# It wasn't my dog, it wasn't my dog's fault, it's not my dog's DNA the science is wrong!



George Sofronidis BSc (Hons)
Managing Director – Orivet Genetic Pet Care



What is the Best Way to Get Started?



**Sourcing DNA Information** 

# What do we hope you get out of the Worskhop?

Dog Attacks = Various Approaches = Various Collection Procedures



I don't get it, it's all too hard. I wouldn't know where to start. "Just stick everything a bag and send."

I've got the kit. I've watched CSI and I sought of know what to do.

It all makes sense and such a simple process!

#### What Tools do You Need?







#### **Law Enforcement**

Police
Councils (Local Laws)
Veterinarians

Collect Evidence Assess the Scene Chain of Custody







#### Science

Laboratory
Tests – Accreditation
Technology

Sign off on samples
Run through
Turnaround Times



#### **Legal Issues**

Sign off on samples Run through













#### What to look for and what is the process for collection?





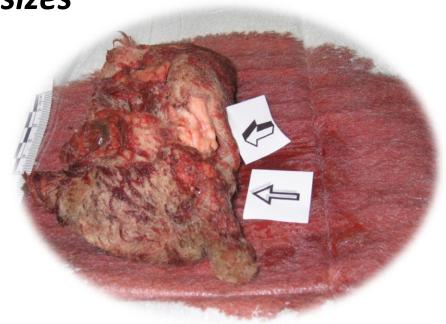
Every case is different, every client is different and every emotion is different!



# All shapes



All sizes





All requests

# I have QUESTION?

My cat's gone missing





Owner thinks their cat has been found as "road kill"

Hi

Sorry to hear about the failed sample. I understand these things can occur. I was quite sure that I had taken an adequate sample.

The puppy is extremely aggressive and we still managed to get the swab in the mouth for 15 seconds while he had a muzzle.

I had originally taken a blood sample for a Sydney based lab but they then said we could not ship blood over from NZ due to Quarantine restrictions.

Is it necessary to take a swab or can blood be used?

Previous Case No: Clinical Particulars	Heiro	Killod Ina daz		
Cilincal Farticulars	HONG	rement vy aus.		
Need prost-	That	saliva m swalls	matches	selva m
featter	, W (	wound edges.		ECESSARY TO FIND
<u> </u>				ON EACH OF THE
Panel Breakdown on Reverse*			BIRDS	, ONE IS ENOUGH





#### 2. Evidence Sample Collection

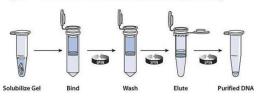
To be completed by sample collecto	or.					Case			
Complete this section for animal DNA ev		the scene that you w	vill be submitting for	DNA analysis.					
Please individually bag and seal all sam	ples.								
Sample Collector's Details						Received			
Name			Company						
Professional Address Telephone									
Sample Details									
Address of where sample was taken									
Relevant Physical or Geograpl	hic Features of I	Note							
(e.g. Sample taken from left corner of p	ark near broken fend	e line)							
Hair Blood Swab	Clothing	Number of samples t	taken:	Other:					
Brief sample description (if applicable)									
Declaration									
I, (insert name)	collected the sa	ample(s) specified abo	ve and completed the	label. The samples we	ere then placed in t	he bag and sealed.			
(Signature of collector completing declaration)  Date									
Chain of Custody									
Chain of Custody			d ava daardu labal	1.4					
Please ensure that each Evidence so Samples that have been sealed and labe		-	•						
Sample sent to Orivet via Cou									
Carrier Consignment number									
Sent by Date of dispatch									
Sample Hand Delivered									
Delivered by			Of						
Date									
I									





#### **Extraction**

Figure 1 — The PureLink™ Gel Extraction Kit procedure is quick and easy

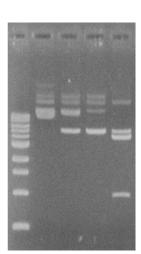




#### **Quantity – How Much Do we Have?**

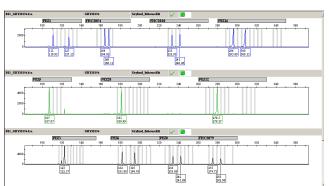




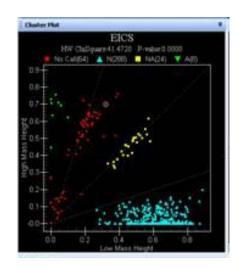


#### **Analysis**



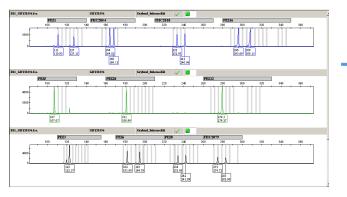


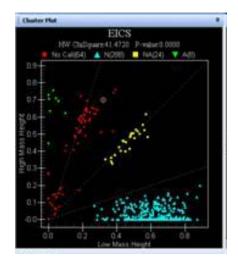




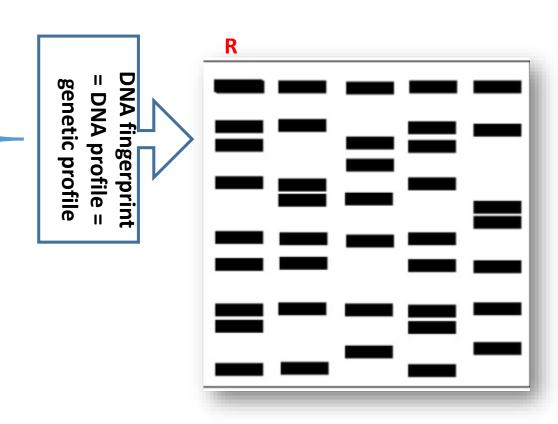


48421681 48421691 48421701 48421711 48421



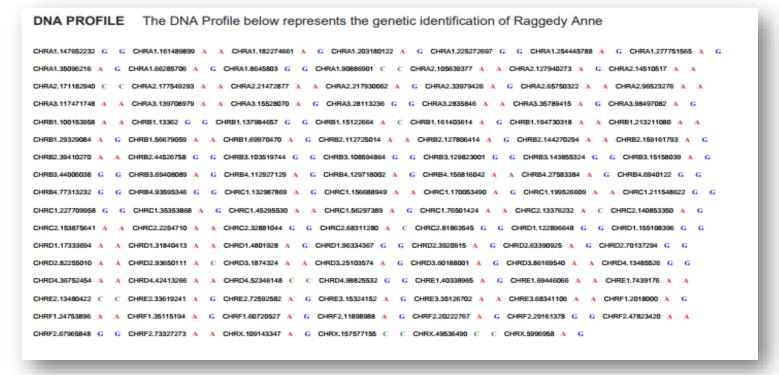


Set of numbers (size of the DNA) molecular weight or a set of letters (DNA code) G, A, T or C



# DNA PROFILE The DNA Profile below represents the genetic identification of Charlie P1\_2 A A P3\_2 A A P3\_3 G G P11\_3 C C P12\_1 A G P24\_2 A A P13\_3 G G P30\_3 A T P13\_1 A C P24\_3 A P31\_1 A P28\_3 T T P31\_3 A G P24\_2 A A P13\_2 A A P13\_3 C C P24\_3 C C P31\_3 A G P24\_1 T T P10\_1 G G P13\_2 A A P33\_3 G G P26\_3 G G P33\_1 G G P14\_1 T T P10\_1 G G P26\_1 A G P34\_3 A C P15\_2 G G P15\_3 A P16\_3

#### "set of genetic values unique to that individual, inherited from each parent"



P1\_2 A G P28\_3 A T P10\_1 G G P10\_3 C C P29\_1 G C P11\_1 C C P11\_2 G G P29\_2 G G P3\_1 G G P3\_2 C A P3\_3 P11\_3 G G P12\_1 G G P24\_2 A A P12\_3 C C P30\_3 T A P13\_1 P24\_3 T T P31\_1 T T P31\_3 C C P25\_1 C C P32\_2 G G P13\_2 T T P13\_3 C A P25\_2 A G P25\_3 G G P32\_3 A G P33\_1 G G P14\_1 A A P26\_1 A A P33\_3 C C P26\_2 A A P14\_2 C G P26\_3 C C P14\_3 C C P15\_1 G A P34\_1 G T P34\_2 T T P34\_3 G G P15\_2 T T P15\_3 G G P16\_3 C G P35\_1 C C P35\_2 G G P36\_1 A A P17\_1 G G P36\_2 C C P37\_2 T C P17\_2 A A P37\_3 A A P38\_1 G G P38\_2 C C P27\_1 G G P17\_3 C C P27\_2 T T P4\_3 T T P18\_2 C C P18\_3 G T P5\_1 C C P19\_1 T T P19\_2 G G P5\_2 C C P19\_3 C C P2\_1 G G P21\_3 C C P22\_2 C C P28\_1 C C P7\_1 G T P7\_2 T T P28\_2 P7\_3 T T P8\_1 G G P21\_3 C C P22\_2 C C P28\_1 C C P7\_1 G T P7\_2 T T P28\_2 P7\_3 T T P8\_1 G G P21\_3 C C P22\_2 C C P8\_3 T T P23\_1 C C P9\_3 T A P23\_2 P23\_3 A A P24\_1 G G

#### **Partial DNA Profiles**

#### **Quantity and Quality of DNA**

Dropout of markers (SNPs) – blank spaces

#### **DNA Testing – Why Do It?**



Practical (Effective means)

# DNA is interrogated

**Social**Direct to consumer

#### DNA Profiling/individual Identification Forensics





Paternity – Dam and Sire verification

Disease diagnosis prevention/risk Prenatal diagnosis







#### Ancestry

Fun tests – not health related "aspects of life"



#### **Traits?**





## Parentage Tool

#### **Under Construction**

DAM & SIRE CONFIRMATION

SINGLE PARENT CONFIRMATION

FORENSIC TOOL

Please select animal barcodes from dropdown menu. You can only select animals listed in your account and any shared animals.

**Examination Sample** 

Reference Sample

17136237

17142041

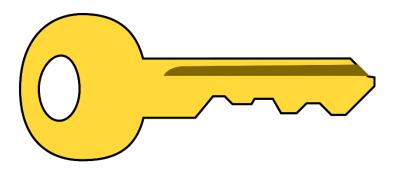
RUN ALGORITHM

Final Result: CANNOT BE EXCLUDED Exclusions: CANNOT BE EXCLUDED Inconclusive: ["snp01","snp11","snp13","snp15","snp19","snp20","snp24","snp26",
"snp37","snp60","snp78"] *Inclusions are up to 99.9998%* depending on breed(s), number of **UPDATE FINAL RESULT** markers and samples submitted. Percentage Summary Match Percentage 87.5 % [77] xclusions = No Match = 100% Exclusion Percentage 0 % [0] DropOuts Percentage:12.5 % [11]

DOWNLOAD PDF

Used to incriminate or exonerate!

success	snp02	AA	AA
success	snp03	GA	GA
success	snp04	СС	CA
success	snp05	СС	CC
success	snp06	СС	σ
success	snp07	GC	GG
success	snp08	GG	GG
success	snp09	GG	GG
success	snp10	СС	CA
warning	snp11		GG
success	snp12	GG	GG
warning	snp13		GG
success	snp14	GG	GG
warning	snp15		СС
success	snp16	π	π
success	snp17	СС	cc
success	snp18	π	GT
warning	snp19		π
warning	snp20		СС
success	snp21	тс	π
success	snp22	GG	GG



What is a Reference Sample?

Origin is known!

**Collection Process** 

Can be collected at any time

Can be more than one sample

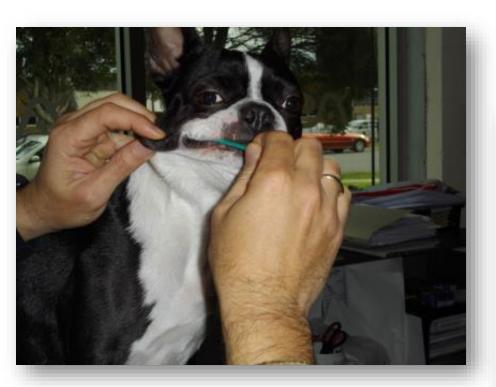
Are you qualified to collect?



#### 1. Reference Sample Collection

o be completed by sample collecto	or.		Case			
his reference sample will be compared	mple from an animal that is suspected o to the sample(s) taken from the scene. sample. Reference sample can be colle		Destinal			
nimal's Details			Received			
Name	Male Female Desexed	Breed/Suspected Breed (please complete only if known)				
COLOUR						
Owner's Details (if known)						
Name						
Address						
Reference Sample Type (must us	se laboratory approved procedure)					
Swab Bloo	od (EDTA) Other					
Declaration (collector)						
, (insert name of collector)						
of (insert professional address/or same)						
took the sample specified at (insert time)		am/pm on (date)				
at (insert place of collection)						
took the sample specified above and com	pleted the label. The samples were then pla	aced in their own bag and sealed.				
Signature of collector completing declaratio	n)		Date			
Chain of Custody						
•	sample is in separate sealed bags a	and are clearly labelled.				
	lled are to be sent to Orivet Genetic Pet	•				
ample sent to Orivet via Cour	ier					
Carrier Consignment number						
Sent by		Date of dispatch				
ample Hand Delivered						
Delivered by		Of				

#### The Collection Process – PINCH, SWIRL and DRY!





Ensure that you pinch the cheek well around the nylon brush.



Using your fingers to keep the cheek pinched, swirl the nylon brush around the pinched cheek for a good 6-10 secs.

#### Positive ID - You MUST cite the number - scan and read and check

Take your time and SCAN from top to bottom





What do you do if you try and try and still cannot find the chip?

Record and note — "chip could not be found (scanned)" next to the chip number

#### **GENETIC ANALYSIS SINGLE REPORT**

#### OWNER'S DETAILS

**George Sofronidis** 

C/O Orivet Genetic Pet Care

St Kilda

Victoria 3182 AU

#### ANIMAL'S DETAILS

Registered Name: Bangers and Mash

Registration Number: 2190705842

Microchip Number: 956008001058738

Date of Birth: 10/2/2014

#### **COLLECTION DETAILS**

Case Number: 17157148

Approved Collection Method: YES

Pet Name: Snagz

Breed: Dachshund
Sex: Intact Male

Colour: Brown

Date of Test: 18/08/2017

Collected By: Jane Smith

Any vet and/or "trained" officer

Scanned and cited



e labresults@orivet.com p +61 3 9534 1544 www.orivet.com



VS



VS

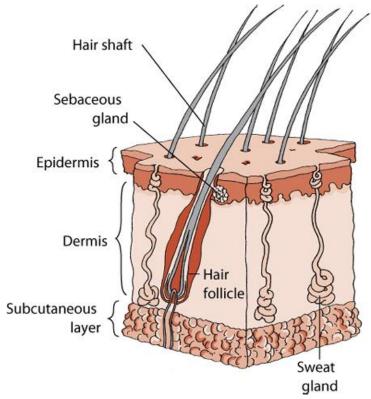


Nylon Brushes or Cotton Swabs









#### Mitochondrial dna (mt dna)

VS

#### nuclear DNA (genomic)

Mitochondrial DNA only has one chromosome, and this is organized like a circular genome (similar to most prokaryotic DNA). This single chromosome is much shorter, and codes for the specific proteins which are used in the metabolic processes

Nuclear DNA is the standard 46 chromosomes which you've heard about, where you inherit 23 from your mom and 23 from your dad. These are very long, with centromeres and telomeres

mtDNA, since it is solely **matrilineal**, doesn't undergo recombination like nuclear DNA--meaning that while nuclear DNA is a shuffle of your parents' DNA, all changes in mtDNA have to come from mutations. Thus, mtDNA is great for trying to detect human ancestry.



You have a cat attacked by what you believe are **two dogs** (neighbour) and dog roaming the street. You submit and have collected a wet swab from a number of wounds near the cat. A hair sample (matted) from the cat and a "wet swab" taken from the neighbour's dog.

**Q:** What reference samples do you need to collect?



You are contacted by a farmer whose chickens have been mauled by what they think is a dog or fox or wild animal.

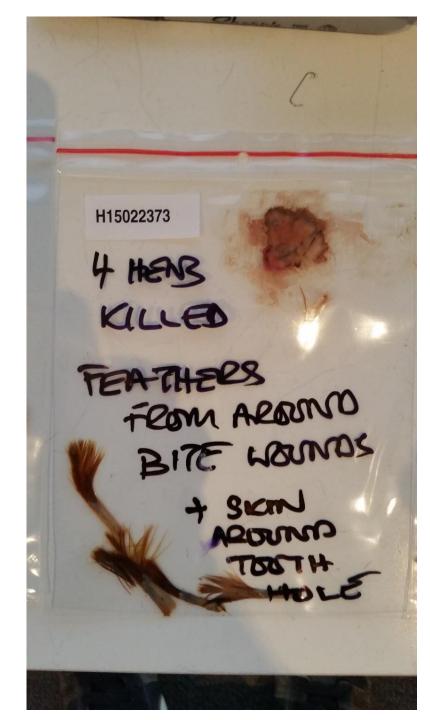
**Q:** What samples should you try and look for?



Find a cat that seems to have been set on fire? Owner thinks it's their cat.

**Q:** What can or can anything be done to help resolve this case?

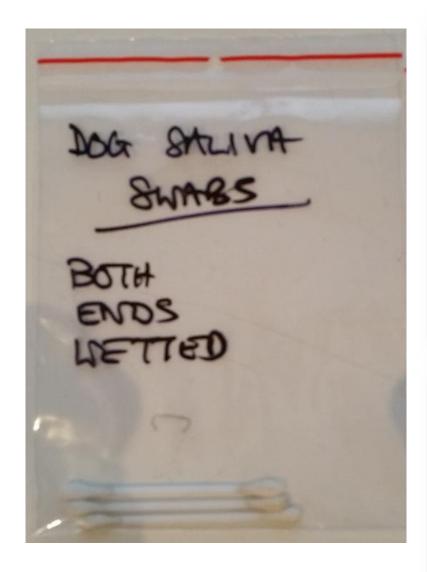












SNP01										
SNP02 TT	SNP03 GA	SNP04 CC	SNP05 GG	SNP06	SNP07	SNP08	SNP09 GG	SNP10 AA	SNP11	
SNP12	SNP13	SNP14 GG	SNP15	SNP16 TT	SNP17 CC	SNP18 TT	SNP19 TT	SNP20	SNP21 CC	SNP22 CC
SNP23	SNP24 AA	SNP25 AG	SNP26	SNP27 AA	SNP28	SNP29 AT	SNP30 GG	SNP31	SNP32 AA	SNP33 GG
SNP34 TC	SNP35 CC	SNP36 GA	SNP37	SNP38	SNP39 TT	SNP40 CT	SNP41 GG	SNP42 GG	SNP43	SNP44 GG
SNP45	SNP46 AG	SNP47 CC	SNP48	SNP49 CA	SNP50 GG	SNP51 GG	SNP52 CC	SNP53 GC	SNP54 TT	SNP55 GG
SNP56 CT	SNP57 CC	SNP58 GG	SNP59 TT	SNP60 TA	SNP61	SNP62 CC	SNP63 TC	SNP64 GG	SNP65 TT	SNP66 TT
SNP67 AA	SNP68	SNP69 TC	SNP70 TC	SNP71	SNP72 GA	SNP73	SNP74	SNP75 CC	SNP76 GT	SNP77
SNP78	SNP79 TT	SNP80 GG	SNP81 GG	SNP82 CC	SNP83 CT	SNP84	SNP85 TT	SNP86 CC	SNP87	SNP88



DNA PROFILE The DNA Profile below represents the genetic identification of Reference H15022373

5.01.110.122	THO BIVITION	o bolott toplooc	into tho gonotic	radrian dator r	110000100111	0022070				
SNP01										
SNP02 TT	SNP03 GA	SNP04 CC	SNP05 GG	SNP06	SNP07	SNP08	SNP09 GG	SNP10 AA	SNP11	
SNP12	SNP13	SNP14 GG	SNP15	SNP16 TT	SNP17 CC	SNP18 TT	SNP19 TT	SNP20	SNP21 CC	SNP22 CC
SNP23 AT	SNP24 AA	SNP25 AG	SNP26	SNP27 AA	SNP28	SNP29	SNP30 GG	SNP31	SNP32 AA	SNP33 GG
SNP34 TC	SNP35 CC	SNP36 GA	SNP37	SNP38	SNP39 TT	SNP40 CT	SNP41 GG	SNP42 GG	SNP43	SNP44 GG
SNP45	SNP46 AG	SNP47 CC	SNP48	SNP49 CA	SNP50 GG	SNP51 GG	SNP52 CC	SNP53 GC	SNP54 TT	SNP55 GG
SNP56 CT	SNP57 CC	SNP58 GG	SNP59 TT	SNP60 TA	SNP61	SNP62 CC	SNP63 TC	SNP64 GG	SNP65 TT	SNP66 TT
SNP67 AA	SNP68	SNP69 TC	SNP70 TC	SNP71	SNP72 GA	SNP73	SNP74	SNP75 CC	SNP76 GT	SNP77
SNP78	SNP79 TT	SNP80 GG	SNP81 GG	SNP82 CC	SNP83 CT	SNP84	SNP85	SNP86 CC	SNP87	SNP88







## Two Examples of mouth Swabs – Reference Samples























#### **Analysis and Conclusion**

The *Examination Sample Barcode 17-136237* had DNA extracted and analysed for canine DNA Profiling. A partial DNA Profile was obtained from the sample submitted and collected by Dr Wong with a total of **58** Single Nucleotide Polymorphisms (SNPs) detected out of a possible 88 SNPs.

The **Reference Sample Barcode 17-142041** had DNA extracted and analysed for canine DNA Profiling. A complete DNA profile was obtained from the sample submitted by Dr Jacobs.

The veterinarian had requested a comparison of the *Examination Sample Barcode 17-136237* against the *Reference Sample Barcode 17-142041*.

The examination sample showed a complete match of 60.35% to the reference sample (35 of the 58 SNPs matched the reference sample) – 60.35% of the DNA profile obtained from the examination sample was identical to the reference sample.

The examination sample had a 29.31% (17 of the 58 SNPs) partial match to the reference sample – 29.31% of the DNA profile obtained from the examination sample had a partial match (50%) to the reference sample.

The examination sample showed an exclusion (did not match) at 6 of the 58 SNPs (10.34%) - 10.34% of the DNA profile obtained from the examination sample had no match to the reference sample.

**Please Note:** Only one reference sample was provided, no reference sample of the "victim" canine was collected or submitted.

#### Summary of SNPs (Results)

Number of Exclusions (not matching)	6 (10.34%)
Number of Matching Sites	35 (60.35%
Partial Match	17 (29.31%
TOTAL	58

Results Reviewed and Confirmed by George Sofronidis

BSc (Hons)

# What to do if you are attacked by a dog!



#### What Your Choice in PETS reveals about YOU (YOUR CLIENT) (Scientific American)

#### Dog Owners are more likely to:

Live with several family members

Senior management

Work as a professor/nurse/IT professional

Be extroverted & agreeable



#### Cat Owners are more likely to be:

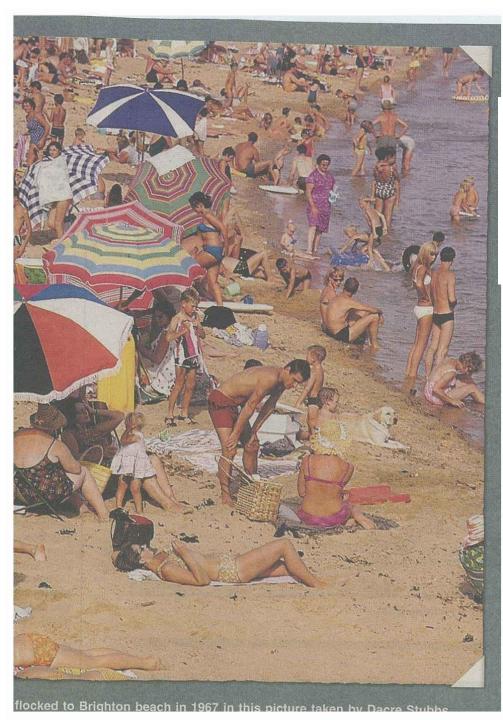
Divorced, widowed or separated

Be college educated

More intelligent

Less socially dominant





# Whose off-leash dog beach is it, anyway? Answer: not your dog's

Neil McMahon

f SHAR





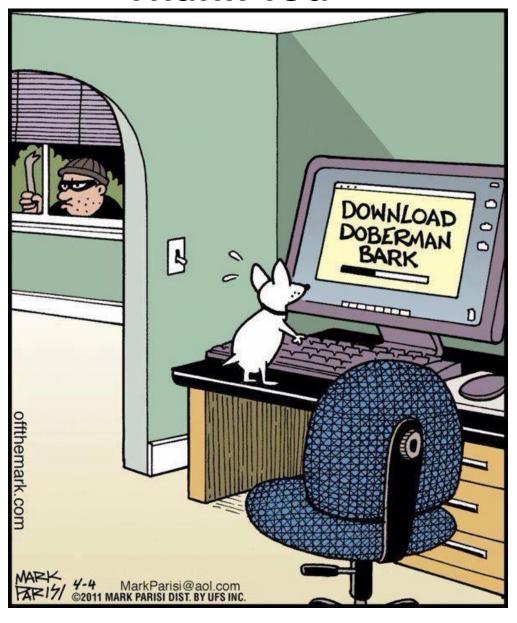


On Saturday night I went to the off-leash dog beach with my puppy for our regular twilight romp on the sand.

Typically here's what happens: Maudie, a seven-month-old English Staffy, runs and wrestles and plays with the rest of the Port Melbourne canines, whose owners assemble on the shore for the last wear-the-buggers-out dog walk of the day. Then we all go home.



# **Thank You**



george@orivet.com