




**IZSAM G.CAPORALE  
TERAMO**

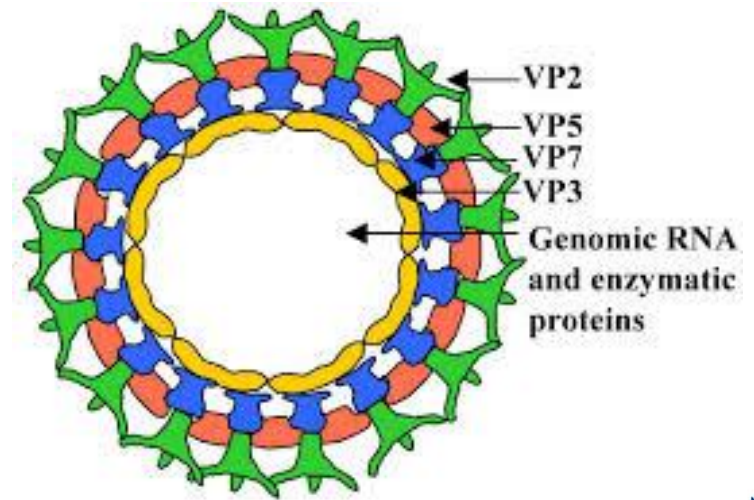
**All-in-One: una nuova piattaforma microarray per la  
identificazione e caratterizzazione del virus della Bluetongue**

**Alessio Lorusso**

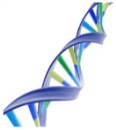
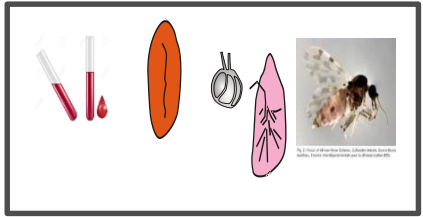


# Bluetongue Virus (BTV)

- 
- BTV, *Orbivirus/Reoviridae*
  - Tanti sierotipi
  - VP2 determina sierotipo
  - 10 segmenti RNA ds



# Diagnosi



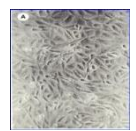
*Serogroup identification*

**ISOLATION**



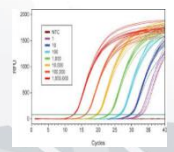
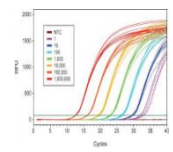
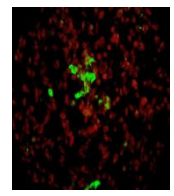
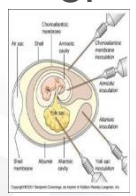
**SEROTYPING**

**Serotype specific RT-PCR**



**KC, BHK, VERO**

or





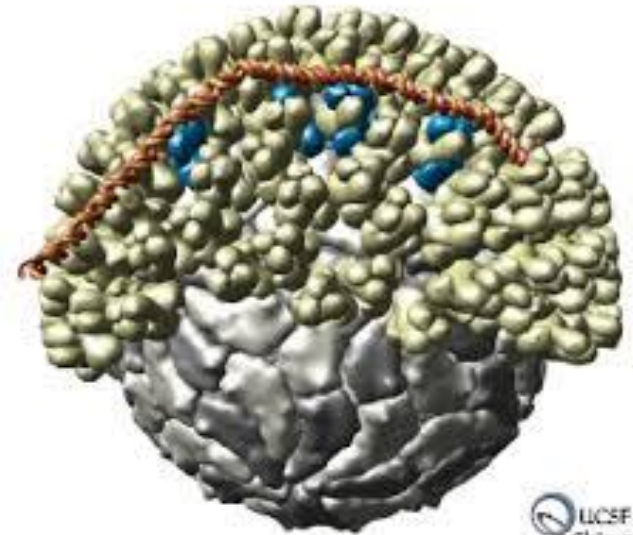
**BREAKING  
NEWS**

**BTV-28**

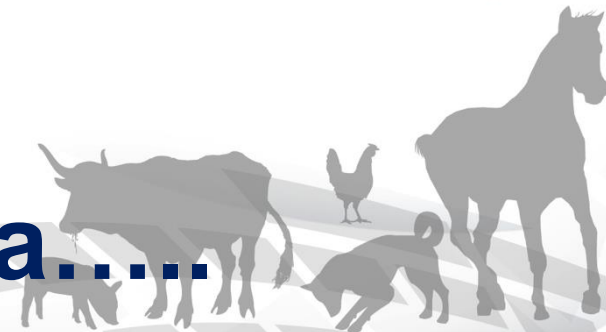
**BTV-29**

**BTV-30 Sardinia**

**more BTVs from Asia.....**



LICSF  
Chimera

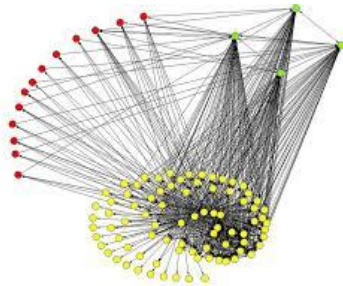




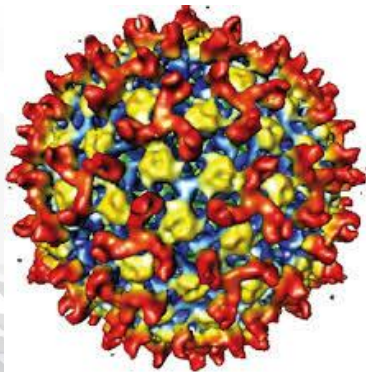
# BTV-27



**Sane**



**3 varianti in VP2 (92% nt id Seg2)**



**Unico sierotipo**

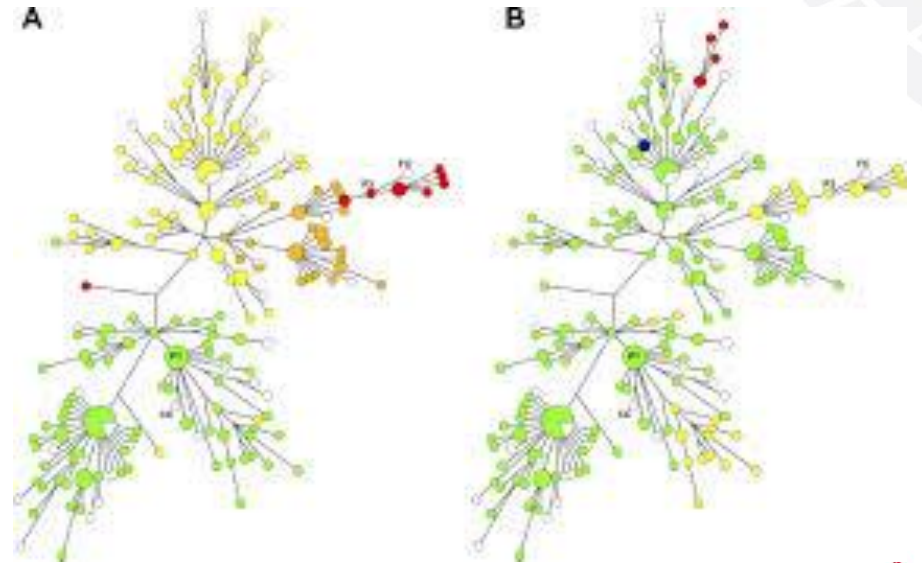
Schulz et al., 2016; Zientara et al., 2014





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# I TEST DIAGNOSTICI SONO EFFETTUATI SULLA BASE DELLA SITUAZIONE EPIDEMIOLOGICA



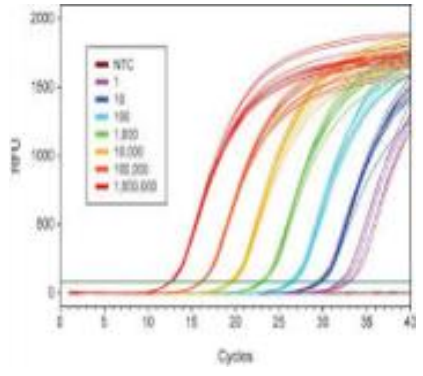
Possiamo perderci qualcosa?

# SI



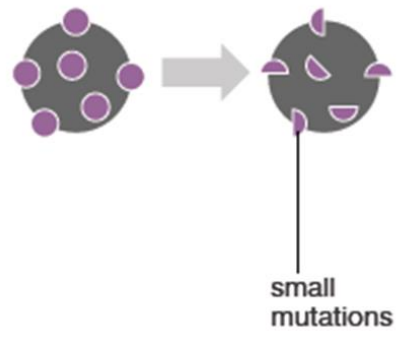


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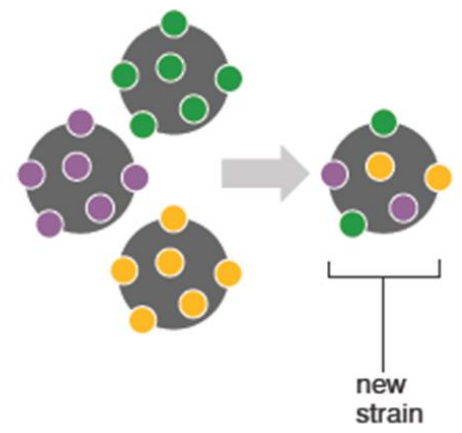


### Mutation

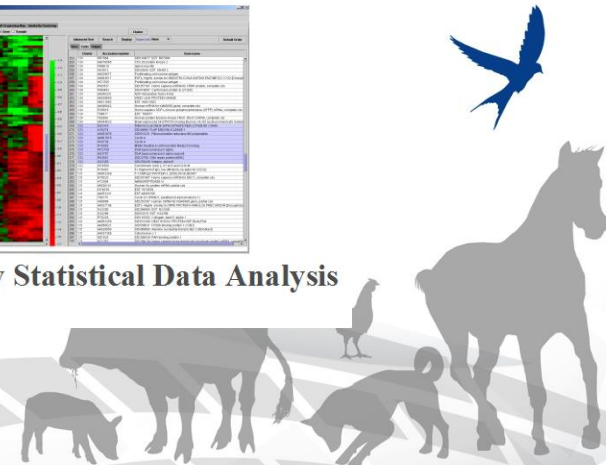
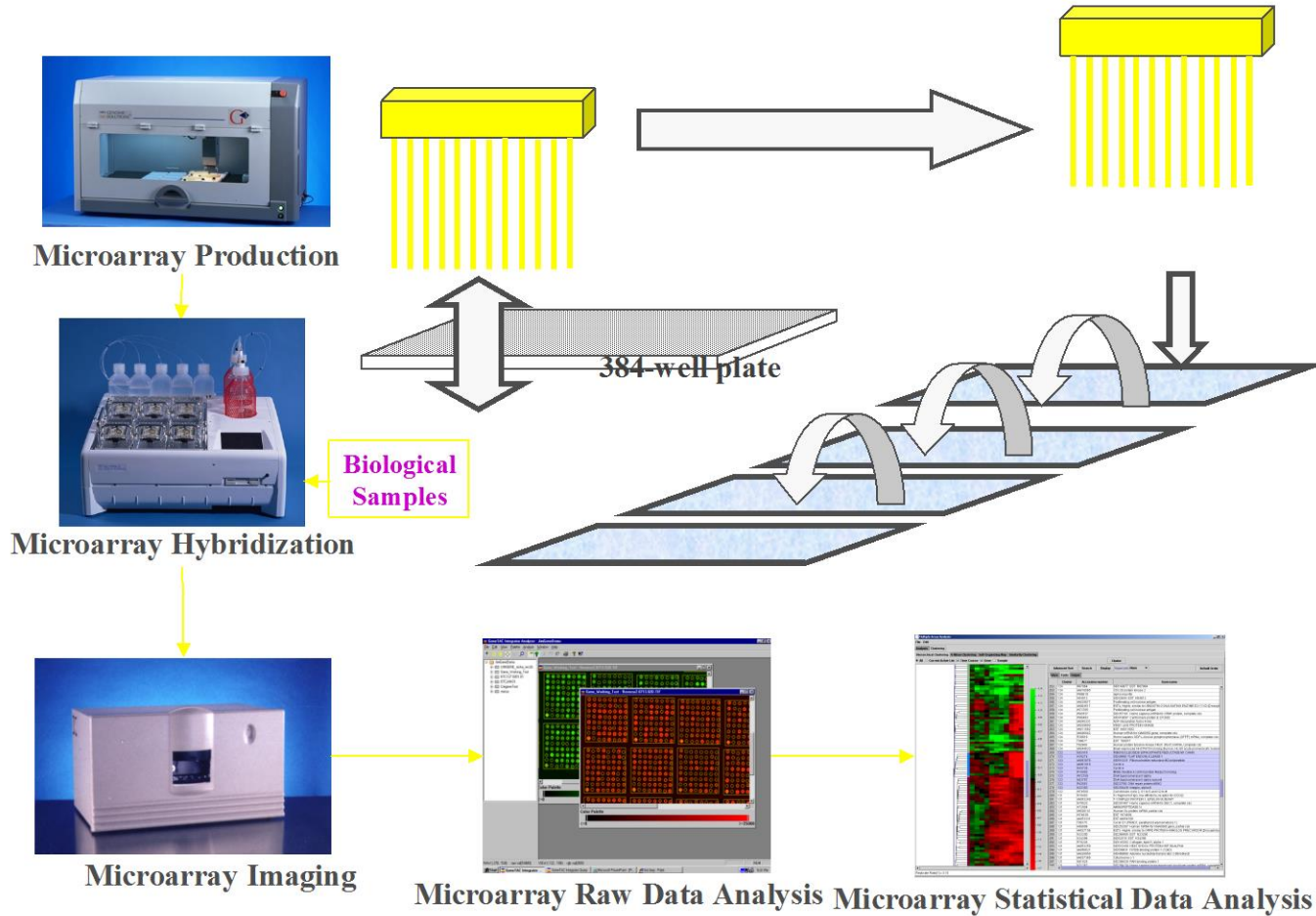
#### Antigenic drift



#### Antigenic shift



# Microarray tradizionali







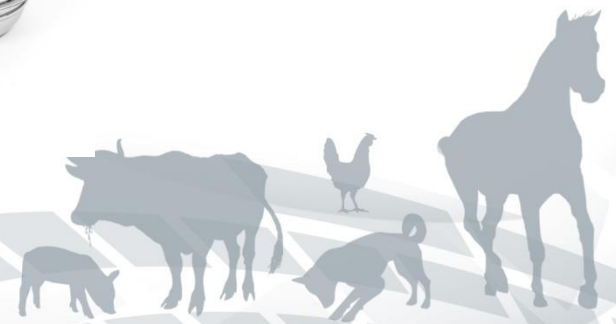
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MESSAGGIO PROMOZIONALE



Per informazioni  
**051.80.60**  
[www.eminflex.it](http://www.eminflex.it)



# nCounter<sup>®</sup> Analysis System

15 Minutes Hands-on Time

No Printing of Slides

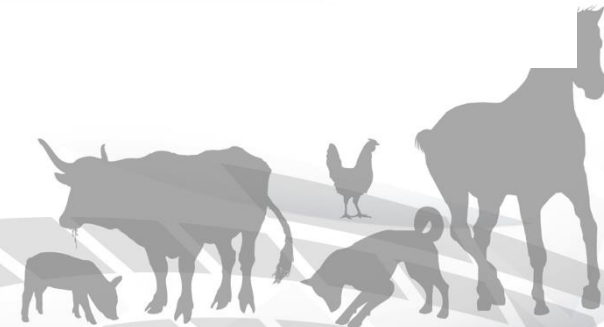
No Amplification

No Imaging Software


No Labelling

No Data Mining

No Washing



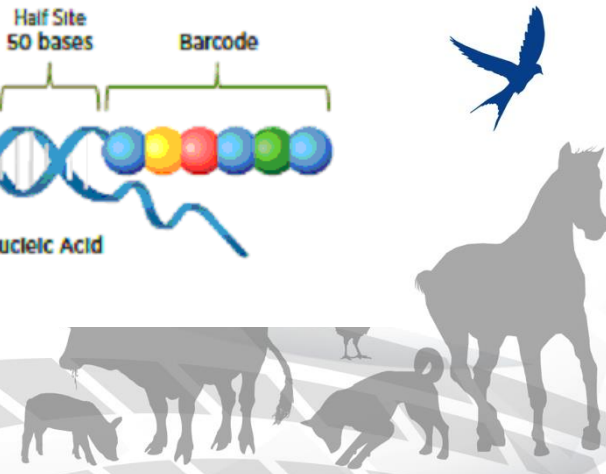
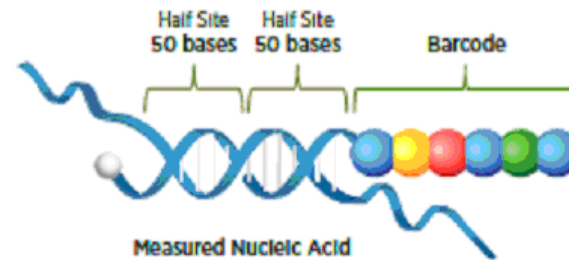
# nCounter<sup>®</sup> Analysis System



Design of probes consists of 100 mer sequences

- 58 capture probes corresponding to specific sequences to the various Segment-2 serotypes
- 2 capture probes one for NS1 and other for NS3 are included as positive controls
- 5 capture probes for *Culicoides* species
- 1 capture probe for actin gene

- The hybridization occurs in solution
- The probe pair consists of a
  - Reporter Probe, which carries the signal on its 5' end
  - Capture Probe which carries a biotin on the 3' end





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**-Fino ad 800 sonde per tubino**

**-Strip da 12**





# PubMed

PubMed comprises more than 26 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.

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- [Journals in NCBI Databases](#)
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- [LinkOut](#)

## Latest Literature

- New articles from highly accessed journals
- [Ann N Y Acad Sci \(1\)](#)
  - [Blood \(4\)](#)
  - [Cancer Res \(9\)](#)

## Trending Articles

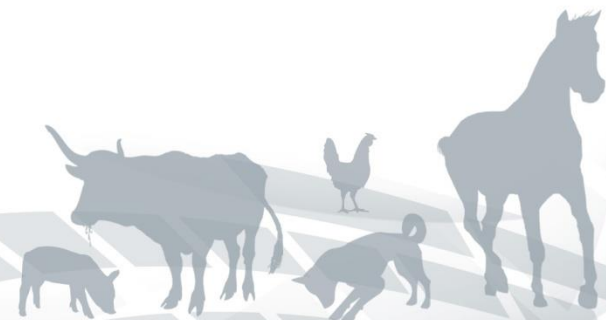
- PubMed records with recent increases in activity
- [CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. Cell. 2016.](#)

## PubMed Commons

- Featured comments
- [Sequencing sepsis: Author P Wolkow replies to Salter concerning approach to identify bacterial in blood. bit.ly/2eIXU3Z Nov 24](#)



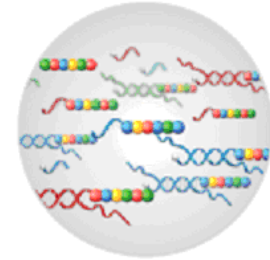
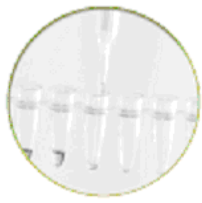
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# nCounter<sup>®</sup> Analysis System

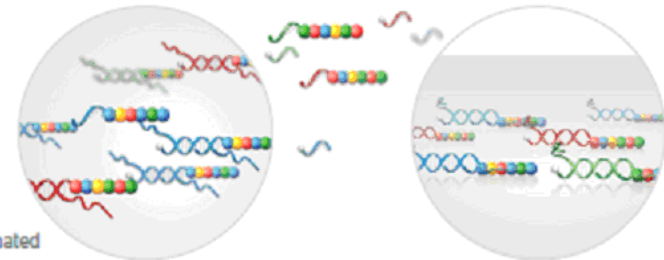
## 1 Hybridize

1. Buffer
2. CodeSet
3. Sample



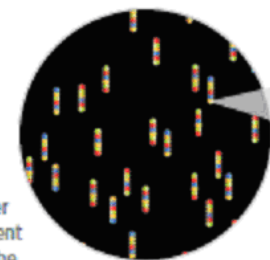
Sample material is mixed with excess CodeSet and hybridized overnight.

## 2 Purify



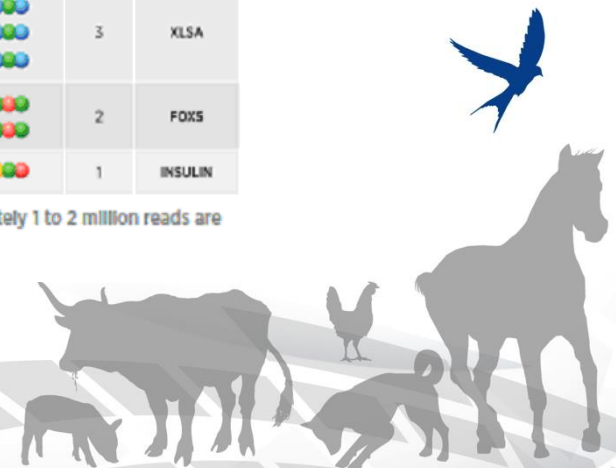
The Prep Station is an automated robotic instrument that removes excess CodeSets and immobilizes CodeSet/RNA complexes in the nCounter Cartridge for data collection.

## 3 Count



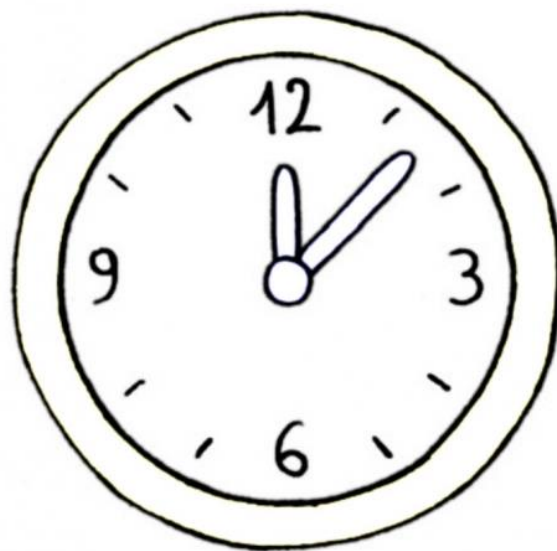
Sample cartridges are placed in the Digital Analyzer for data collection. Fluorescent barcodes on the surface of the cartridge are counted and a running total of each target is tabulated. Approximately 1 to 2 million reads are generated in each lane of the flow cell (12 lanes per cell).

Barcode	Counts	Identity
	3	XLSA
	2	FOX5
	1	INSULIN





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**18 ore**

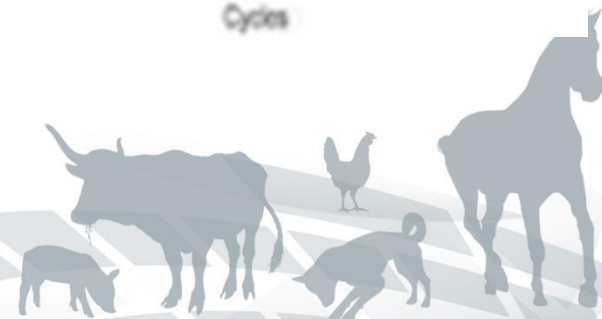
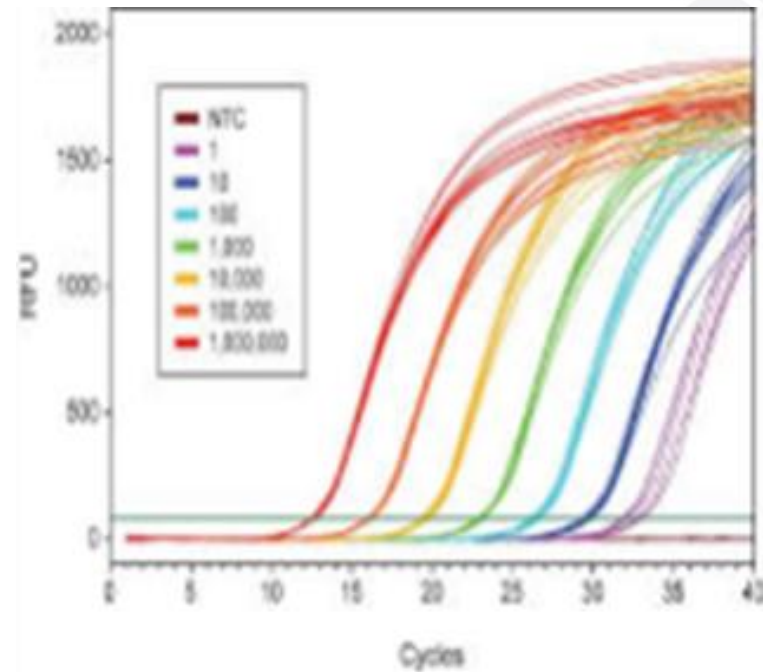






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# Due diversi studi

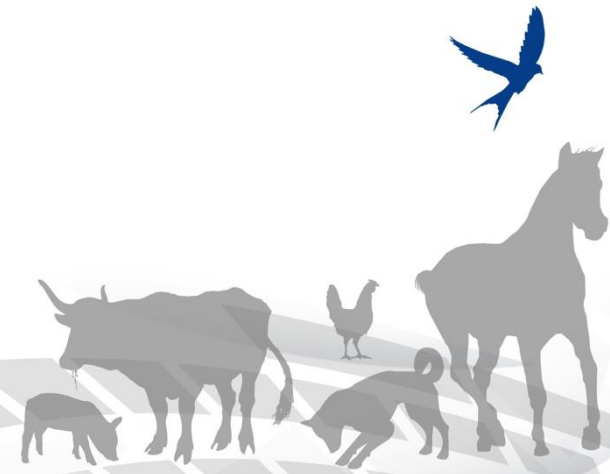


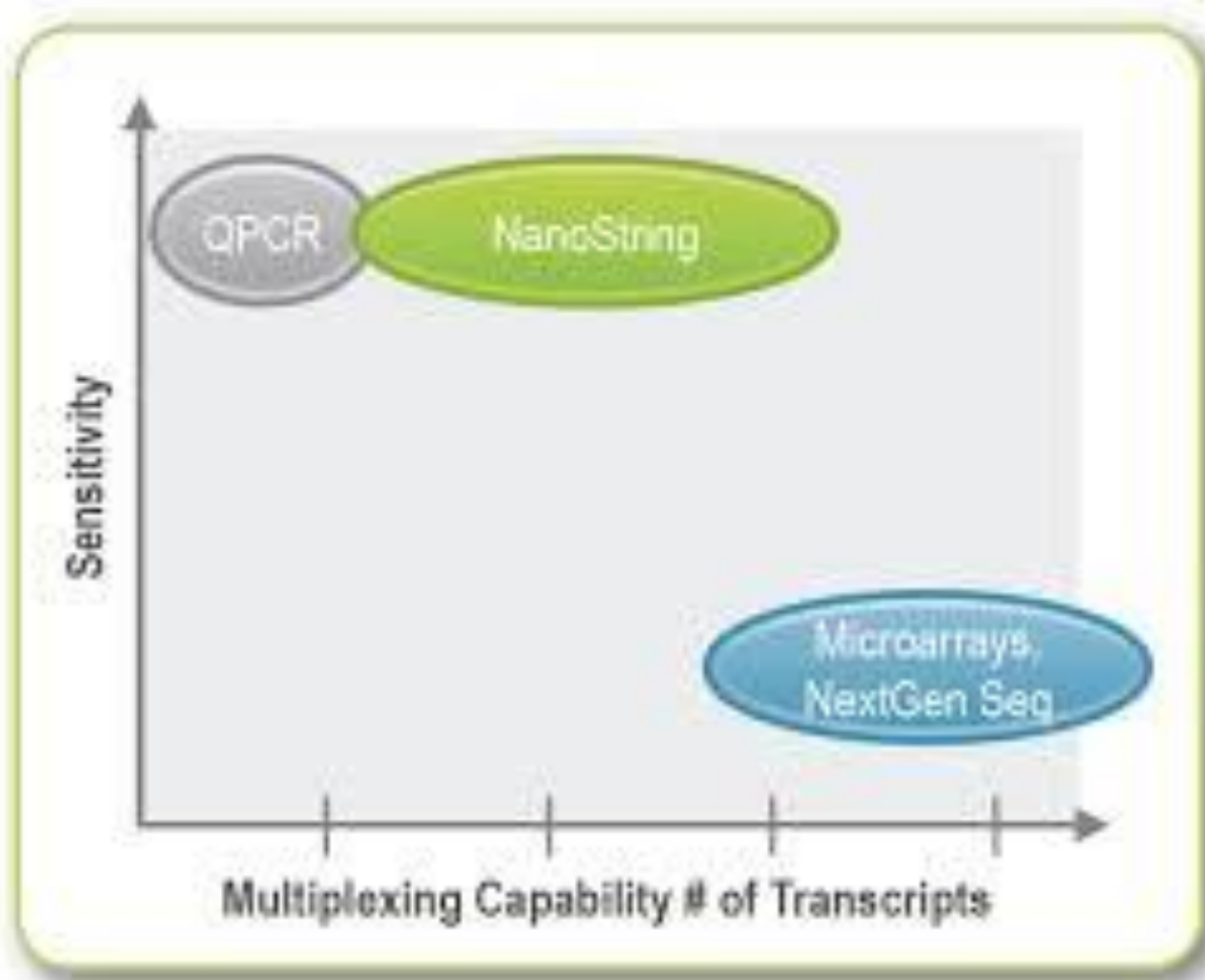
# *nCounter*<sup>®</sup> Analysis System



- **Identificati:**

- **25 (no BTV-25 e 27) isolati di referenza**
- **BTV da tessuti biologici**
- **BTV-1 da Culicoides**
- **Co-infections (BTV-1, BTV-4)**



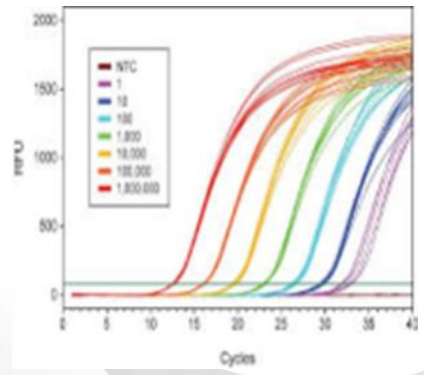




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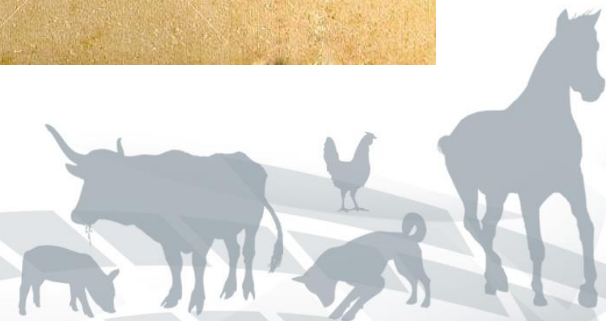
# APPROCCIO IDEALE IN IZSAM

15 Minutes Hands-on Time





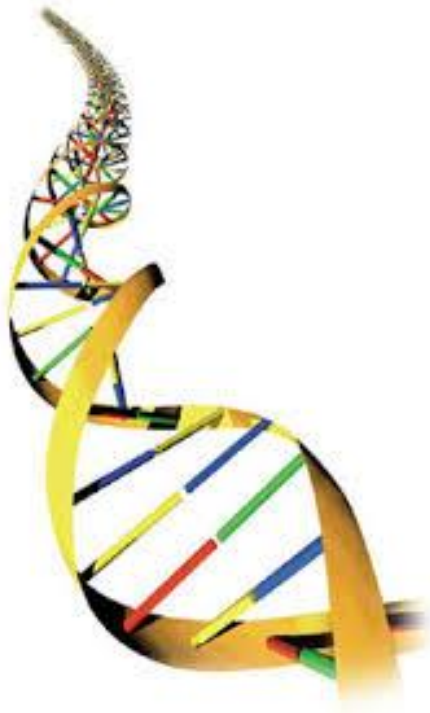
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# Genoma Plastico = Diagnosi Plastica



## NUOVE SONDE





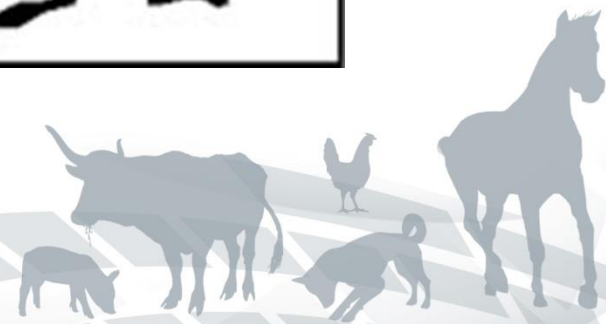
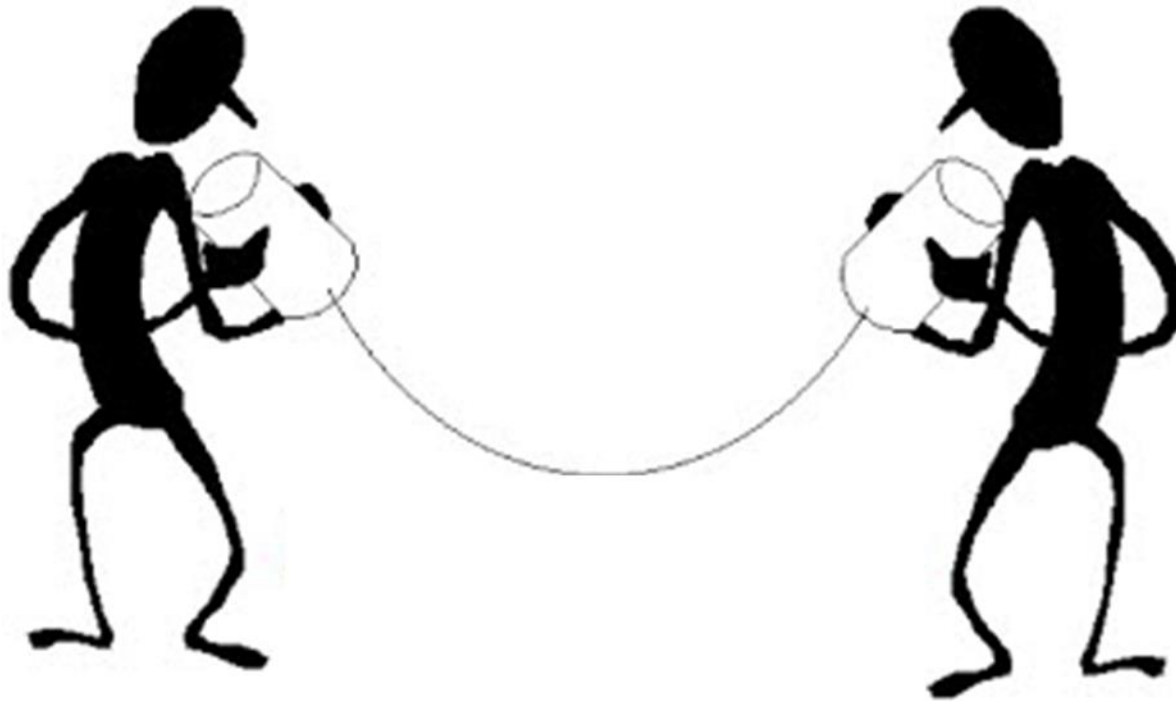
# Ulteriori usi

- **Co-infezioni**
- **Espressioni geni di virulenza nel patogeno e nell'ospite**





***Se continui a fare quello che hai sempre fatto,  
otterrai gli stessi risultati.***





# VIRUS DISCOVERY

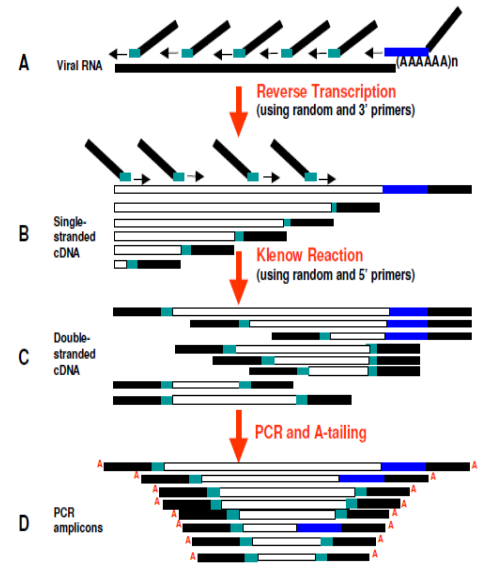


# Whole Genome Sequencing by NGS



→ RNA →

2 blood samples (from F1 and F2)  
with the lowest Ct RT-qPCR<sub>1-27</sub> values



←

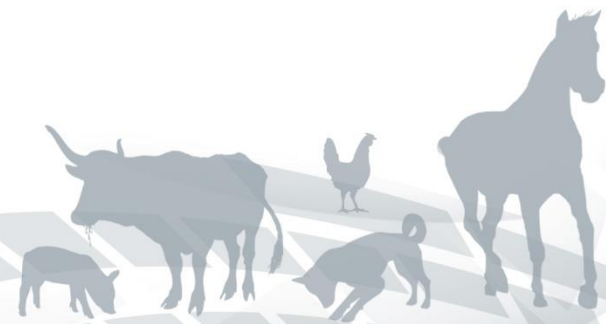


# COSA SAREBBE L'IZSAM SENZA BLUETONGUE ?

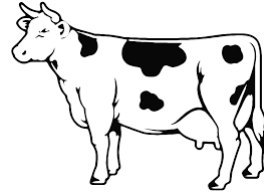
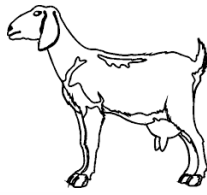
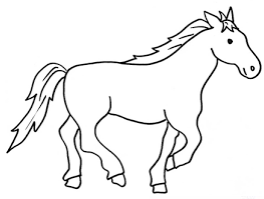




# Applicabile alla diagnostica !







15 Minutes Hands-on Time

No Amplification

No Printing of Slides

No Imaging Software

No Labelling

No Data Mining

No Washing



Nanostring Microarray

NGS, Illumina

RC 2016

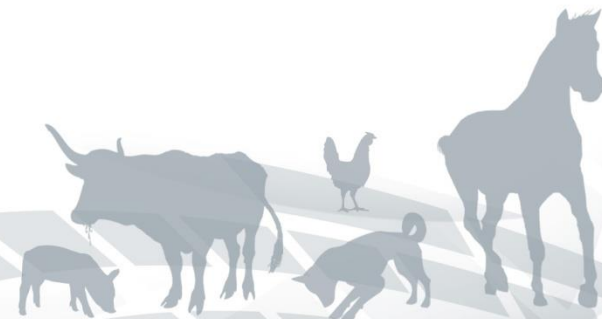


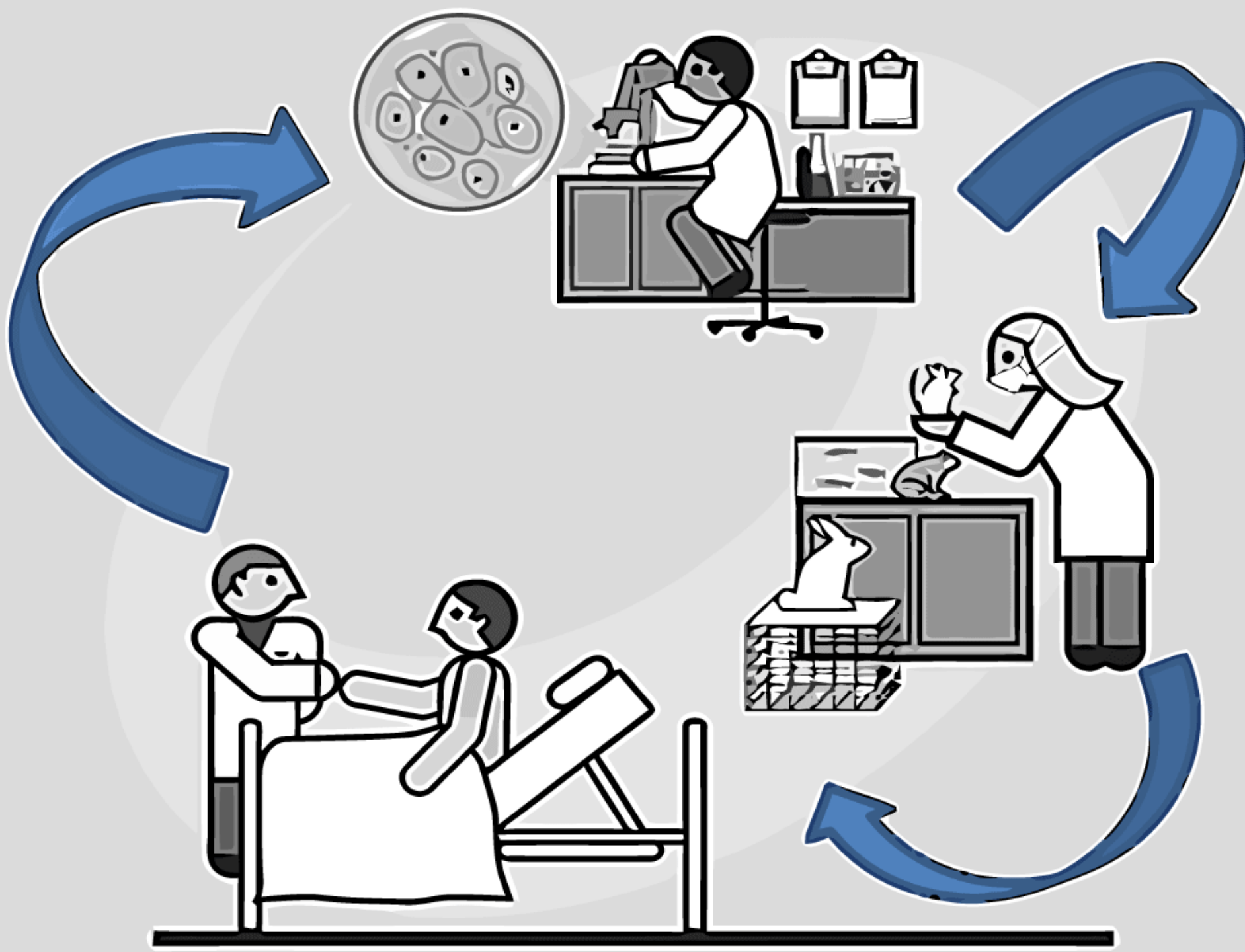
**-Fino ad 800 sonde per tubino**

**-Strip da 12**

**-4 strip al giorno**

**-36800 target al giorno**









# **RICHIESTE TANTO AL CHILO**



# **INTERPRETAZIONE DEI RISULTATI !!!**



# **PIU' RICERCA**



Thanks!

