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OXFORD INSECT TECHNOLOGIES

Eliminating tetracycline contamination.

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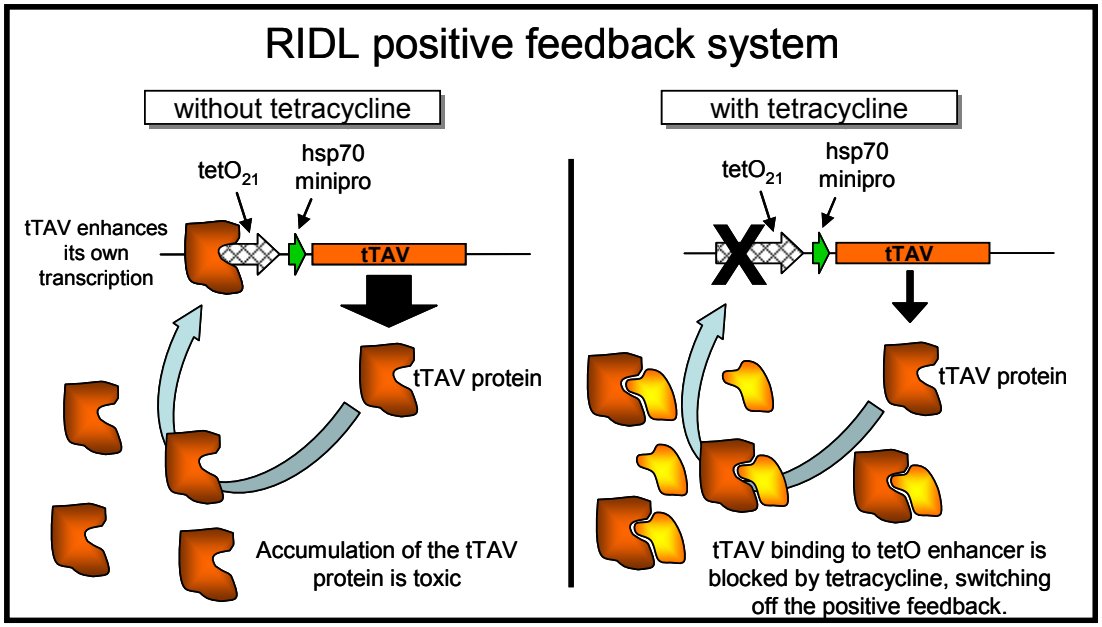
I
The problems with tetracycline contamination.

The molecular technology used in RIDL is based on a positive feedback system that we can control using tetracycline. The figure below shows the positive feedback system found in OX513. Without tetracycline present small amounts of tTAV expressed from the hsp70 minimal promoter are produced. The tTAV then binds to the tetO sites found in front of the hsp70 minimal promoter and enhance its expression. This in turn produces more tTAV which binds to the remaining tetO binding sites forming a positive feedback system. Tetracycline binds to tTAV and prevents it from binding to tetO. Therefore, the small amount of tTAV produced by expression from the hsp70 minimal promoter is prevented from forming a positive feedback system.



Tetracycline is very efficient at binding to the tTAV and switching off the enhancement effect. Therefore, even small amounts of tetracycline can repress the RIDL system.

This was highlighted by a difference in results seen between our laboratory and a collaborator. They were getting 15% survival of a transgenic line and we were getting 3%! After a lot of testing and comparing experimental design it was found that they used a cat food to feed the larvae and this cat food contained chicken. It is known that tetracycline is routinely used to prevent infections in chickens, especially in the cheap, mass produced, chicken used for animal food. The chicken is heat treated before being used, but this does not remove all of the tetracycline. This meant that a small amount of tetracycline was being added from the food to the larvae and repressing the lethal system.

ii
Larval rearing

To rear larvae that are free from tetracycline contamination we recommend the following procedures to be followed;

- Wash hands before commencing any work/experiments.
- Always use the same larval diet as we use (details of this can be found in the rearing document).
- Use distilled/deionised water for rearing.
- Either have separate trays for non-tetracycline rearing, which are clearly marked (we spray the sides of our trays with black paint). Or use a new (disposable) tray each time.
- Wash non-tet trays in a separate sink to the tet trays.
- Keep all tet contaminated accessories (pipettes, tray covers, sieves, weight boats etc…) away from non-tet experiments.
- To reduce the chances of spills of tet water into non-tet trays keep them separated as much as possible.

iii
Adults

With adults we do not provide a source of tetracycline (i.e. in the sugar water) because this can contaminate the eggs of the next generation in several ways;

- Direct transfer to the egg paper by residual amounts on the insects legs.
- Transfer to the eggs from the female.
- Transfer through the faeces of a bloodmeal to the egg paper.

iv
Eggs

Always wash your hand before handling non-tet egg papers so as not to transfer any tet residue on your hands. Contamination of eggs mainly comes from the adults, but it is possible to reduce the residual contamination of tet on the eggs to a minimum by washing the eggs in distilled water. This is done by removing the eggs and washing through filter paper, you can use a vacuum filter for this to speed things up. However this is not required if you do not feed tet sugar water to the adults.

v
Producing tetracycline free offspring

When a line needs to be reared without tetracycline, it is best to do the following to get tetracycline-free offspring to analyse;

1. Rear your transgenic line on tetracycline and separate out about 10 males.
1. Cross these males with wild type females and collect the eggs.
2. Rear the eggs without tetracycline and analyse.

Do not use females from your transgenic line because these can pass tetracycline accumulated as larvae to the embryos.