

GENETIC TESTING FOR PLN-ASSOCIATED VARIANT GENES



DIRECTIONS FOR TESTING A LITTER



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Introduction

- After years of research supported by hundreds of Wheatens, their owners and breeders, Drs. Meryl Littman and Paula Henthorn at Penn Vet have identified mutations associated with PLN in two genes
- Some breeders have expressed an interest in testing their litters....either to be able to know the results before placing puppies or to assist in ongoing research.
- This guide is to assist those breeders and in some instances their veterinarians, as testing puppies can provide more challenges than testing adult dogs.
- There is a reduced fee when submitting a litter at the same time:
 - When submitting samples on **more than 3 puppies** from a single litter, the fee per puppy is \$100.00.
 - The **maximum** litter fee, regardless of the number of puppies submitted is \$500.00.
- Continue to watch the website for more information

<http://www.scwtca.org/health/dnatest.htm>





Guidelines for Swabbing Puppies

- Puppies can be swabbed using the same type of sterile cytology brushes as used for adult dogs.
- However, just as when swabbing any dog, you must be sure not to contaminate the sample.
- This is a greater challenge for a litter of puppies...there's lots of ways to contaminate the samples...
 - The dam's milk (and her DNA) in the pup's cheeks
 - Shared water and toys
 - Urine and feces
 - Hair, skin and saliva from littermates or mom
 - Food resulting from weaned puppies frequent feeding schedule
- To avoid contamination,
 - Separate the puppies from the dam, food, shared toys and water and each other for 3 hours before swabbing.
 - It's best to swab puppies that have been weaned and away from the dam overnight.
 - Carefully follow the other instructions in the Step By Step Guide





Guidelines for Taking Blood Samples

- Because of the difficulties of swabbing, some breeders may choose a blood draw.
- Blood can be drawn during the docking of tails and removal of dewclaws as follows
 - 0.5 ml may be drawn from **healthy** puppies weighing 120-500 grams (4.25 to 17.5 oz)
 - Breeders should always follow their veterinarian's advice
- 1 – 2 ml may be drawn from larger puppies or adults
- Use care with squirmy puppies!
- EDTA (lavender or “purple top”) tubes are used for these samples.





Submitting Blood Samples

- Label EDTA (purple top) tube with owner's last name and animal's call name (or AKC#)
- DO NOT FREEZE the blood.
 - The blood sample must be kept cold but not frozen.
 - Place tubes in a Ziploc® (or similar) sealed plastic bag, then in bubble wrap for protection.
 - In the summer, consider sending the bubble-wrapped sample (with an ice pack) inside a white Styrofoam box for overnight or 2-day delivery.
 - Your veterinarian may have special Styrofoam containers or cardboard mailers to send blood tubes.
- Complete the required submission information on the PennGen site (see *next page*) and mail with sample, along with a copy of the dog's registration.
- Mail EDTA purple top tube(s) and forms. All samples for DNA testing can be shipped by regular mail, but PennGen recommends sending by registered or higher, so that you can track the delivery of your samples.
- **INTERNATIONAL SHIPMENTS:** Please see the PennGen site for instructions. <http://research.vet.upenn.edu/penngen> Select *Instructions for Sample Submission* and then the *International Shipping* tab.





Tips on using PennGen for litters

- The PennGen website is to be used for submission of all samples. Note the following tips for creating the puppies' records.
- Create the Animal Record for the first puppy
 - If the puppy is not yet registered, you can use the collar color ("Red Girl") or call name ("Poopsie").
 - Keep good records!! You can later have PennGen update the information but you'll need to know who Red Girl became!
 - When selecting the test, it will show the full price without the discount for the litter. Select it anyway.
 - PennGen will apply the discount and when you receive notice your results are ready, you will be charged the correct price.
 - In the "Comments" section, note that this puppy is part of a litter submission and include the number of puppies being submitted.
 - You have the option at the end of the page to "Create the Animal Record and Create More from the Same Litter". This will open up another Animal Record with some of the information already completed.



Access the PennGen site at:

<http://research.vet.upenn.edu/penngen>





Submitting Samples

Whether you are submitting blood or swabs, please remember to include the following:

- ✓ Completed ***and signed*** submission form ***for each puppy*** from the PennGen site.
- ✓ **Label** each tube or swab sleeve with the puppy's call name and your last name.
 - Think ahead! What you call a puppy in a litter may change so keep track of who “red collar” becomes!
- ✓ A **4-generation pedigree**.
- ✓ If the litter or puppy has been registered, a copy of the registration form.





Questions?

- ***Why should I test my litter?***
 - Some wish to know the status of the puppies before placement.
- ***Will the puppies be harmed by taking that amount of blood?***
 - If your veterinarian follows the guidelines (0.5 ml for healthy puppies weighing 120 – 500 gms) they should be fine...however ***always*** follow your own veterinarian's advice.
- ***If I submit 2 puppies now and 2 more later, can I get the discount?***
 - Yes...but please make sure
 - ✓ You provide the information on the previous puppies submitted
 - ✓ Everyone has the same litter number, date of birth and sire and dam
 - ✓ You submit everyone within 1 month.
- ***Why do you need a pedigree if I give you the sire & dam's names?***
 - A pedigree helps researchers understand more about inheritance of PLN
 - Additionally, it helps make sure that the database is correct...so we know if "CH Wheaten Fido", "CH Wheaten's Fido" and CH Wheaten's Phideaux" are the same dog.
- ***If I submit samples before I register the litter, do you want the registration information later?***
 - Yes...please include a copy of the results when you send Penn the information.





For more information

Check the SCWTCA website for:

- Audio of Dr. Meryl Littman's presentation on May 5, 2012
- Step By Step Guides
- FAQs for owners and breeders
- Research abstract presented at Tufts in 2011
- Contact information for more assistance
- ...and watch for more resources

<http://www.scwtca.org/health/dnatest.htm>

