**Dissection of pupal abdomens for fluorescent imaging**

1. With a wet brush, take a few pupae of the correct stage and pre-screen them in a few droplets of water in a Petri dish for the presence of fluorescence
2. Fill a well of a 9-well glass dish with 1 mL of 70% ethanol
3. Place a transgenic pupa for a minute into the ethanol
4. Take the pupa out and dry it briefly on a paper towel
5. Place the pupa sideways on a slide with double-sided tape stuck to one surface, the head facing to your right (if you are right-handed)
6. With the sharpest forceps in your right hand, open the pupal case above the head and gently pull the body out, while holding the bottom of the puparium with the left forceps
7. Put the body back into the ethanol and remove the clear membrane that may still be attached to the body
8. Use dissection scissors and forceps to cut the wings and legs off
9. Put the body briefly on a paper towel (gently)!
10. Have a very clean slide with a small drop of Halocarbon oil 27 ready
11. Place the body immediately into the oil drop
12. Align your body so that either the dorsal side, the dorsolateral side, or the lateral side faces up (all three sides need to be imaged, but due to photo bleaching you may need several pupae to complete all orientations)

Using the fluorescent scope, take image stacks of about 25 images, starting from the top and ending at the bottom focal plane. Place all images of the same construct into the same folder. Adhere to the following file name nomenclature, do not put extra spacebars and do not omit spacebars:

1 gut y2 3kb in gut lateral a
1 gut y2 3kb in gut lateral b
...
1 gut y2 3kb in gut lateral y
1 gut y2 3kb in gut lateral za
1 gut y2 3kb in gut lateral zb
...
1 gut y2 3kb in gut lateral zy
1 gut y2 3kb in gut lateral zza, and so on

**File name components:**

**1 = pupa ID number.** Any images taken of any body part in any orientation of that particular pupa start with that number. The next pupa imaged and stored in the same folder gets a “2”, and so on.

**gut y2 3kb = construct origin, gene, and name.** This construct was cloned from *D.* ***gut****tifera* DNA, belongs to the ***y*** gene, and contains the genomic region is **2 3 kb**.

**in gut = transgenic host species.** This is the species of pupa that you dissect. (mel = melanogaster)

**lateral = body position.** This is the body position. If you image a wing, type “**wing**” instead.

**a, b, … y, za, zb, … zy, zza, zzb, zzc = image order for Helicon Focus.** These letters go from top to bottom.