repressor would not be bound in either case, and the CAP would not be bound in the sample loaded into lane 3. There is less transcription in lane 3 compared to lane 2, but since there is some transcription seen in lane 3, we can conclude that the removal of the CAP (because cAMP levels are low) is not entirely effective at preventing transcription. Overall, the results indicate that the binding of the *lac* repressor is much more effective at preventing transcription of the *lac* operon compared to the removal of the catabolite activator protein.

E3. In the normal strain, the *lac* operon would be fully induced, so there would be maximal expression of the mRNA (lane 1). The same thing would happen in lane 2, since the *lac* repressor has been inactivated. In lane 3, we would not see any mRNA because the *lac* repressor does not bind to allolactose. Therefore, the *lac* repressor would remain bound to the *lac* operator even in the presence of lactose. In lane 4, the CAP would not activate transcription although the repressor would not prevent transcription. You would probably see a little bit of transcription, but not as much as the maximal level seen in lane 1.

In media without lactose, you would see a band only in lane 2. The only strain that would be induced would be the one that lacked a functional *lac* repressor.

E4. A. Yes, if you do not sonicate, then ß-galactosidase will not be released from the cell, and not much yellow color will be observed. (Note: You may observe a little yellow color because some of ß-ONPG may be taken into the cell.)

B. No, you should still get yellow color in the first two tubes even if you forgot to add lactose because the unmated strain does not have a functional *lac* repressor.

C. Yes, if you forgot to add ß-ONPG you could not get yellow color because the cleavage of ß-ONPG by ß-galactosidase is what produces the yellow color.

E5. The data indicate that two operators are needed because you need O_1 plus either O_2 or O_3 to get a high level of repression. If all three operators were needed, the deletion of any one of them should have prevented high levels of repression. This was not observed.

E6. You could mate a strain that has an F′ factor carrying a normal *lac* operon and a normal *lacI* gene to this mutant strain. Since the mutation is in the operator site, you would still continue to get expression of ß-galactosidase, even in the absence of lactose.

E7. It appears to be a mutation in the operator site so that a repressor protein cannot bind there and prevent *lac* operon expression in the absence of lactose.

E8. In this case, things are more complex because AraC acts as a repressor and an activator protein. If AraC were missing due to mutation, there would not be repression or activation of the *ara* operon in the presence or absence of arabinose. It would be expressed constitutively at low levels. The introduction of a normal *araC* gene into the bacterium on an F′ factor would restore normal regulation (i.e., a *trans*-effect).

E9. The results suggest that there is a mutation in the AraC protein so it cannot bind arabinose although it still binds to the operator sites correctly.

E10. Antisense RNA prevents the translation of the mRNA to which it is complementary. If the polypeptide that is encoded by the mRNA has an important function during early embryonic stages, the embryo may not develop properly. In this case, one may observe gross developmental abnormalities. For example, if the polypeptide is important in the formation of anterior structures, one may see that the head does not develop properly when the antisense RNA is injected into the fertilized oocyte. In contrast, if the polypeptide does not play an important role during the early stages of development, the embryo should develop properly.