# Summer School 2015

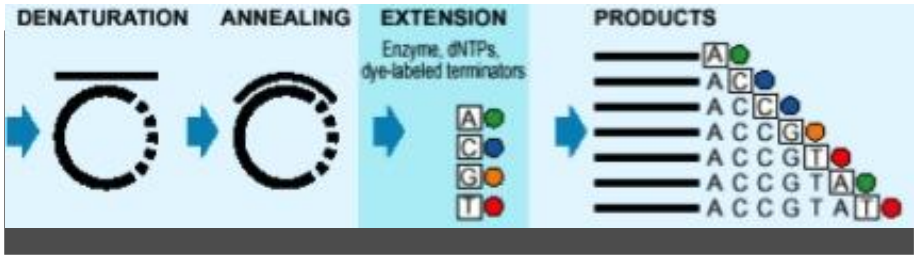
## Alloreattività e trapianti nell'uomo: le nuove metodiche di studio e i trapianti alternativi

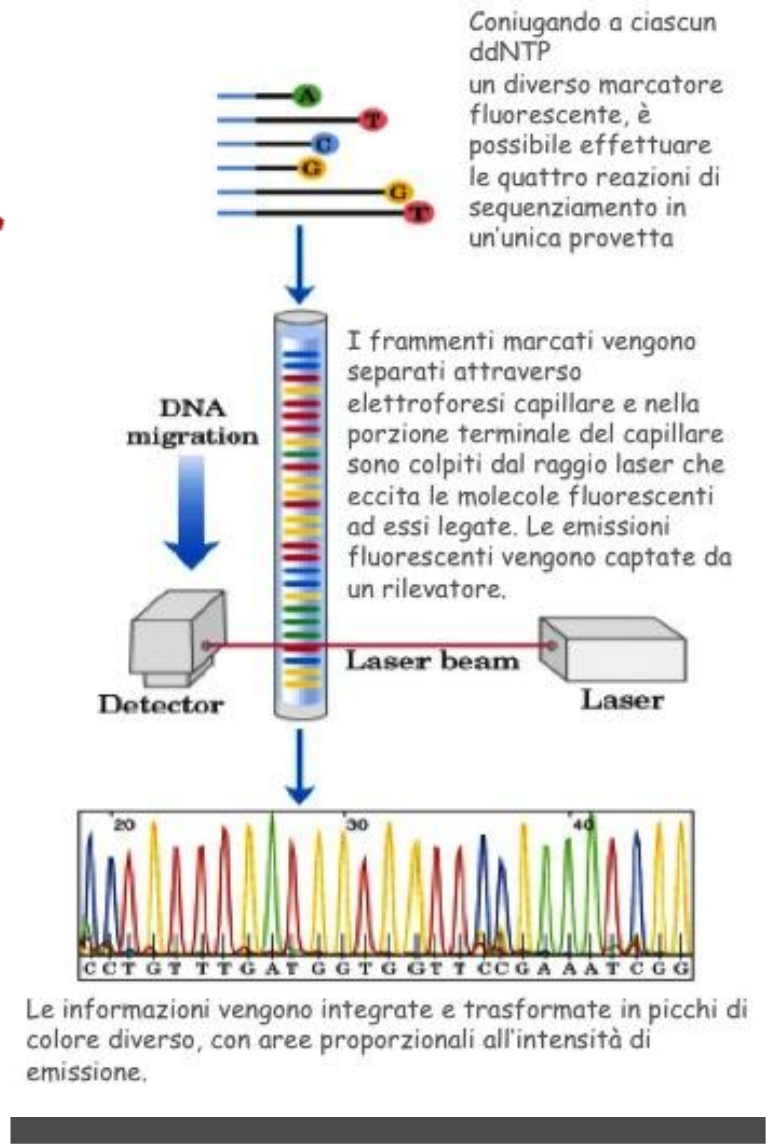
## Next Generation Sequencing : Principi

Alessandra Santoro

04 - 06 giugno 2015 Villaggio Cala la Luna Favignana (TP)

# Sequenziamento di Sanger




- *Read di 300-600nt*
  *X*
  *96 read*

***0.6Mb***

- ***Sensibilità 20%***

# *Next generation sequencing*

- *Sequenziamento dell'intero genoma di uno o più organismi*
- *Alta velocità di analisi di un'elevata mole di dati*
- *Sensibilità pari all'1%*

***30 Mb***

***Notevoli potenzialità diagnostiche***

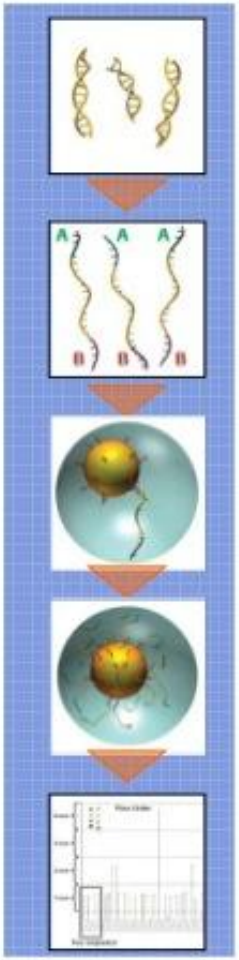
# *Diverse tecnologie*

- **Ion torrent**

- **Genome analyzer Illumina**

- **454 Roche tecnology**

# Tre fasi...



1. **Amplicon Library Prep**

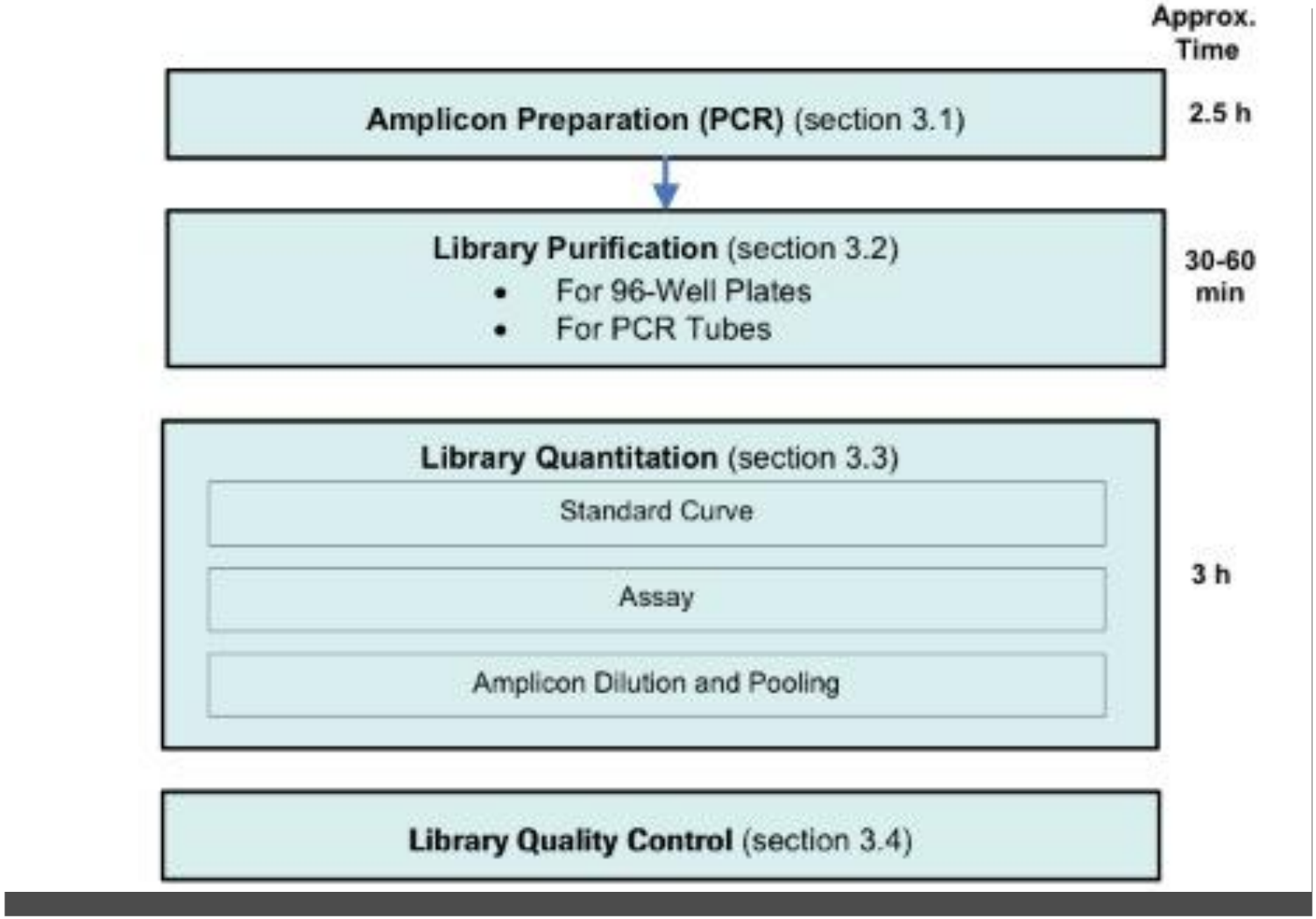
2. **emPCR amplification- Clonal amplification of library fragments**

3. **Sequencing- Prepare PicoTiterPlate device and start run**

www.454.com

454 SEQUENCING

# 1: Ampliconi → Libreria di ampliconi



## Molte variabili...

- **Numero di ampliconi**
- **Lunghezza degli ampliconi**
- **Tipo di sequenziamento (bidirezionale)**

## Due diversi approcci

**GS junior titanium**

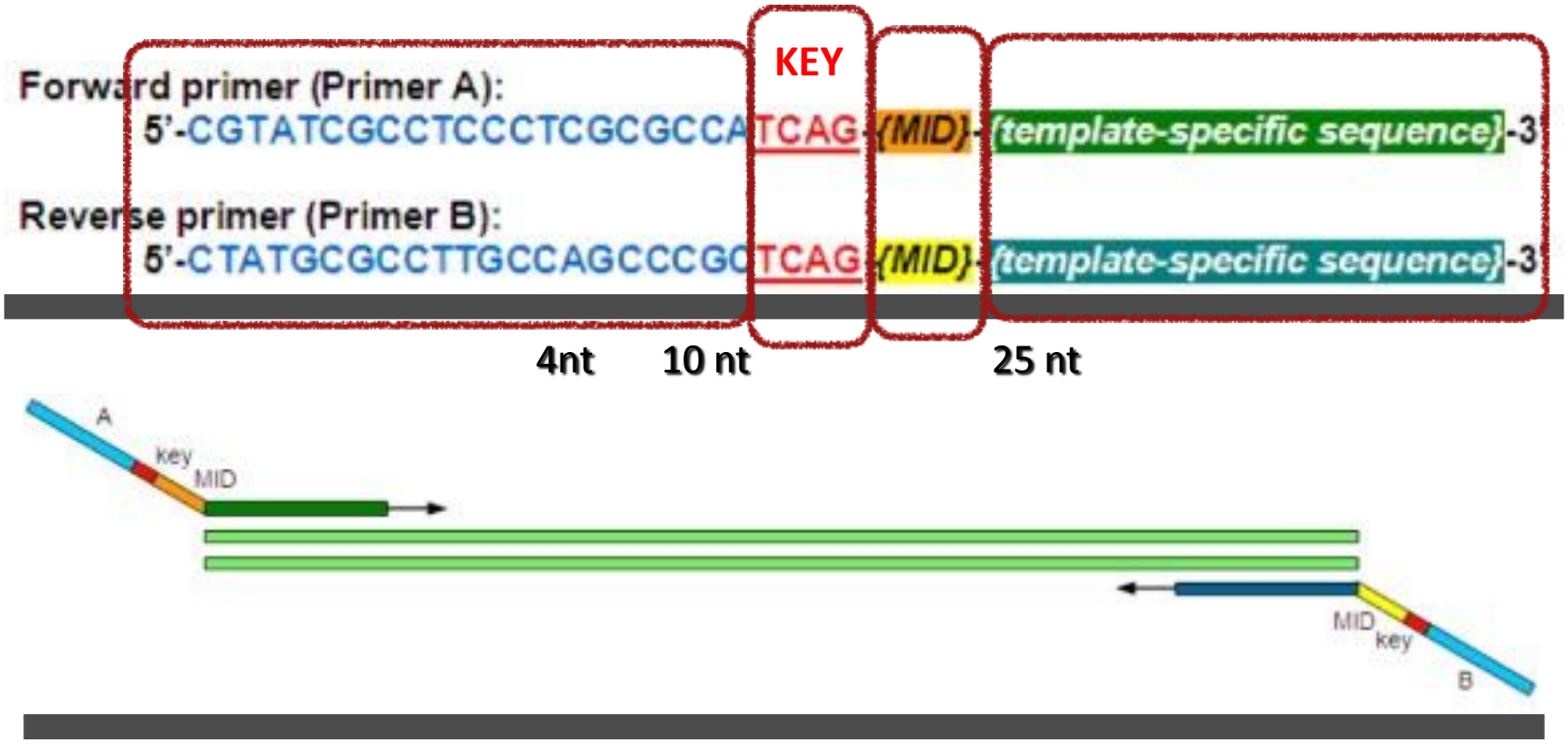
**Basic**

**Universal tailed**

# BASIC amplicon sequencing

- **Semplice**
- **Pochi campioni e/o ampliconi**

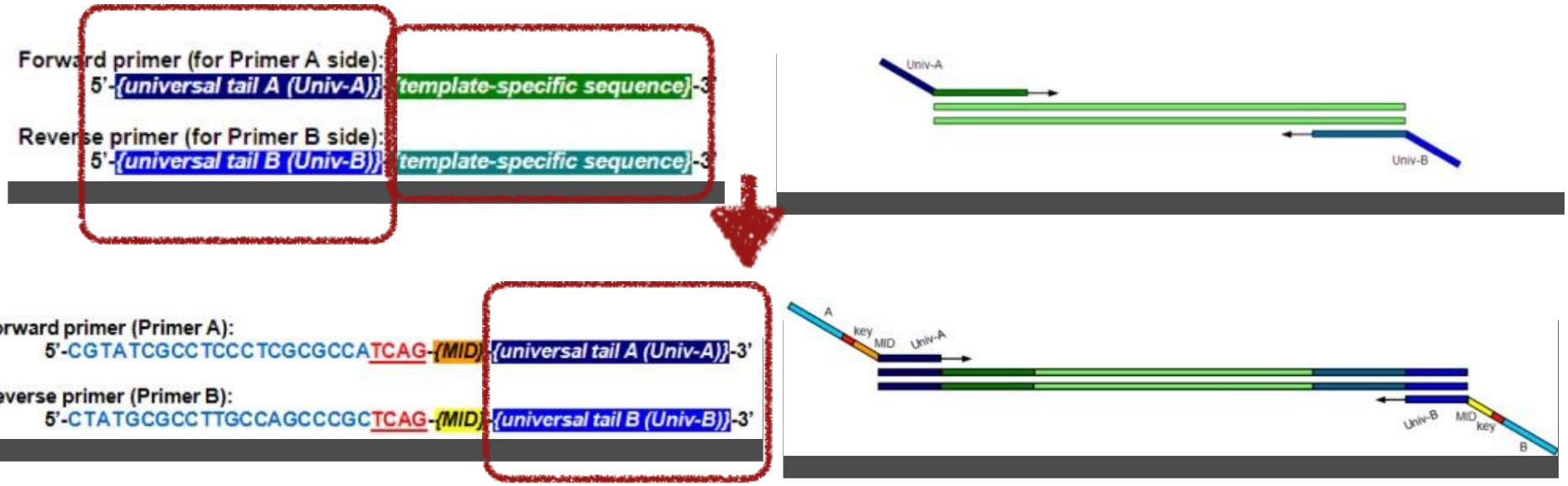
- **Dispendioso in termini di tempo e di lavoro**



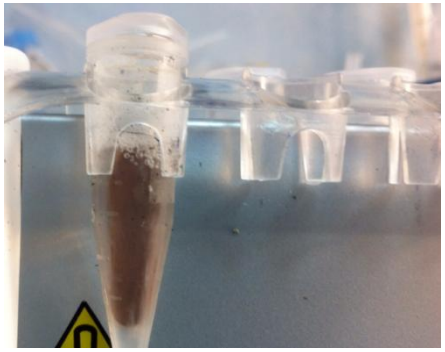
# Universal tailed amplicon library preparation

- **Tanti campioni o ampliconi**
- **Riduzione numero di primers**

- **Parte della sequenza non è informativa**



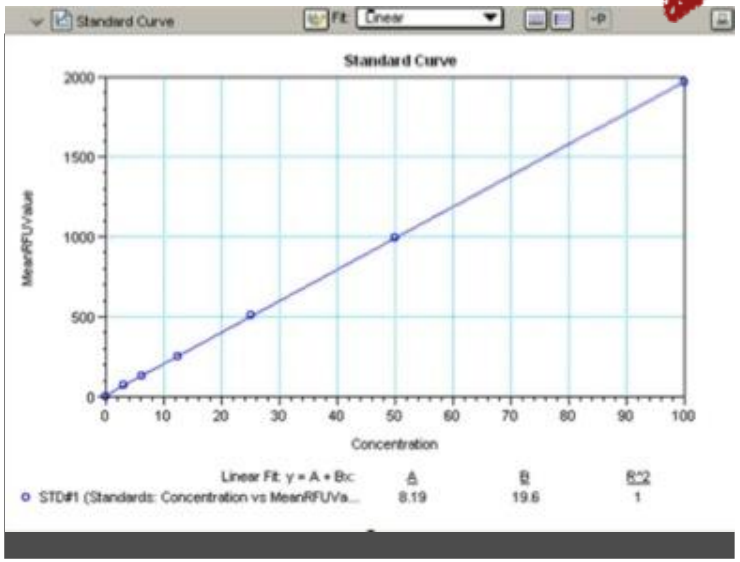
## Purificazione



**AMPure XP beads magnetiche**

**Legano in modo aspecifico gli ampliconi**
**Amplicone : AMPure 1:1 - 1:0.7**

## Quantizzazione




**Quant-it Picogreen** **10E9**

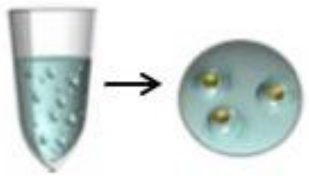
| Tube # | Well | DNA Concentration |
|---|---|---|
| Tube 1 | A12 | 100.00ng/well |
| Tube 2 | B12 | 50.00ng/well |
| Tube 3 | C12 | 25.00ng/well |
| Tube 4 | D12 | 12.50ng/well |
| Tube 5 | E12 | 6.25 ng/well |
| Tube 6 | F12 | 3.13 ng/well |
| Tube 7 | G12 | 1.56 ng/well |
| Tube 8 | H12 | 0.00 ng/well |

## Pooling dei campioni

**10E6**

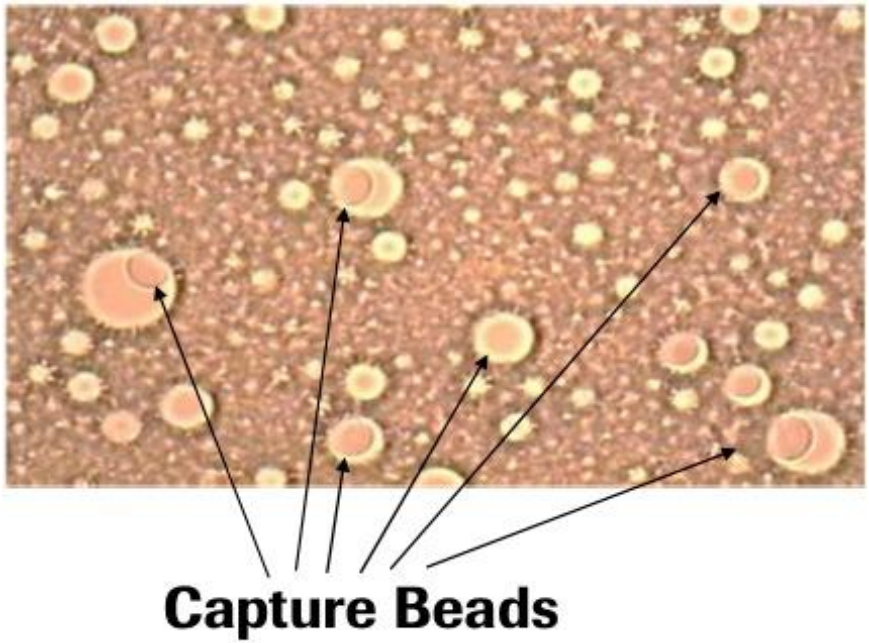
**Molecole DNA / Beads = 1:1,4 - 1:0.8**

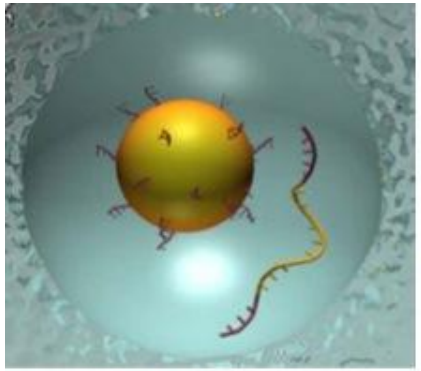
# 2:Emulsion-PCR amplification



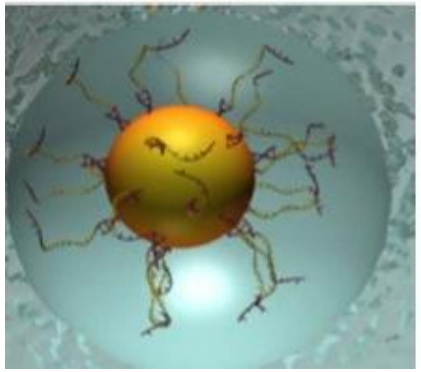
Mix DNA with Capture Beads

Emulsify DNA Capture Beads and PCR reagents in water-in-oil micoreactors



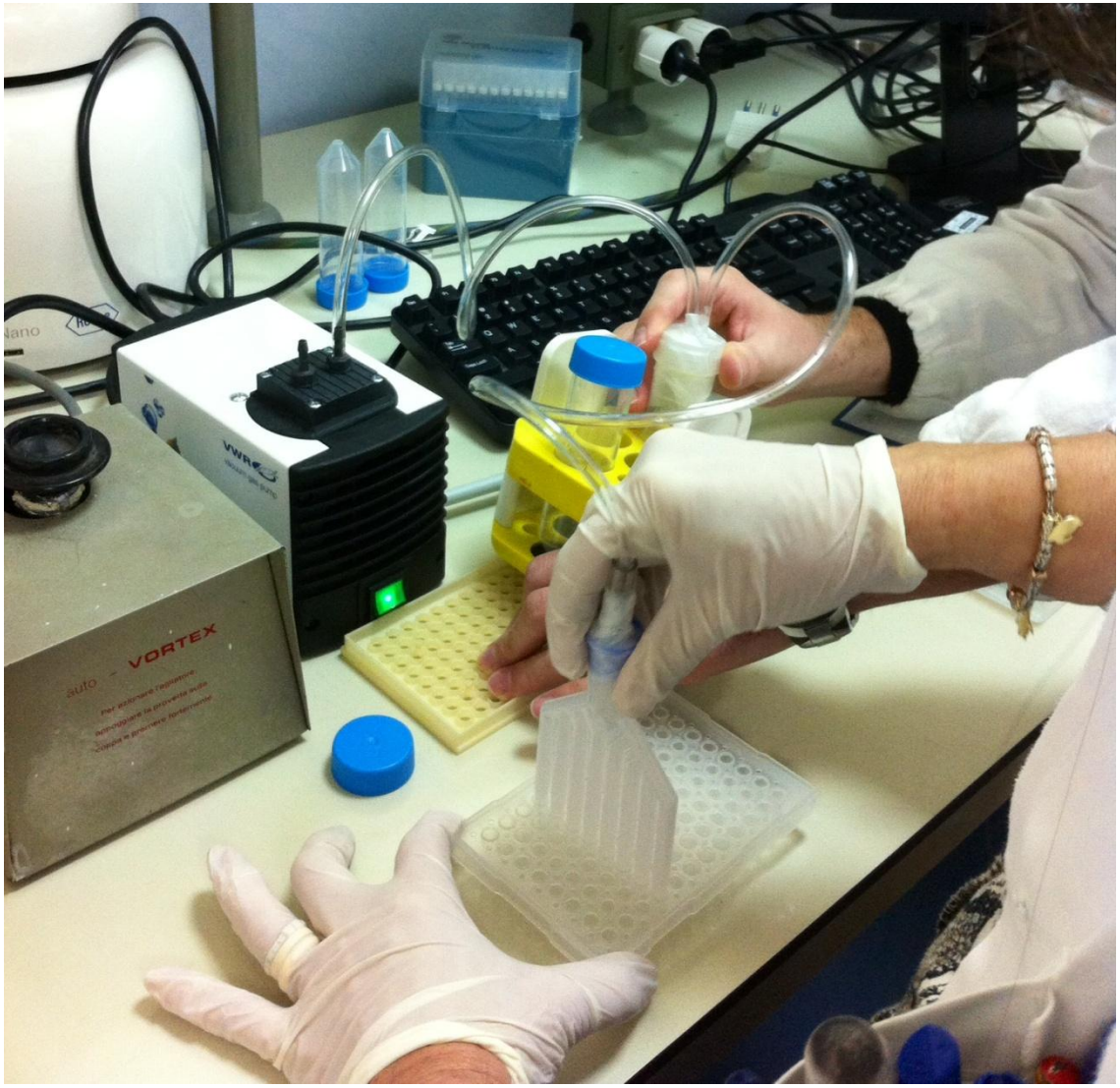



Before PCR



After PCR

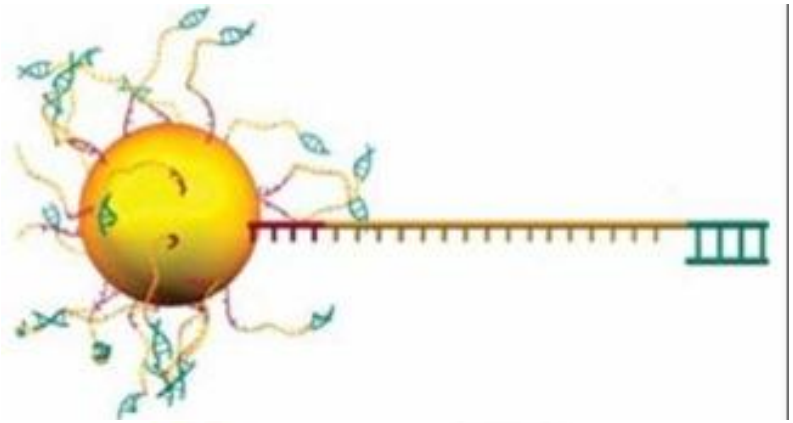
# Recupero

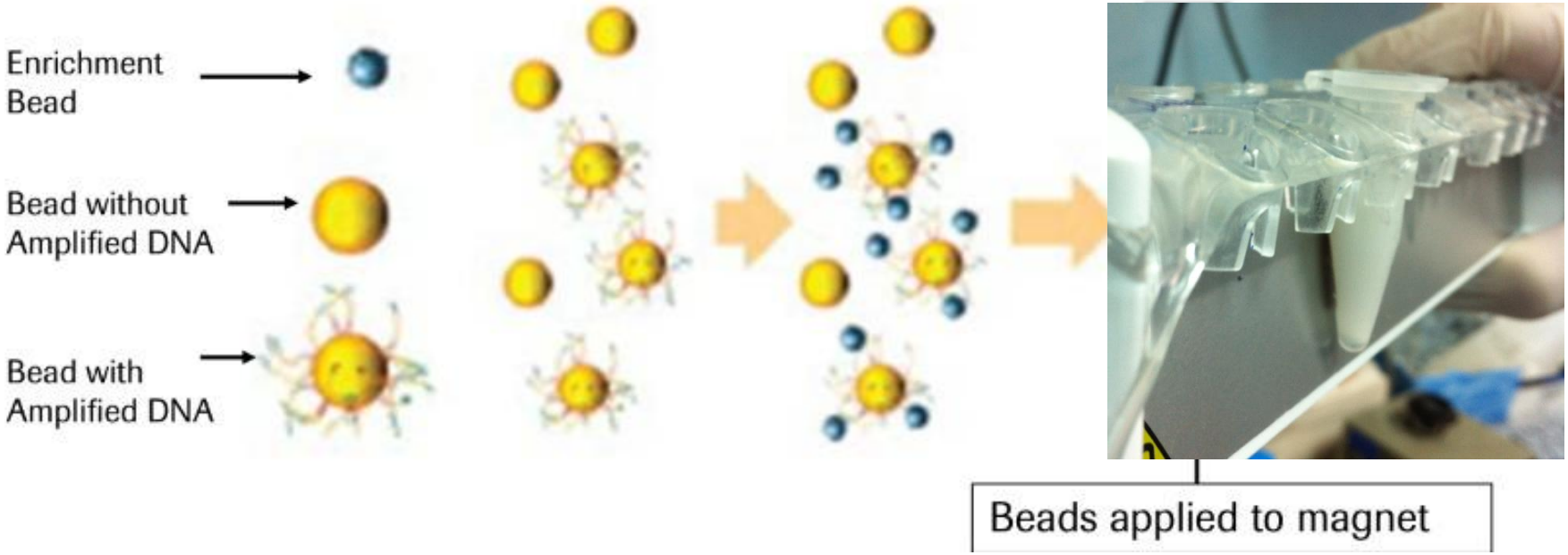



**Lavaggi per eliminare l'emulsione**

# Arricchimento

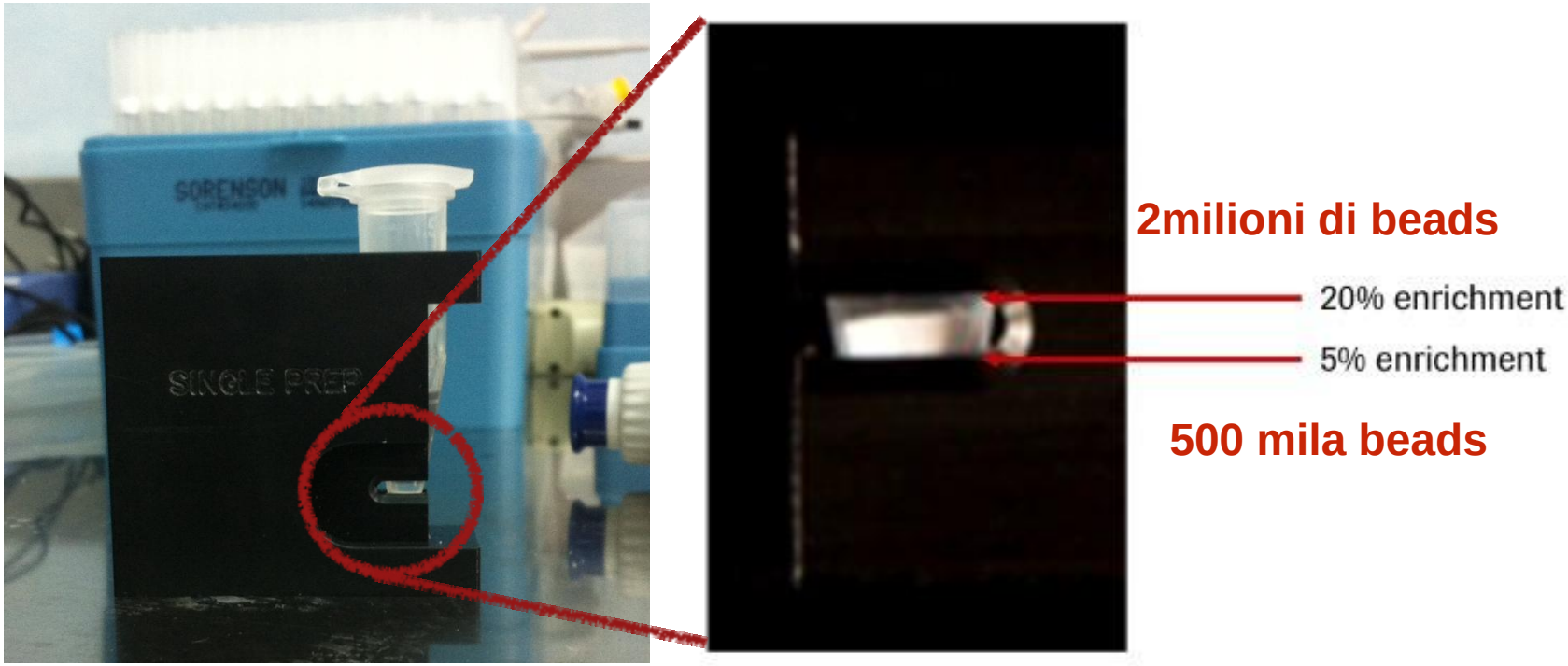
**Denaturarazione dei ds di DNA amplificati**



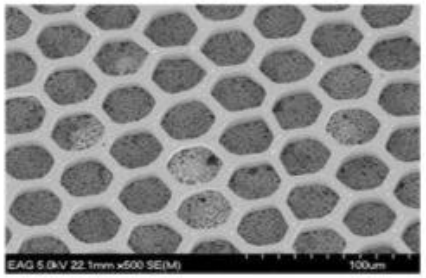



**Annealing dei primer per il sequenziamento**

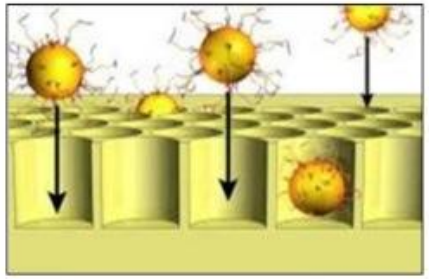
# Beads counting



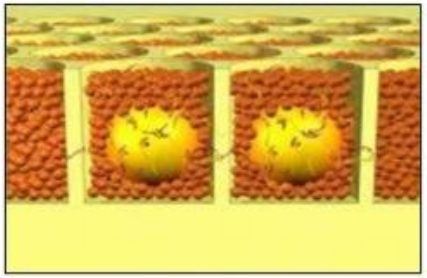
# 3: Sequenziamento



PicoTiterPlate device



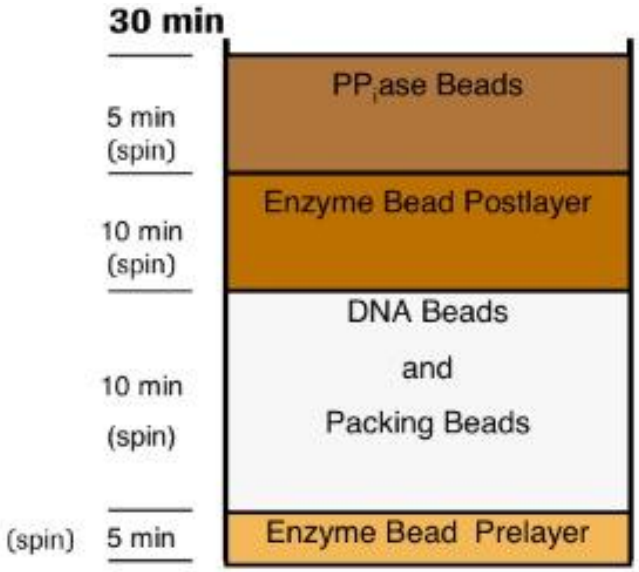
DNA beads are loaded into the wells of the PTP device

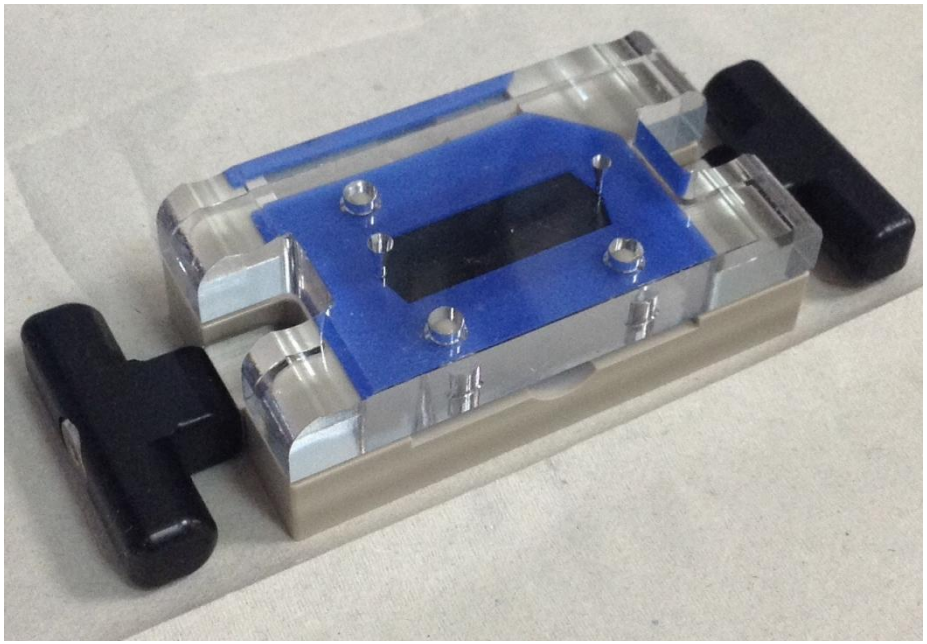


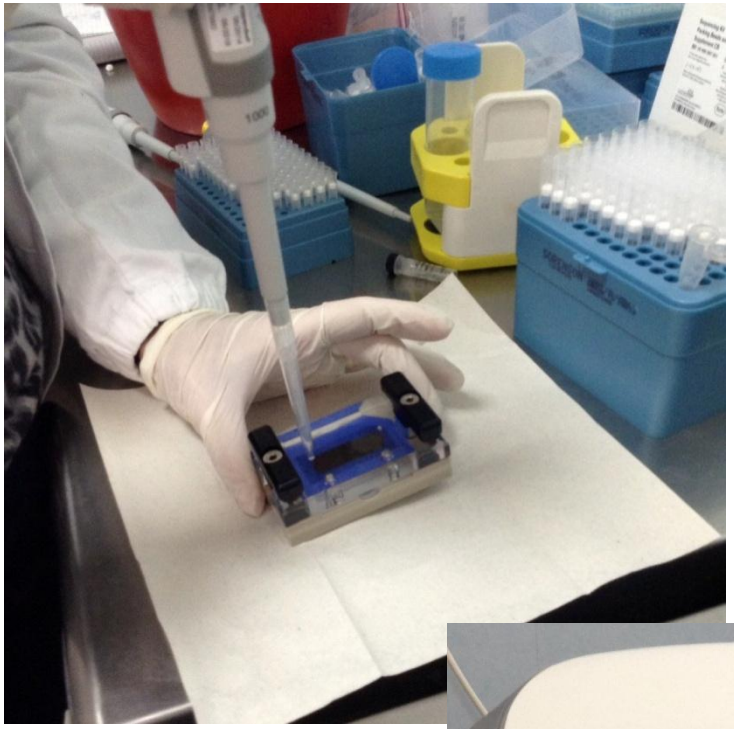
DNA beads packed into wells with surrounding beads and sequencing enzymes.

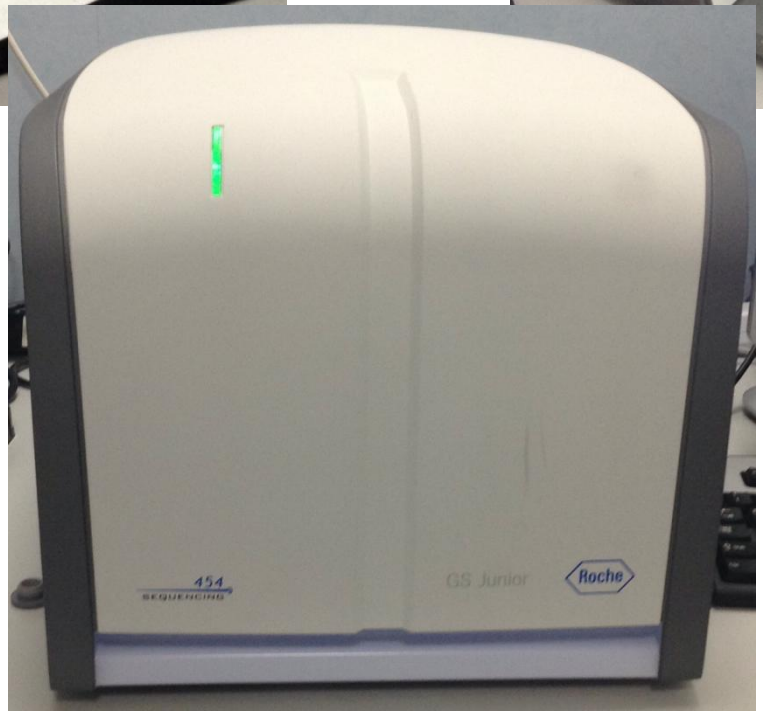
**PicoTiter plate**

**1,5 milioni di pozzetti**

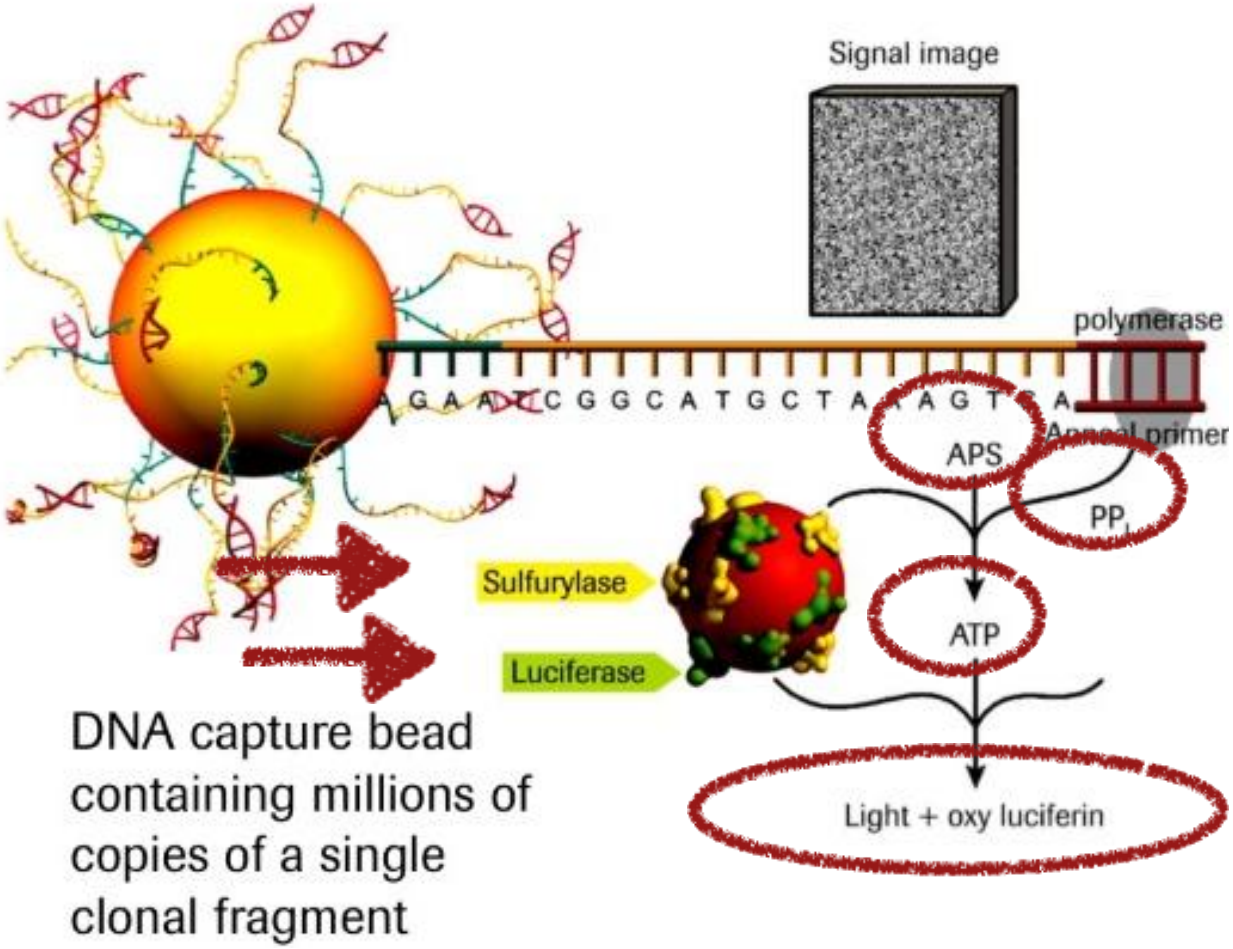


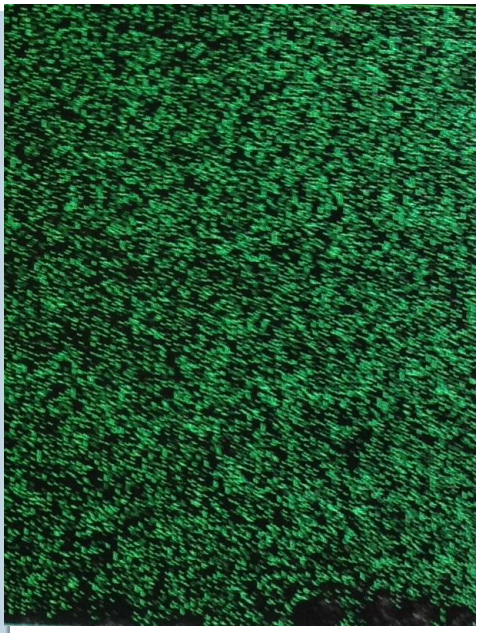



454
SEQUENCING
GS Junior
Roche

L'innovazione del sistema 454-Roche sta nel fatto che con questa tecnica non vengono più utilizzati nucleotidi fluorescenti ma sfrutta il pirofosfato inorganico (PPi), prodotto di scarto della DNA polimerasi. Il pirofosfato inorganico è il substrato dell'enzima sulfurilasi che produce ATP a partire da PPi e AMP. A sua volta l'ATP viene prelevato dalla lucefirasi che lo utilizza per ossidare la luciferina. Grazie a questa ultima reazione si produce un segnale luminoso. I nucleotidi vengono aggiunti uno per volta proprio perché la fluorescenza è uguale per ciascuno di essi. Il segnale è detectato tramite un fotodetector



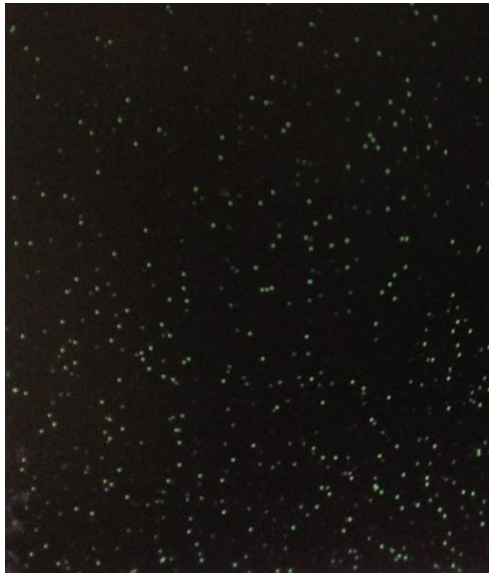

RAW DATA (A/T/C/G)



PASSED

FAILED

SEQ NO KEY



CTR PASSED

CTR FAILED

# Quali parametri considerare?

| Control DNA 400 bp >98% | Raw wells | <20% dot+ mixed | <40% % short quality | % seq passed | n° seq passed |
|---|---|---|---|---|---|
| >60- 62%accettabile bene >80% | attorno 200,000-2500000 | <6% + <15-20% | < 40% | | > 60.000 |
| | | | | | |
| 69,84 | 237.000 | 6% | 47% | 47% | 107.250 |
| 60,64 | 258.000 | 4,20% | 54% | 41% | 101.149 |
| 63,68 | 298.000 | 8% | 53% | 38% | 109.102 |
| 69,43 | 268.000 | 13% | 58% | 28% | 70.747 |
| 62.66 | 259.000 | 27% | 50% | 22% | 53.113 |
| 81.88 | 260.000 | 8% | 40,60% | 51,50% | 129.206 |
| 75,59% | 240.000 | 9% | 55% | 35% | 78.558 |
| 68,24% | 259.173 | 6% | 37,20% | 52,86% | 131.651 |
| 53,10% | 255.402 | 2%+ 12% | 38% | 47% | 117.848 |
| 79,00% | 218.181 | 2%+3% | 30% | 63,70% | 133.167 |
| 75,50% | 274.347 | 2%+ 3,5% | 43,00% | 49,30% | 130.594 |
| 79,70% | 258.624 | 1%+ 5,3% | 46,50% | 46,80% | 115.570 |
| 72,50% | 223.898 | 2,3%+ 3,5% | 43,40% | 46,80% | 98.697 |
| 81,10% | 233.732 | 3,7%+ 26,4% | 37,70% | 30,40% | 65.790 |

| ampure | purificazione camp. | primer * PCR di emulsione | n° di molec * capture beads | % di arrichimento camp. |
|---|---|---|---|---|
| | | | | accettabile 5-20% |
| 1x | 1 | 40 microL | 0.8 molecole | 10/12 % |
| 0.7x | 2 | 10 microL primer +30 microL H2O | 1.4 | 10/12 % |
| 0.7x | 2 | 40 microL | 1.4 | 12% |
| 1x | 2 | 30 microL primer +10 microL H2O | 1.4 | 15% |
| 0.7x | 2 | 40 microL | 1.4 | 18-20% |
| 0.7x | 2 | 20 microL primer +20 microL H2O | 1.4 | 15% |
| 0.7x | 2 | 20 microL primer +20 microL H2O | 1.4 | 8% |
| 0.7x | 2 | 20 microL primer +20 microL H2O | 0,8 | 5% |
| 0.7x | 2 | 20 microL primer +20 microL H2O | 0,8 | 14% |

# 70.000- 100.000 SEQUENZE

**AMPLICONI**  **SENSIBILITA'**

**10 ampliconi**

**10000 Seq X amplicone**
**(0.1% di sensibilità )**

**100 ampliconi**

**1000 Seq X amplicone**
**(1% di sensibilità )**

**1000 ampliconi**

**100 Seq X amplicone**
**(10% di sensibilità )**

# HLA Typing Publication from The

Erlich *et al. BMC Genomics* 2011, **12**:42
http://www.biomedcentral.com/1471-2164/12/42

BMC Genomics

**METHODOLOGY ARTICLE** **Open Access**

## Next-generation sequencing for HLA typing of class I loci

Rachel L Erlich[1†], Xiaoming Jia[1,2†], Scott Anderson[1], Eric Banks[1], Xiaojiang Gao[3,4], Mary Carrington[3,4], Namrata Gupta[1], Mark A DePristo[1], Matthew R Henn[1], Niall J Lennon[1], Paul IW de Bakker[1,5,6,7*]

**Abstract**

**Background:** Comprehensive sequence characterization across the MHC is important for successful organ transplantation and genetic association studies. To this end, we have developed an automated sample preparation, molecular barcoding and multiplexing protocol for the amplification and sequence-determination of class I HLA loci. We have coupled this process to a novel HLA calling algorithm to determine the most likely pair of alleles at each locus.

**Results:** We have benchmarked our protocol with 270 HapMap individuals from four worldwide populations with 96.4% accuracy at 4-digit resolution. A variation of this initial protocol, more suitable for large sample sizes, in which molecular barcodes are added during PCR rather than library construction, was tested on 95 HapMap individuals with 98.6% accuracy at 4-digit resolution.

**Conclusions:** Next-generation sequencing on the 454 FLX Titanium platform is a reliable, efficient, and scalable technology for HLA typing.

# Other HLA Genotyping Publications

- **A novel single cDNA amplicon pyrosequencing method for high-throughput, cost-effective sequence-based HLA class I genotyping.** Lank SM, Wiseman RW, Dudley DM, O'Connor DH. (2010) Human Immunology 71(10): 1011-7.

- **Next-Generation Sequencing: The Solution for High-Resolution, Unambiguous HLA Typing.** Lind C, Ferriola D, Mackiewicz K, Heron S, Rogers M, Slavich L, Walker R, Hsiao T, McLaughlin L, D'Arcy M, Gai X, Goodridge D, Sayer D, Monos D. (2010) Human Immunology 71(10): 1033-42.

- **High-resolution, high-throughput HLA genotyping by next-generation sequencing.** Bentley G, Higuchi R, Hoglund B, Goodridge D, Sayer D, Trachtenberg EA, Erlich HA. (2009) Tissue Antigens 74(5): 393-403.

- **Rapid High-throughput HLA Typing by Massively-parallel Pyrosequencing for High-Resolution Allele Identification.** Gabriel C, Danzer M, Hackl C, Kopal G, Hufnagl P, Hofer K, Polin H, Stabentheiner S, Pröll J. (2009) Human Immunology 70(11): 960-4.

# GS GType HLA Primer Sets

## *HLA Typing with the GS FLX and GS Junior Systems*

- Two Primer Sets
  - Plate 1 for Medium Resolution
  - Plate 2 for High Resolution (when used with Plate 1)
- Multiplex up to 10 samples per primer plate
- Designed for HLA genotyping on the GS FLX and GS Junior Systems
- Output compatible with 454 System-specific Conexio software – leading provider of HLA Sanger sequencing software
- For life science research use only. Not for use in diagnostic procedures.


**GS GType HLA MR Primer Set (blue label ) contains four 96-well plates**

**GS GType HLA HR Primer Set (yellow label ) contains four 96-well plates**

# GS GType HLA Primer Sets

## *Target Exons*

- Exons from the HLA Class I and Class II loci targeted in the GS GType MR and HR Primer Sets
- MR plates are used to sequence the exons in column one, or can be used in combination with HR plates to sequence all exons listed

| *High Resolution (MR + HR)* | |
|---|---|
| ***Medium Resolution (MR)*** | |
| **GS GType MR Primer Set** | **GS GType HR Primer Set** |
| HLA-A exons 2, 3 | HLA-A exon 4 |
| HLA-B exons 2, 3 | HLA-B exon 4 |
| HLA-C exons 2, 3 | HLA-C exon 4 |
| DQB1 exon 2 | DQB1 exon 3 |
| DRB1,3,4,5 exon 2 | DPB1 exon 2 |
| | DQA1 exon 2 |

# GS GType HLA MR & HR Primer Sets

Samples 1-10

Negative Control

Blank for PicoGreen Stds

Plate Map

**Medium Resolution Plate**

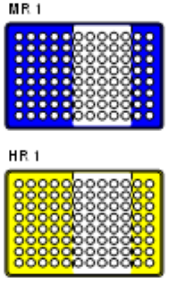
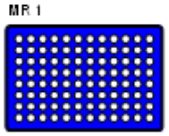
| | MID1 | MID3 | MID4 | MID6 | MID7 | MID8 | MID9 | MID10 | MID13 | MID16 | MID11 | Blank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | HLA-A2 | |
| B | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | HLA-A3 | |
| C | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | HLA-B2 | |
| D | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | HLA-B3 | |
| E | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | HLA-C2 | |
| F | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | HLA-C3 | |
| G | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | DQB1-2 | |
| H | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | DRBx-2 | |

**High Resolution Plate**

| | MID1 | MID3 | MID4 | MID6 | MID7 | MID8 | MID9 | MID10 | MID13 | MID16 | MID11 | Blank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | HLA-A4 | |
| B | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | HLA-B4 | |
| C | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | HLA-C4 | |
| D | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | DPB1-2 | |
| E | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | DQA1-2 | |
| F | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | DQB1-3 | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

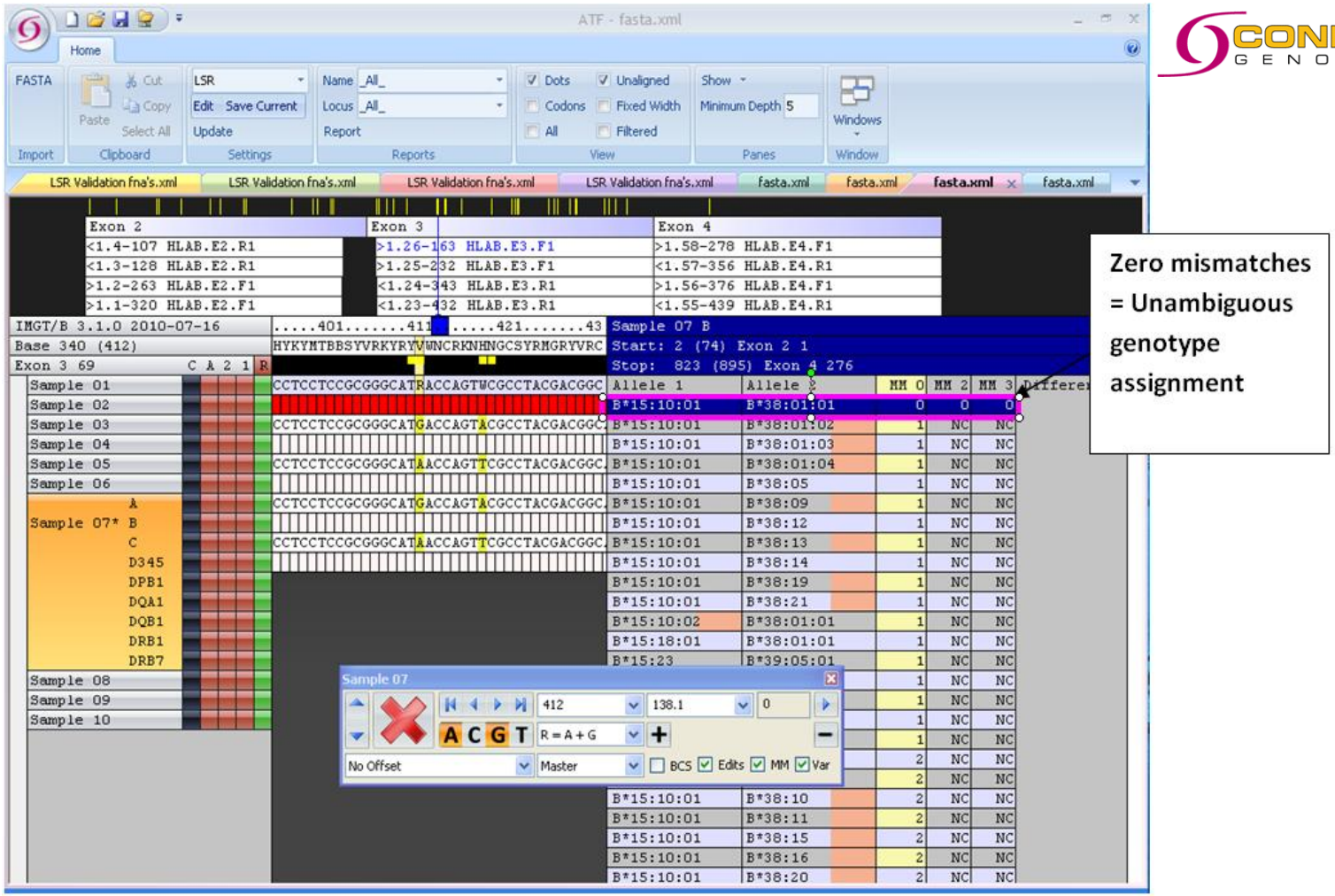
# Options for Sequencing

## *GS Junior System*

| | | |  Number of Samples |
|---|---|---|---|
| **HR on GS Junior**<br>½ MR plate + ½ HR plate =<br>5 samples @ 14 Amplicons / sample |  |  Single size gasket<br>5 HR samples<br>(or 10 HR samples<br>on two Runs) | **5** |
| **MR on GS Junior**<br>1 MR plate =<br>10 samples @ 8 Amplicons / sample |  |  Single size gasket<br>10 MR samples | **10** |

# ATF 454 Analysis Software from Conexio

## *Automatic Allele Assignment*




Example shown: For HLA-B, the Conexio ATF interface shows an unambiguous genotype assignment (zero mismatches in columns MM0, MM2, MM3) for B*15:10:01/B*38:01:01.

## Stato mutazionale Abl (resistenza TKI)

**ABL cDNA**
**4 ampliconi** → **5 samples 20 ampliconi**
**Sensibilità <1%**

## Stato mutazionale RUNX1

**Esoni 3-8**
**7 Ampliconi** → **12 samples 84 ampliconi**
**Sensibilità 1%**

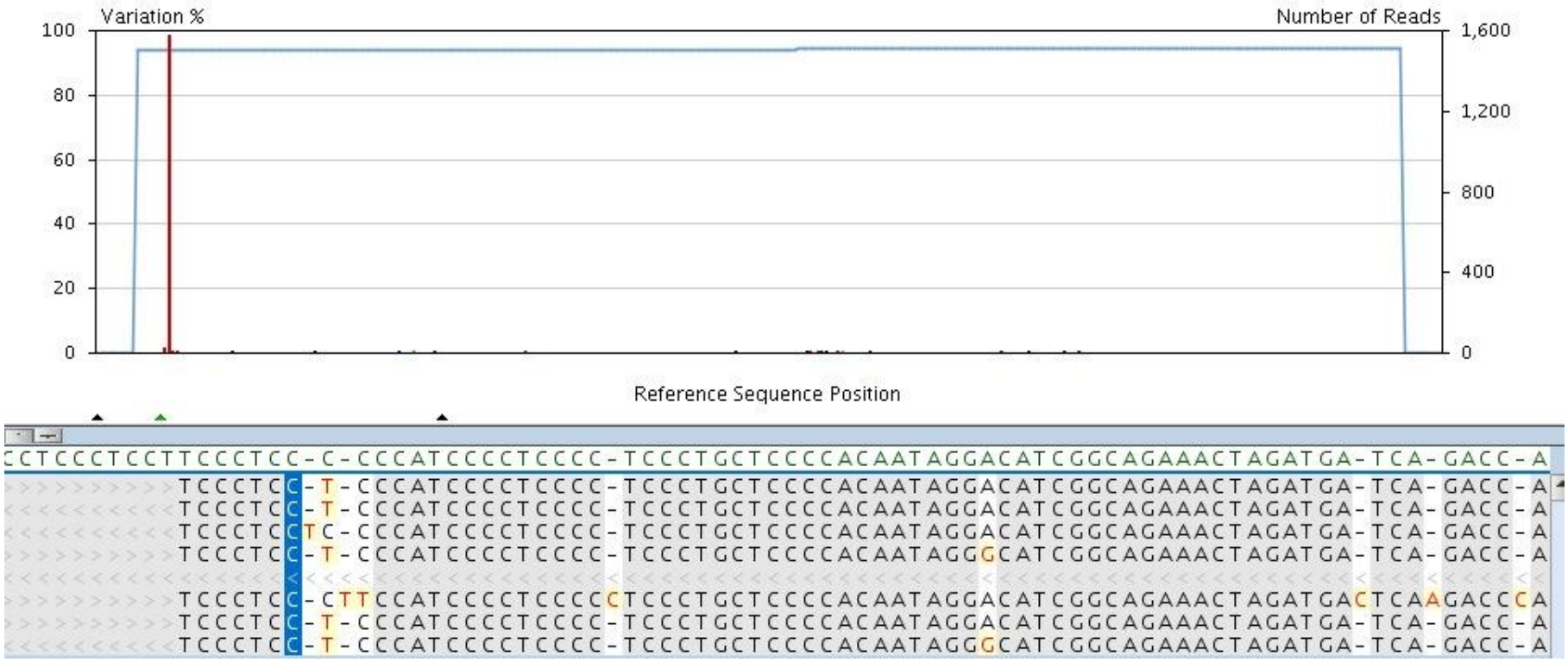
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MID1 | MID2 | MID3 | MID4 | MID19 | MID6 | MID8 | MID10 | MID13 | MID18 | MID15 | MID16 |
| A | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 |
| B | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 |
| C | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 |
| D | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 |
| E | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 | Exon 3 |
| F | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 | Ex 8.1 |
| G | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 | Ex 8.2 |
| H | | | | | | | | | | | MID 17 Exon 7 NC* | MID 17 Ex 8.2 NC* |

## Stato mutazionale TP53

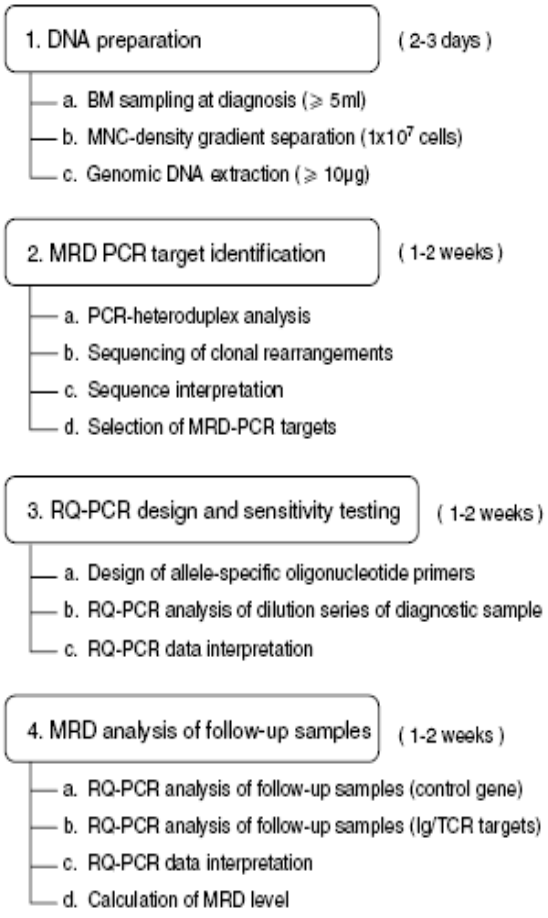
**Esoni 4-11**
**8 ampliconi** → **11 samples 88 ampliconi**
**Sensibilità 1%**

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Neg. Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MID1 | MID2 | MID3 | MID4 | MID5 | MID6 | MID7 | MID8 | MID10 | MID14 | MID15 | MID15 |
| A | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 | Exon 4 |
| B | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 | Exon 5 |
| C | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 | Exon 6 |
| D | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 | Exon 7 |
| E | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 | Exon 8 |
| F | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 | Exon 9 |
| G | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 | Exon 10 |
| H | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 | Exon 11 |

# Software AVA



# MRD Detection in ALL Patients




**Detection and selection of clonal Ig/TCR gene rearrangements at diagnosis**:

Ig/TCR PCR

Sequencing of clonal rearrangements

Sequence interpretation

Selection of MRD-PCR targets

***Guidelines BIOMED***

↓

**RQ-PCR sensitivity testing**:

Design of allele-specific oligonucleotide primers

RQ-PCR analysis of dilution series of diagnostic sample

Q-PCR data interpretation: quantitative range and sensitivity

***Guidelines ESG-MRD-ALL***

↓

**MRD analysis of follow-up samples**

Control gene RQ-PCR analysis

MRD-PCR target RQ-PCR analysis

RQ-PCR MRD data interpretation

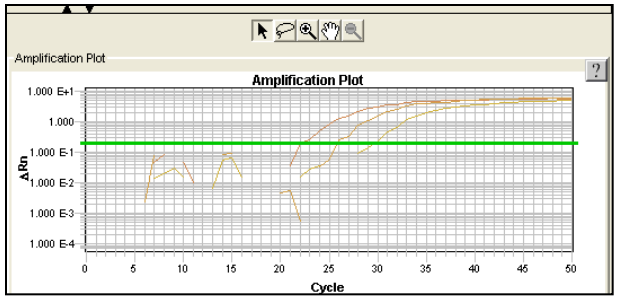
***Guidelines ESG-MRD-ALL***

# Approccio “standard” BIOMED 2-RQPCR

**Screening**
- IGH
- DH
- IGK-Kde

**Selezione di 2 marcatori**

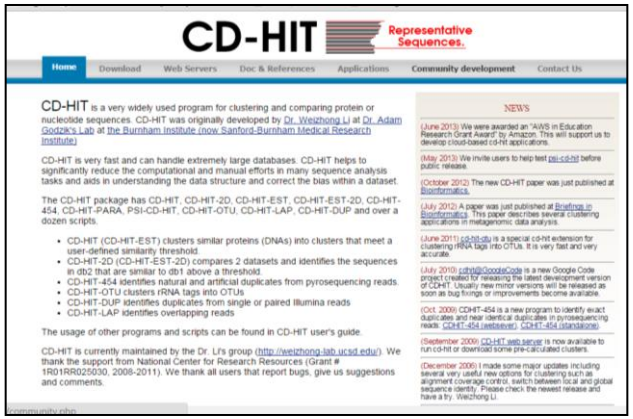
**MRD**
- Oligo paziente specifico
- Amplificazione specifica con metodologia TaqMan

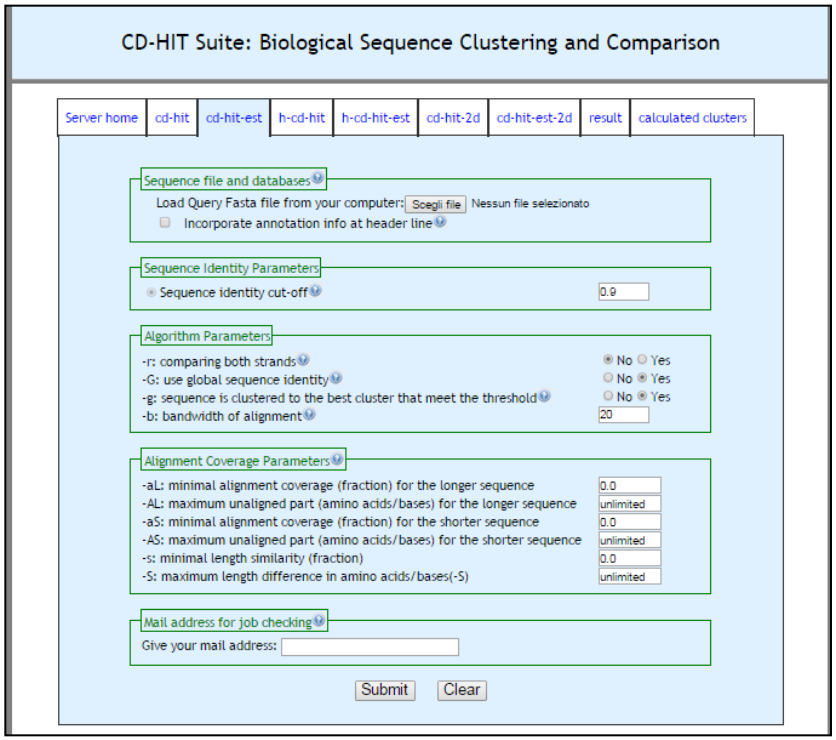



# Approccio “NGS”

**Screening**
- IGH
- DH
- IGK-Kde

**MRD**
- IGH
- DH
- IGK-Kde




Huang Y at al.
Bioinformatics, 2010(26): 680-682

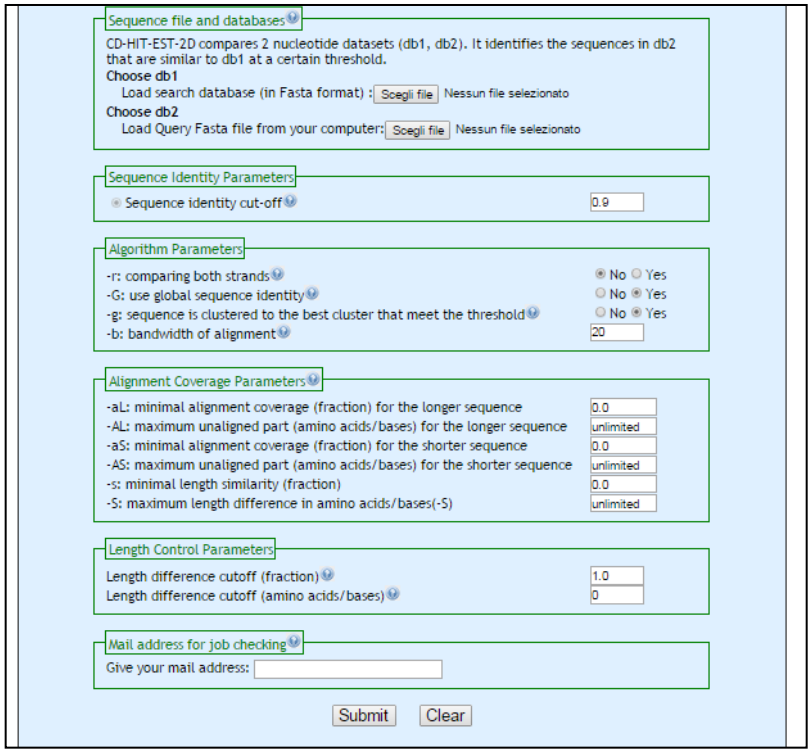
**19 cluster**

Cluster 0, No. sequences: 17822
Representative: I6JKX5O01CVONS
>I6JKX5O01CVONS length=545
xy=1063_3590

VH4-55*02 DH3-09*01JH6*03
VH4-55*02 DH3-09*01JH6*03
IGKV1-37*01
IGKV1-37*01-Kde
IGKV4-1*01
IGKV4-1*01-Kde
IGKV2-30*01
IGKV2-30*01-Kde

Vk2-30-Kde
Vk2-30-Kde
Vk3D-20
Vk3-20-Kde
DH2-2*01-JH5*01
VH6-1*02DH2-2*02JH5*02
VH3-11*06 DH5-18*01JH4*02
VH3-11*06 DH5-18*01JH4*02

VH2-5*02 DH3-22*01JH4*02
VH2-5*02 DH3-22*01JH4*02
IGKV1D-33*01
IGKV1D-33*01-kde




# Cd-hit-est-2d

11/ 13.000

```
0.98
1. JCKBR3K01AJN8J, length: 331, identity: +/98% VH6-1*01 DH7-27*01 JH5*02
2. JCKBR3K01CCQCL, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
3. JCKBR3K01COX03, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
4. JCKBR3K01C30TM, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
5. JCKBR3K01C2FEG, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
6. JCKBR3K01AFLGD, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
7. JCKBR3K01CF19P, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
8. JCKBR3K01A6NAM, length: 331, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
9. JCKBR3K01CUNYJ, length: 331, identity: +/98% VH6-1*01 DH7-27*01 JH5*02
10 JCKBR3K01DDP2P, length: 330, identity: +/99% VH6-1*01 DH7-27*01 JH5*02
11. JCKBR3K01BBOOL, length: 330, identity: +/99% VH6-1*01 DH7-27*01JH5*02

0.97
1. JCKBR3K01AYXLV, length: 338, identity: +/97% VH6-1*01 DH7-27*01 JH5*02
2. JCKBR3K01AJN8J, length: 331, identity: +/98%
3. JCKBR3K01CCQCL, length: 331, identity: +/99%
4. JCKBR3K01COX03, length: 331, identity: +/99%
5. JCKBR3K01C30TM, length: 331, identity: +/99%
6. JCKBR3K01C2FEG, length: 331, identity: +/99%
7. JCKBR3K01AFLGD, length: 331, identity: +/99%
8. JCKBR3K01CF19P, length: 331, identity: +/99%
9. JCKBR3K01A6NAM, length: 331, identity: +/99%
10. JCKBR3K01CUNYJ, length: 331, identity: +/98%
11. JCKBR3K01DDP2P, length: 330, identity: +/99%
12. JCKBR3K01BBOOL, length: 330, identity: +/99%
```

EuroMRD-QC: 5x10E-4

**Screening** → **VH6**

Leukemia. 2014 Jun;28(6):1299-307. doi: 10.1038/leu.2013.375. Epub 2013 Dec 17.

**Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders.**

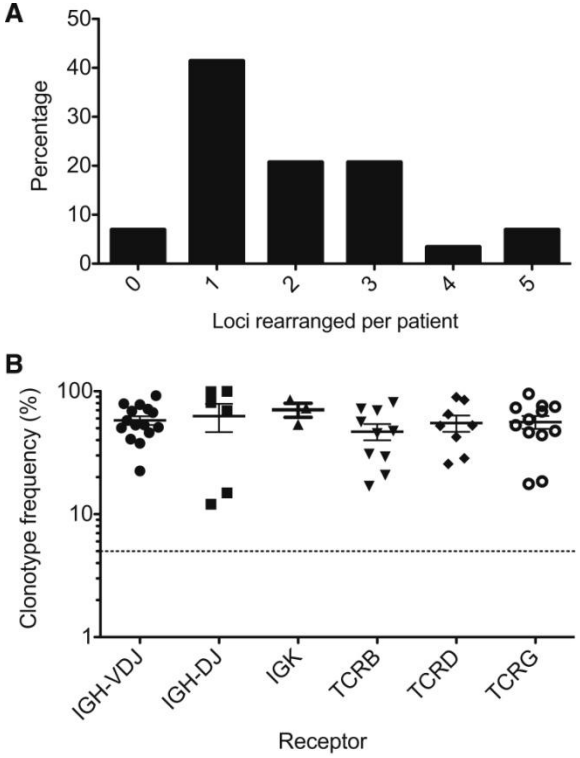
Ladetto M[1], Brüggemann M[2], Monitillo L[1], Ferrero S[1], Pepin F[3], Drandi D[1], Barbero D[1], Palumbo A[1], Passera R[4], Boccadoro M[1], Ritgen M[2], Gökbuget N[5], Zheng J[3], Carlton V[3], Trautmann H[2], Faham M[3], Pott C[2].

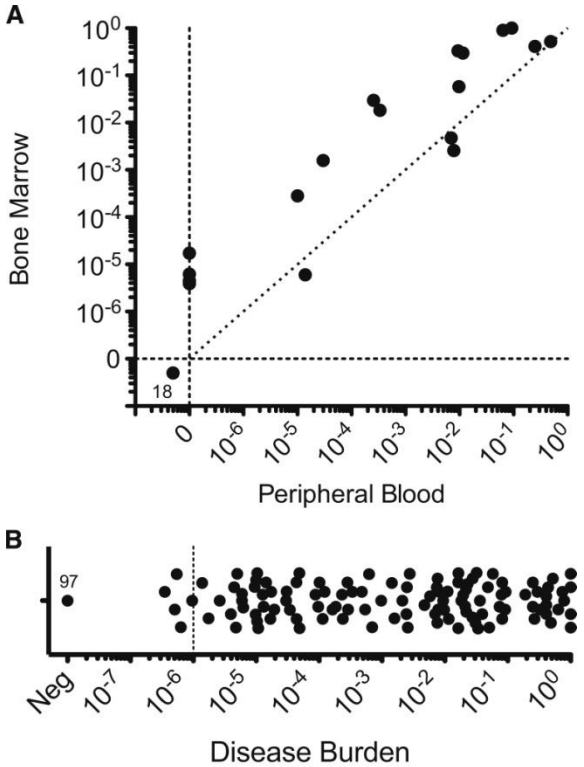
NGS showed at least the same level of sensitivity as allele-specific oligonucleotides-PCR, without the need for patient-specific reagents. We conclude that NGS is an effective tool for MRD monitoring in ALL, MCL and MM. Prospective comparative analysis of unselected cases is required to validate the clinical impact of NGS-based MRD assessment

*Immunoglobulin and T Cell Receptor Gene High-Throughput Sequencing Quantifies Minimal Residual Disease in Acute Lymphoblastic Leukemia and Predicts Post-Transplantation Relapse and Survival*

*Aaron C. Logan, Nikita Vashi, Malek Faham, Victoria Carlton, Katherine Kong, Ismael Buño, Jianbiao Zheng, Martin Moorhead, Mark Klinger, Bing Zhang, Amna Waqar, James L. Zehnder, David B. Miklos*

*Biology of Blood and Marrow Transplant*
Volume 20, Issue 9, Pages 1307-1313 (September 2014)
DOI: 10.1016/j.bbmt.2014.04.018



Blood. 2015 May 28;125(22):3501-8. doi: 10.1182/blood-2014-12-615757. Epub 2015 Apr 10.

**IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients.**

Pulsipher MA[1], Carlson C[2], Langholz B[3], Wall DA[4], Schultz KR[5], Bunin N[6], Kirsch I[7], Gastier-Foster JM[8], Borowitz M[9], Desmarais C[7], Williamson D[7], Kalos M[10], Grupp SA[11].

- Absence of detectable IgH-V(D)J NGS-MRD pre-HCT defines good-risk patients potentially eligible for less intense treatment approaches. Post-HCT NGS-MRD is highly predictive of relapse and survival, suggesting a role for this technique in defining patients early who would be eligible for post-HCT interventions.

## Altre applicazioni...

- **Sequenziamento del trascrittoma-RNAseq**
- **Sequenziamento di porzioni di DNA legate da proteine regolatorie-ChIPseq**
- **Metilazione**