

29° CONGRESSO  
NAZIONALE  
S.I.S.A.



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## Study of miRNome to identify new molecular causes of Familial Hypercholesterolemia

Presentation of the project

Bando borsa di studio "Andrea Mezzetti" 2015

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Università degli Studi  
di Napoli Federico II



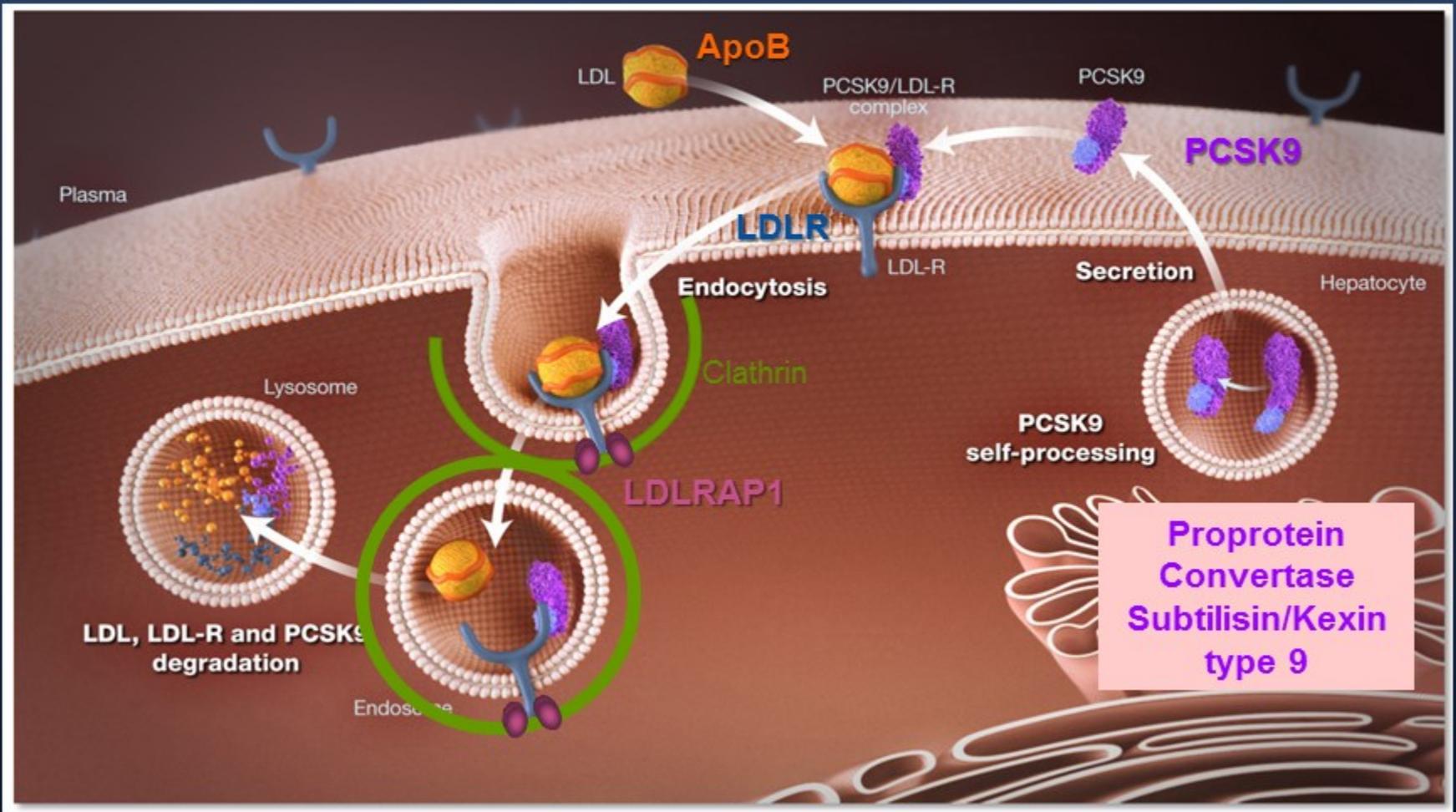
Università degli Studi  
di Salerno

# Familial Hypercholesterolemia (FH)

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- increased levels of LDL-cholesterol
- premature atherosclerosis and increased cardiovascular risk
- Tendon xanthomas, corneal arcus
- autosomal dominant and rarely autosomal recessive
- 2 forms
  - heterozygous
  - homozygous/compound heterozygous

# Genetics of Familial Hypercholesterolemia

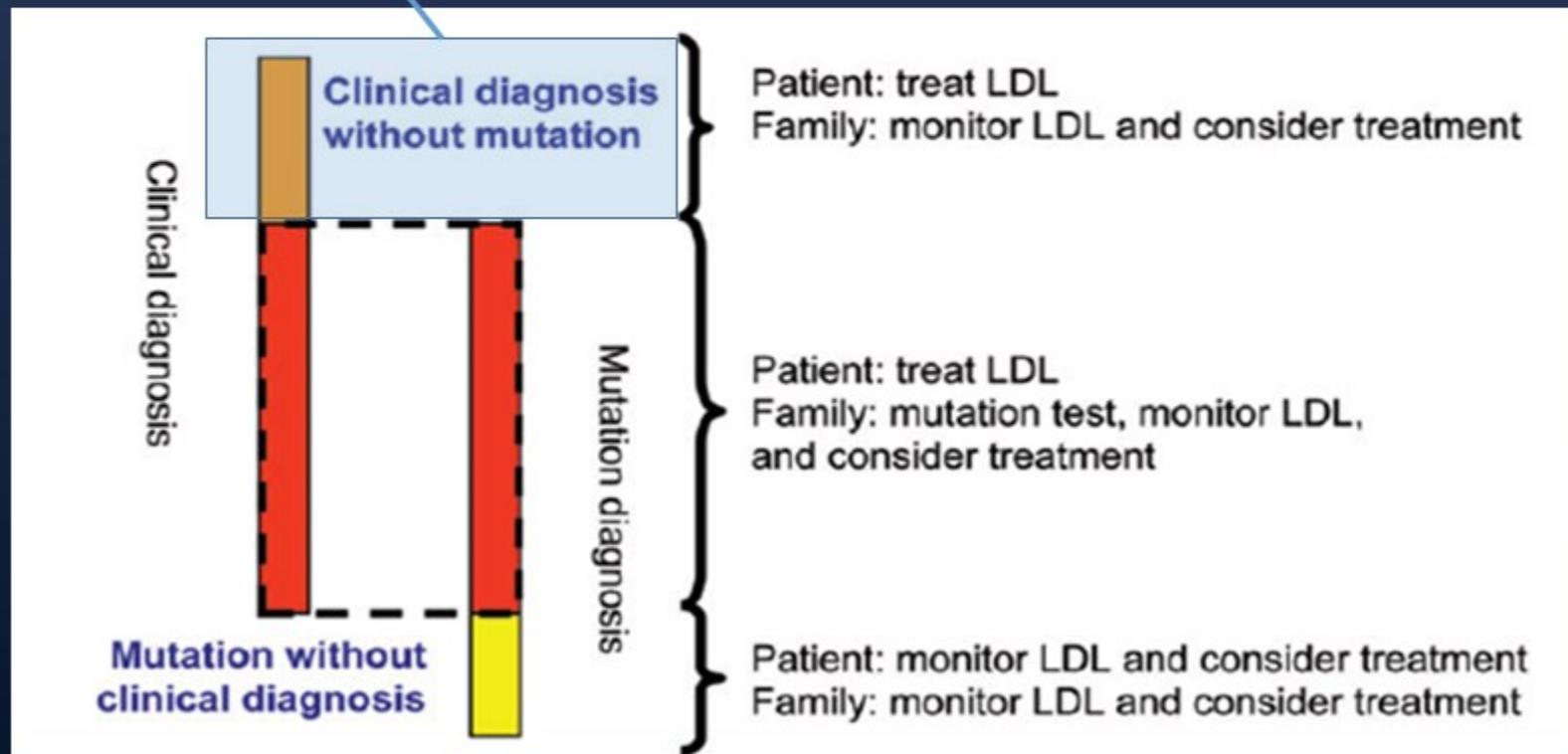


Adapted from Horton JD, et al. J Lipid Res. 2009;50 Suppl:S172-7

# Molecular and clinical diagnosis

~ 20-30%

Varret et al. 2008 (several studies) → 12-72%  
van der Graaf et al. 2011 (The Netherlands) → 5%



# Causes of mutation absence

## Methodological limits

- Patient recruitment
- Research of known mutations
- Large rearrangements/Splicing alterations

## Defects in other genes

- Cholesterol 25-Hydroxylase (CH25H)
- Insulin induced gene 2 (INSIG2)
- Signal transducing adaptor family member 1 (STAP1)

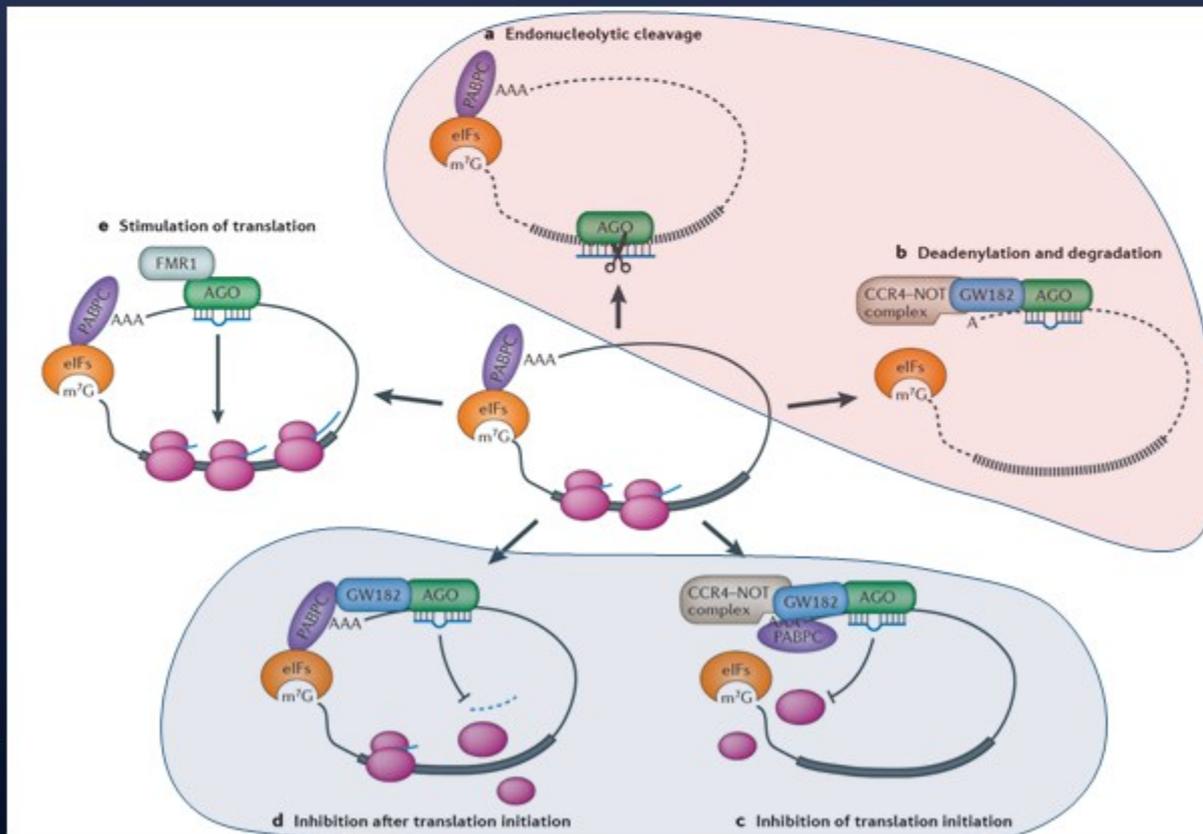
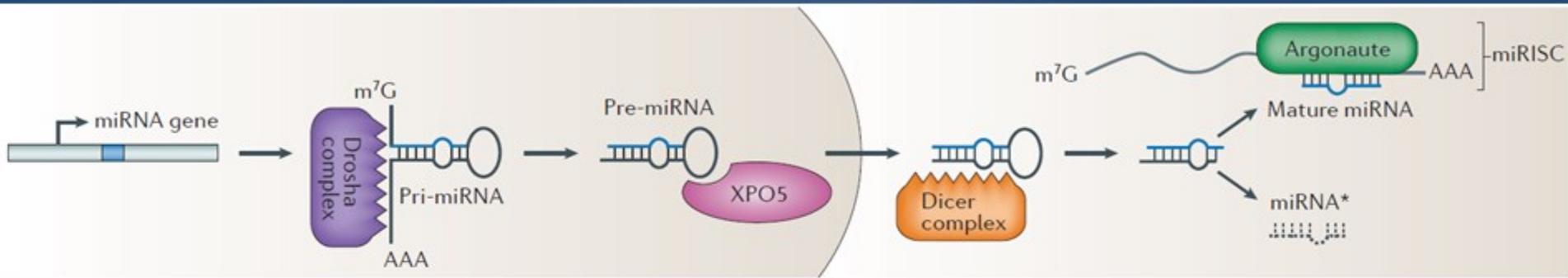
## Polygenic hypothesis

- Gene scores calculated on the basis of the genotype at specific loci

## Additional genetic mechanisms

- Regulation of gene expression:
  - Epigenetics
  - miRNA alterations

# miRNA synthesis and action



Degradation

Translation inhibition

# Aim of the project

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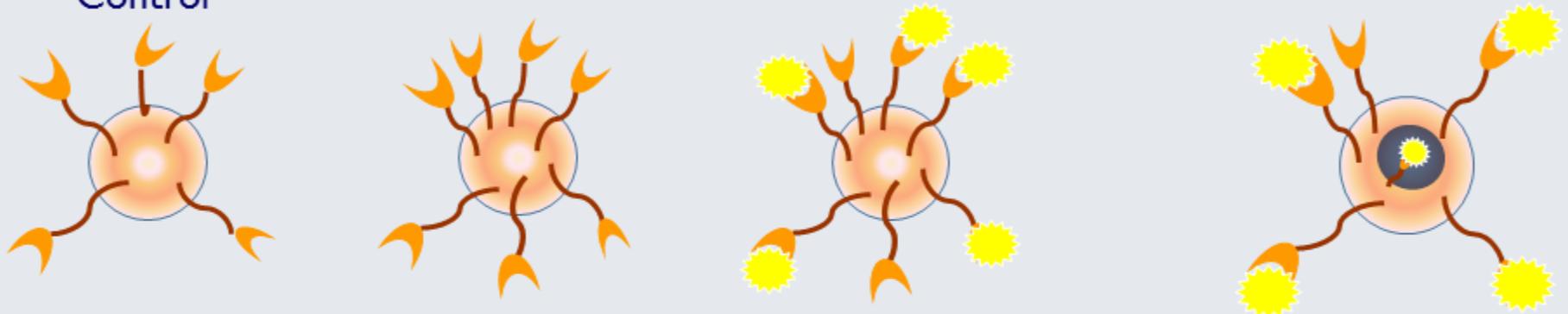
Identify molecular alterations responsible of FH in patients without mutations by a combined approach of:

1. Functional characterization and expression quantification of LDLR
2. Identification of miRNA alterations

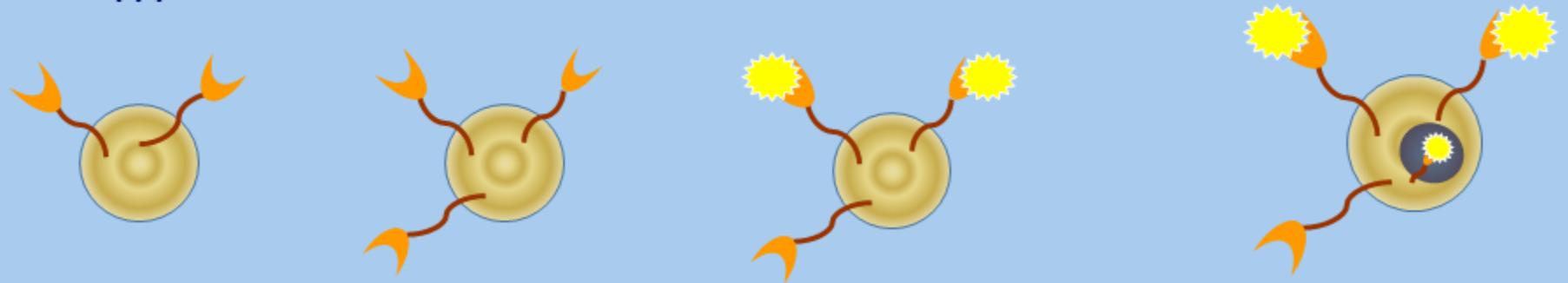


# Functional assay of LDLR

Control



FH



Incubation of T-lymphocytes with mitogens  
and lipoprotein deficient serum for 48h

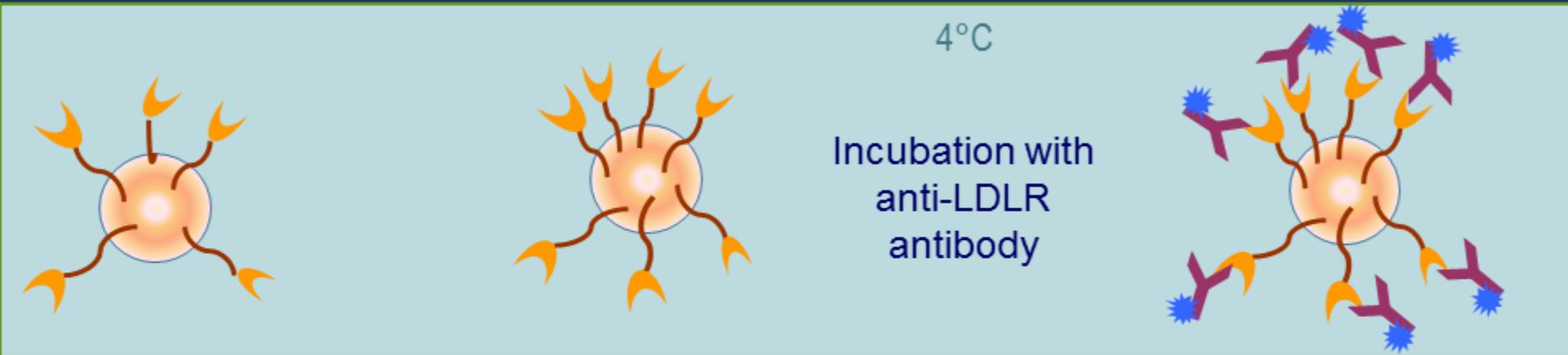
Incubation with Dil-LDL  
for 3 hours at 37°C





# Evaluation of LDLR expression

- Quantification of membrane LDLR



Incubation of T-lymphocytes with mitogens and lipoprotein deficient serum for 48h

- Quantification of LDLR mRNA
  - real time PCR with a TaqMan® probe spanning exons 2-3
  - analysis by comparative Ct method



# Functional characterization and expression of LDLR

- Verify if the disease is due to altered LDLR function
- Divide the population in 3 groups:
  1. normal LDLR activity
  2. decreased activity with decreased expression of LDLR
  3. decreased activity with normal expression of LDLR
- Allow a better evaluation of results from miRNA analysis



# smallRNA-Seq

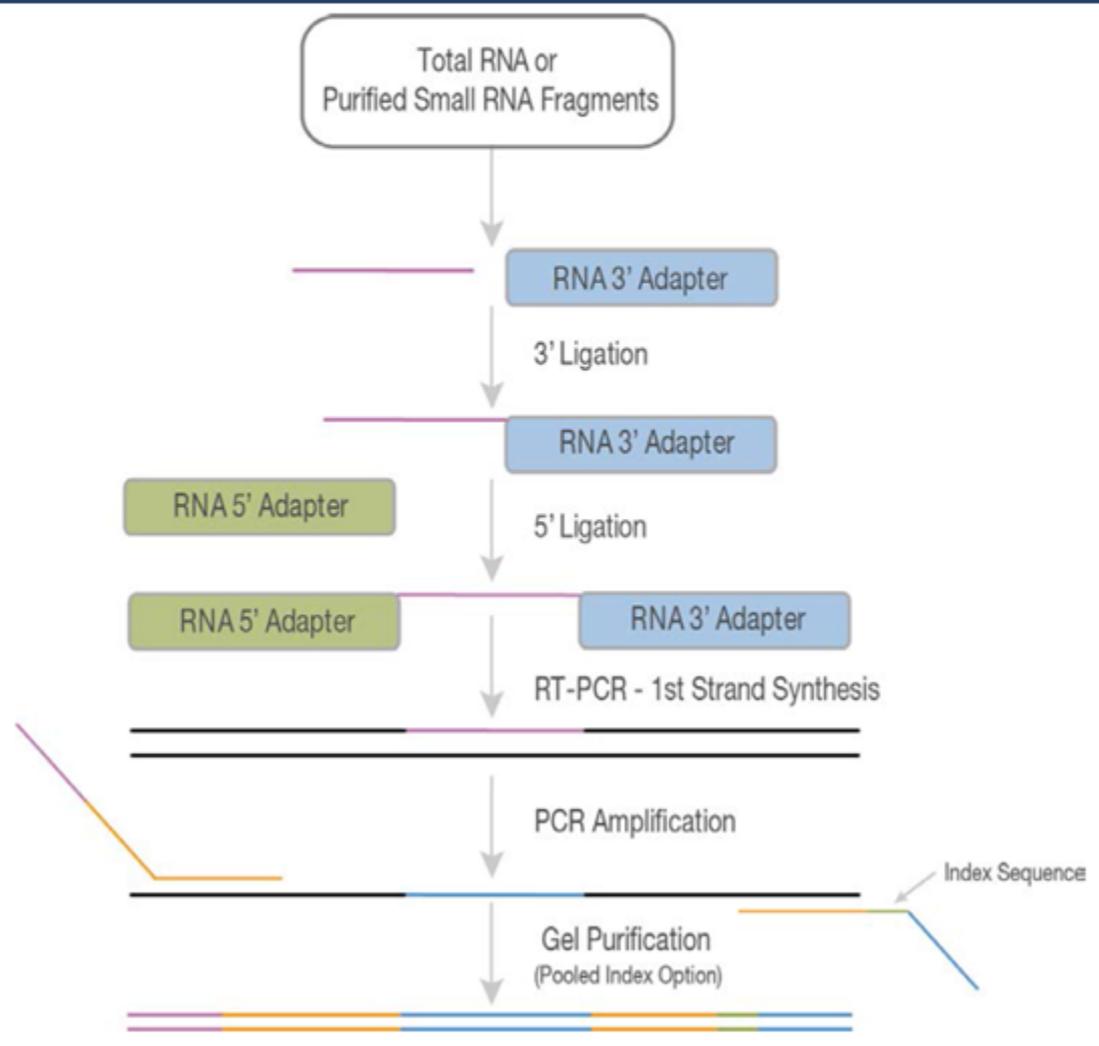
## Small RNA analysis by massive parallel sequencing

Total RNA  
isolation

Library  
preparation

mirVana  
miRNA  
Isolation Kit

TruSeq Small  
RNA Library  
Prep Kit





# smallRNA-Seq

Small RNA analysis by massive parallel sequencing



mirVana  
miRNA  
Isolation Kit

TruSeq Small  
RNA Library  
Prep Kit

MiSeq 2500  
(Illumina)

iMir



# Bioinformatic analysis – iMir software



Giurato *et al.* *BMC Bioinformatics* 2013, **14**:362  
<http://www.biomedcentral.com/1471-2105/14/362>



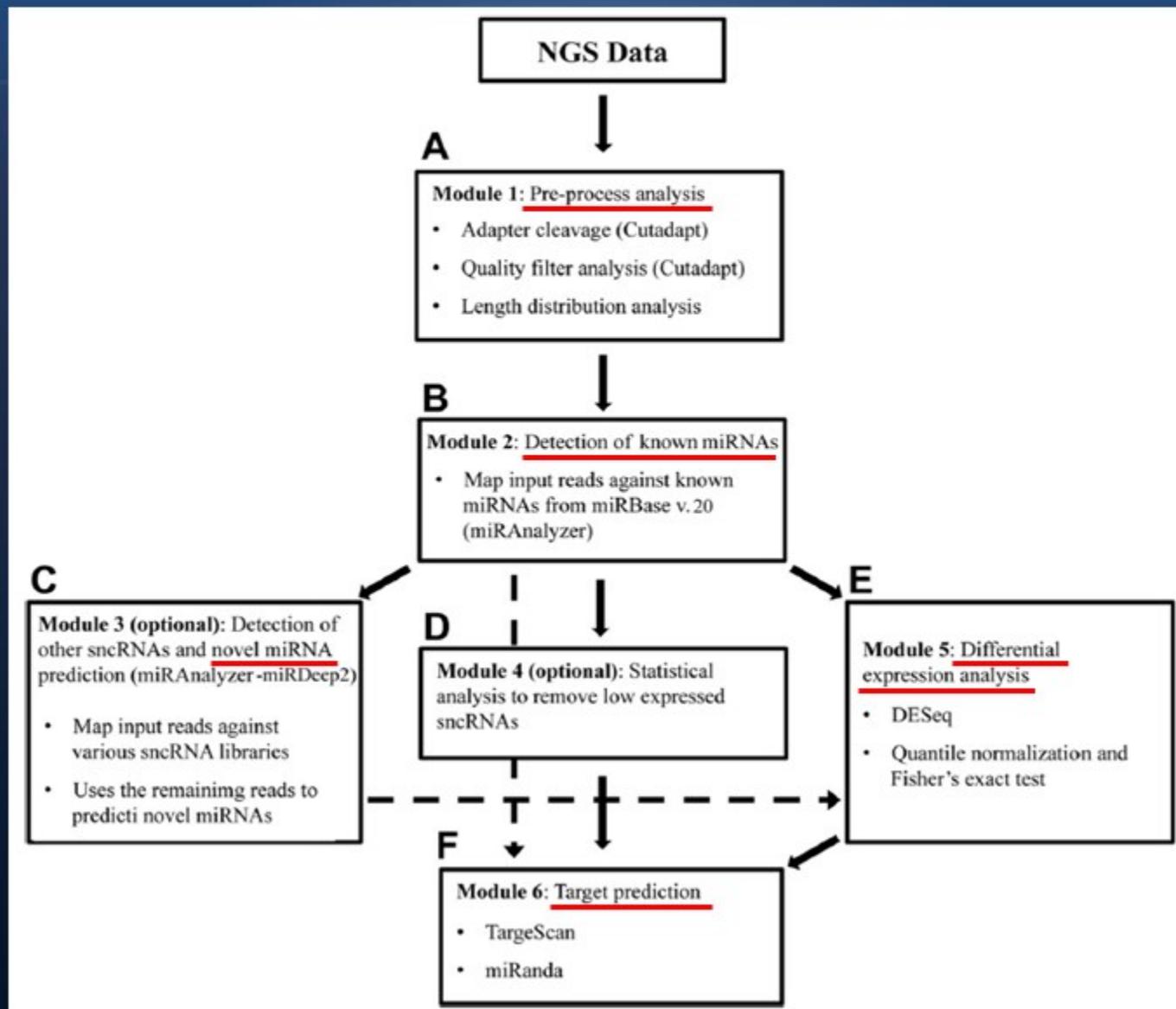
**SOFTWARE**

**Open Access**

## iMir: An integrated pipeline for high-throughput analysis of small non-coding RNA data obtained by smallRNA-Seq

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# Bioinformatic analysis – iMir software



# miRNome sequencing

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Evaluate all miRNAs present in cells:

- Quantify miRNA's amounts in cells
  - Increased levels of a miRNA cause the expression decrease of the target genes and vice-versa
- Analyse alterations of miRNA sequences
  - A variant in a miRNA sequence can result in a different match with the mRNA sequence leading to
    - Degradation instead of translational repression (or the opposite)
    - Different target genes

# Identification of pathways and quantification of target gene expression

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## 1. Pathway identification

- Ingenuity Pathway Analysis (IPA) platform
- Analysis taking into account the LDLR function/expression

## 2. Quantification of:

- mRNA of target genes by real time PCR
- proteins by western blot

# Possible expected outcomes

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- Identify new genetic markers of the FH to improve the molecular diagnosis
  - miRNA levels
  - altered miRNA sequences
- Identify new players in the development of FH that could be used as target for innovative therapy
  - miRNAs
  - genes identified by pathway analysis

# Acknowledgements

- Fondazione S.I.S.A. per la promozione della ricerca sulle malattie da arteriosclerosi



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- Università degli Studi di Salerno  
Prof. Alessandro Weisz

