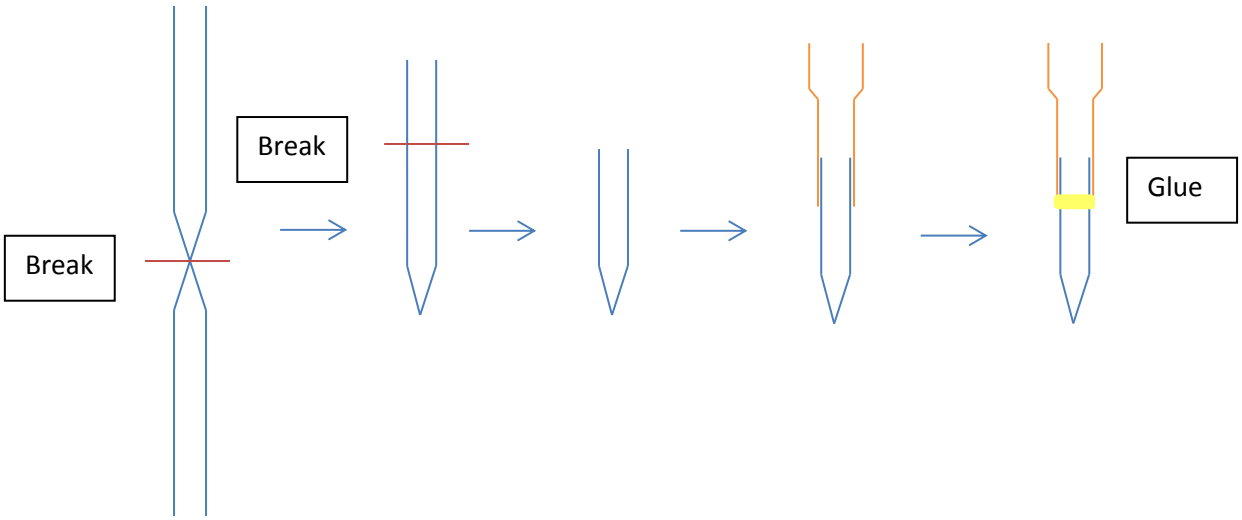
dsRNA injection in Tsetse flies

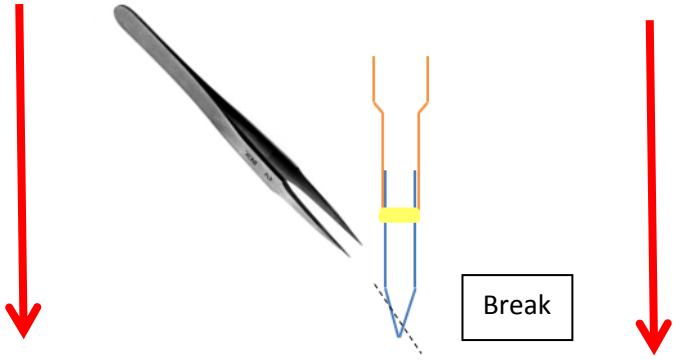
1. **Needle preparation**

   Glass capillary tubes: length- 10cm, diameter- 1mm. Using a needle puller (Narishige), clamp glass capillary tube into position at top and bottom using the screw catches, ensure tight enough to prevent the tube slipping when the weight becomes engaged. The central ring filament heats up and the weight pulls down on the softened glass, stretching it until the point of snapping – creating smaller needles with a closed end. A yellow, non-filter pipette tip should then be cut with the aim of the diameter of the tip fitting the needle with a 'snug fit' when inserted. The back end of the needle should be snapped to shorten it so when the needle is placed inside the pipette tip it is short enough to allow the delivery mechanism to still engage. The two should be glued together around the top using epoxy adhesive i.e. araldite, to create a solid, secure seal keeping the two together. Needles should be left in a petri dish on a raised plasticine bed to prevent the tip touching the bottom of the dish – must be left to set for at least 12 hours.



   The second stage of needle preparation is to break the point of the needle to create an open end suitable for drawing up fluid to inject. This is done using a pair of sharp tweezers to break off the very end and should be held down towards the ground to avoid shattered glass hitting you and also to prevent glass from entering the needle. By flicking the needle gently this should remove any small fragments that have entered. Breaking the tip is easier when looking against a dark background. The aim is for a smooth sharp point, it should not be too thin or platelets will block the end due to agglutination but equally should not be too large or will inflict an un-necessarily large hole in the tsetse fly. Twisting the needle should be avoided as this will introduce hairline fractures into the glass, these stop fluid coming up the tube

and the outside of the needle become wet due to leakage and will not dispense correctly. When the needle is broken the shape of the tip should be examined under the microscope. A needle with a double point should be altered to remove one of the two points or the correct volume of fluid will not be taken up and air will enter the needle.

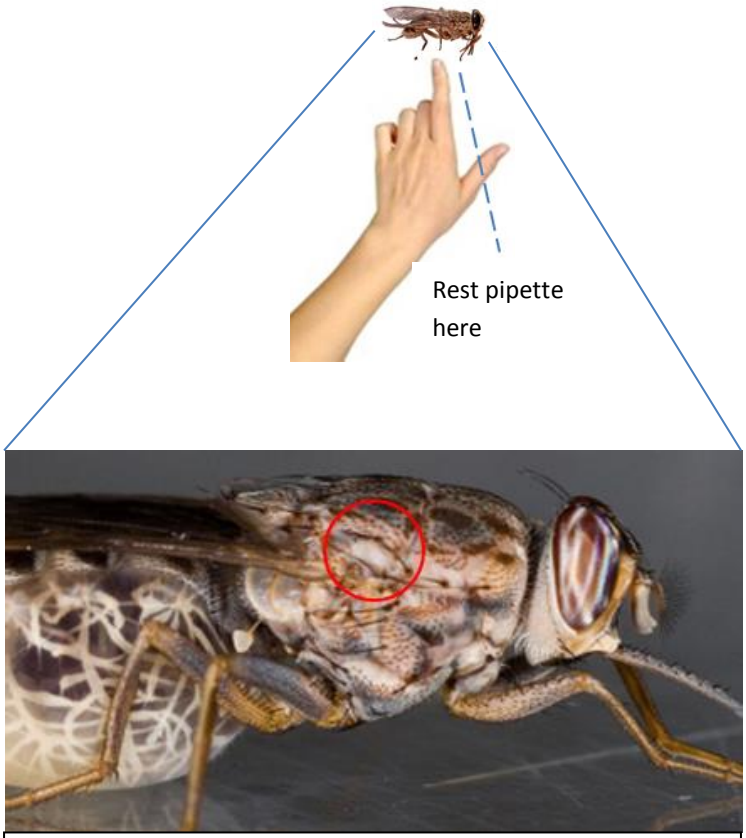


2. **Fly handling**

Flies should be handled with great care to reduce mortality. If using teneral flies they should be injected 24hours after emergence as they still have some milk but are harder to inject as more “squishy”, which is reflected in the higher mortality rate (~25%). If using older flies they should be fed the day before injection because of dehydration caused by the hole from needle injection. The blood meal gives fat bodies which improves survival (mortality rate ~5%). Flies should be kept on ice for the minimum time possible – no more than 10 minutes or they fail to recover, increasing mortality. Flies should be removed from the insectary and transferred to the cold room to lower their metabolism and ‘stun’ them. Once knocked-down they should be transferred in small groups to Petri dishes with no more than 5 tsetse per dish, where they can stay on ice for only 10 minutes – giving a rough guide time of 2 minutes per fly injection. Ice used should be cold and fresh with no water in the bucket when tipped or the flies warm up and buzz – causing difficulties when injecting. Petri dishes with flies waiting to be injected should be kept in the ‘wet draw’ (wet blue roll lining a drawer). The aim of the wet draw is to increase the humidity around the flies, stopping dehydration and improving success rates. If there are more than one group of flies to be injected e.g. positive and negative controls, do one group at a time and keep all other groups in the insectary until needed.

3. **Injections**

For injections, 2µL of fluid should be used, this is equivalent to half the flies' body volume and any larger would place strain on the flies. The microscope should be operated with no bottom light – as it warms up the flies too fast. The dsRNA should be mixed every time between injections to avoid precipitation and prevent drying out. Placed on parafilm loading should be done with a high angle to ensure all fluid is taken up. Care must be taken to avoid touching the parafilm with the tip of the needle as this could cause contamination of the sample and potentially block the needle tip. If there is excess fluid on the needle this should be removed using blue roll as it is absorbent and non-toxic. Flies should be removed gently from their Petri dish on ice and placed onto the dissection microscope on their sides. Using a forefinger, the legs of the tsetse should be trapped to keep it still; the injection site should be aimed at the light pocket on the side of the thorax just above the thin line where there is less muscle. To ease the process and to stop hands from shaking, the thumb can be extended to support the pipette. These steps must be done as quickly as possible before the fly warms up from the heat of fingers and the lights from the microscope. Once injected, they should be quickly transferred to their cage on the wet blue roll to recover. Mortality should be observed after 24 hours and after 7 days.



Injection site is the cream coloured pocket just above the thin line to the side of the wing