Pharmacogenomic technologies

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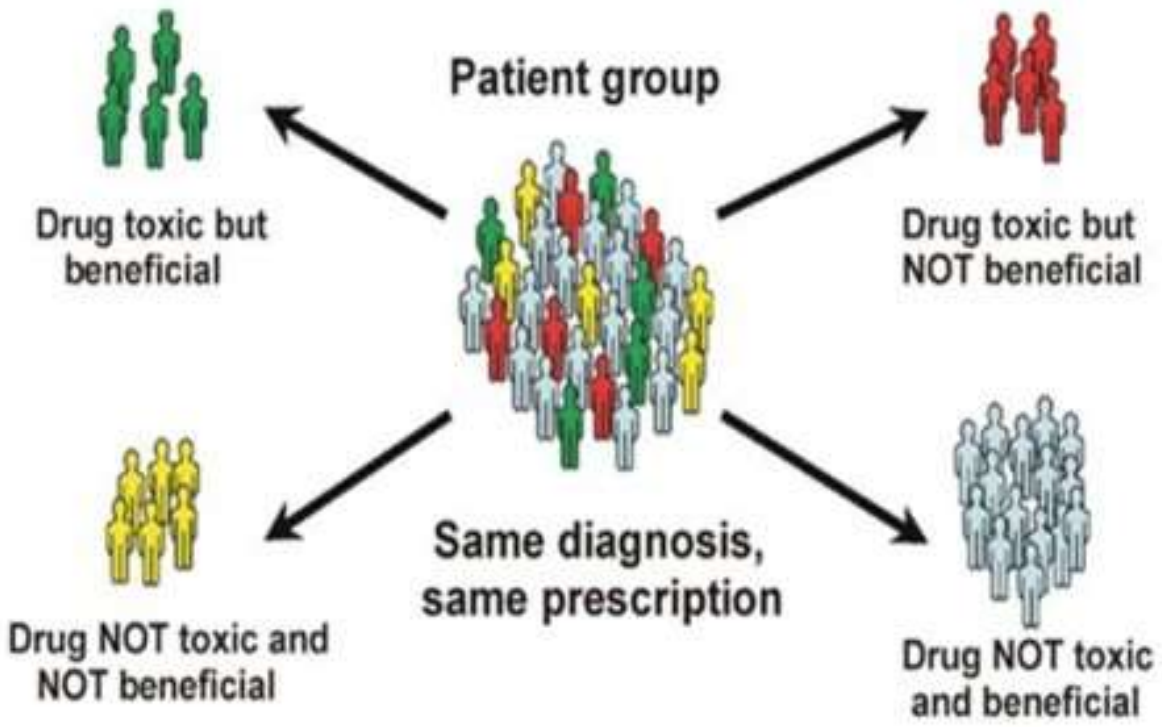
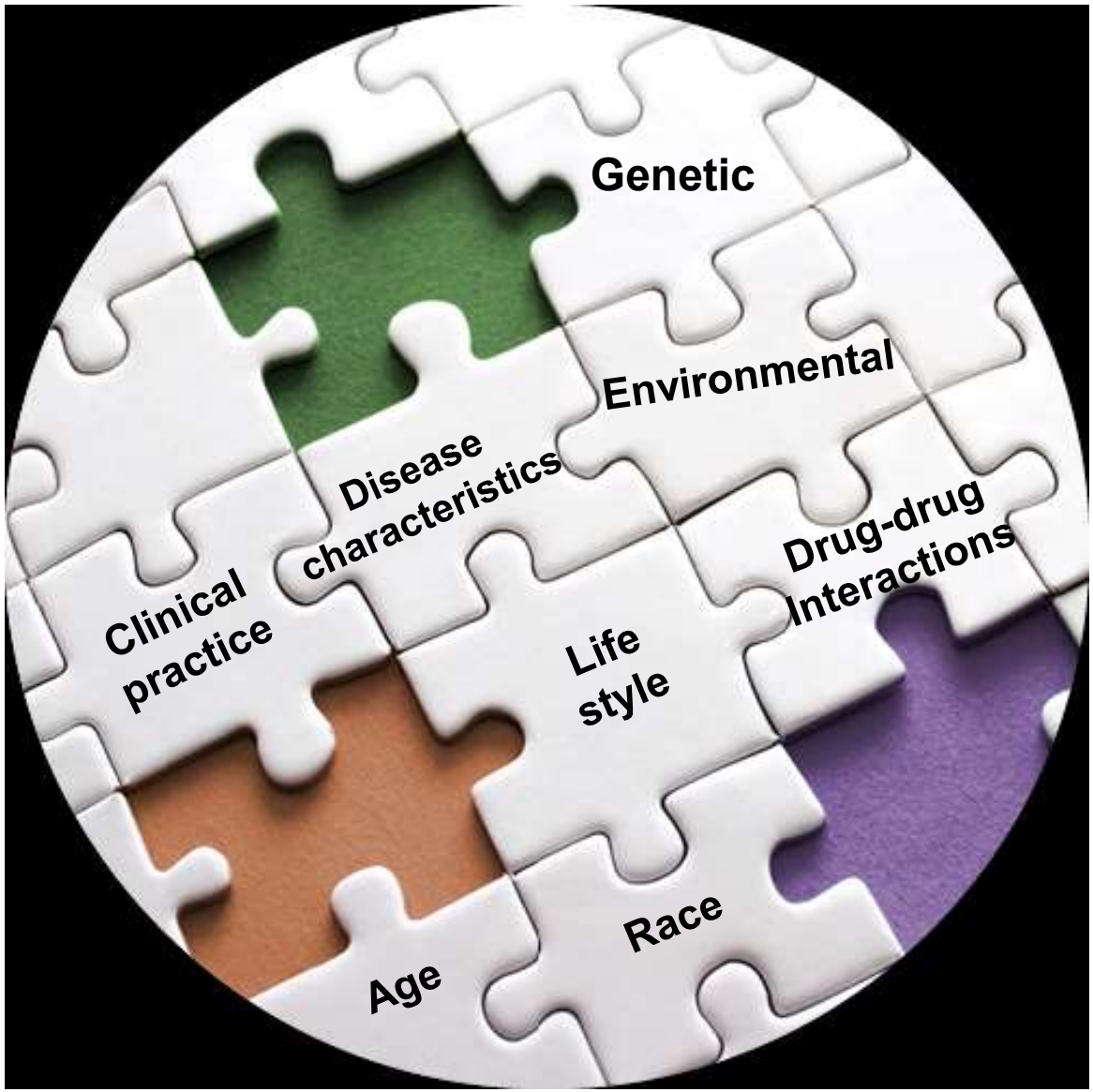
Pharmacogenomics

contribution of genetic factors to the interindividual variability in drug efficacy and safety

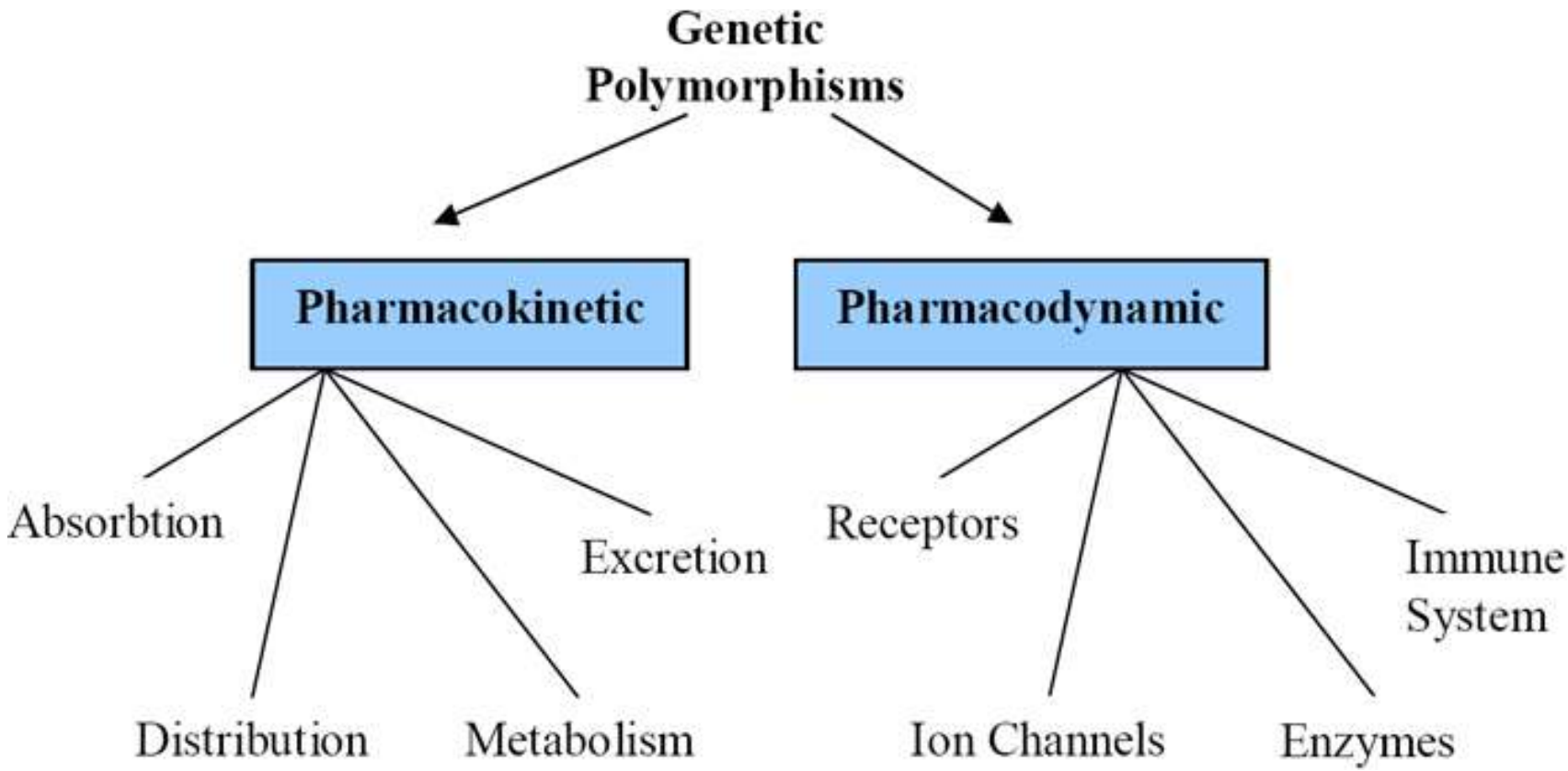


Pharmacogenomics

contribution of genetic factors to the interindividual variability in drug efficacy and safety



Pharmacogenetics



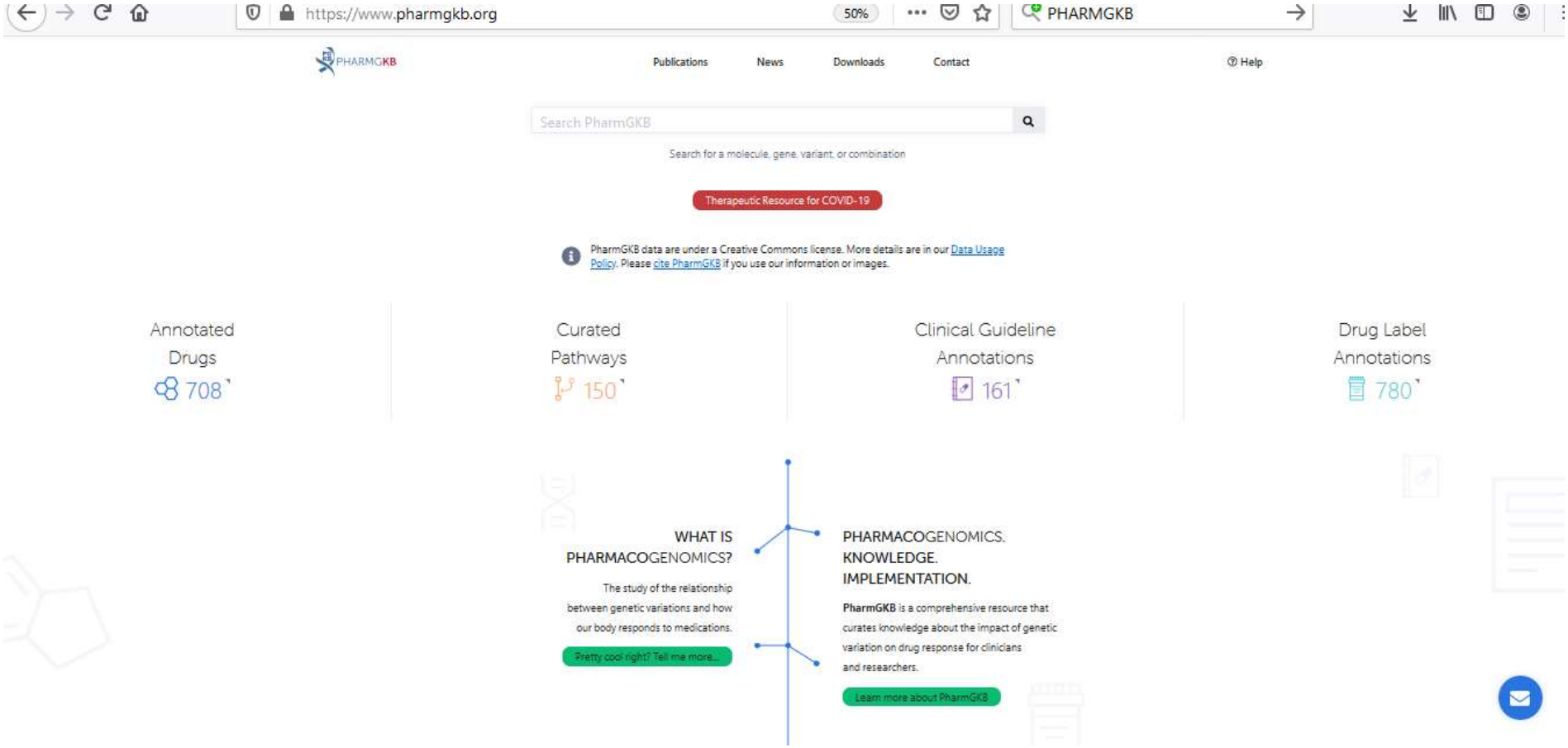
Pharmacogenomics and Personalized Medicine

By: Jill U. Adams, Ph.D. (*Freelance science writer in Albany, NY*)

Types of DNA sequence variation

- SNP: Single Nucleotide Polymorphism
- RFLP: Restriction Fragment Lenght Polymorphism
- VNRT: Variable Number of Tandem Repeats
- CNV: Copy Number Variations
- SSR: Simple Sequence Repeat
- Insertions or Deletions
- Rearrangement

PharmGKB: The Pharmacogenomics Knowledge Base



Tecniche di genotipizzazione

- **A basso rendimento (low throughput)**
  - **PCR-RFLP**
  - **PCR allele specifica**
  - **Ibridazione oligo (ASO)**

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

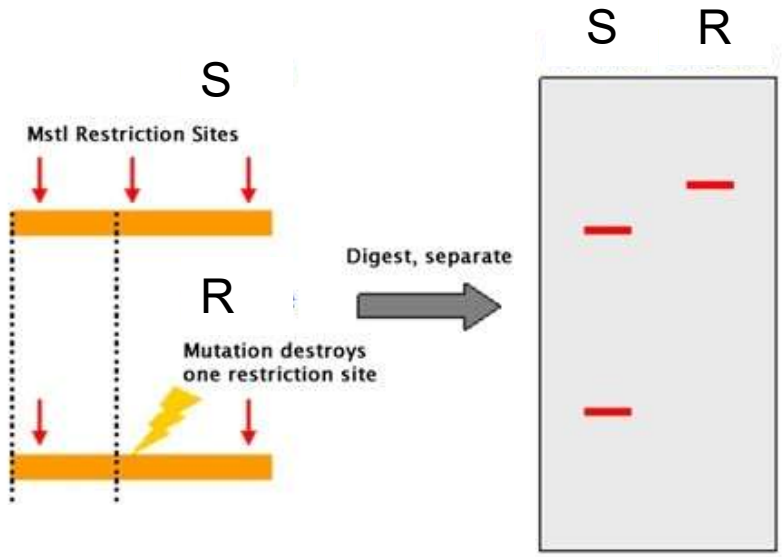
Amplificazione DNA (PCR)

Digestione dell'amplificato con enzima di restrizione (http://rebase.neb.com/rebase/rebase.html)
creazione /abolizione sito di taglio
frammentazione anomala

Elettroforesi su gel di agarosio
colorazione con GEL RED o
etidio bromuro

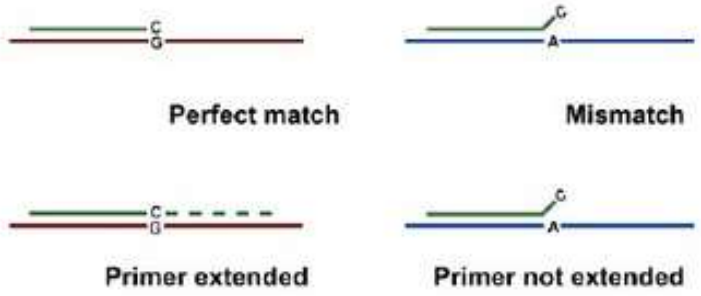
Pattern di frammenti caratteristici
creazione sito di taglio: + 1 banda
abolizione sito di taglio: - 1 banda

Gli enzimi di restrizione sono endonucleasi di origine batterica in grado di riconoscere specifiche sequenze di 4-8 nucleotidi e di tagliare in quelle posizioni il DNA.

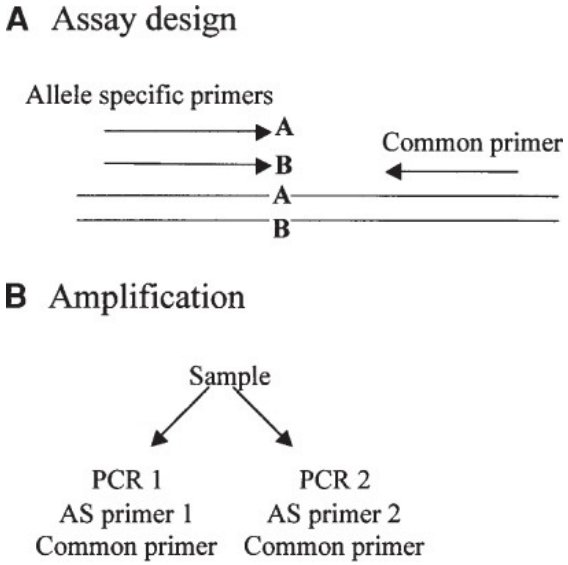


Allele-specific PCR

Reazione di PCR nella quale vengono utilizzati separatamente primers allele-specifici che differiscono per il nucleotide all'estremità 3' del filamento.
La sintesi dell'amplificato nella reazione di PCR dipende dal corretto appaiamento dell'estremità 3'.



Per ogni campione vengono allestite due reazioni di PCR utilizzando in ogni reazione il primer comune in combinazione con quello corrispondente alla sequenza wt e a quella mutata.

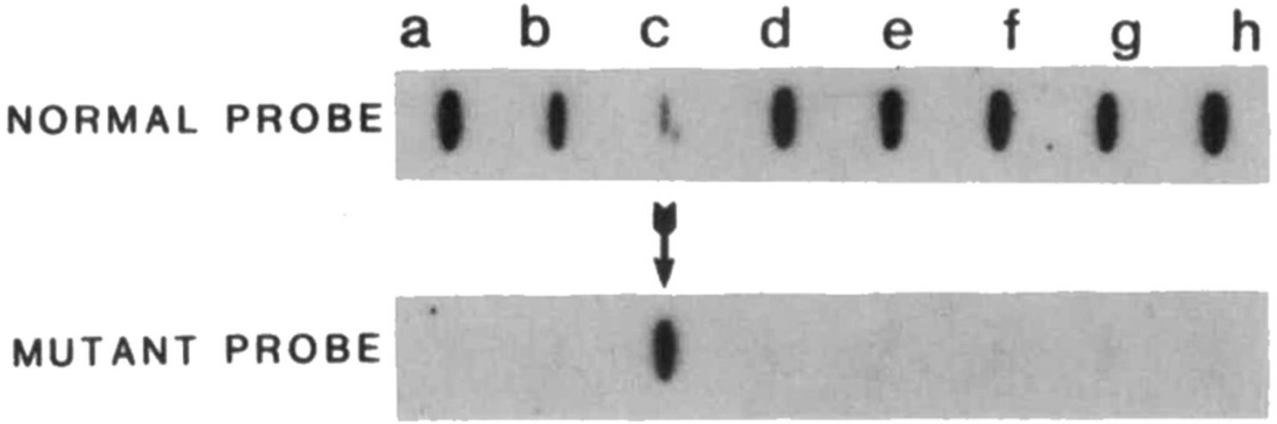


La presenza degli amplificati viene evidenziata mediante elettroforesi su gel di agarosio e colorazione con gel red.

Allele-specific oligonucleotides PCR (ASO)

- La presenza di una mutazione viene riconosciuta facendo reagire il DNA amplificato con sonde oligonucleotidiche complementari alla sequenza mutata o normale (separatamente).

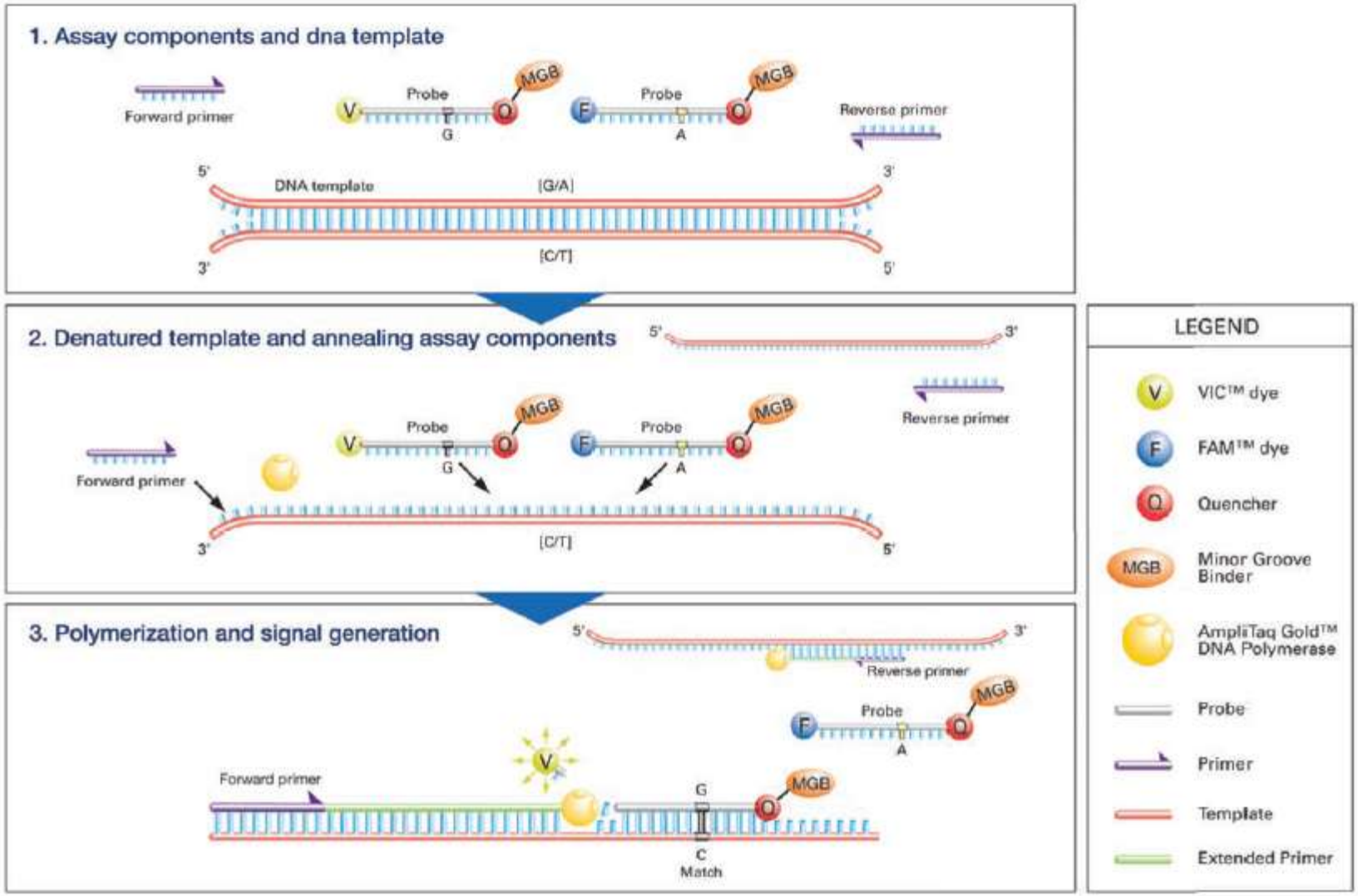
- Solo in presenza del 100% di omologia tra le sequenze si avrà l'ibridazione della sonda con il DNA.



Tecniche di genotipizzazione

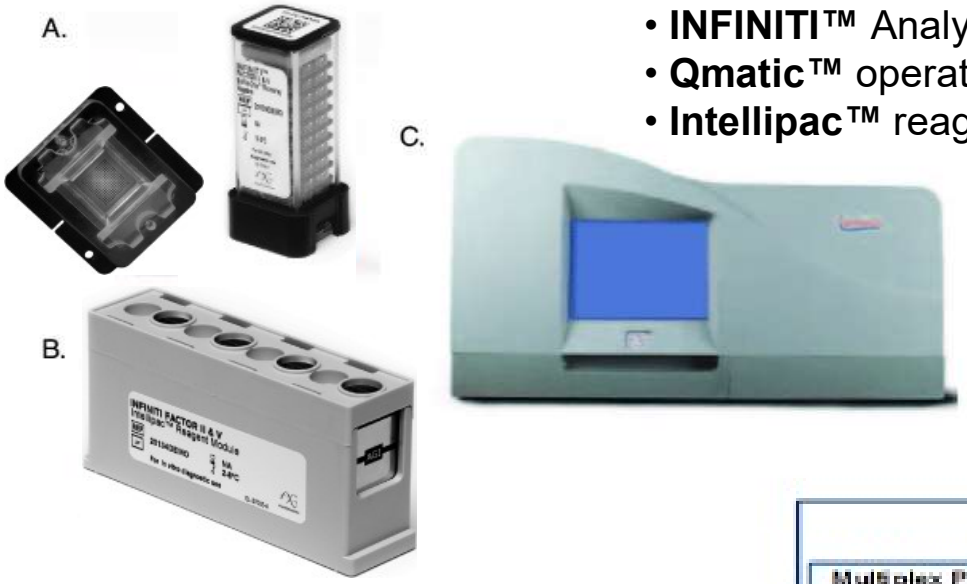
- **A medio rendimento (medium throughput)**
  - **Taqman® (Applied Biosystem)**
  - **Infiniti™ (Autogenomics)**
  - **Pyrosequencing® (QIAGEN)**
  - **Invader® Assay (Hologic)**

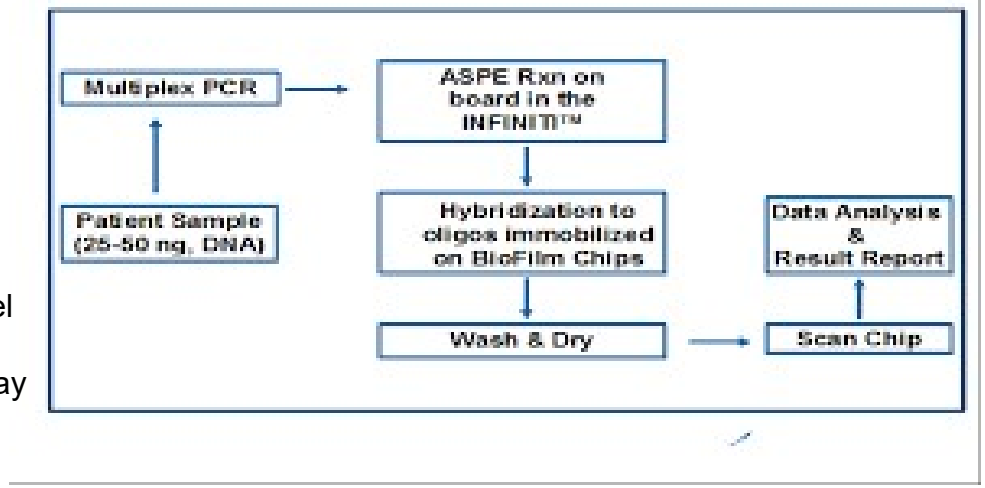
TaqMan® SNP Genotyping Assays



Infiniti™ (Autogenomics)

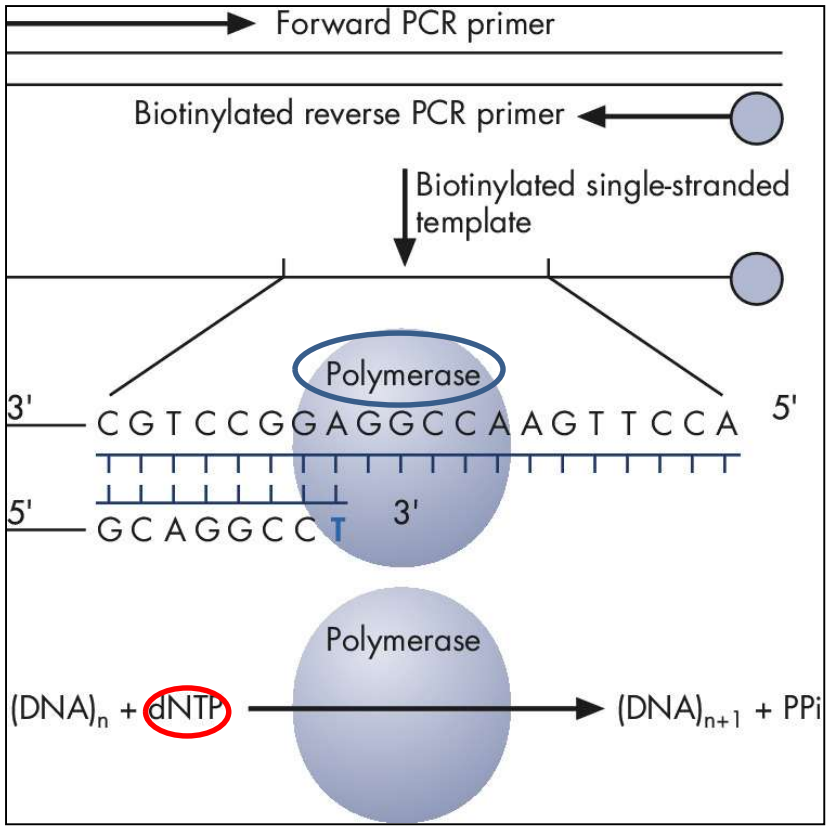
- **BioFilmChip™** microarray (A)
- **INFINITI™** Analyzer with sample to result automation (C)
- **Qmatic™** operating software with applications interface
- **Intellipac™** reagent management module (B)

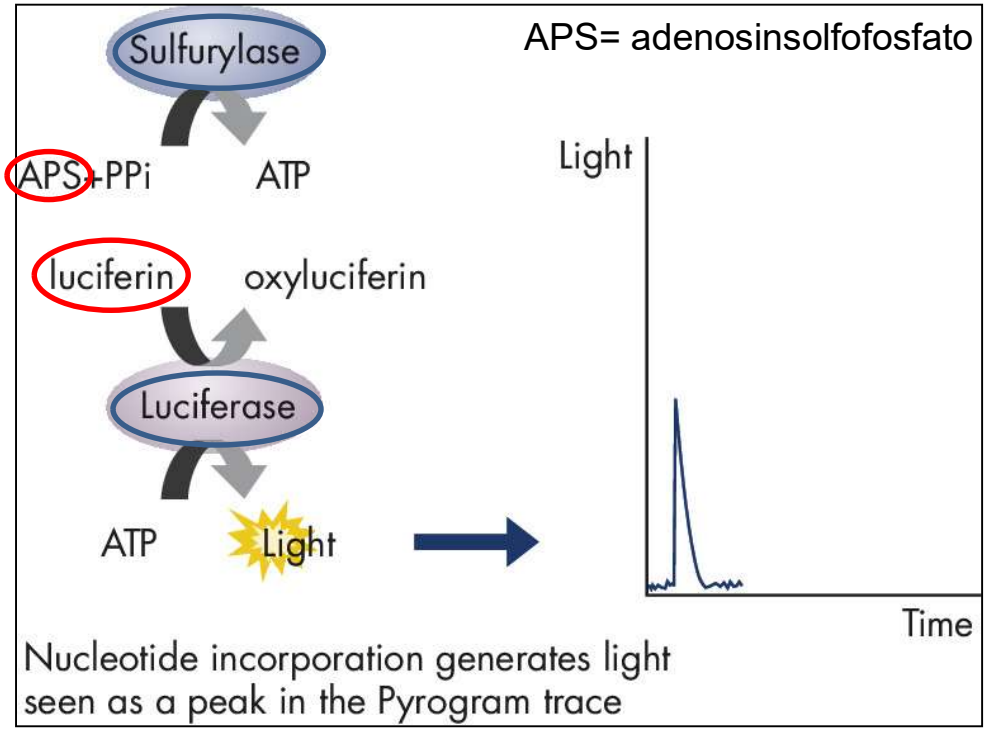


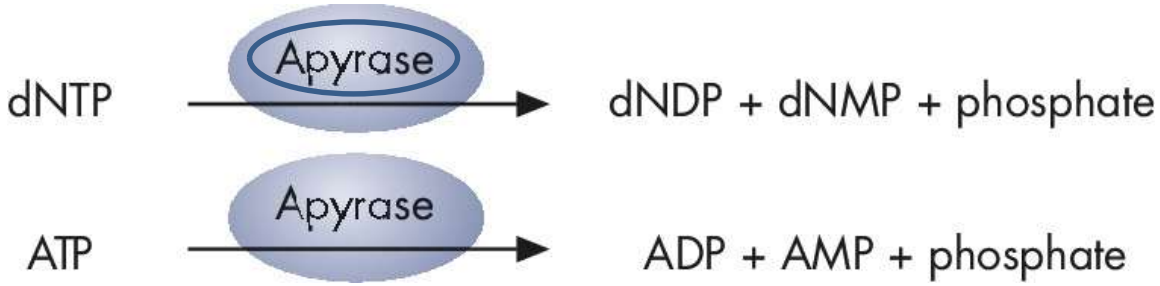


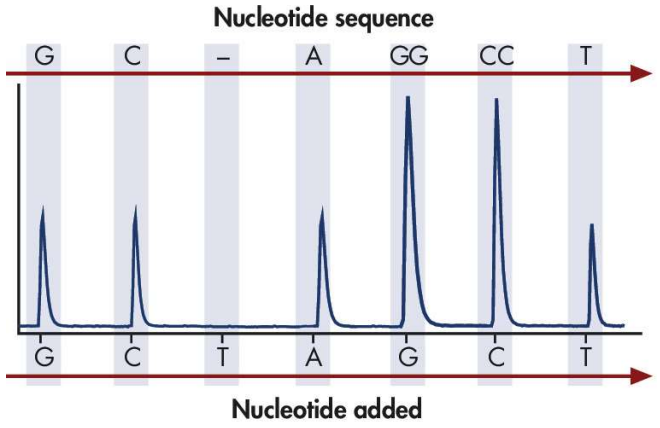
Protocollo del test:
(a) Estrazione del DNA da campioni di sangue umano
(b) Amplificazione mediante PCR
(c) Estensione del primer allele specifico con incorporazione del marcato (ASPE)
(d) Ibridazione dei primer esteso e fluorescente su un microarray e successivo lavaggio
(e) Scansione del microarray
(f) Rilevazione del segnale e analisi (determinazione del genotipo)

Pyrosequencing® (QIAGEN)





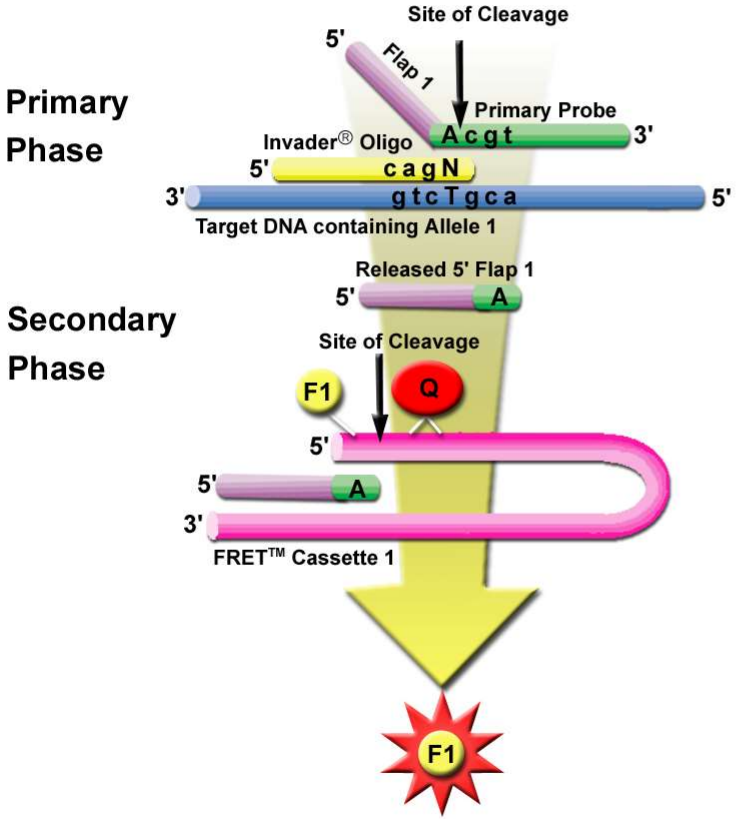




Invader® Assay (Hologic)

A

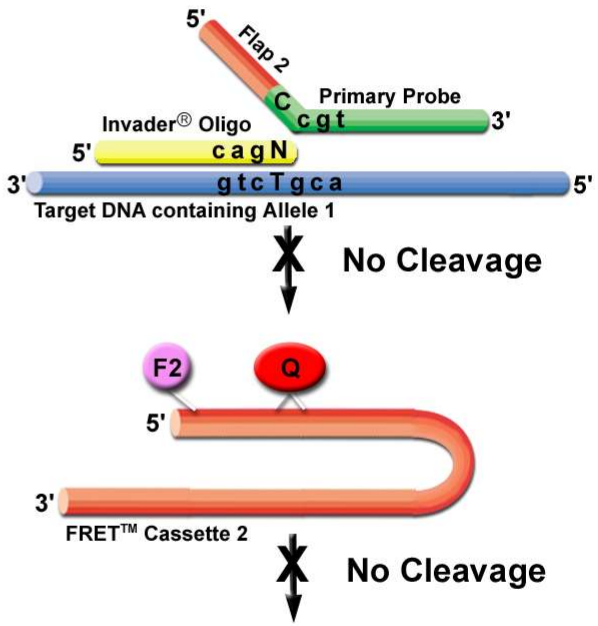
Invasive structure forms from single-base overlap between Invader® Oligo and Primary Probe when hybridized to Target DNA containing Allele 1.



"Perfect Match" = Invasion = Signal for Allele 1

B

Mismatch between Primary Probe and Target DNA containing Allele 1 prevents single-base overlap and no invasive structure is formed.



Any Mismatch = No Invasion = No Signal

Tecniche di genotipizzazione

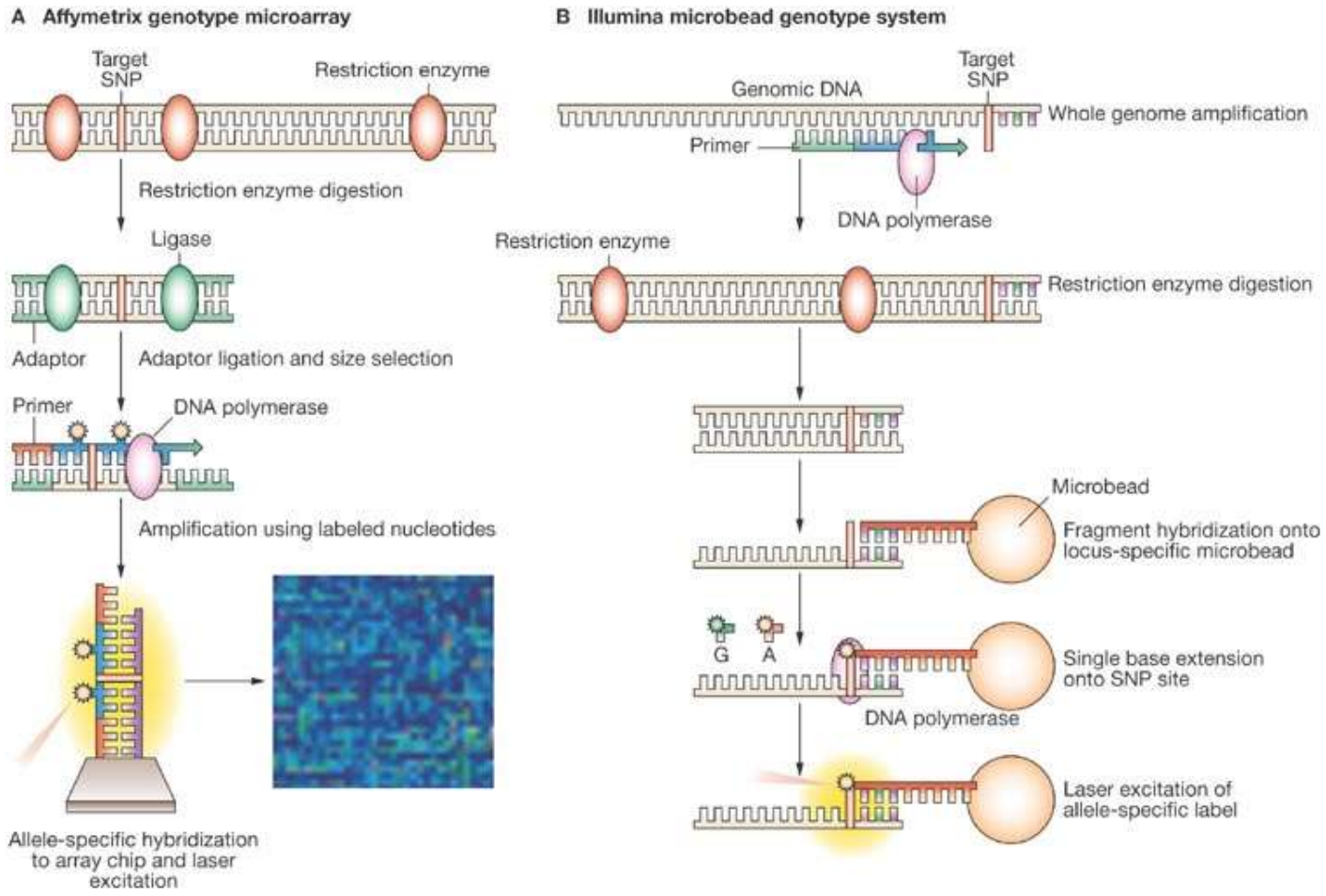
- **Ad altissimo rendimento (super-high throughput)**
  - **Genome-wide Human SNP Array 6.0 (Affymetrix)**

    1.8 million markers, including 946,000 probes for the detection of copy number variants and 906,600 SNPs

  - **BeadArray™ System (Illumina)**

    1.2 million markers

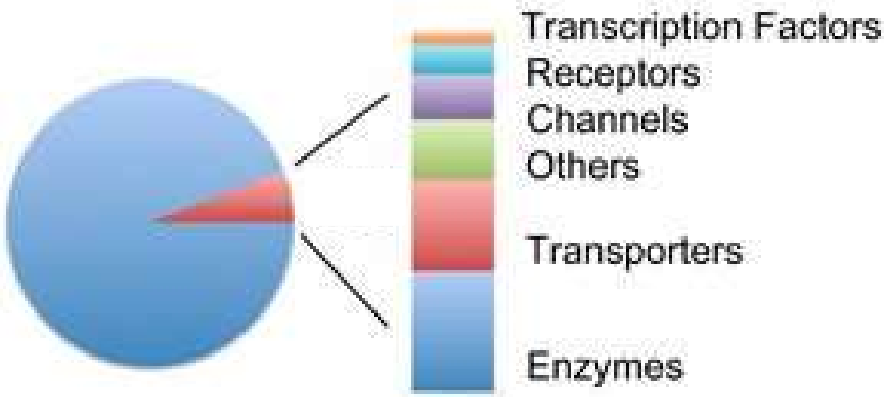
Two ultra-high-throughput single nucleotide polymorphism genotyping platforms for use in genome-wide association analyses.



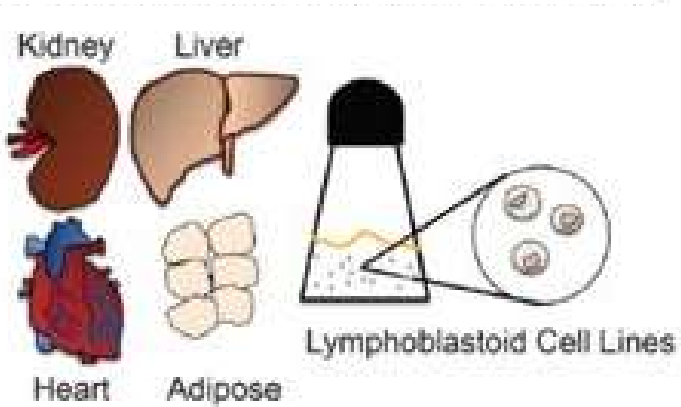
Walker EJ and Siminovitch KA (2007) Primer: genomic and proteomic tools for the molecular dissection of disease *Nat Clin Pract Rheumatol* 3: 580–589 doi:10.1038/ncprheum0595

Transcriptomic variation of pharmacogenes

Pharmacogene candidates



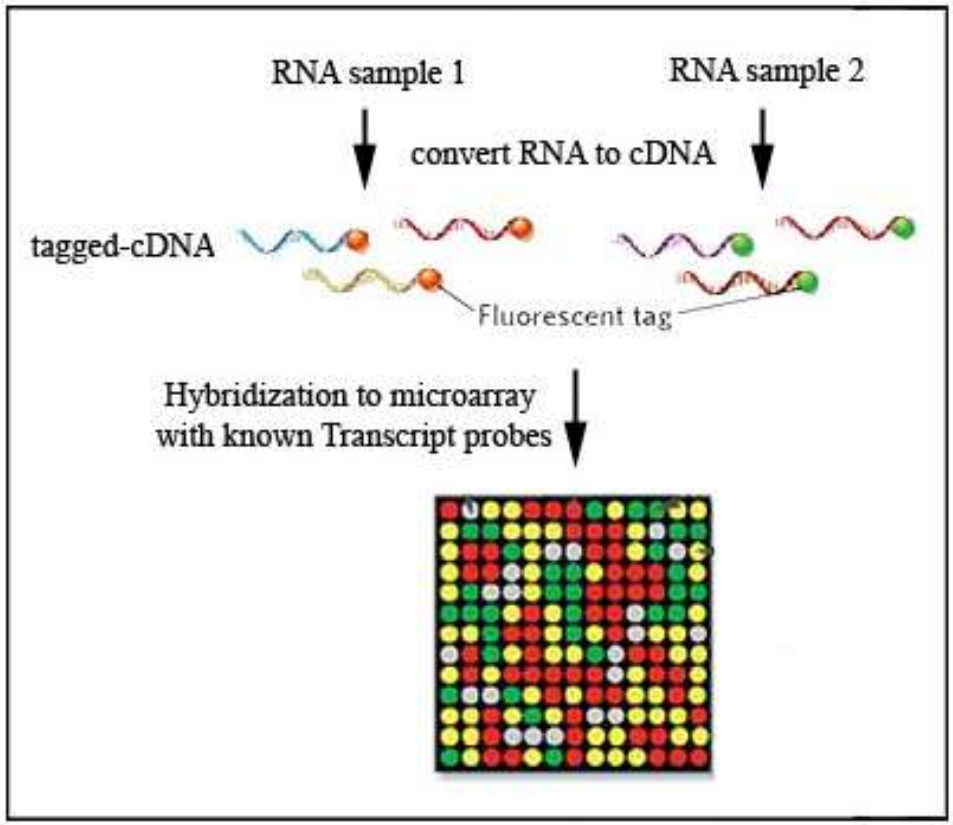
High-troughput screening



Realtime PCR
SYBR green
TaqMan Technology

MICROARRAY
Next Generation Sequencing
Roche 454
Illumina Hi-Seq
Ion Torrent
Nanopore Technologies

Microarray



relative intensity
=
expression levels

Low sensitivity
Low dynamic range
known transcript only
No alternative splicing information
lower cost

Perché RNA-sequencing?

MICROARRAY:

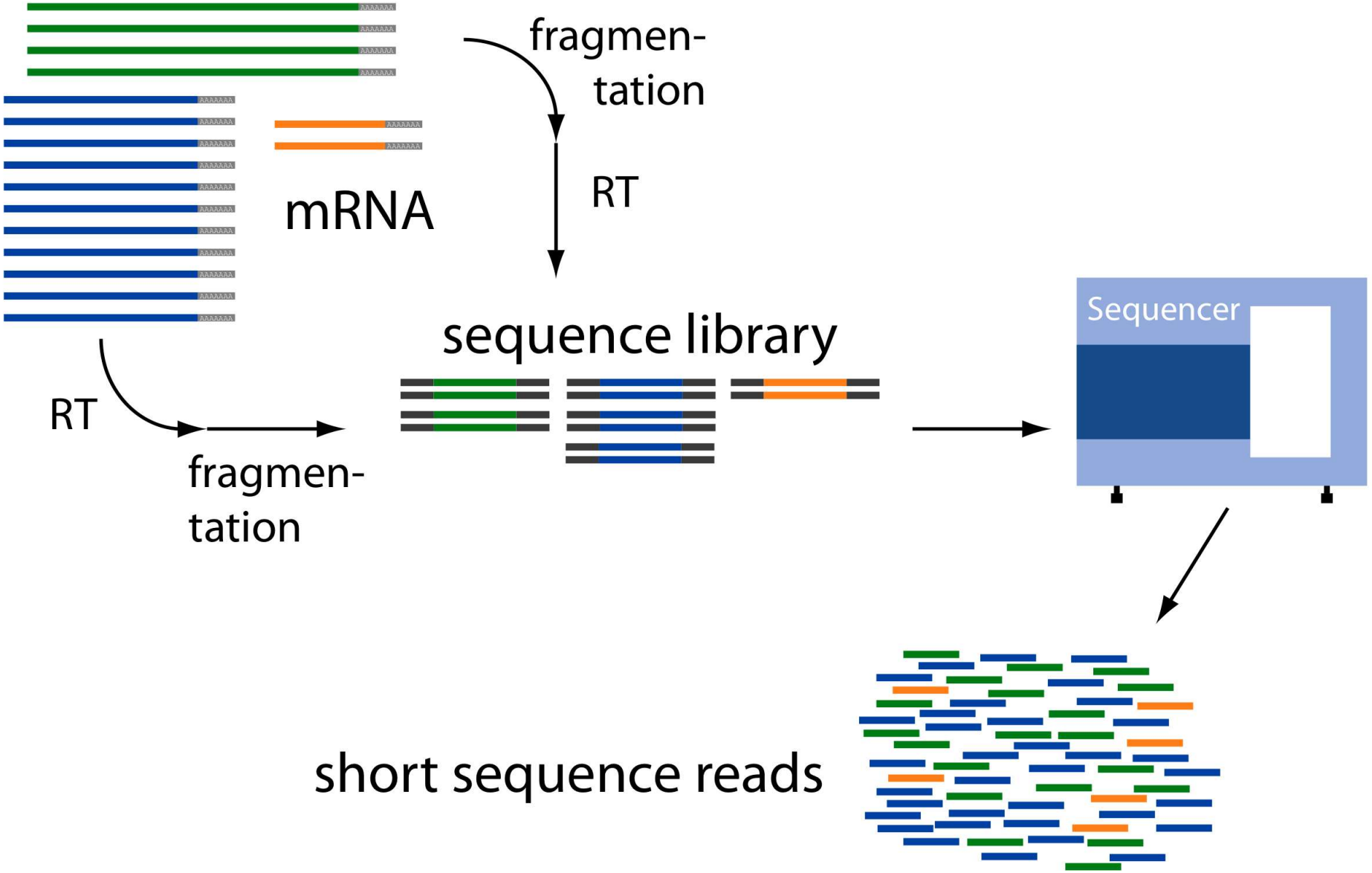
1. Limitato alle seq. spottate sul chip (non ho il trascrittoma completo);
2. Si basa su segnali di ibridazione competitiva strettamente dipendenti da come sono preparate le librerie (variabilità di fondo = scarsa affidabilità statistica);
3. Si basa sull'ibridazione di sonde corte che legano solo una specifica porzione del trascritto quindi non ho un'informazione riguardo alle possibili varianti (es. splicing alternativi).

RNA-seq:

1. Non ho limitazioni, posso analizzare tutte le seq. espresse;
2. Posso discriminare un campione dall'altro, ottengo dei valori di espressione assoluti e direttamente comparabili (direttamente proporzionali al numero di conte = statisticamente affidabile);
3. La mappatura delle reads avviene sul genoma quindi è possibile rilevare le possibili varianti di un singolo trascritto;
4. Maggiori applicazioni-potenzialità-informazioni

I COSTI SONO COMPARABILI

RNA-sequencing: la tecnica

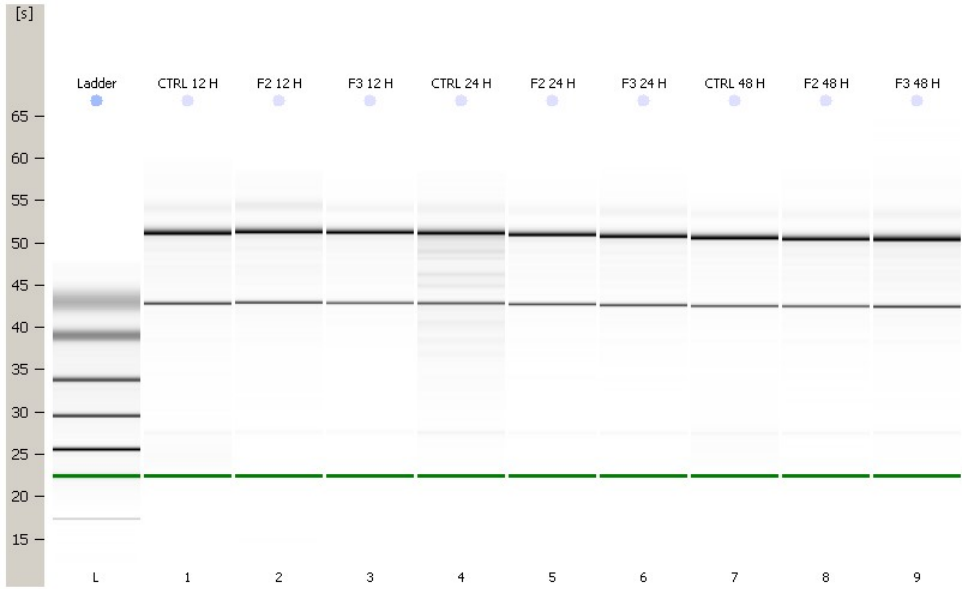


RNA-seq: preparazione campioni

VALUTAZIONE DELLA QUALITA' DELL'RNA MEDIANTE ELETTROFORESI CAPILLARE SU CHIP (BIOANALYZER dell'AGILENT)

Il campione di RNA:

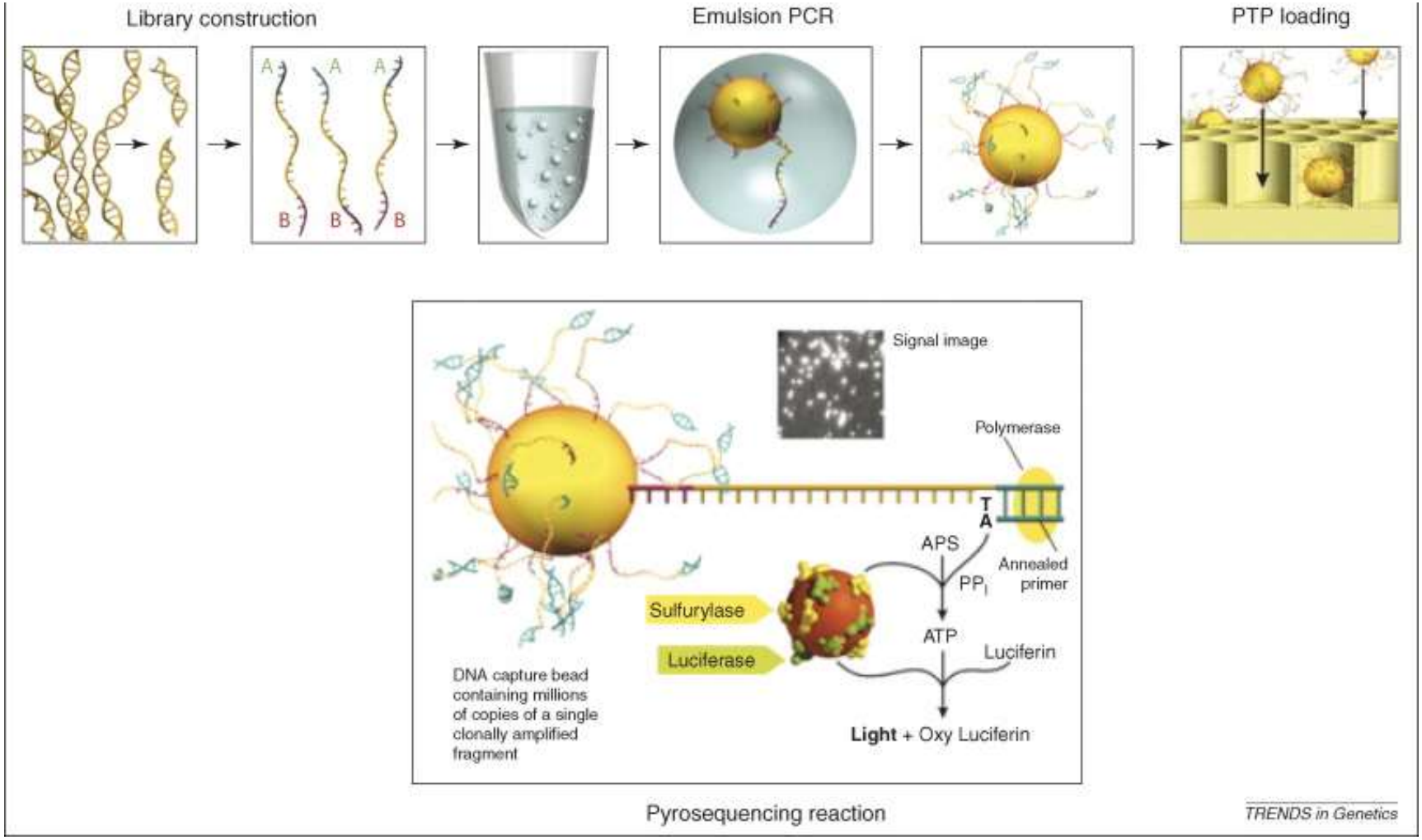
- **non deve essere degradato**
- **deve avere un rapporto A260/A280 compreso tra 1.8 e 2**
- **deve avere un rapporto A260/A230 compreso tra 2 e 2,2**
- **1-2 µg di RNA**



RNA INTEGRITY NUMBER ≥ 7!!!
DATO DAL RAPPORTO DELL'INTENSITA' DELLE 2 BANDE DI rRNA RISPETTO AL RUMORE DI FONDO

NGS platform: Roche 454

Sequencing method: Pyrosequencing
Library amplification method: Emulsion PCR

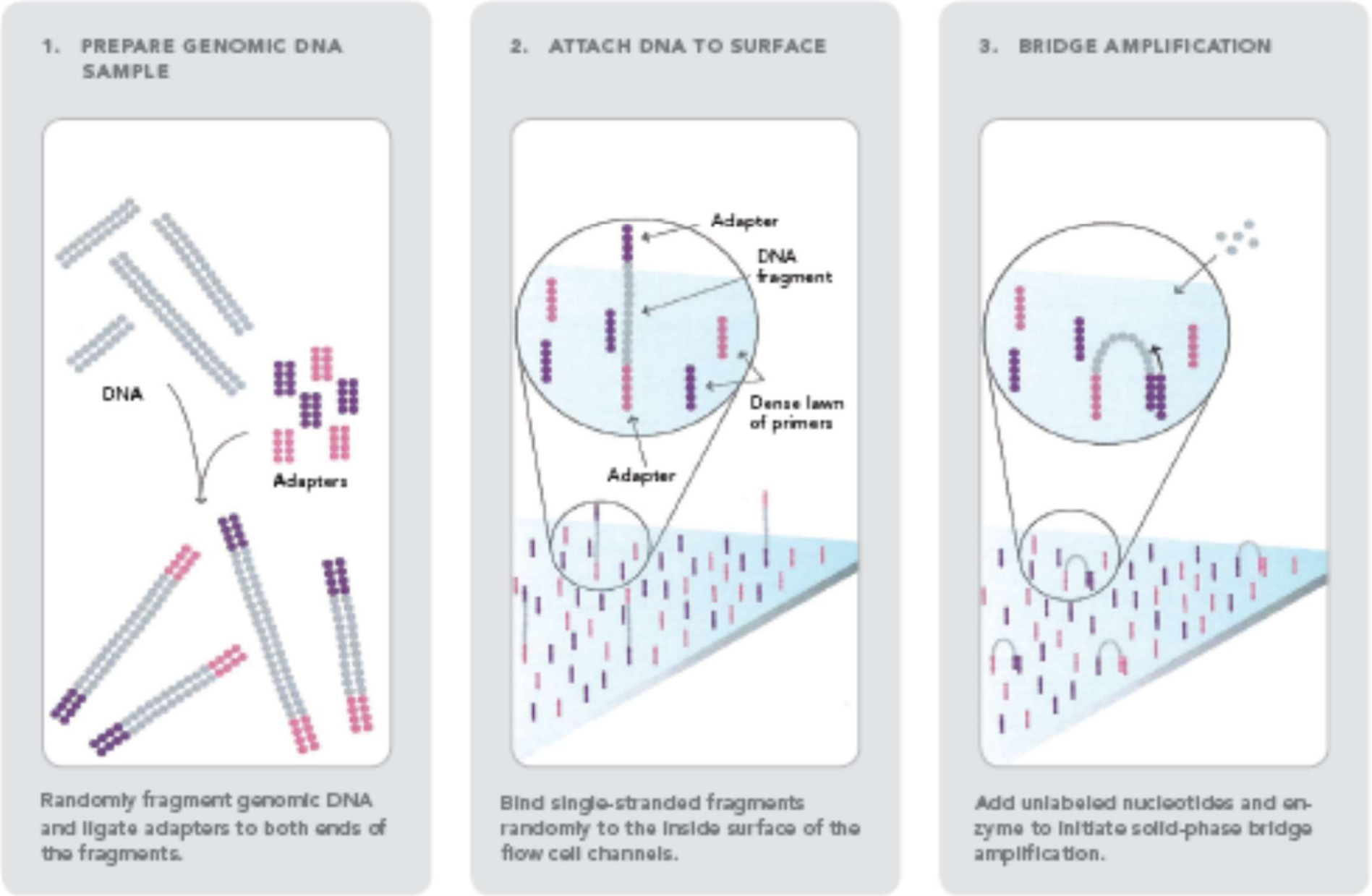


NGS platform: Illumina platform

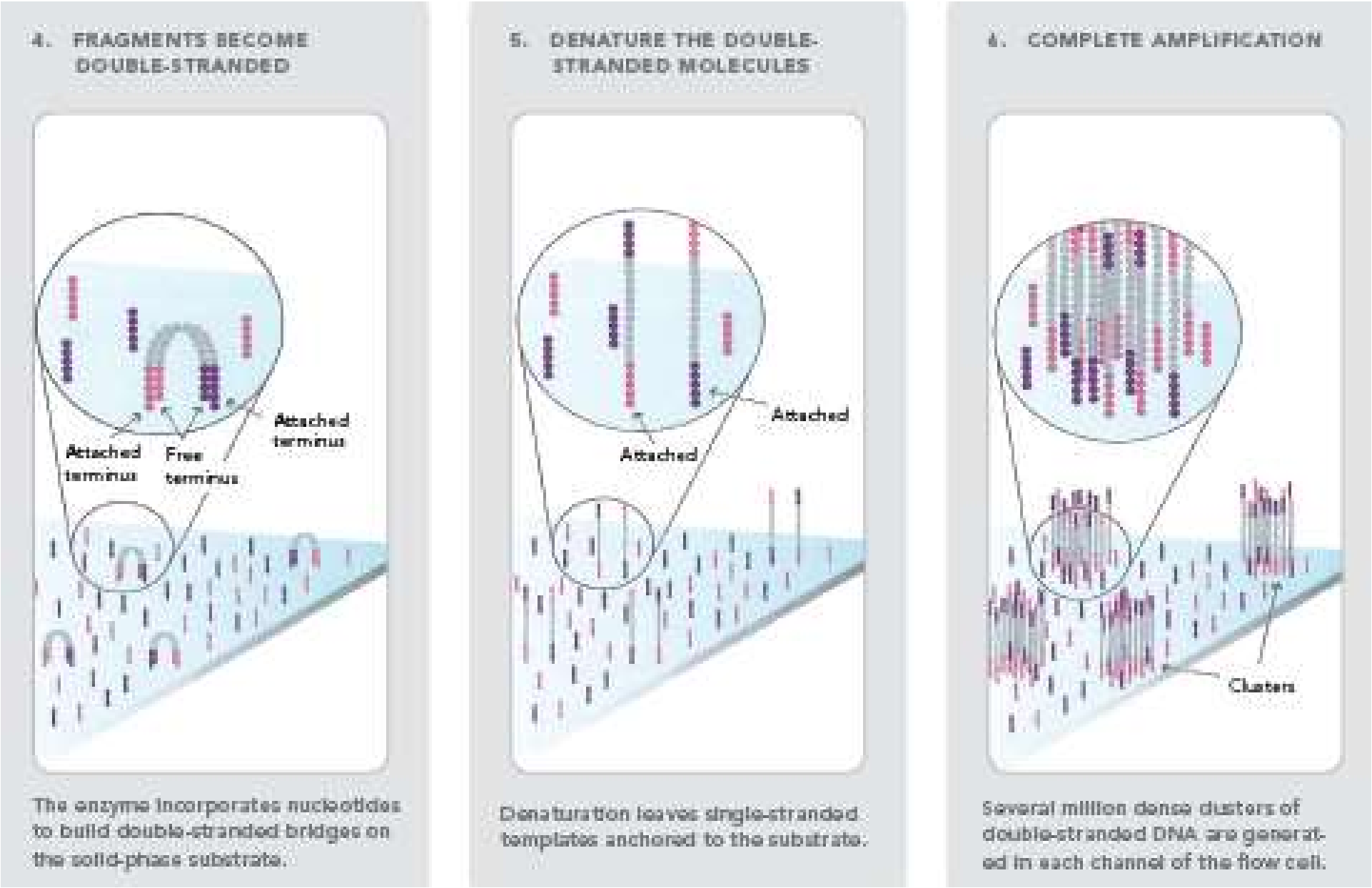
Illumina (Solexa) HiSeQ 2000 Flow Cell

- 1 flow cell
- 8 canali
- Ogni canale può correre fino a 12 differenti librerie (Multiplexed Sequencing).
- Input richiesto: 0.1–1.0 µg (single- and paired-end reads), 10 µg (Mate Pair reads).
- 1.4-mm larghezza del canale.
- Si basa sul legame casuale di frammenti di oligo DNA fissati sul vetrino, una superficie ottica trasparente (flow cell).
- 96-120 milioni di reads (clusters) per lane

NGS platform: Illumina platform

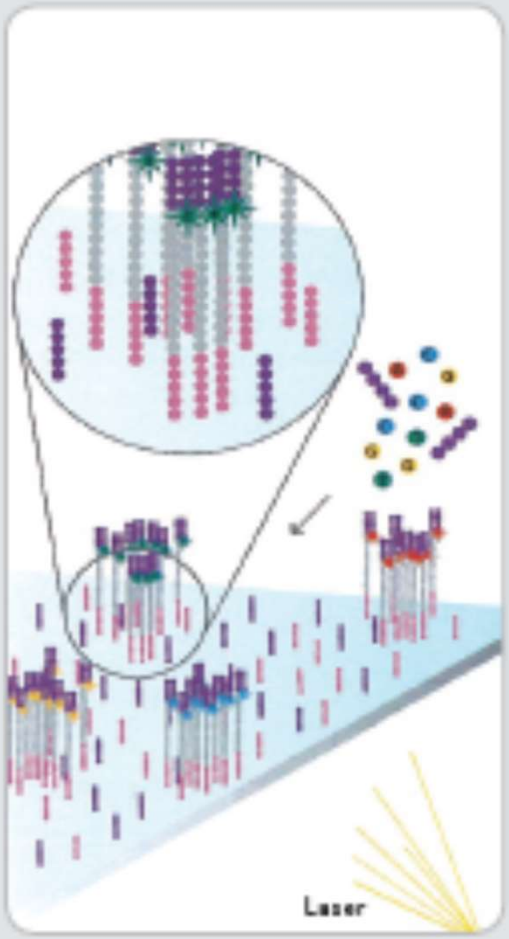


NGS platform: Illumina platform



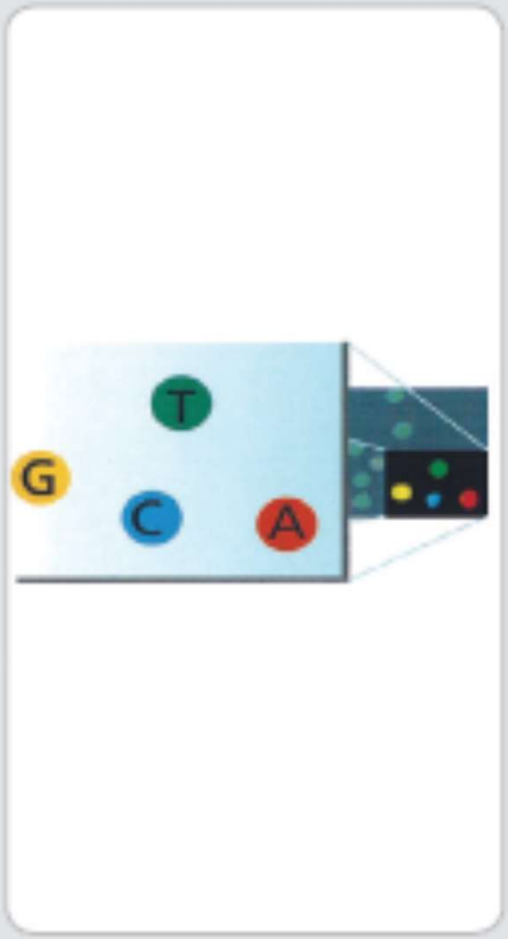
NGS platform: Illumina platform

7. DETERMINE FIRST BASE



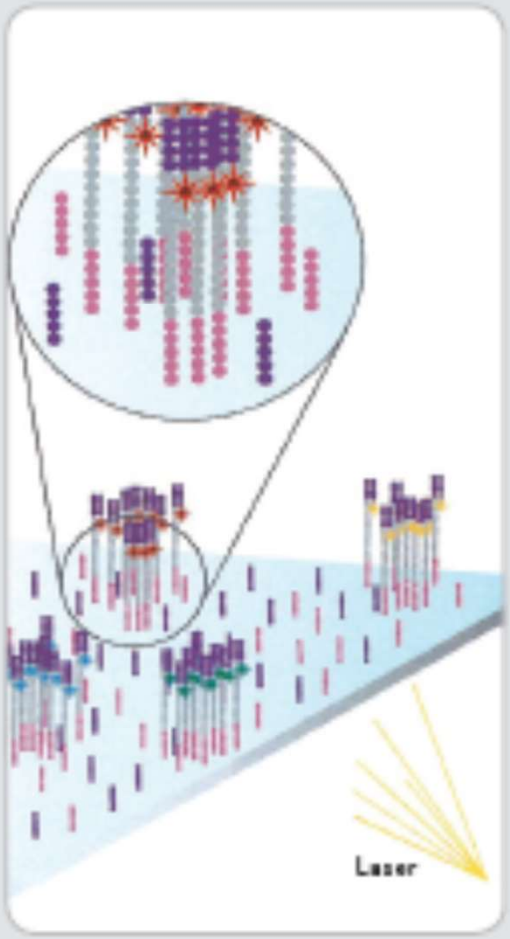
The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

8. IMAGE FIRST BASE



After laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified.

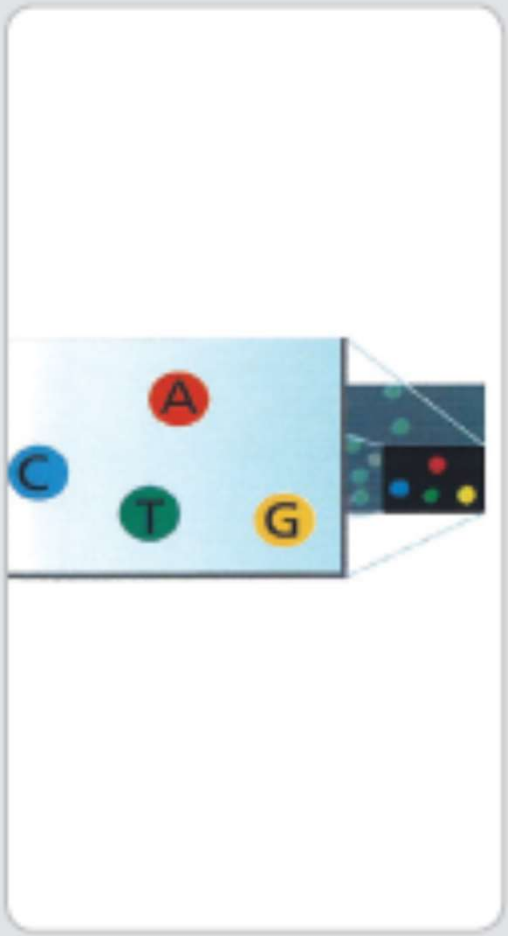
9. DETERMINE SECOND BASE



The next cycle repeats the incorporation of four labeled reversible terminators, primers, and DNA polymerase.

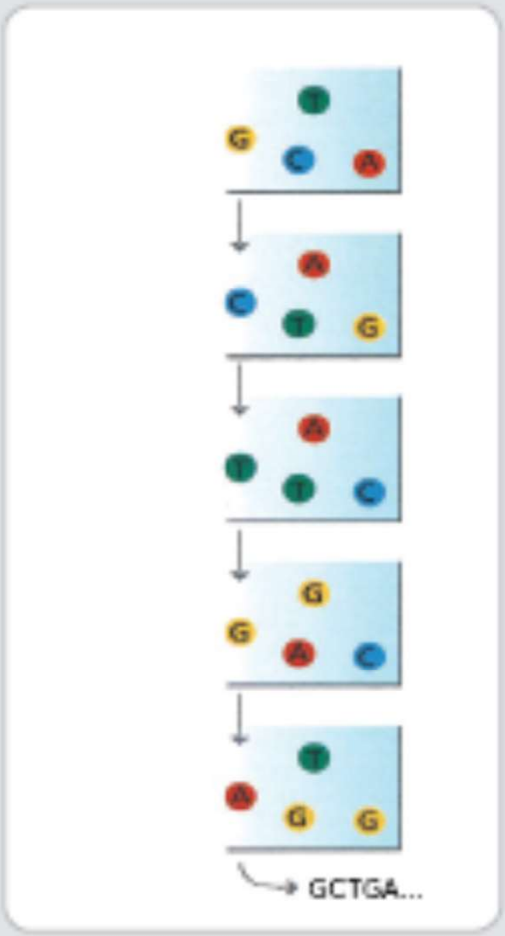
Sequencing Technology Overview

10. IMAGE SECOND CHEMISTRY CYCLE



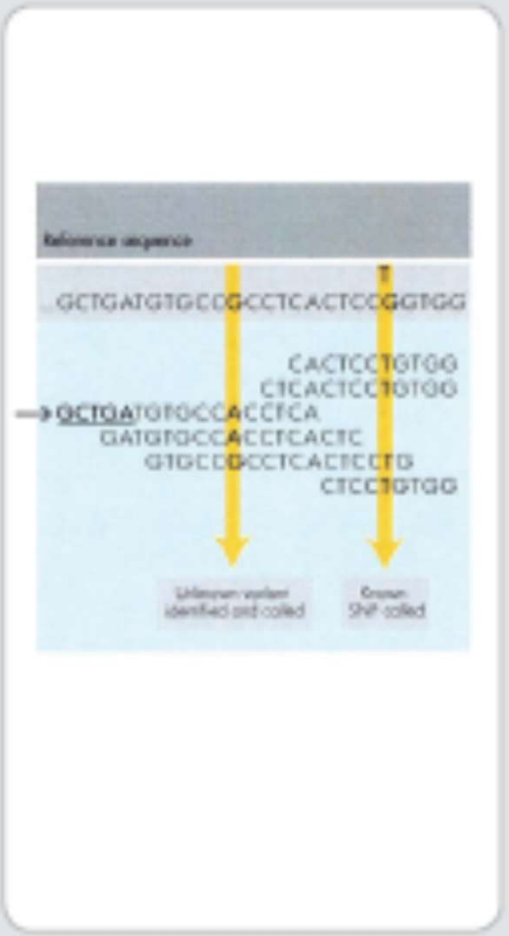
After laser excitation, the image is captured as before, and the identity of the second base is recorded.

11. SEQUENCING OVER MULTIPLE CHEMISTRY CYCLES



The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

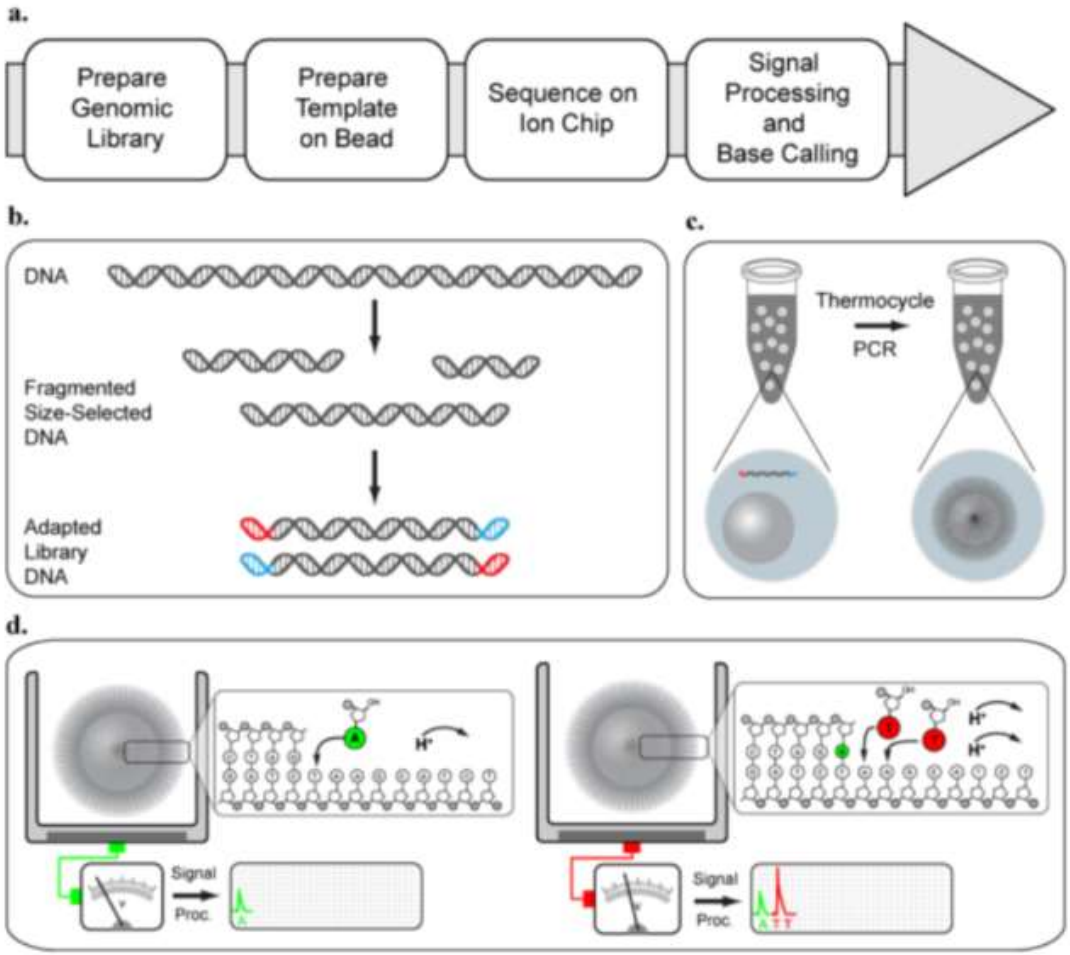
12. ALIGN DATA

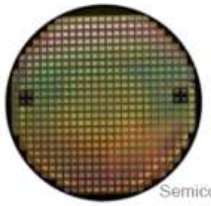


The data are aligned and compared to a reference, and sequencing differences are identified.

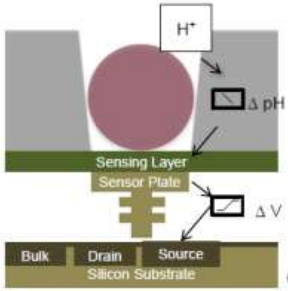
NGS platform: Ion Torrent platform

Sequencing method: Ion semiconductor
Library amplification method: Emulsion PCR

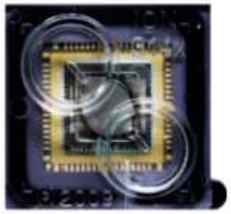




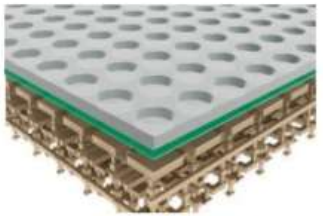
Wafer
Semiconductor Manufacturing



Single Sensor
Chemical to Digital Sequence



Chip
Semiconductor Packaging

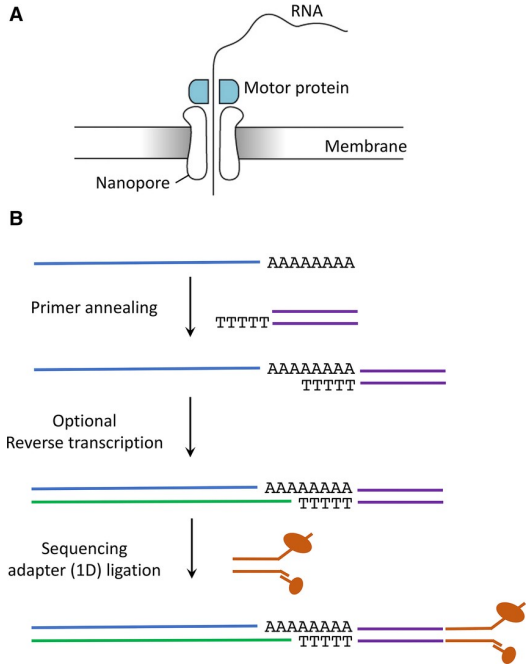


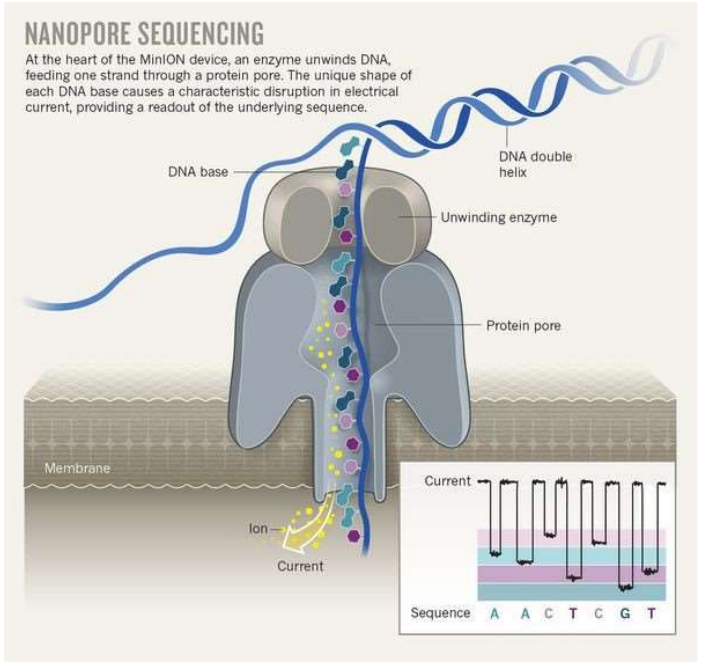
Millions of Sensors
Semiconductor Design

NGS platform: Nanopore Technologies

The technology produces full-length transcripts!
Direct RNA sequencing

A protein nanopore is set in an electrically resistant polymer membrane. An ionic current is passed through the nanopore by setting a voltage across this membrane. If an analyte passes through the pore or near its aperture, this event creates a characteristic disruption in current. Measurement of that current makes it possible to identify the molecule in question.





A strand of RNA is passed through a nanopore. The current is changed as the bases G, A, T and C pass through the pore in different combinations!

NGS platform: Nanopore Technologies

MinION

- Pocket-sized, portable device for biological analysis
- Up to 512 nanopore channels
- Simple 10-min sample prep available
- Real-time analysis for rapid, efficient workflows
- Adaptable to direct DNA or RNA sequencing
- MinIT available to support IT/software needs

Choose MinION if you:

- would like access to sequencing for $1,000
- want to sequence immediately, not wait
- want to sequence outside a lab
- need 10–20Gb per 48 hrs
- want to avoid CapEx investments.

Coming soon

SmidgION

- Designed to be our smallest sequencing device so far
- Same nanopore sensing technology as MinION and PromethION
- Designed for use with a smartphone in any location

RNA-seq: analisi dei dati

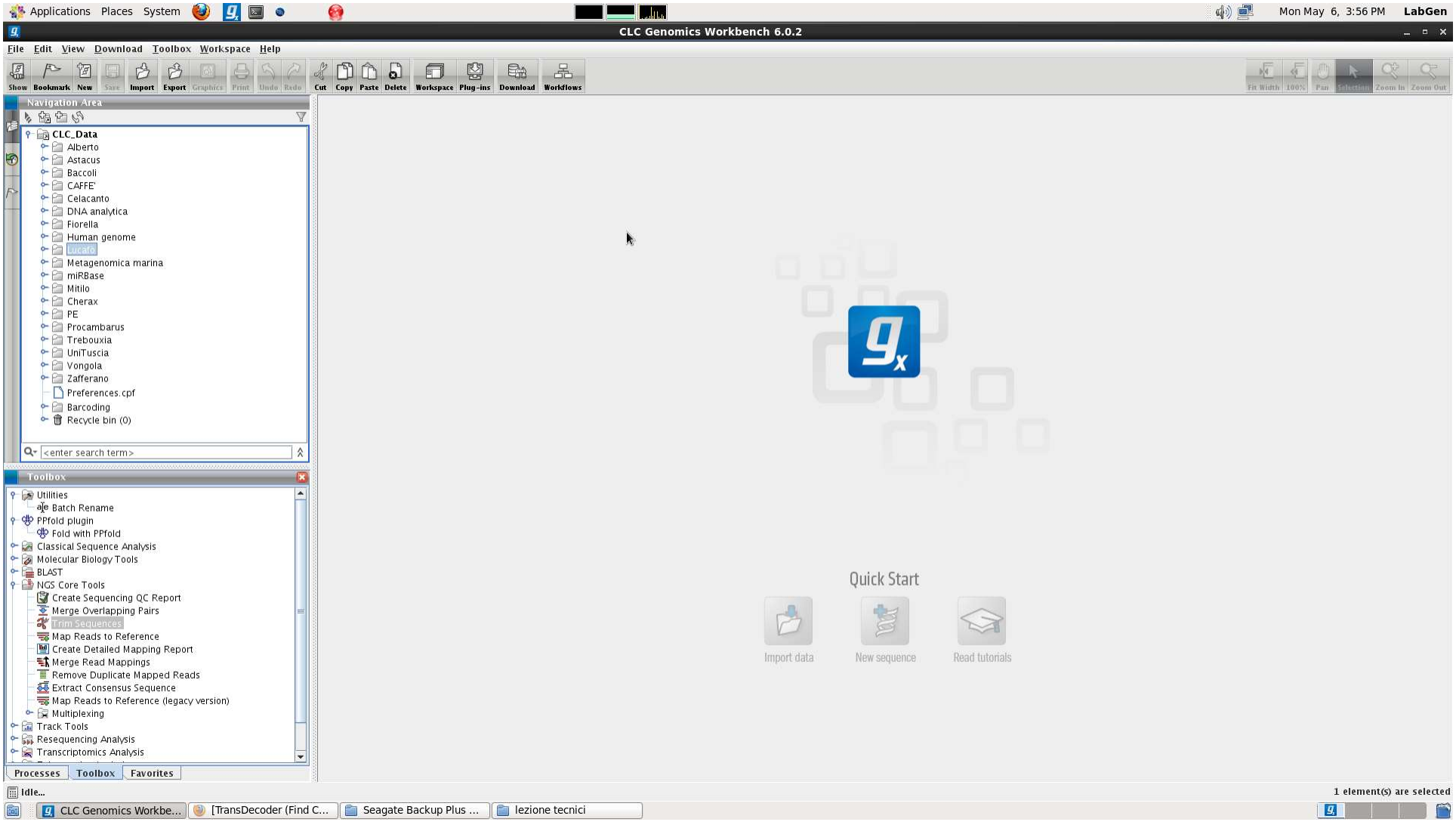
- Sequenza fornita in output dal sequenziatore = READ
- Formato dei file di output: FASTQ;
- Software: CLC Genomics Workbench (A comprehensive and user-friendly analysis package for analyzing, comparing, and visualizing next generation sequencing data)

TAPPE PRINCIPALI:

1) TRIMMING
2) ALLINEAMENTO (MAPPAGGIO)
3) CONTA DELLE READS
4) ANALISI STATISTICA
5) ANALISI SECONDARIE

CLC Genomics Workbench

TRIMMING



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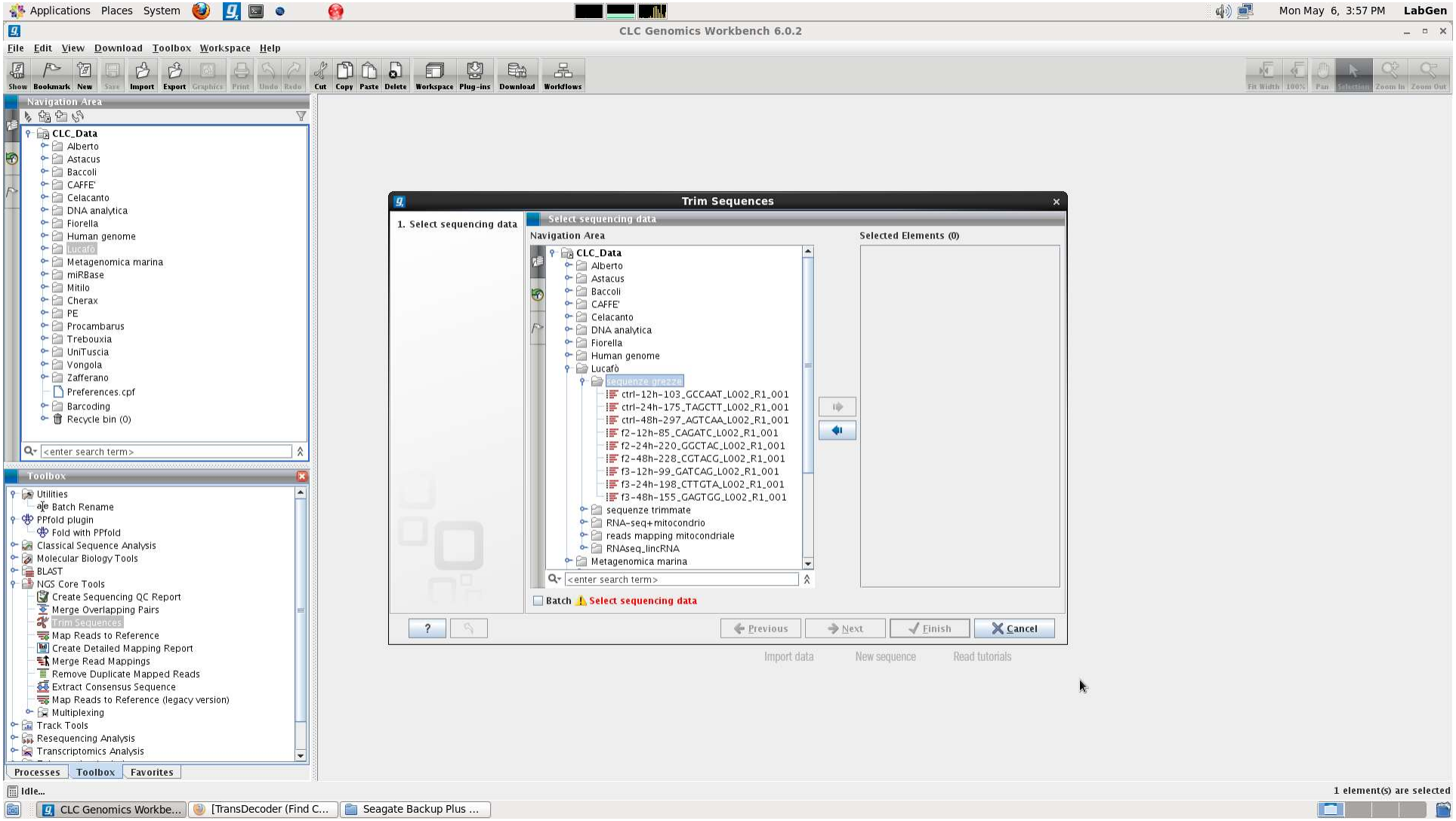
Applications Places System
Mon May 6, 3:57 PM LabGen
CLC Genomics Workbench 6.0.2
File Edit View Download Toolbox Workspace Help
Show Bookmark New Save Import Export Graphics Print Undo Redo Cut Copy Paste Delete Workspace Plug-ins Download Workflows
Navigation Area
CLC_Data
Alberto
Astacus
Baccoli
CAFFE'
Celacanto
DNA analytica
Fiorella
Human genome
Lucafò
Metagenomica marina
miRBase
Mitilo
Cherax
PE
Procambarus
Trebouxia
UniTuscia
Vongola
Zafferano
Preferences.cpf
Barcoding
Recycle bin (0)
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Toolbox
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Fold with PPfold
Classical Sequence Analysis
Molecular Biology Tools
BLAST
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Remove Duplicate Mapped Reads
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Multiplexing
Track Tools
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Trim Sequences
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DNA analytica
Fiorella
Human genome
Lucafò
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ctrl-12h-103_GCCAAT_L002_R1_001
ctrl-24h-175_TAGCTT_L002_R1_001
ctrl-48h-297_AGTCAA_L002_R1_001
f2-12h-85_CAGATC_L002_R1_001
f2-24h-220_GGCTAC_L002_R1_001
f2-48h-228_CGTACG_L002_R1_001
f3-12h-99_GATCAG_L002_R1_001
f3-24h-198_CTTGTA_L002_R1_001
f3-48h-155_GAGTGG_L002_R1_001
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Metagenomica marina
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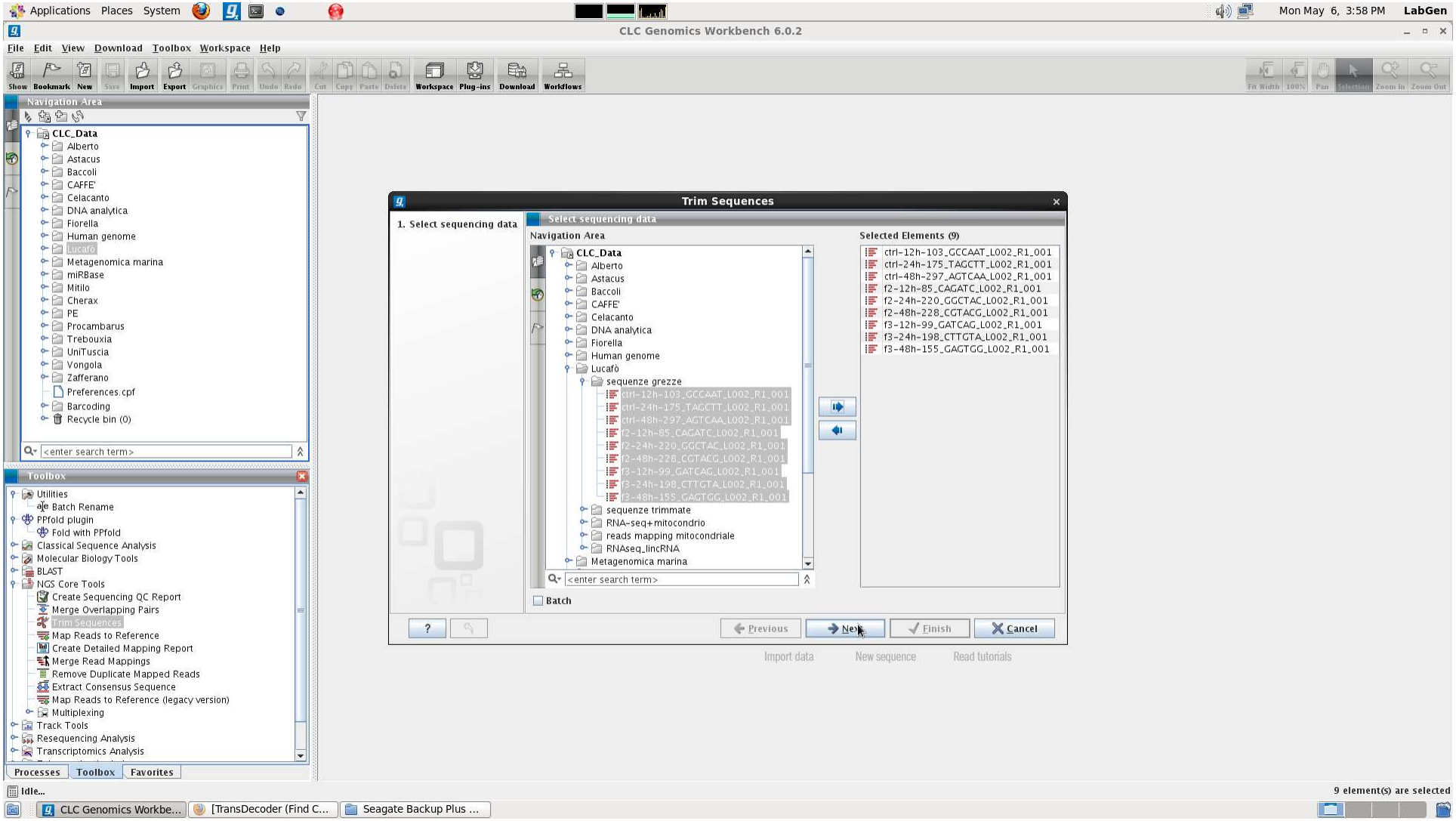
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reads mapping mitocondriale
RNAseq_lincRNA
Metagenomica marina
<enter search term>
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ctrl-24h-175_TAGCTT_L002_R1_001
ctrl-48h-297_AGTCAA_L002_R1_001
f2-12h-85_CAGATC_L002_R1_001
f2-24h-220_GGCTAC_L002_R1_001
f2-48h-228_CGTACG_L002_R1_001
f3-12h-99_GATCAG_L002_R1_001
f3-24h-198_CTTGTA_L002_R1_001
f3-48h-155_GAGTGG_L002_R1_001
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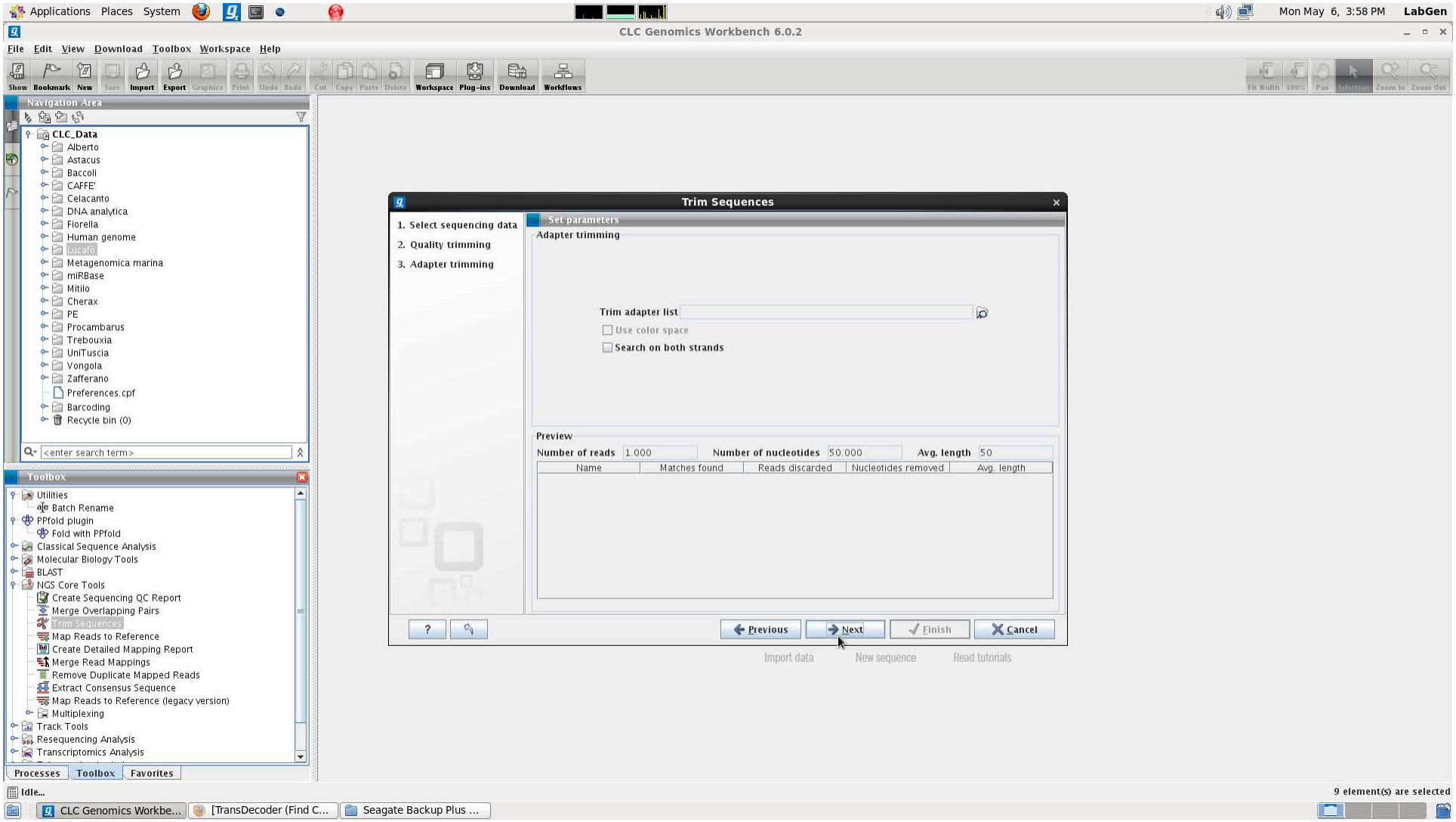
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2. Quality trimming
3. Adapter trimming
Set parameters
Adapter trimming
Trim adapter list
Use color space
Search on both strands
Preview
Number of reads 1.000
Number of nucleotides 50.000
Avg. length 50
Name
Matches found
Reads discarded
Nucleotides removed
Avg. length
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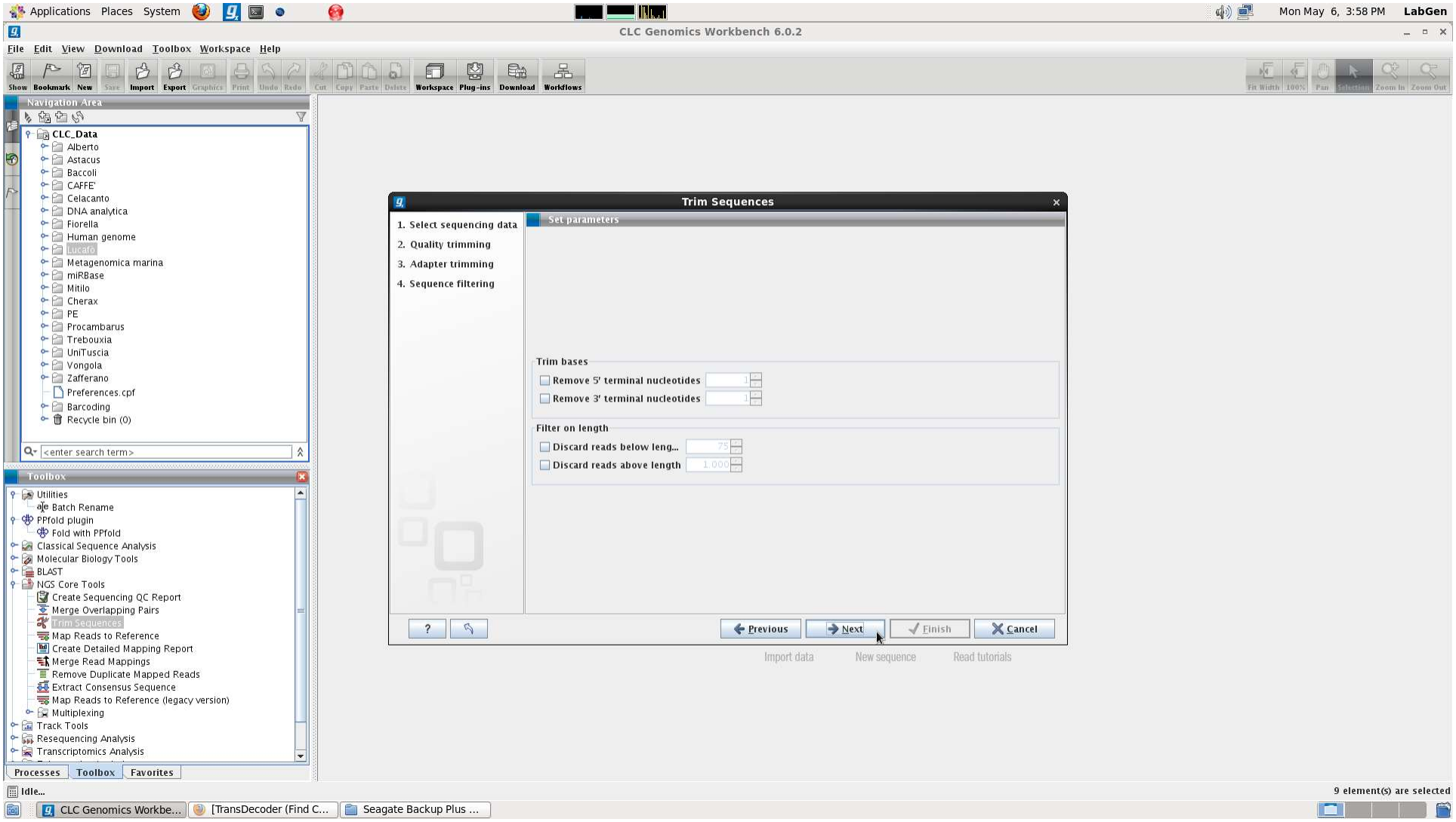
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2. Quality trimming
3. Adapter trimming
4. Sequence filtering
Set parameters
Trim bases
Remove 5' terminal nucleotides
Remove 3' terminal nucleotides
Filter on length
Discard reads below leng...
Discard reads above length
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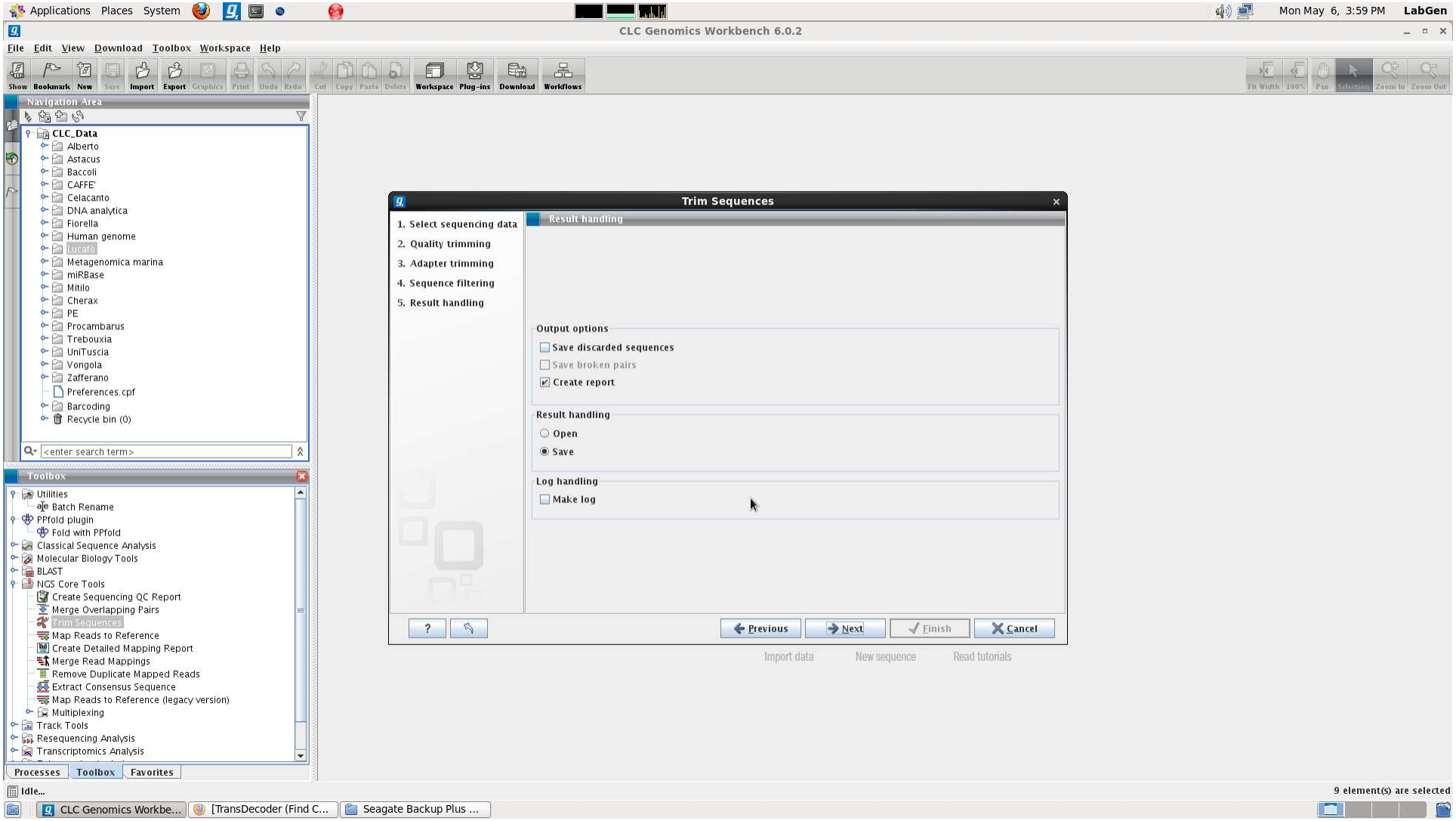
TRIMMING


Applications Places System
Mon May 6, 3:59 PM LabGen
CLC Genomics Workbench 6.0.2
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Fit Width 100% Pan Selection Zoom In Zoom Out
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3. Adapter trimming
4. Sequence filtering
5. Result handling
Result handling
Output options
Save discarded sequences
Save broken pairs
Create report
Result handling
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Save
Log handling
Make log
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9 element(s) are selected
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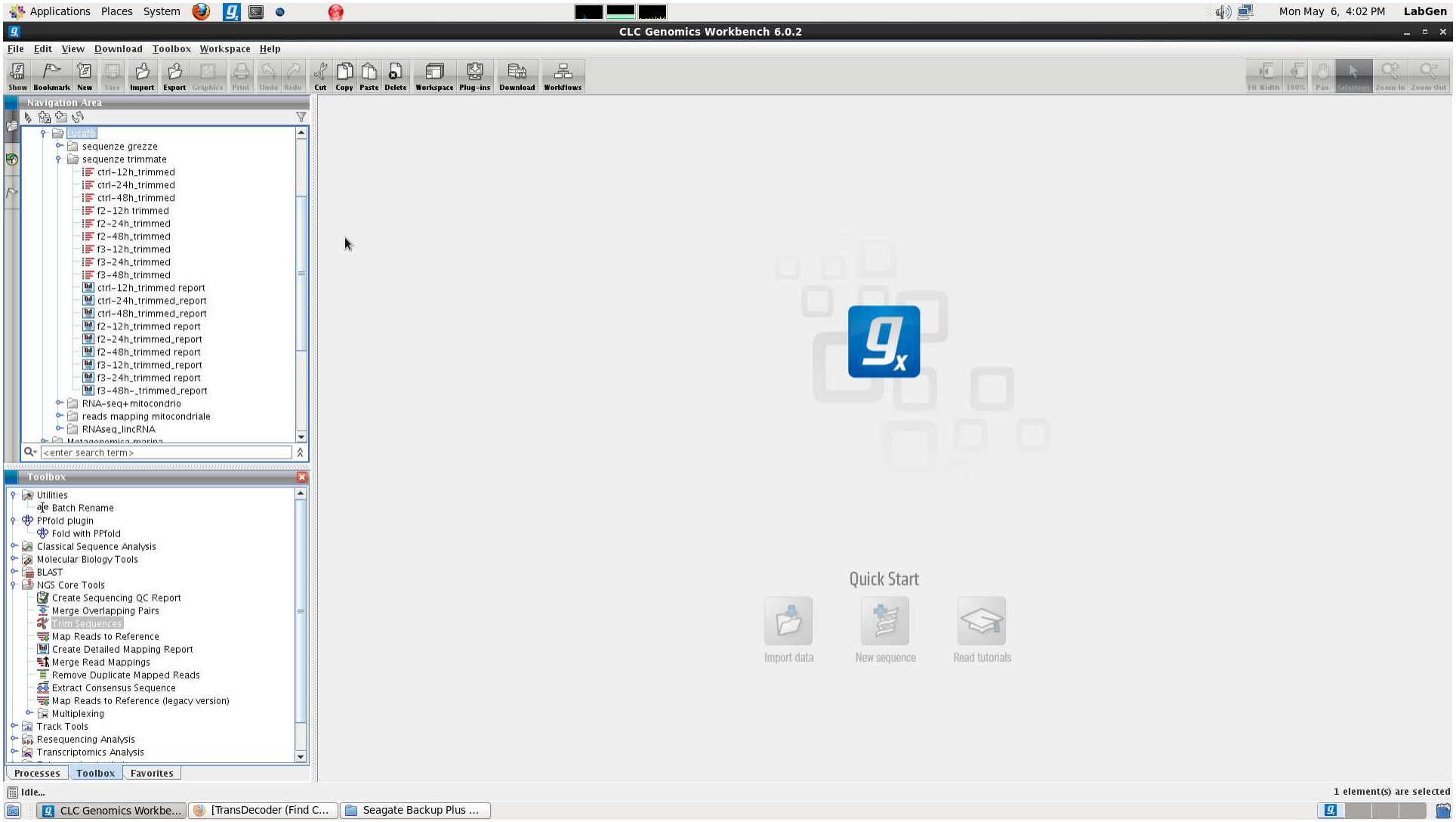
Seagate Backup Plus ...

TRIMMING


Applications Places System
Mon May 6, 4:02 PM LabGen
CLC Genomics Workbench 6.0.2
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Show Bookmark New Save Import Export Graphics Print Undo Redo Cut Copy Paste Delete Workspace Plug-ins Download Workflows
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sequenze trimmate
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ctrl-24h_trimmed
ctrl-48h_trimmed
f2-12h trimmed
f2-24h_trimmed
f2-48h_trimmed
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f3-24h_trimmed
f3-48h_trimmed
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ctrl-24h_trimmed_report
ctrl-48h_trimmed_report
f2-12h_trimmed report
f2-24h_trimmed_report
f2-48h_trimmed report
f3-12h_trimmed_report
f3-24h_trimmed report
f3-48h-_trimmed_report
RNA-seq+mitocondrio
reads mapping mitocondriale
RNAseq_lincRNA
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Transcriptomics Analysis
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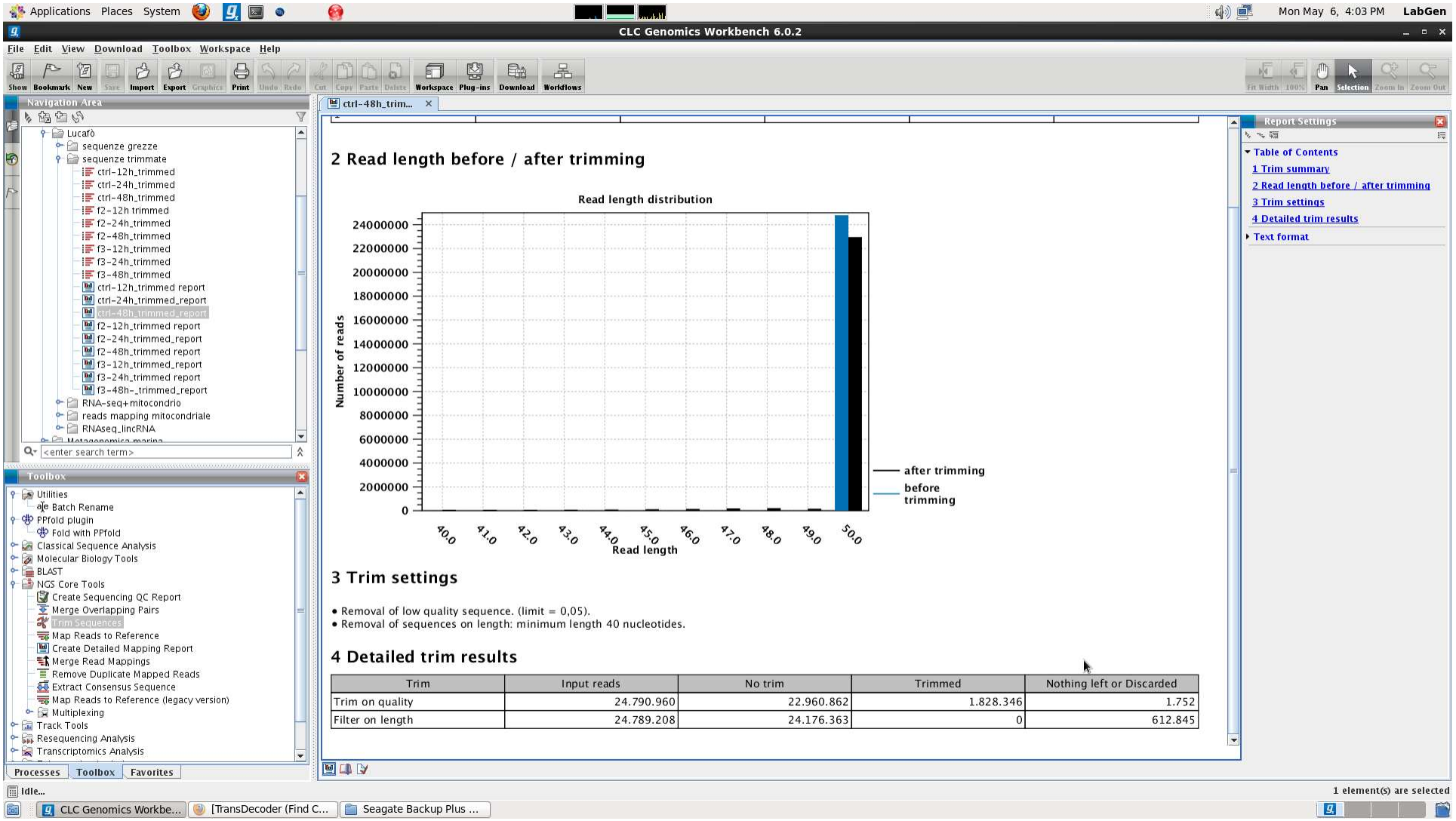
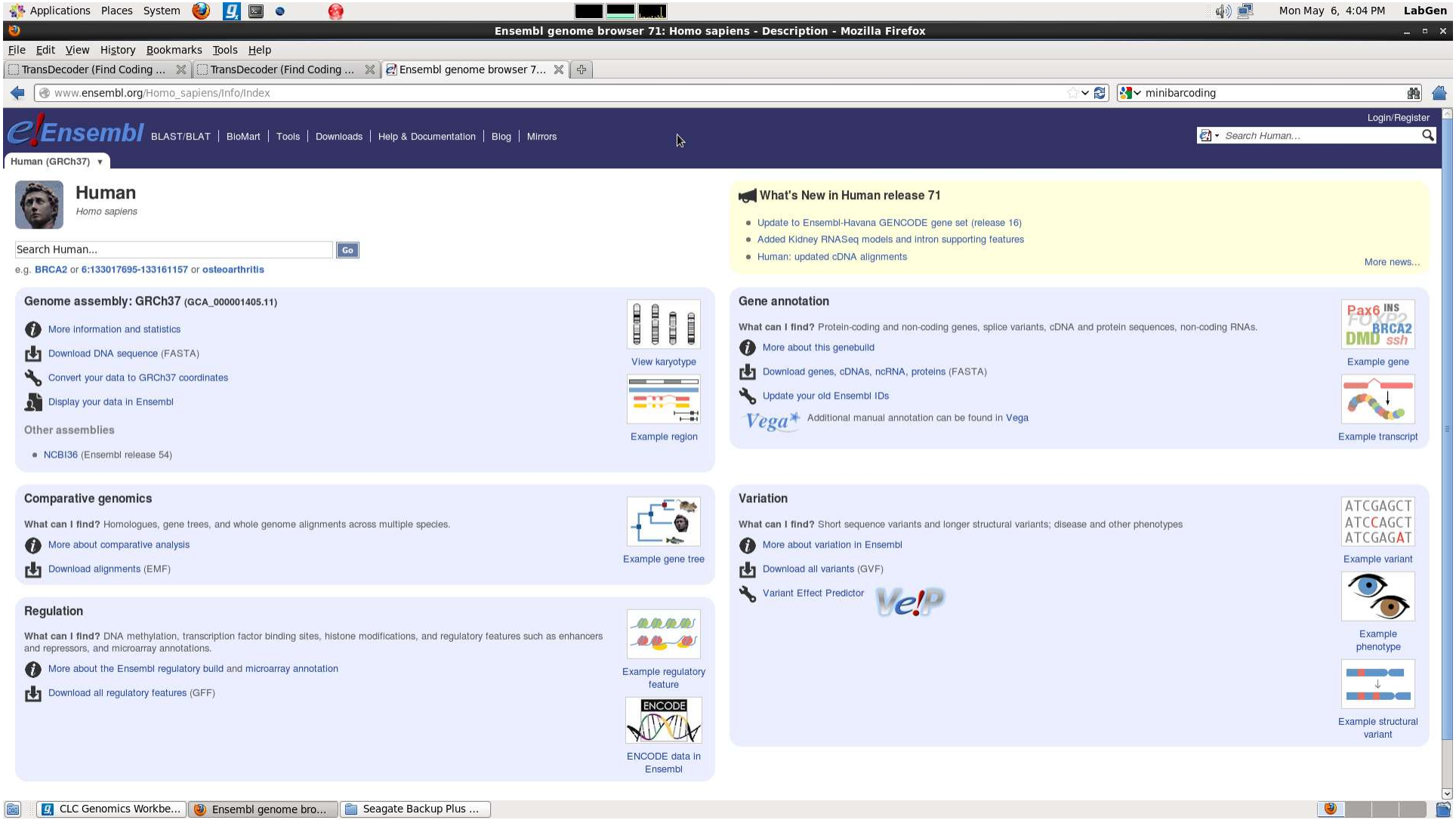
Quick Start
Import data
New sequence
Read tutorials
Idle...
1 element(s) are selected
CLC Genomics Workbe...
[TransDecoder (Find C...
Seagate Backup Plus ...

TRIMMING: report finale

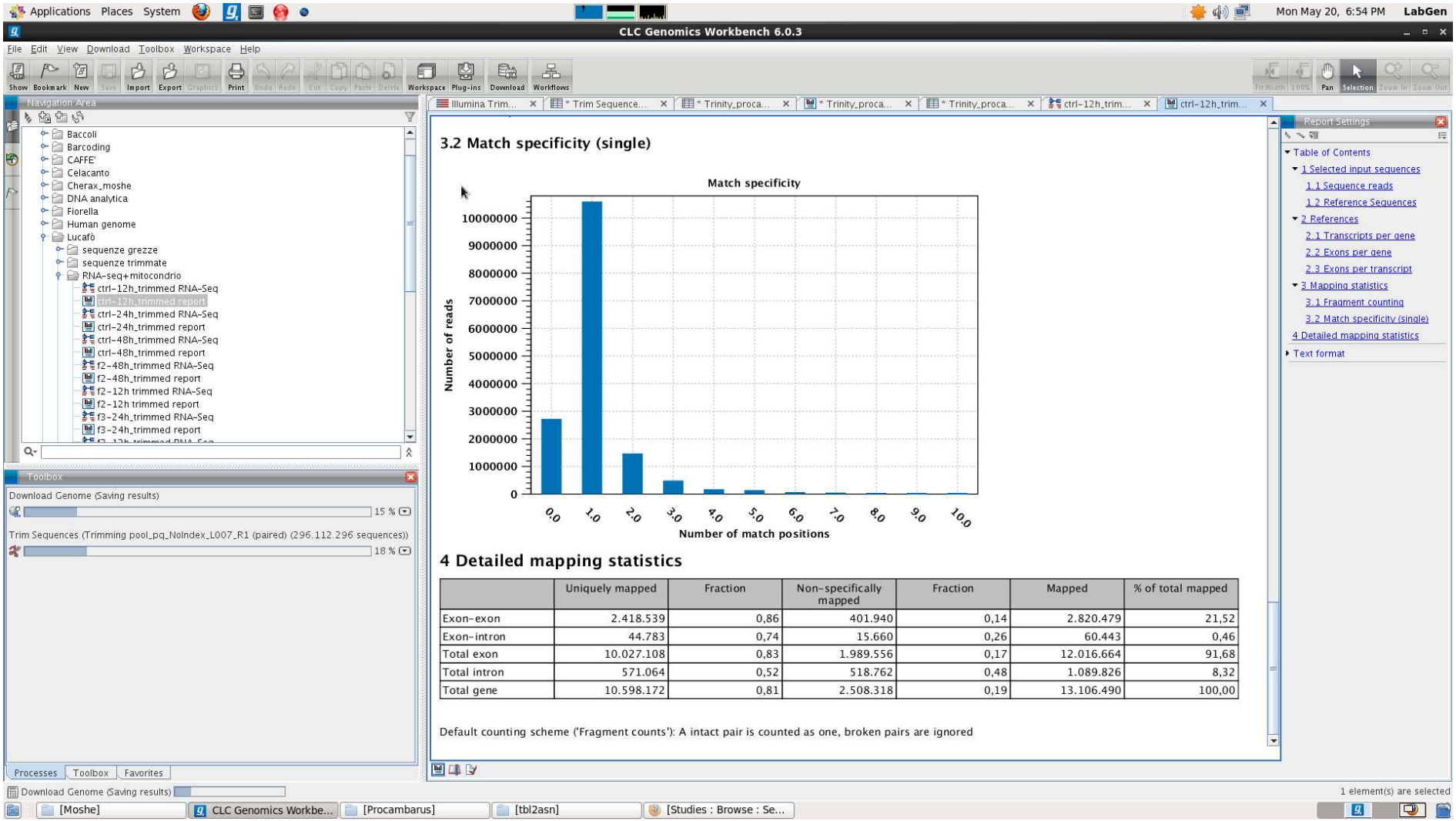


Trim	Input reads	No trim	Trimmed	Nothing left or Discarded
Trim on quality	24.790.960	22.960.862	1.828.346	1.752
Filter on length	24.789.208	24.176.363	0	612.845

2) ALLINEAMENTO

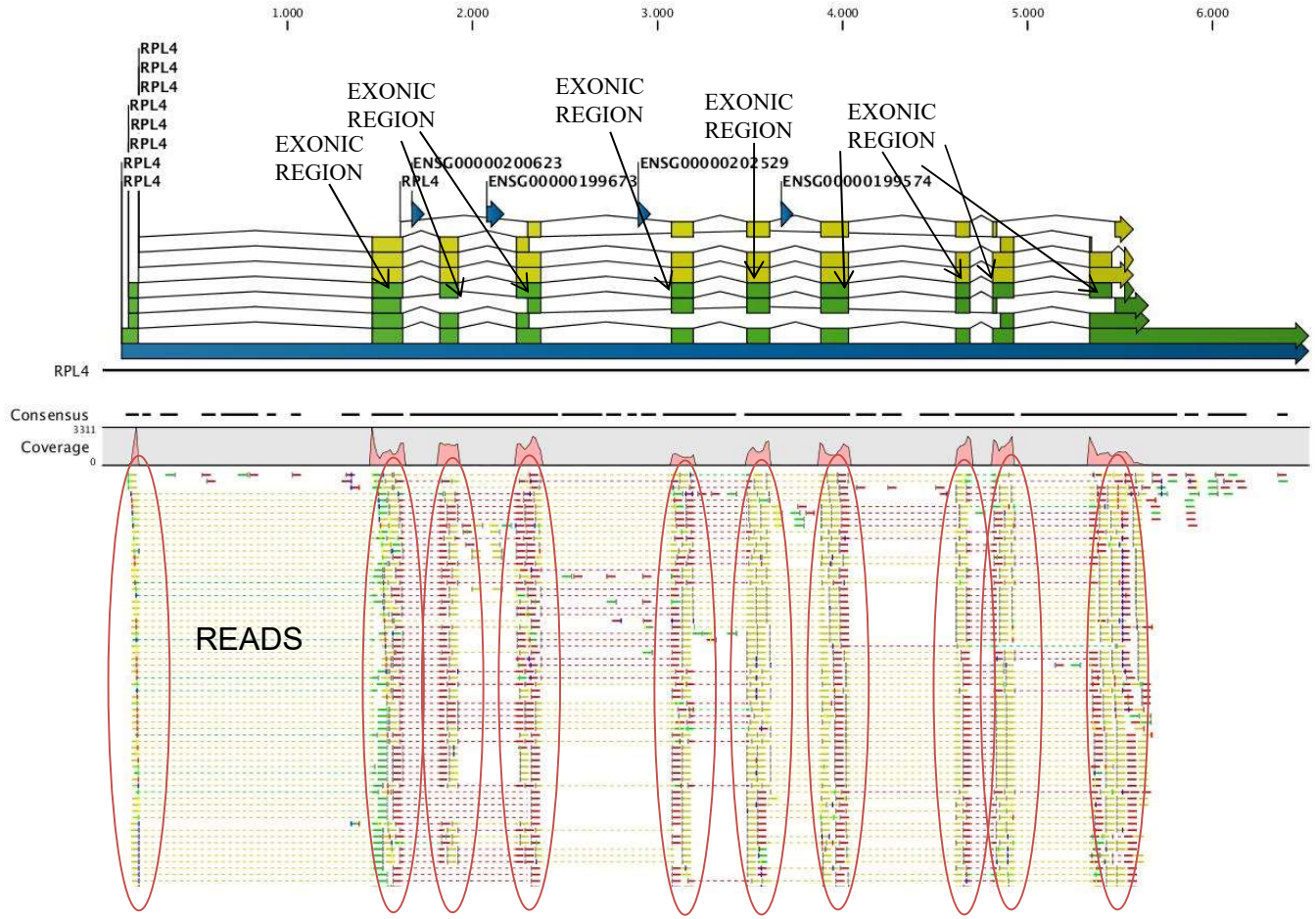


2) ALLINEAMENTO



	Uniquely mapped	Fraction	Non-specifically mapped	Fraction	Mapped	% of total mapped
Exon-exon	2.418.539	0,86	401.940	0,14	2.820.479	21,52
Exon-intron	44.783	0,74	15.660	0,26	60.443	0,46
Total exon	10.027.108	0,83	1.989.556	0,17	12.016.664	91,68
Total intron	571.064	0,52	518.762	0,48	1.089.826	8,32
Total gene	10.598.172	0,81	2.508.318	0,19	13.106.490	100,00

2) ALLINEAMENTO



Read: Sequenza fornita in output dal sequenziatore, che identifica l'ordine in cui si susseguono le basi nei frammenti di cDNA. Il numero di basi che la compongono rappresenta la sua lunghezza (misurata in nt o bp).

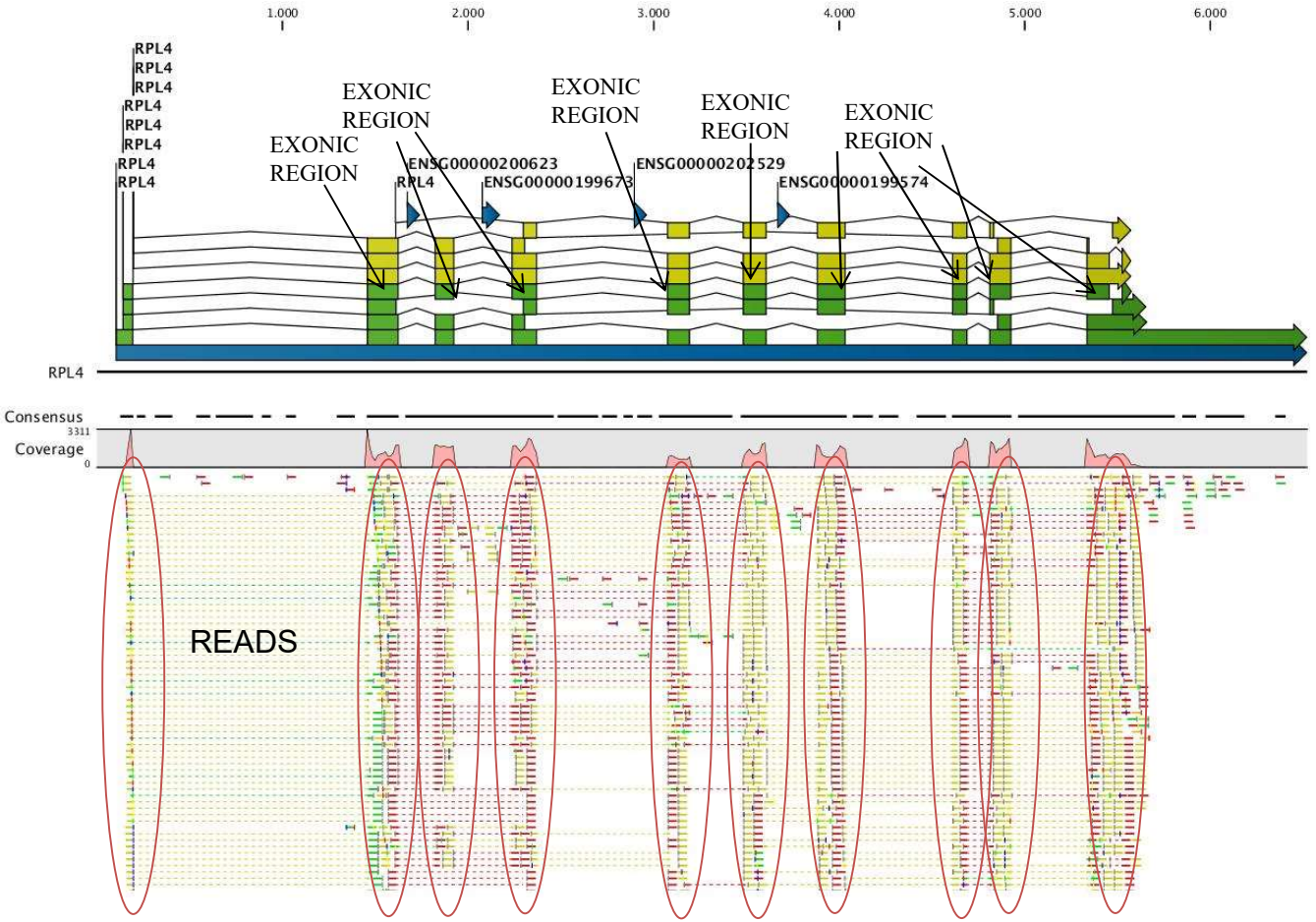
3) CONTA

Numero di read mappate su una feature biologica di interesse (gene, trascritto, esone). Il numero di reads è proporzionale all'abbondanza della feature, quindi del suo livello di espressione!

RPKM (Reads Per Kilobase per Million mapped reads) :

Indice del livello di espressione

RPKM= total read/read mappate[milioni]*lungh. gene [kb]

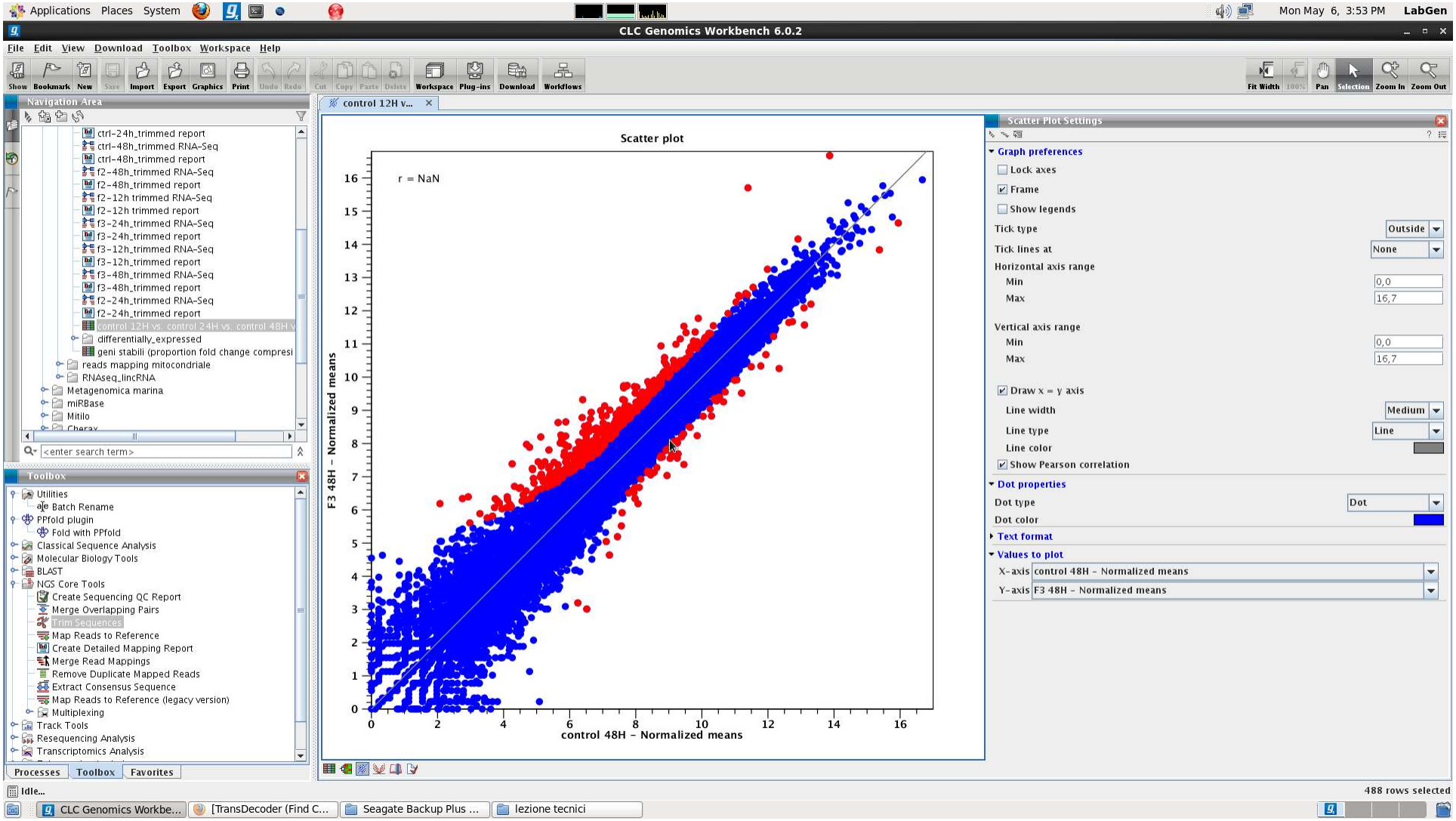


4) ANALISI STATISTICA:
Kal's Z-test (cutoff e-value di 1e-10)

Feature ID	Proportions fold change	FDR p-value correction
RP5-1182A14.1	-1,46	1,19E-12
MLL2	-1,37	0
IFRD1	-1,33	1,48E-12
SEC61A1	-1,32	1,19E-12
DST	-1,28	1,44E-12
MLL3	-1,28	2,02E-12
UBR4	-1,27	0
UGGT1	-1,27	5,78E-13
DHX9	-1,27	0
BCLAF1	-1,26	1,44E-12
DNAJC10	-1,26	1,30E-11
POLR1A	-1,26	2,50E-12
CREBBP	-1,25	1,44E-12
RANBP2	-1,25	0
PRPF19	-1,25	0
HSP90AA1	-1,25	0
TFF1	-1,24	1,84E-12
CCDC47	-1,23	1,57E-12
NPM1	-1,23	8,24E-13
SLC26A2	-1,23	0
HYOU1	-1,22	0
DDX21	-1,22	0
HSPA5	-1,22	0
HSPH1	-1,22	1,57E-12
UTP20	-1,22	7,44E-12
ANKRD11	-1,21	3,15E-12
MKI67	-1,2	0
AMAC1L3	-1,2	2,24E-12
SON	-1,19	0
SRRT	-1,19	1,73E-12
TMED9	-1,19	2,80E-11
SRCAP	-1,19	3,13E-12
PTMA	-1,18	7,48E-13
MTDH	-1,18	0

Feature ID	Proportions fold change	FDR p-value correction
ATP7B	2	3,65E-13
COX1	2	0
VEGFB	2	2,72E-13
SYT15	2,01	1,89E-11
GABBR1	2,01	8,80E-14
AC010336.1	2,02	0
TMPRSS13	2,03	0
RAB31	2,03	1,85E-13
SYT7	2,03	1,31E-12
GBAS	2,04	0
BRWD3	2,05	6,49E-13
EHD2	2,05	0
AF042090.2	2,06	2,11E-13
GADD45B	2,06	7,06E-11
RNR1	2,07	0
MOSPD3	2,08	4,42E-12
ACSF2	2,08	1,85E-13
ID2	2,09	0
TMC4	2,09	0
NCRNA00263	2,11	2,09E-11
EPOR	2,14	1,27E-12
SELENBP1	2,14	1,03E-12
ZNF704	2,15	0
CRELD1	2,15	4,29E-11
ATG16L2	2,16	0
AHNAK	2,17	0
CKB	2,17	1,45E-11
HMGCL	2,19	2,52E-12
INPP5J	2,21	0
SLC2A10	2,22	5,63E-11
DDAH2	2,22	5,31E-13
LTBP4	2,23	0
PYROXD2	2,23	4,74E-14

SCATTER PLOT



5) ANALISI SECONDARIE: CARATTERIZZAZIONE ONTOLOGICA

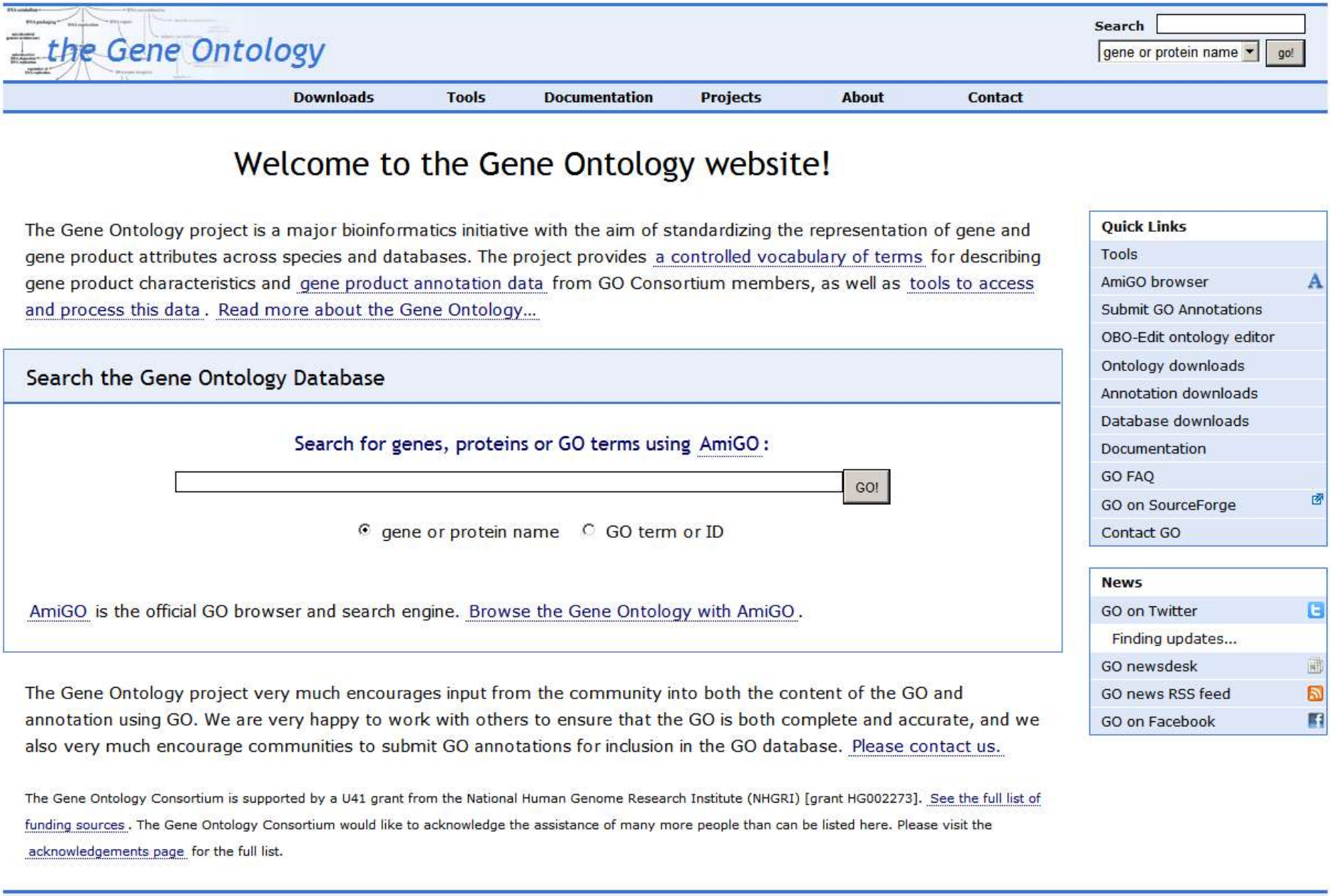
DAL DATO GREZZO AL SIGNIFICATO BIOLOGICO

Il progetto Gene Ontology fornisce una ontologia di termini definiti che rappresentano le proprietà dei prodotti dei geni
L'ontologia è suddivisa in tre ambiti:

- *Molecular function*;
- *Biological process;*
- *Cellular component*;

http://www.geneontology.org/

http://www.geneontology.org/



5) ANALISI SECONDARIE: CARATTERIZZAZIONE ONTOLOGICA

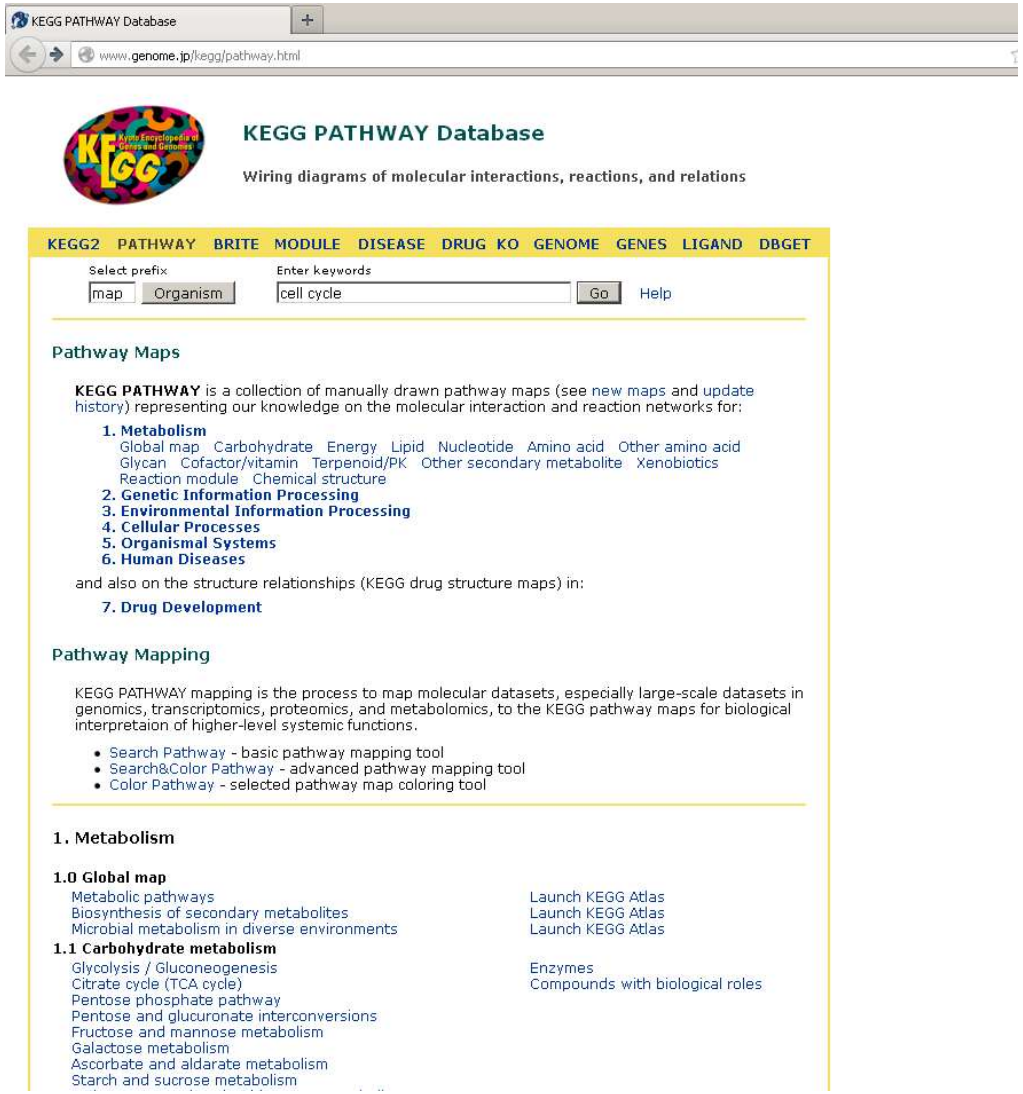
DAL DATO GREZZO AL SIGNIFICATO BIOLOGICO

GO term	Biological Process	F2 - 12H	F2 - 24H	F2 - 48H	F3 - 12H	F3 - 24H	F3 - 48H
75	cell cycle checkpoint (Reactome:REACT_1538 [TAS])	NS	NS	**	****	***	****
82	G1/S transition of mitotic cell cycle (PMID:8681378 [TAS])	NS	NS	**	***	***	****
84	S phase of mitotic cell cycle (PMID:21196493 [IMP])	NS	NS	**	****	***	****
85	G2 phase of mitotic cell cycle (PMID:9154802 [TAS])	NS	NS	*	*	*	*
86	G2/M transition of mitotic cell cycle (Reactome:REACT_2203 [TAS])	NS	NS	NS	NS	*	*
122	negative regulation of transcription from RNA polymerase II promoter (PMID:10973986 [IDA])	NS	NS	NS	NS	NS	*

GO term	Molecular Function	F2 - 12H	F2 - 24H	F2 - 48H	F3 - 12H	F3 - 24H	F3 - 48H
3723	RNA binding (PMID:3323886 [TAS])	**	**	**	***	***	***
4003	ATP-dependent DNA helicase activity (GO_REF:0000002 [IEA] InterPro:IPR000212)	NS	*	NS	NS	*	**
4004	ATP-dependent RNA helicase activity (PMID:7610041 [TAS])	*	*	*	**	*	**
5515	protein binding (PMID:12681488 [IPI] UniProtKB:Q7Z465)	****	****	***	***	***	***
5524	ATP binding (GO_REF:0000037 [IEA] UniProtKB-KW:KW-0067)	*	**	***	***	***	****
5525	GTP binding (PMID:17107948 [IDA])	NS	*	NS	*	NS	*

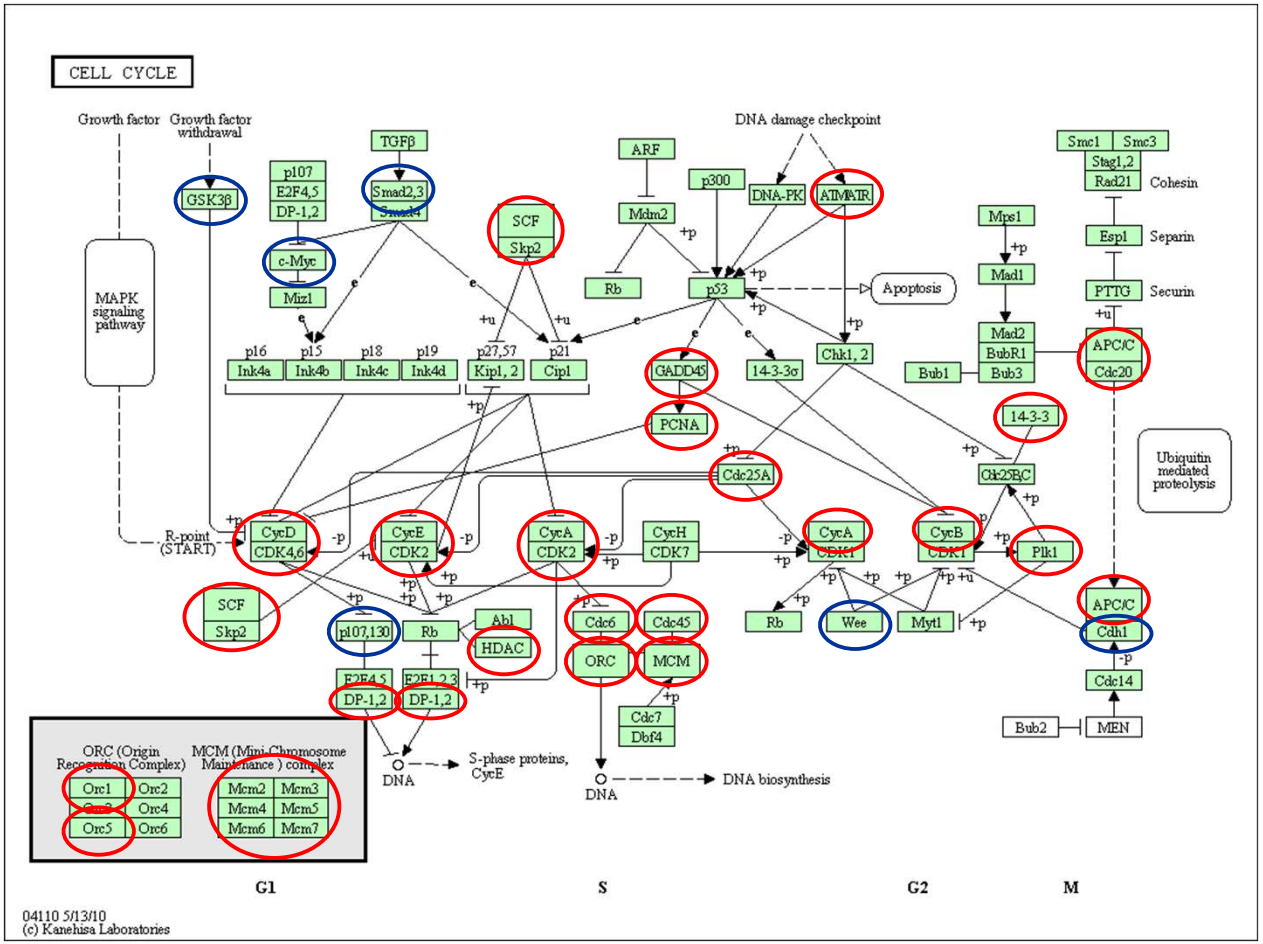
GO term	Cellular Compartment	F2 - 12H	F2 - 24H	F2 - 48H	F3 - 12H	F3 - 24H	F3 - 48H
502	proteasome complex (PMID:8811196 [TAS])	*	*	**	****	****	***
5605	basal lamina (GO_REF:0000019 [IEA] Ensembl:ENSMUSP00000005532)	NS	NS	*	**	*	NS
5606	laminin-1 complex (PMID:10964500 [NAS])	NS	NS	NS	**	NS	NS
5634	nucleus (PMID:19188445 [IDA])	*	NS	**	***	**	***
5641	nuclear envelope lumen (PMID:8245774 [IDA])	NS	*	NS	**	**	**
5643	nuclear pore (PMID:11024021 [NAS])	NS	NS	*	**	*	**

5) ANALISI SECONDARIE: PATHWAY ALTERATE



http://www.genome.jp/kegg/pathway.html

5) ANALISI SECONDARIE: PATHWAY ALTERATE



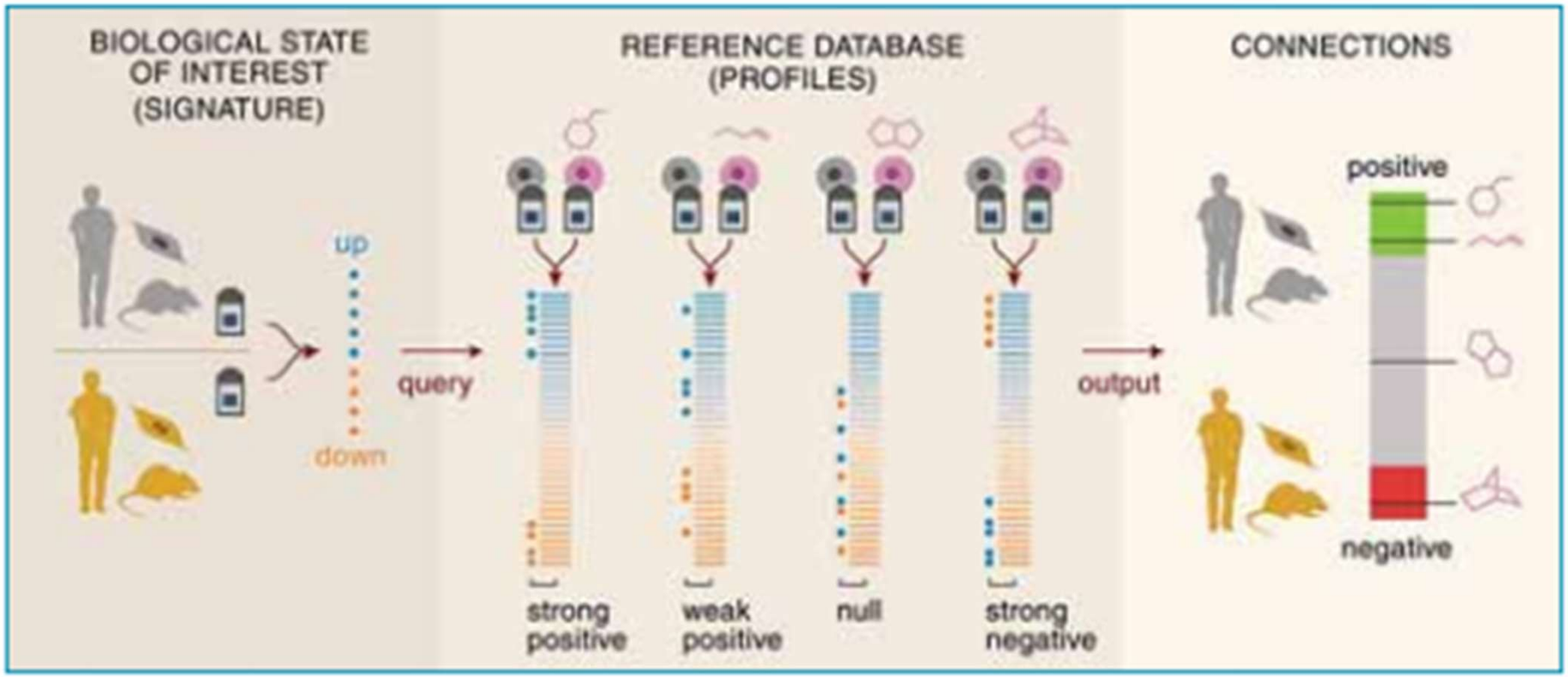
Down regulated genes

Up regulated genes

http://www.genome.jp/kegg/pathway.html

5) ANALISI SECONDARIE: CONNECTIVITY MAP

http://www.broadinstitute.org/cmap/



5) ANALISI SECONDARIE: CONNECTIVITY MAP

http://www.broadinstitute.org/cmap/

CMAP NAME	SPECIFITY	P-VALUE					
		F2 - 12 H	**F2** - 24 H	**F2** - 48 H	**F3** - 12 H	**F3** - 24 H	**F3** - 48 H
Wortmannin	**	ns	0	0	0	0	0
Trichostatin A	ns	ns	0	0	0	0	0
Quinostatin	*	ns	0,00404	0	0.00002	0.00002	0
Syrosingopine	**	ns	0.00258	0.00109	0.00012	0.02386	0.00036
Rescinnamine	*	ns	0.02604	0.00505	0.00382	0.00264	0.00078
Tonzonium bromide	*	ns	0.03074	0.00419	0.00658	0.00097	0.00074
Clomifene	*	ns	0.00734	0.03754	0.00197	0.00734	0.00221
Fluphenazine	ns	ns	0.05081	0.00183	0.00325	0.00377	0.00423
Flunarizine	*	ns	0.06261	0.00249	0.01998	0.0041	0.00505
Homochlorcyclizine	*	ns	0.04123	0.00366	0.0585	0.00853	0.00207
Sirolimus	*	ns	0	ns	0	0	0
Fulvestrant	*	ns	ns	0.00006	0	0	0
Vorinostat	ns	ns	0.00228	ns	0	0.00018	0
Trifluoperazine	ns	ns	0.00332	ns	0.0004	0.00689	0.00012
Amiodarone	*	ns	ns	0.01693	0.00112	0.00326	0.00054
0297417-0002B	*	ns	0.05036	ns	0.00145	0.00091	0.00052
Rottlerin	*	ns	ns	0.09434	0.0001	0.0001	0.0001
Etoposide	*	ns	ns	0.08817	0.00618	0.00563	0.00414
Pyrvinium	**	ns	ns	0.09941	0.0003	0.00579	0.0001
LY-294002	*	ns	ns	ns	0	0	0
Thioridazine	*	ns	ns	ns	0	0.00022	0
Prochlorperazine	*	ns	ns	ns	0	0.00268	0
Valproic acid	*	ns	0.0001	ns	0.00556	ns	0.0001

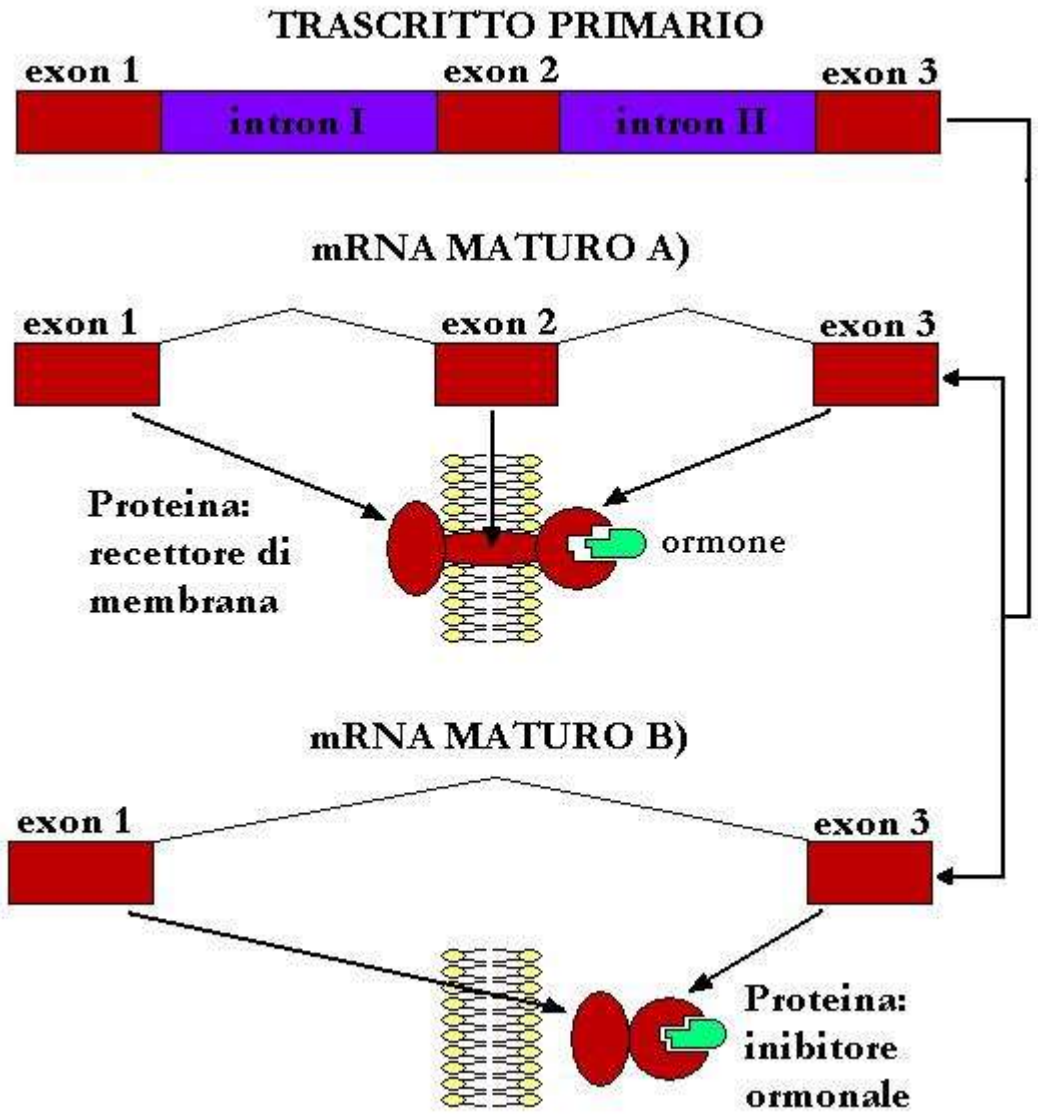
Connectivity Map analysis summary. * = significant specificity (<0.1), ** = highly significant specificity (<0.01).

= inhibitors of PI3K signal transduction pathway;

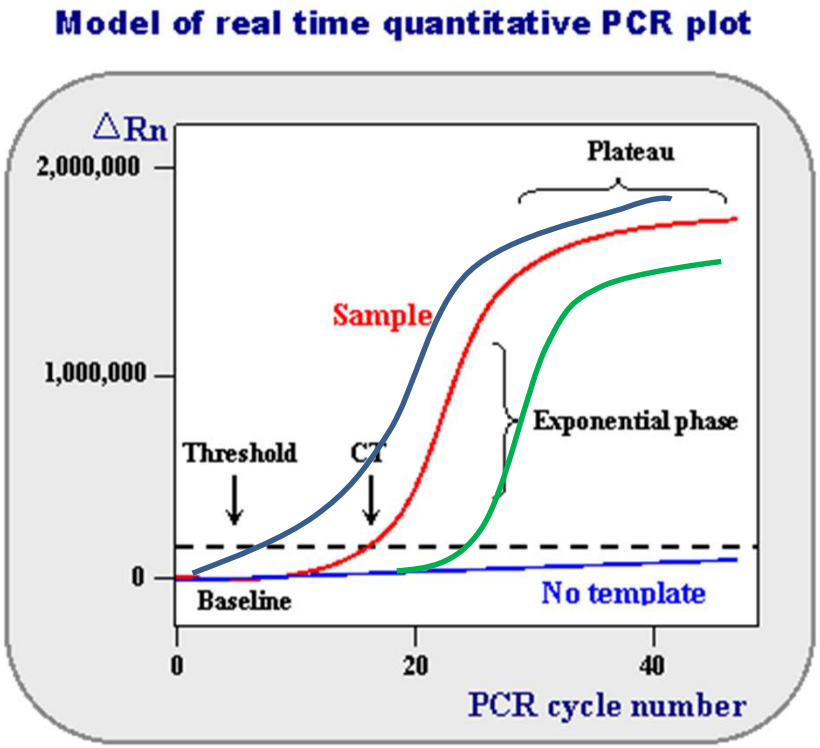
= inhibitors of histone deacetylase enzymes ;

= antipsychotic drugs

RNA-sequencing: splicing-alternativo



Quantitative Real-time PCR



Cycle
(40 Cycles)

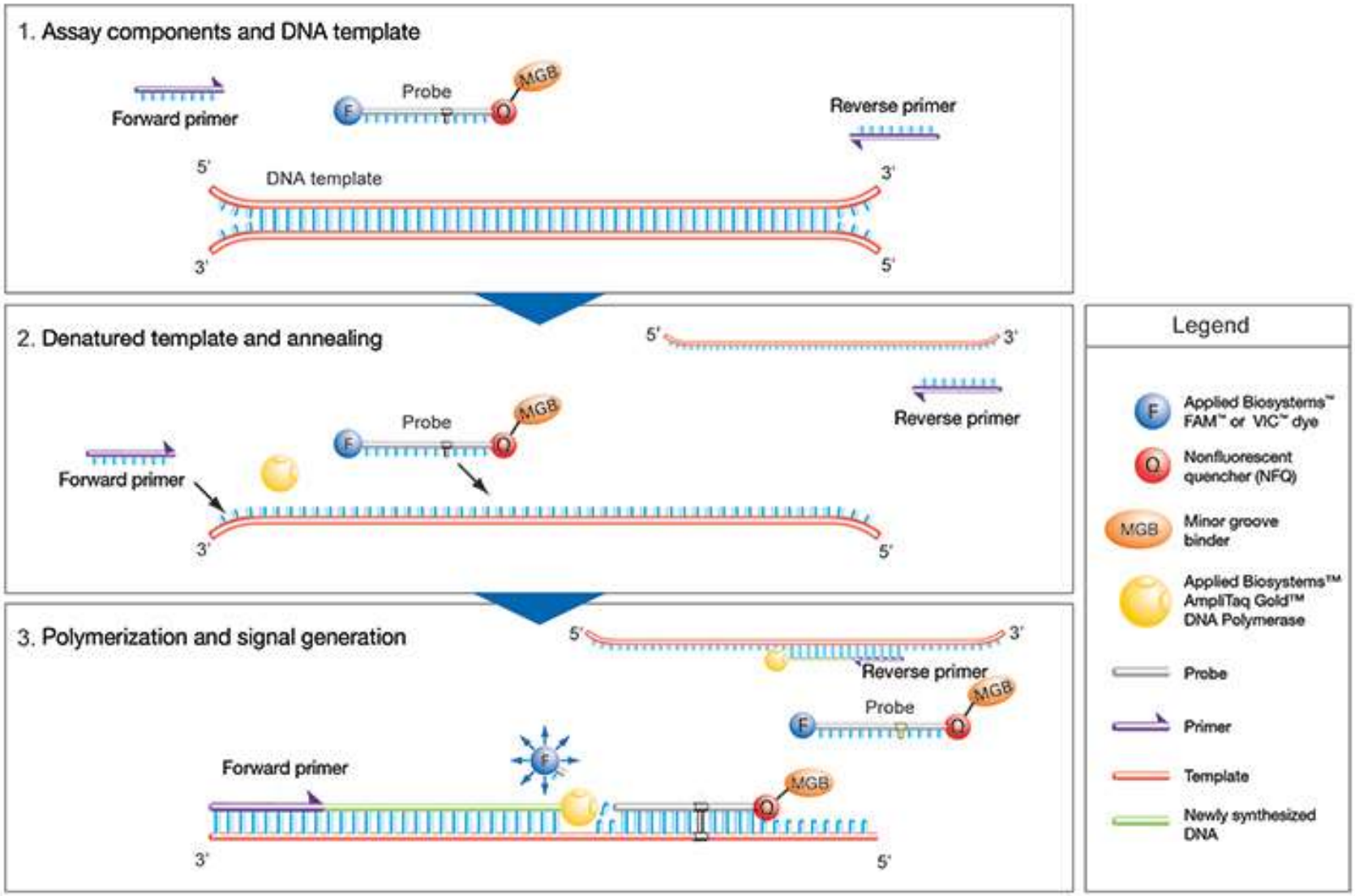
Baseline
is defined as PCR cycles in which a reporter fluorescent signal is accumulating but is beneath the limits of detection of the instrument.

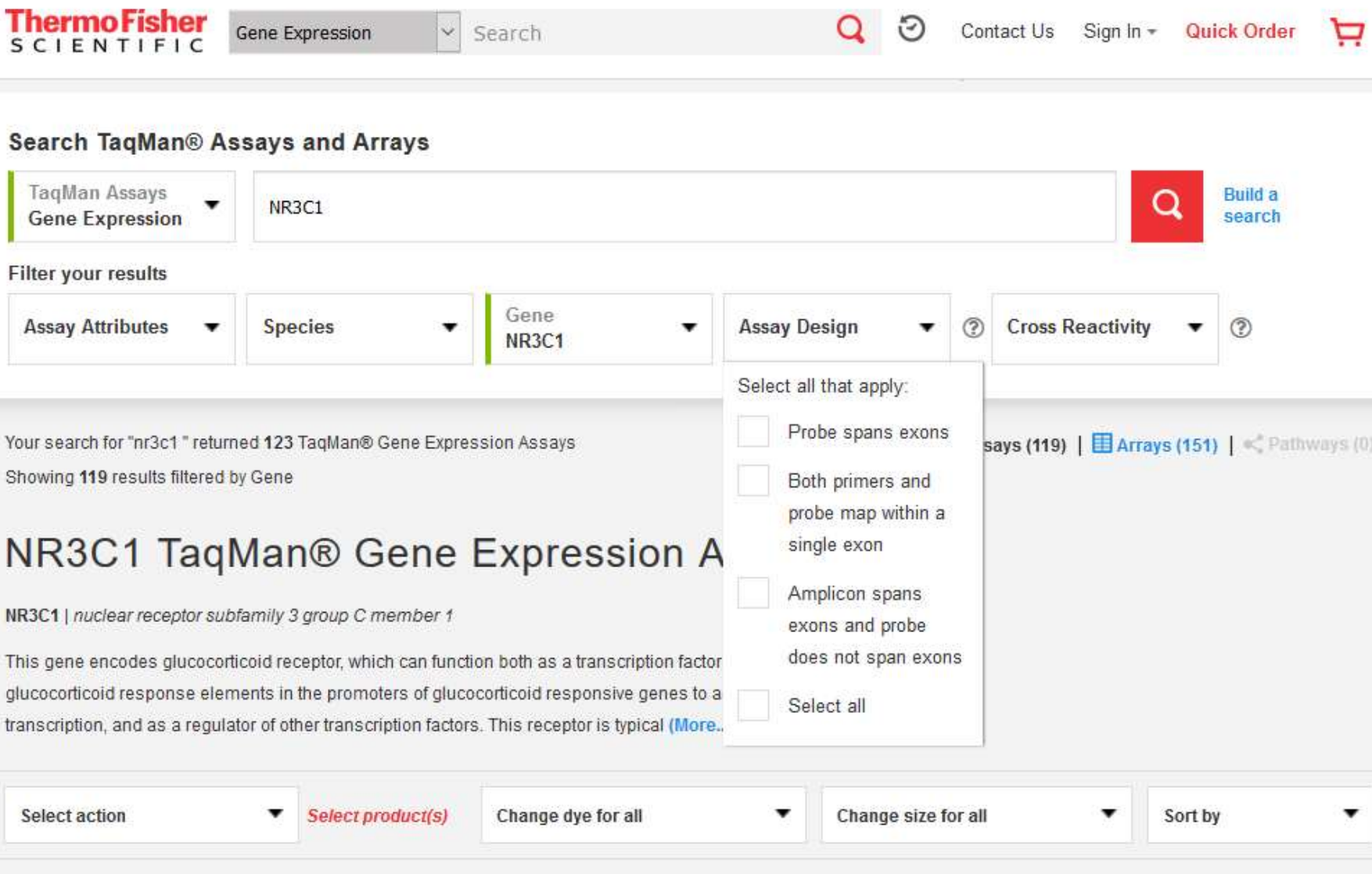
ΔRn
is an increment of fluorescent signal at each time point. The ΔRn values are plotted versus the cycle number.

Threshold
is an arbitrary level of fluorescence chosen on the basis of the baseline variability. A signal that is detected above the threshold is considered a real signal that can be used to define the threshold cycle (Ct) for a sample. Threshold can be adjusted for each experiment so that it is in the region of exponential amplification across all plots.

Ct is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data.

Gene Expression Analysis Using TaqMan Assays




ThermoFisher SCIENTIFIC
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TaqMan Assays
Gene Expression
NR3C1
Build a search
Filter your results
Assay Attributes
Species
Gene
NR3C1
Assay Design
Cross Reactivity
Select all that apply:
Probe spans exons
Both primers and probe map within a single exon
Amplicon spans exons and probe does not span exons
Select all
Your search for "nr3c1 " returned 123 TaqMan® Gene Expression Assays
Showing 119 results filtered by Gene
says (119) | Arrays (151) | Pathways (0)
NR3C1 TaqMan® Gene Expression A
NR3C1 | nuclear receptor subfamily 3 group C member 1
This gene encodes glucocorticoid receptor, which can function both as a transcription factor
glucocorticoid response elements in the promoters of glucocorticoid responsive genes to a
transcription, and as a regulator of other transcription factors. This receptor is typical (More..
Select action
Select product(s)
Change dye for all
Change size for all
Sort by

Quantitative Real-time PCR

SAMPLE	18S Ct	NR3C1 Ct
Patient S1	11,4	23,2
Patient S2	11,3	23,4
Patient S3	10,9	23,5
Patient R1	10,5	28,5
Patient R2	10,4	28,8
Patient R3	10,1	28,4

S= sensitive

R= resistant

The *Livak* method or delta delta CT method

$\Delta Ct_{PS} = Ct_{target} - Ct_{calibrator}$ 23,4-11,2=**12,2**

$\Delta Ct_{PR} = Ct_{target} - Ct_{calibrator}$ 28,6-10,3=**18,3**

$\Delta\Delta Ct = \Delta Ct_{PR} - \Delta Ct_{PS}$ 18,3-12,2=**6,1**

Relative expression= $2^{-(\Delta\Delta Ct)}$ $2^{-6,1}$=**0,015**

Patients R presented low levels of *NR3C1*