


miRNAs studies in livestock, a summary of the EADGENE workshop  
Anne-Sophie Lequarré, University of Liège, Belgium

**EADGENE** European Animal Disease Genomics Network of Excellence for Animal Health and Food Safety

**Animal Disease Genomics: Opportunities and Applications**  
10th - 11th June 2008, Edinburgh, UK



**miRNAs studies in livestock, a summary of the EADGENE workshop**

Anne-Sophie Lequarré

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**EADGENE miRNA workshop 3<sup>rd</sup> of March 2008**

- **Bryan Cullen**, Duke University Medical Centre. *The biology of miRNAs*
- **Zissimos Mourelatos**, University of Pennsylvania. *Current protocols for the study of miRNA mediated gene regulation.*
- **Artemis Hatzigeorgiou**, Alexander Fleming Centre, Athens. *Computational approaches for the functional analysis of microRNAs*

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**EADGENE miRNA workshop 3<sup>rd</sup> of March 2008**

- **Laura Bailey**, Roslin Institute. *Micro-regulation of ES cell differentiation*
- **Nezha Sdassy**, INRA, Jouy. *miRNAs in the development of mouse mammary gland*
- **Florian Caiment**, ULg. *miRNA editing in the ovine DLK1-GTL2 imprinted domain.*
- **Denis Baurain**, ULg. *Patrocles: a database of miRNA-mediated gene regulation.*
- **Valentina Mariani**, PTP + INRA. *Host-virus miRNAs regulation: pig dendritic cells infected by PrV*
- **Benoît Muylkens**, INRA, Tours. *Host and virus microRNA during herpesvirus induced lymphomagenesis*

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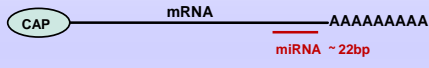
**EADGENE miRNA workshop 3<sup>rd</sup> of March 2008**



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**EADGENE miRNA world, a short summary**

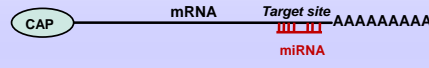
• Principle:



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**EADGENE miRNA world, a short summary**

• Principle:



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### miRNA world, a short summary

- Principle:

Cleavage      Translation repression

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### miRNA world, a short summary

- Principle:

- Discovered in C Elegans (lin4 & let7)
- Exist in all eutherians except sponge and yeast

Cleavage      Translation repression

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### miRNA world, a short summary

Illustration adapted from Cullen B. Nature Genetics Supplement: *Viruses and miRNAs* Vol 38 June 2006

Nucleus      Cytoplasm

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### miRNA world, a short summary

Located in coding and non coding Genes, mostly within introns

Often clustered and transcribed in one polycistronic unit

Nucleus      Cytoplasm

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### miRNA world, a short summary

Illustration adapted from Cullen B., nature genetics supplement. *Viruses And miRNAs*. Vol 38 June 2006

cytoplasm

High homology      mRNA binding      Partial homology

mRNA degradation      mRNA translation inhibition

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### miRNA world, a short summary

ds miRNA is unwinded and one strand is degraded (miRNA\*)

Mostly in plants      Mostly in animals

cytoplasm

High homology      mRNA binding      Partial homology

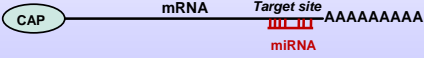
mRNA degradation      mRNA translation inhibition

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### miRNA world, a short summary



- Each miRNA has many possible targets (~200)
- One mRNA can have several target sites for different miRNAs
- About 1000 existing miRNAs

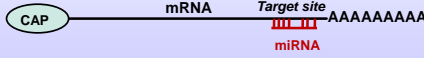
↓

One **THIRD** of genes are **regulated by miRNAs!**

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### miRNA world, a short summary




- Key role during development and cell differentiation
- Fine tuning cell proliferation and apoptosis
- Often dysregulated in cancers

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### miRNA detection, wet lab methods

- Cloning small RNAs
- High throughput sequencing of small RNAs
- PCR, northern blotting



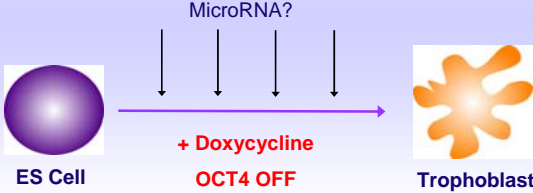
- Micro-arrays profiling
- Immunoprecipitation RISC or argonaute

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### New miRNAs discovery

• **Micro-RNA Regulation of ES Cell Differentiation**  
 Laura Bailey, D McBride, W Carré, L Sutherland, A Law, T Burdon and M Clinton  
 ROSLIN Institute

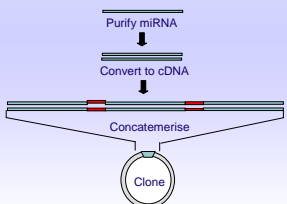


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### New miRNAs discovery

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 ROSLIN Institute



Identified >400 sequences

- 121 previously identified miRNAs
- 91 established novel miRNAs
- ~ 200 candidate novel micros

10% differentially expressed

- miR-30C decreases on differentiation
- miR-467a increases on differentiation
- miRNAs clustered on an intron of *Sfmbt2* also increase

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### New miRNAs discovery

• **miRNAs in the development of mouse mammary gland**  
 N. SDASSI, L. SILVERI, B. PASSET, L. GALIO, J.L. VILOTTE, E. DEVINOY, F. LE PROVOST  
 INRA-Jouy-en-Josas

Mammary gland = organ that undergoes cycles of cell division, differentiation and dedifferentiation in the adult

↓

Role miRNAs?


- Expression pattern of 10 miRNAs during mammary gland development
- Search for miRNA specific for the mammary gland and constitution of library
- DICER deletion (Cre-LoxP) and check biogenesis of miRNA at # stages

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### miRNA detection, computer methods

- miRNA
  - Search for ~ hairpin structure in coding, non coding regions
  - Filter through pattern of evolutionary conservation
- Target
  - Search in 3'UTR for imperfect complementary sequence to miRNA especially the **7-8bp of the SEED** (Lewis-human)
  - Search for 7-8 bp motif in 3'UTR **overrepresented** and conserved interspecies (Xie mammals)
  - Abundance of adenosine stretch flanking complementary sites



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### miRNA polymorphism

- **Patrocles: a database of polymorphic miRNA-mediated gene regulation** **ULg**  
 S. Hiard, D. Baurain, W. Coppieters, X. Tordoir, C. Charlier and M. Georges

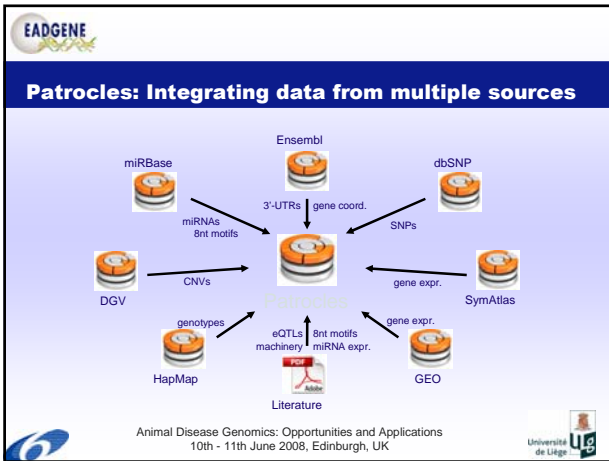
- 1 – Targets (1)
- 2 – miRNAs (100s)
- 3 – Silencing machinery (overall)

Patrocles database

- Subtle variation in expression (hypo/hyper)
- Possibly no phenotypic expression
- Risk factor in complex diseases?

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### Patrocles: Integrating data from multiple sources

Unit Animal Genomics <http://www.patrocles.org/>

The database of polymorphic miRNA-target interactions

Win the name PATROCLETS

To a large extent, Patrocles was named by a factor because he went to battle wearing the armour of his friend Achilles. Likewise, the miRNA target database has inevitably become the target of miRNAs because of its structure using a target-mechanism motif (some) from genuine target genes.

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### Part 1 – Polymorphic targets

Species

Target sites

- Source
- id
- motif

Target genes

- id
- position (Xsome + bp)

SNPs

- effect
- status
- id

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### Part 2 – Polymorphic miRNAs

The screenshot shows the Patrocles web interface with the following fields and annotations:

- Species:** Indicated by an arrow pointing to the 'Select species' dropdown menu.
- miRNA:** Indicated by an arrow pointing to the 'Select miRNA' dropdown menu, with sub-points for 'id' and 'position'.
- SNP:** Indicated by an arrow pointing to the 'Select polymorphisms' dropdown menu, with sub-points for 'position' and 'status'.

### Part 3 – Custom 3'-UTR sequences

The screenshot shows the 'Compare 2 sequences' and 'Analyse 1 sequence' options. A table displays the 3'-UTR of the MSTN allele (Texel vs. WT) with associated miRNAs:

Start	Motif	Source	miRNA
1234	AACATTCC	X	miR-613
1235	ACATTCCA	M	miR-1
		X	miR-206
			miR-613

Below the table is a list of miRNAs with their IDs and sources.

### Mutations in miRNA affecting phenotypes

**miRNA and editing in the Callipyge locus**  
 F Caimen and M Georges

**ULg** The callipyge muscular hypertrophy

Increase in proportion and diameter of fast twitch fibers.

### Non-mendelian inheritance: Polar overdominance

Genotypes shown: +/+ (normal), C/+ (normal), +/C (normal), and C/C (Callipyge).

Legend: C: callipyge, +: normal

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### mutation : DLK1-GTL2 imprinted domain

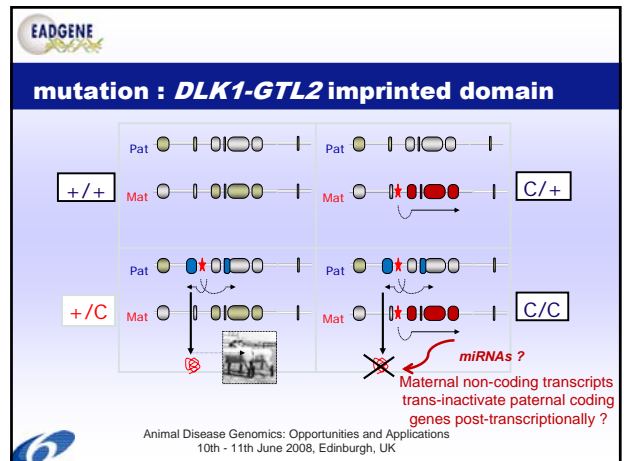
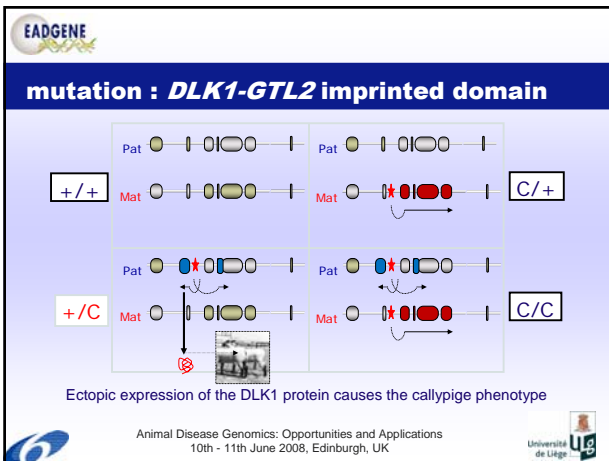
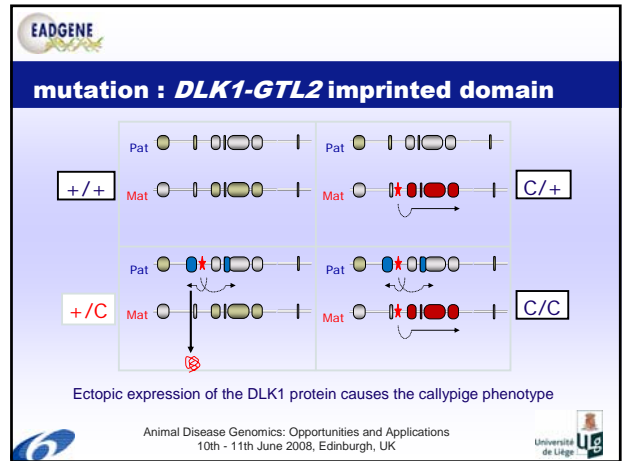
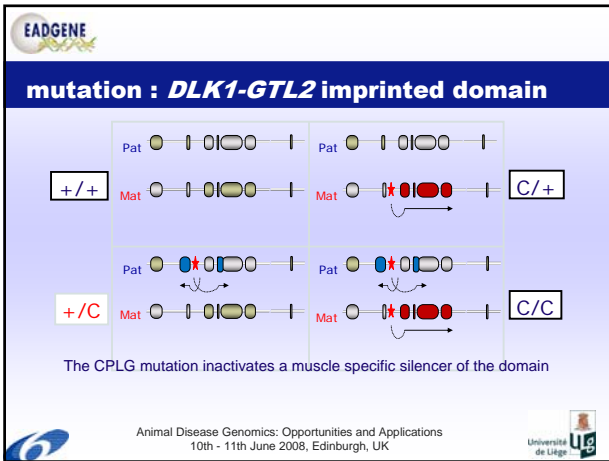
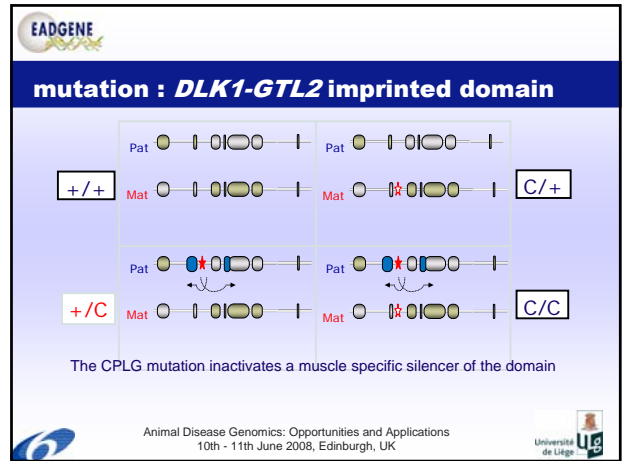
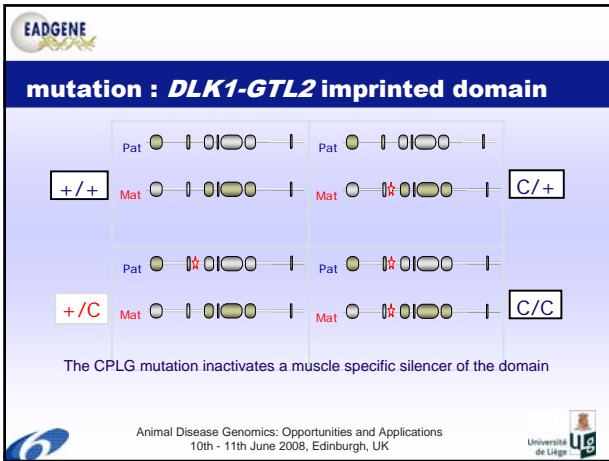
The diagram shows the paternal (Pat) and maternal (Mat) alleles of the DLK1-GTL2 imprinted domain. The paternal allele contains the *Begain*, *DLK1*, and *Peg11* genes. The maternal allele contains the *Gtl2*, *Anti-PEG11*, *Meg8*, and *Mirg* genes. The *IG-DMR* is located between *DLK1* and *Gtl2*. The *DLK1* gene is expressed from the paternal allele, while the *Gtl2* gene is expressed from the maternal allele. The *Mirg* gene is expressed from the maternal allele and produces *C/D* snoRNAs and *miR-127/136*.

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### mutation : DLK1-GTL2 imprinted domain

The diagram shows the paternal (Pat) and maternal (Mat) alleles of the DLK1-GTL2 imprinted domain. A mutation (indicated by a red star) is present in the *DLK1* gene on the paternal allele. This mutation affects the expression of the *DLK1* gene, leading to a loss of function.

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**mutation : *DLK1-GTL2* imprinted domain**

Pat Mat Pat Mat Pat Mat Pat Mat

+/+ C/+ +/C C/C

miRNAs ?

But no maternal miRNA have A target site in DLK1 mRNA !

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**mutation : *DLK1-GTL2* imprinted domain**

- RNA editing :
- Post transcriptional processing mechanism of the RNA sequence
- In mammals, mainly A→I editing
- Editing affecting the seed reported for orthologous miRNAs in mice

DNA extraction  
 Sample  
 RNA extraction  
 cDNA Synthesis  
 PCR Purification Sequencing

Pri-miR

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**mutation : *DLK1-GTL2* imprinted domain**

- Some pre-miRNAs of the domain widely edited in ovine skeletal muscle
- Unfortunately, miRNAs editing doesn't improve *DLK1* targeting
- Editing affects pre-miRNA processing in ovine skeletal muscle
- CLPG genotype influences the level of editing

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**miRNA encoded by Viruses**

- Advantages
  - Non-immunogenic
  - Small amount of genomic space
  - Powerful regulators of gene expression
  - 127 viral miRNAs detected: mostly herpes virus and a few in small DNA tumour viruses.

Down expression of host cell gene products interfering with viral replication cycle

Down regulation of viral messengers to induce a different viral cycle (latency)

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**miRNA encoded by Viruses**

Illustration from Cullen B. Nature Genetics Supplement: Viruses and miRNAs. June 2006

a

mRNA degradation

AAAA

Viral dsDNA genome

miRNA processing

RISC

Viral miRNA inhibits viral mRNA expression

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**miRNA encoded by Viruses**

b

Viral genome

Host cell genome

Viral pri-miRNA

Host cell mRNA

miRNA processing

RISC

Host cell mRNA degradation or translation inhibition

Viral miRNA inhibits host cell mRNA expression

Illustration from Cullen B. Nature Genetics Supplement: Viruses and miRNAs. June 2006

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### miRNA encoded by Viruses

**C**

Viral genome → Viral mRNA → miRNA processing → RISC → Host cell miRNA inhibits viral mRNA expression

Host cell genome → Host cell pri-miRNA → miRNA processing → RISC

Viral mRNA degradation or translation inhibition

Host cell miRNA inhibits viral mRNA expression

Illustration from Cullen B. Nature Genetics Supplement: Viruses and miRNAs. June 2006

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### miRNA and Virus

- Exploring the host-virus miRNAs regulation layer: pig dendritic cells (DCs) infected by PrV (Pseudorabies virus)  
 Mariani V, Malinverni R, Genini S and Giuffra E **PTP**  
 Fiori L, Lefèvre F, Rogel-Gallard C and Chardon P **INRA-Jouy-en-Josas**

**Aims:** - to identify new pig miRNAs

- to identify host and pathogen miRNAs involved in immune defense (host) and virulence (pathogen).

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### miRNA and PrV Virus in pig

Pig miRNAs within miRBASE: **54**  
 → all predicted *in silico*  
 → few biologically confirmed

**Aim:** Identify miRNAs differentially expressed in PrV infected (herpesvirus) pig Dendritic Cells (Ag presenting cells) → miRCURY™ LNA Arrays (Exiqon)

↓

**47** miRNA probes differentially expressed in time course analysis:  
 22 vertebrata, 5 plants, 2 viral, 1 arthropod, 17 not annotated

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### miRNA and PrV Virus in pig

Results validated by Real-Time PCR  
 → 2 viral miRNAs amplified → **mgHV-mir-x** and **ebv-mir-x**

On PrV draft : no ebv-mir-x! → Viral miRNA homologue to host genome  
 → genome sequence has still gaps ?

- Clone + seq miRNAs differentially expressed + validation
- Connection transcriptomic + miRNA expression studies
- Use tools to evaluate gene targets (Patrocles?).
- Bioinformatic tool to predict viral pre-miRNAs

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### microRNA during Gallid herpesvirus 2 infection

- Host and virus microRNA expression signatures during herpesvirus induced lymphomagenesis  
 B. MUYLKENS, S. TRAPP, Y. LEVERNE, G. DAMBRINE, S. PFEFFER, D. RASSCHAERT

**Gallid herpesvirus 2 : GaHV-2**

- responsible for Marek's disease, major pathogen in the chicken industry
- Infection of CD4+ T cell lymphoma and model for virus induced lymphoma

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### microRNA during Gallid herpesvirus 2 infection

Latency  
 One transcript : LAT  
 Mechanism of action ?

Oncogenesis  
 Major oncogene : Meq  
 Oncogenesis associated gene : vTR, USP

What is the role of GaHV-2 encoded miRNAs in the regulation of the viral and cellular cycles ?

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### microRNA during *Gallid herpesvirus 2* infection

Lytic infection (B Ly, M0)

Reactivation

D0

D5-7

D14...

D21

D7

Latent infection (CD4+ T Ly)

Development of lymphoma - CD4 + T cells

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### microRNA during *Gallid herpesvirus 2* infection

8 different cell populations: *in vitro* and *in vivo*

- Size fractionation (19-24 nt)
- Ligation of 3' and 5' adaptors
- RT, PCR1, PCR2 (BanI)
- BanI digestion, concatemerization, TA cloning
- « Large » scale sequencing (~400 concatemers per library)

Small RNA extraction (Word) => **15,163** small RNAs

Assembly (Contig Assembly Program, Bioedit)

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### microRNA during *Gallid herpesvirus 2* infection

- Identification of novel chicken miRNAs + validation
- Localization of additional loci encoding viral miRNAs
- Misexpression of cellular miRNA during GaHV-2 induced lymphomagenesis
  - miRNA-21 ↑ : *oncogenic miRNA* that down regulates several tumor suppressor genes

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### Part of the ULg miRNA team

Thanks for your attention !

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