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PANMEP
PROGRAMA ANDALUZ DE FORMACIÓN EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

EXPERTO UNIVERSITARIO EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

TÉCNICAS

FORMACIÓN IAVANTE
Fundación Progreso y Salud

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COLABORA

Janssen
PHARMACEUTICAL COMPANIES OF Johnson & Johnson

ORGANIZAN

UNIA Universidad Internacional de Andalucía

Junta de Andalucía
Consejería de Salud y Consumo

INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS

Que información nos da el RNA-Seq

Recogida de muestras

Estabilización del ARN

Extracción de ARN

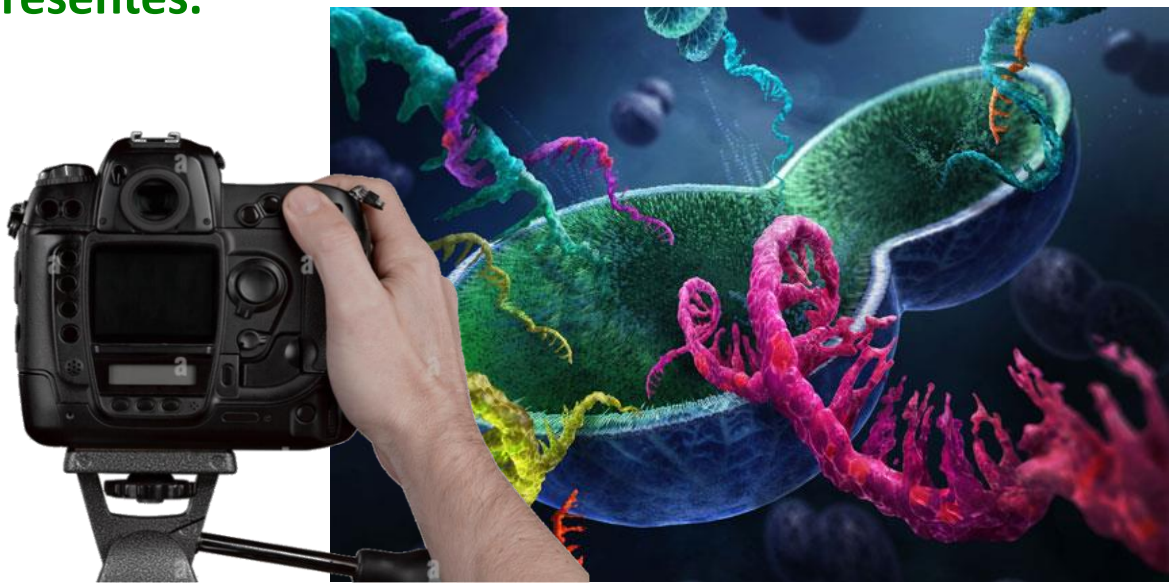
Cuantificación y calidad del ARN

Protocolo para realizar una librería de RNA-Seq

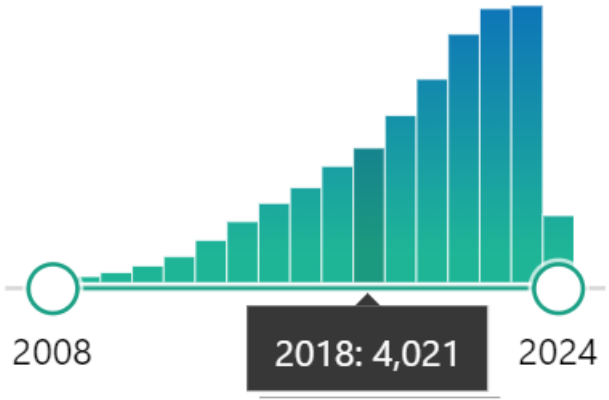
Secuenciación

INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS

RNA-Seq es una técnica que permite conocer el ARN presente (todo o parte de este) en una muestra biológica y cuantificar cada una de las moléculas presentes.



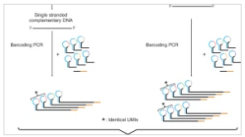
INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS



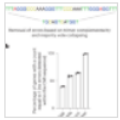
Digital RNA sequencing using unique molecular identifiers enables ultrasensitive RNA mutation analysis

Digital RNA sequencing using unique molecular identifiers and well-performing reverse transcription allows detection of mutant allele frequencies < 0.1%.

Manuel Luna Santamaría, Daniel Andersson ... Anders Ståhlberg

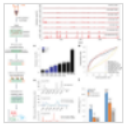


Homotrimer barcodes enable accurate counting of RNA molecules during high-throughput RNA sequencing



We pinpoint PCR artifacts as the primary source of inaccurate quantification in both short- and long-read RNA sequencing, a problem that intensifies with an increase in PCR cycles in both bulk and single-cell sequencing contexts. To overcome this challenge, we engineered a novel unique molecular identifier (UMI) barcode composed of homotrimer nucleotide blocks. This design facilitates accurate quantification of RNA molecules, substantially improving molecular counting.

RNA structure profiling at single-cell resolution reveals new determinants of cell identity

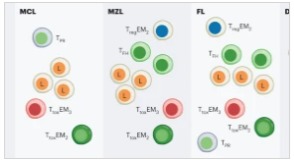


aneous determination of
llowing new insights into

Mapping T cell landscapes in B cell lymphomas

As a major microenvironmental component in B cell lymphomas, T cells are highly relevant for current immunotherapeutic treatment strategies of such tumours. A study now provides an unprecedented multimodal insight into the composition and features of T cell subsets of the four main types of nodal B cell non-Hodgkin lymphoma.

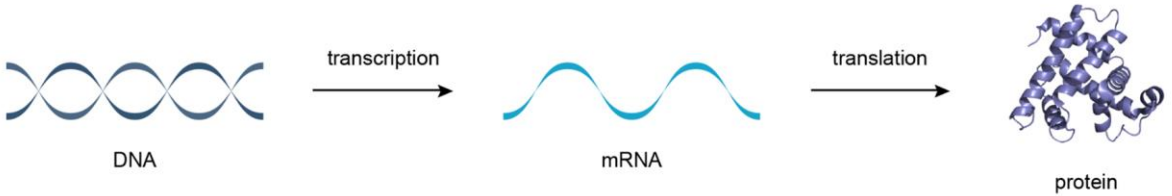
Ralf Küppers

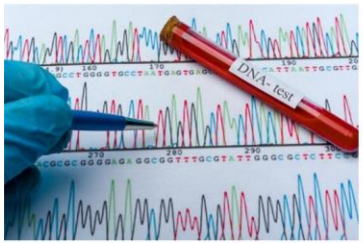


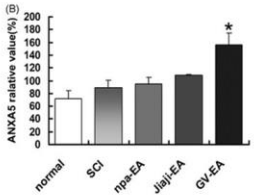
INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS

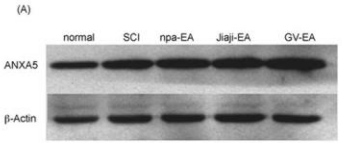
Dogma central de la biología molecular:

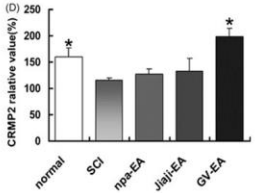
Un GEN -> Una PROTEINA (F. Crick 1958)

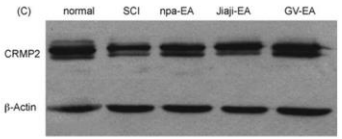




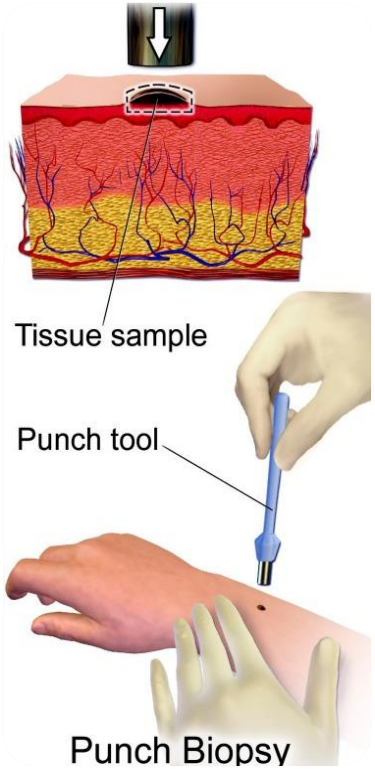


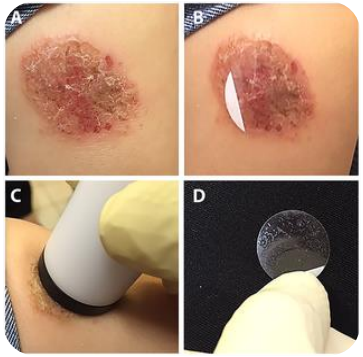


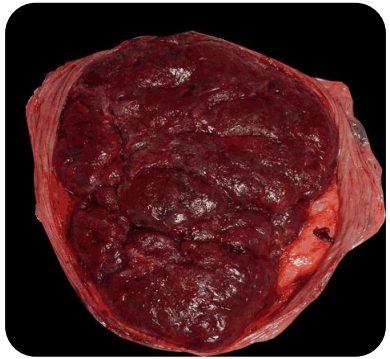




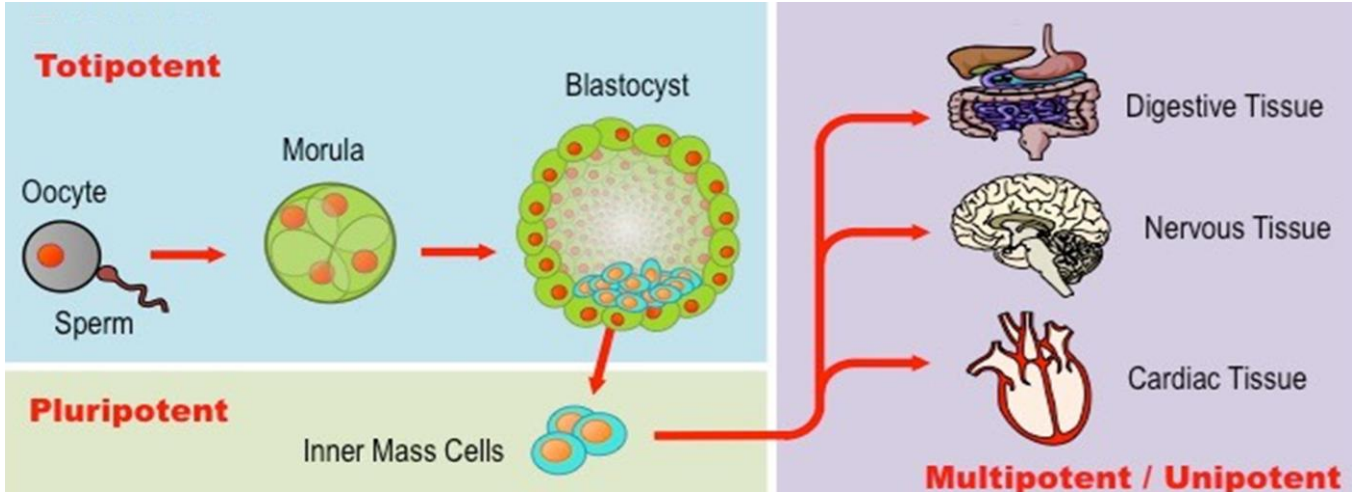
Recogida de muestras

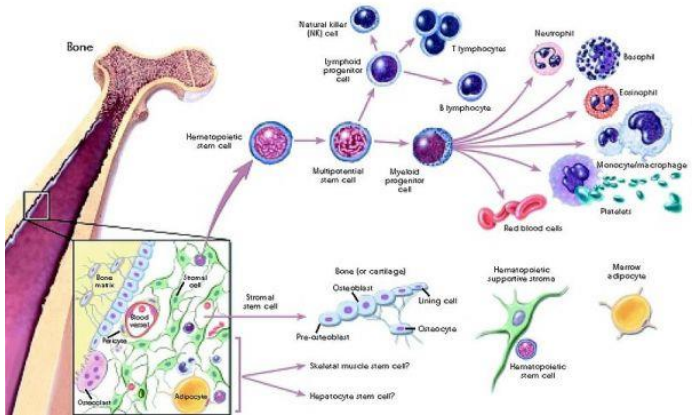


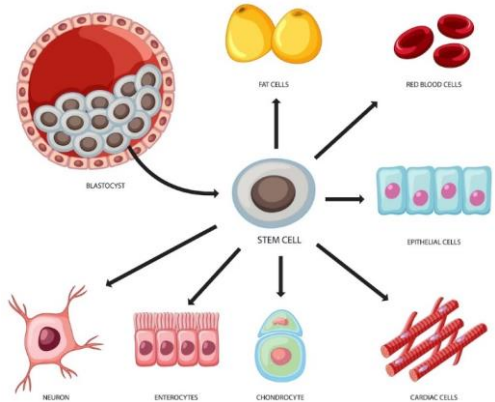




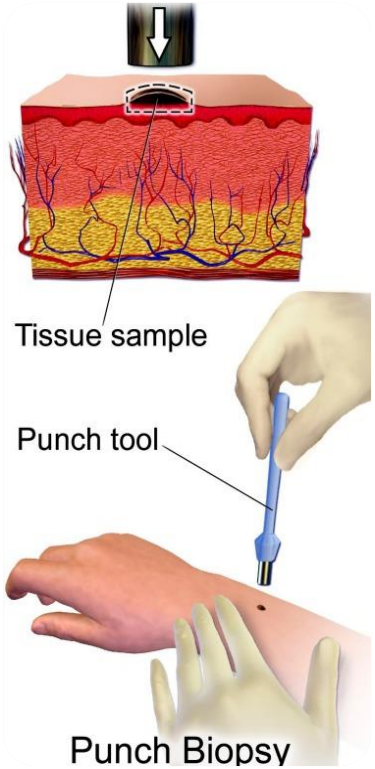
Patrones de expresión génica específicos de tejidos y células

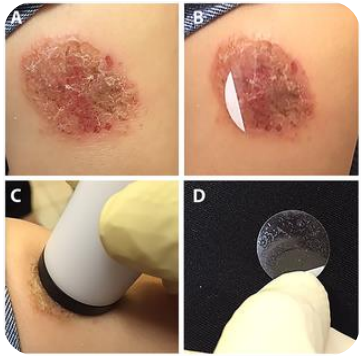


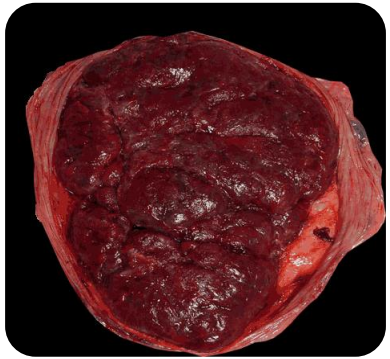




Recogida de muestras

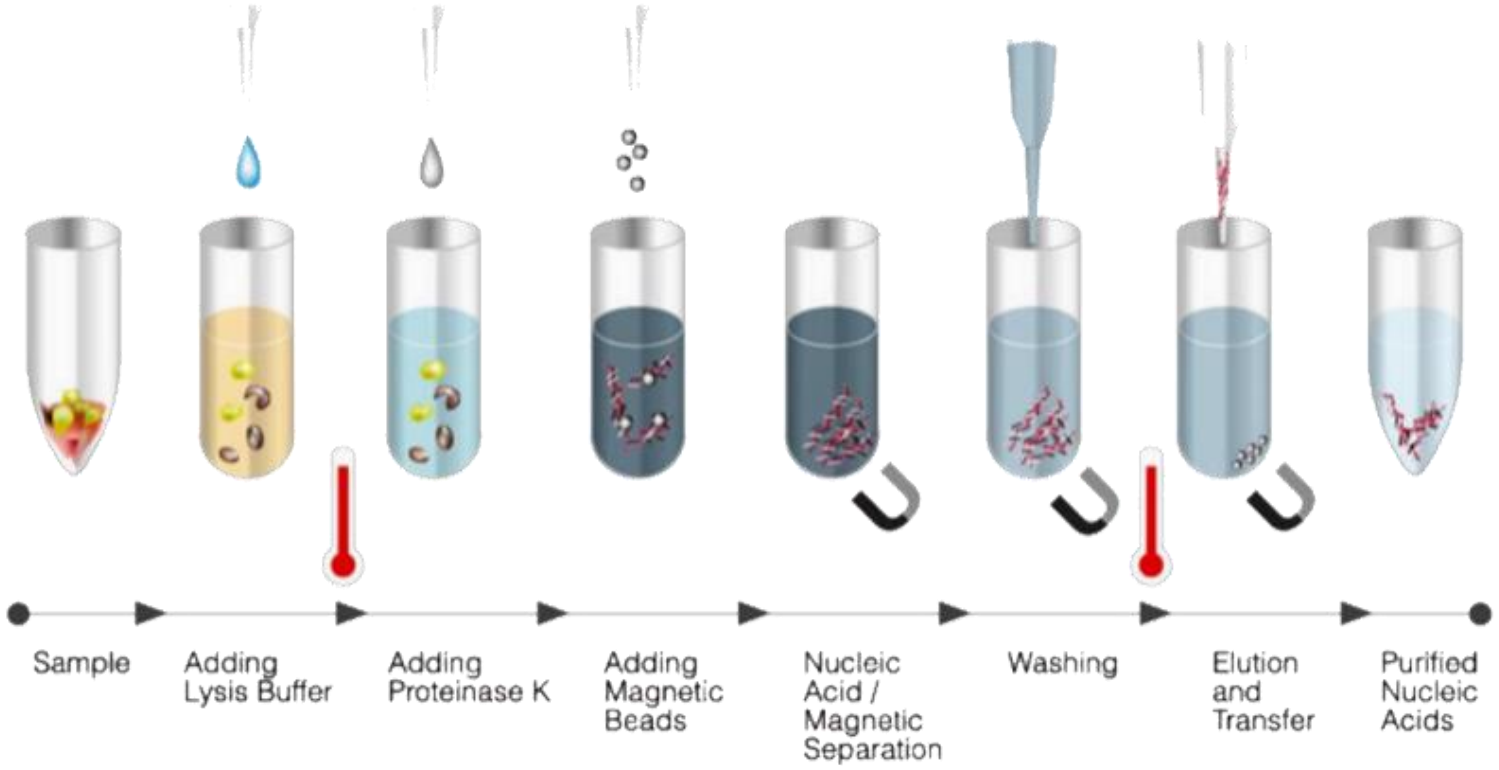






Estabilización del ARN

Extracción de ARN



Extracción de ARN

Cuantificación y calidad del ARN: RIN

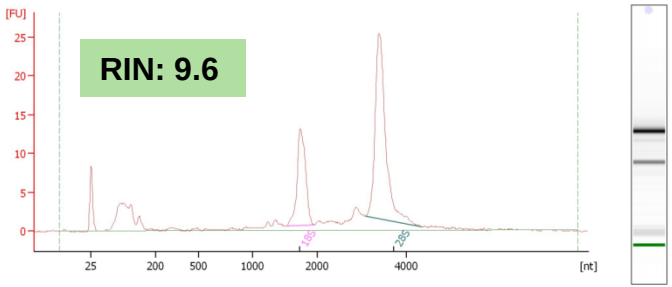
TapeStation

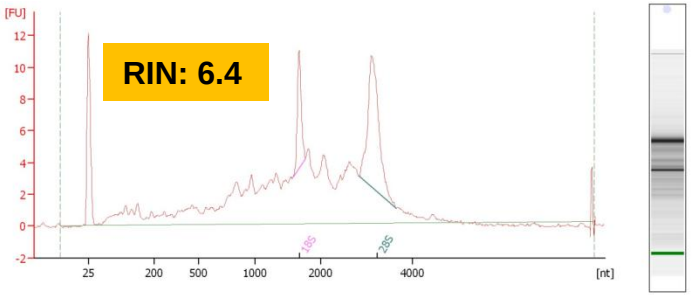
Nanodrop

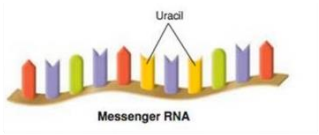
NanoQuant

Qubit

Bioanalyzer

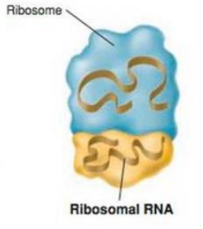






RNAs

- mRNAs
- Noncoding RNAs
  - ncRNAs Housekeeping
    - Ribosomal RNAs (rRNAs)
    - Transfer RNAs (tRNAs)
    - small nuclear RNAs (snRNAs)
    - Small Nucleolar RNAs (snoRNAs)
  - Regulatory ncRNAs
    - Small ncRNAs
      - micoRNAs (miRNAs)
      - PIWI-interacting (piRNAs)
      - Short interfering (siRNAs)
      - tRNA halves (tiRNAs)
    - Long ncRNAs
      - Large Intergenic ncRNA
      - Circulating Large ncRNA



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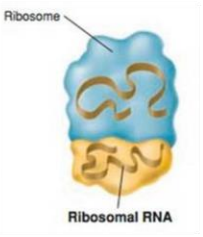

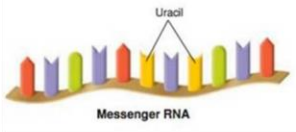

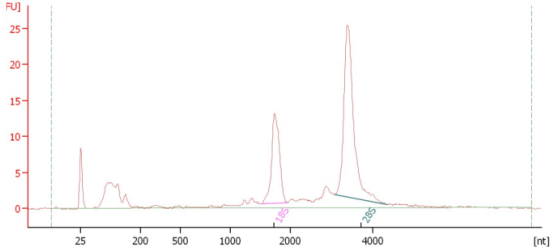

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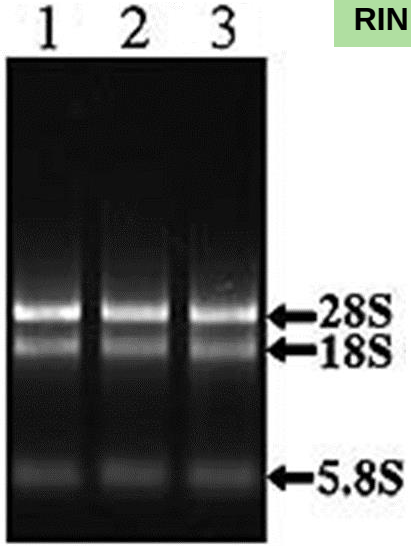
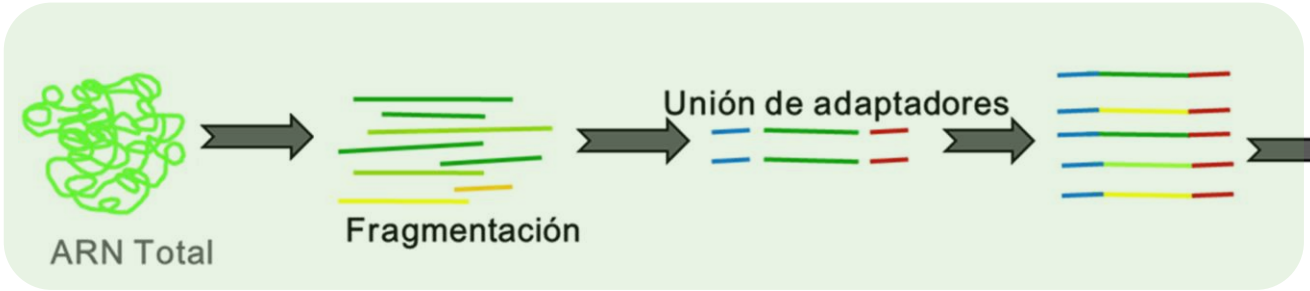
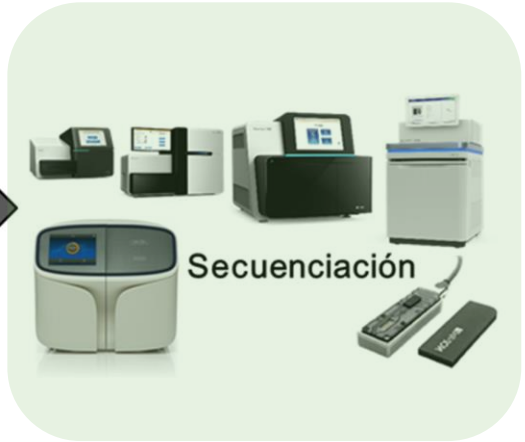


Librería de RNA



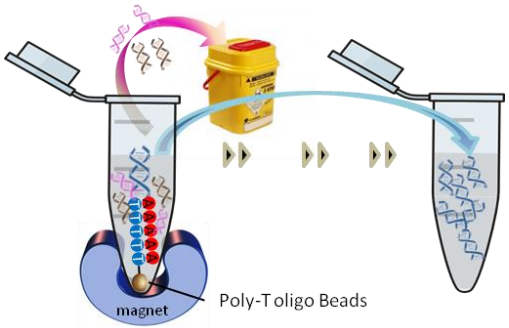


1. Total RNA ▸ Deplete Ribosomal RNA ▸ Fragment RNA
   mRNA ▸ Purify polyA mRNA ▸ Fragment mRNA
2. First Strand cDNA and Second Strand cDNA
3. Adenilate 3´Ends
4. Ligate adaptors and index
5. Enrich DNA fragment
6. Validate, Normalize and Pool Libraries

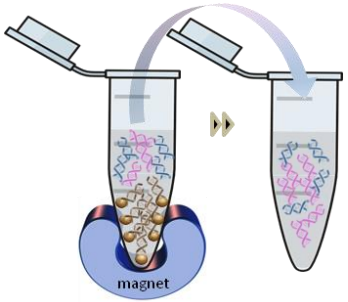
1 **Total RNA ▸ Deplete Ribosomal RNA ▸ Fragment RNA**

mRNA ▸ Purify polyA mRNA ▸ Fragment mRNA

mRNA Libraries



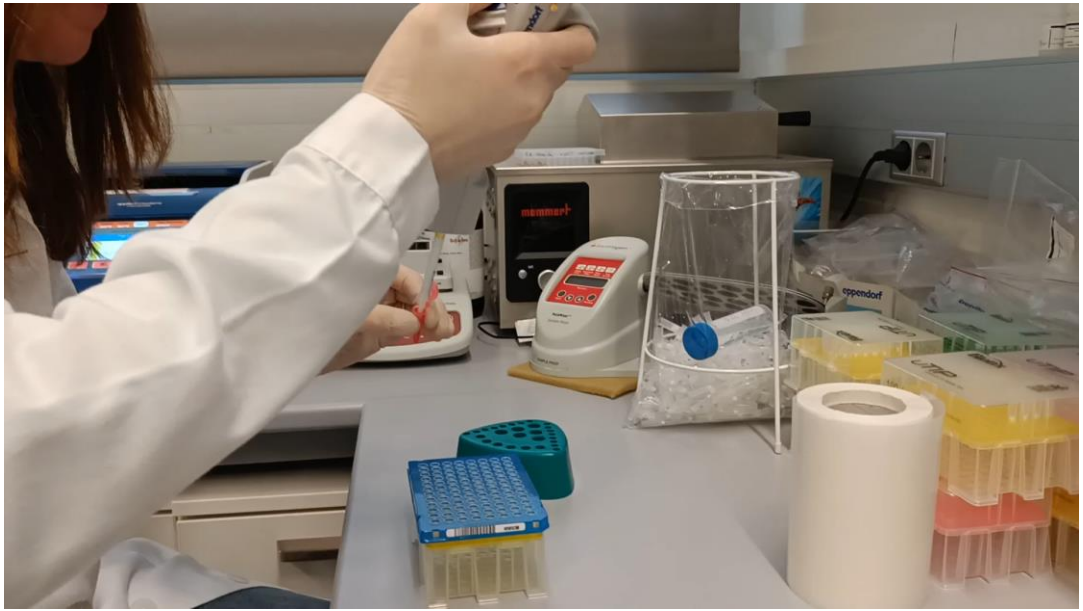
Total RNA Libraries



Fragment

1 Total RNA ▸ Deplete Ribosomal RNA ▸ Fragment RNA
mRNA ▸ Purify polyA mRNA ▸ Fragment mRNA

2 **First Strand and Second Strand cDNA**



1 Total RNA ▸ Deplete Ribosomal RNA ▸ Fragment RNA
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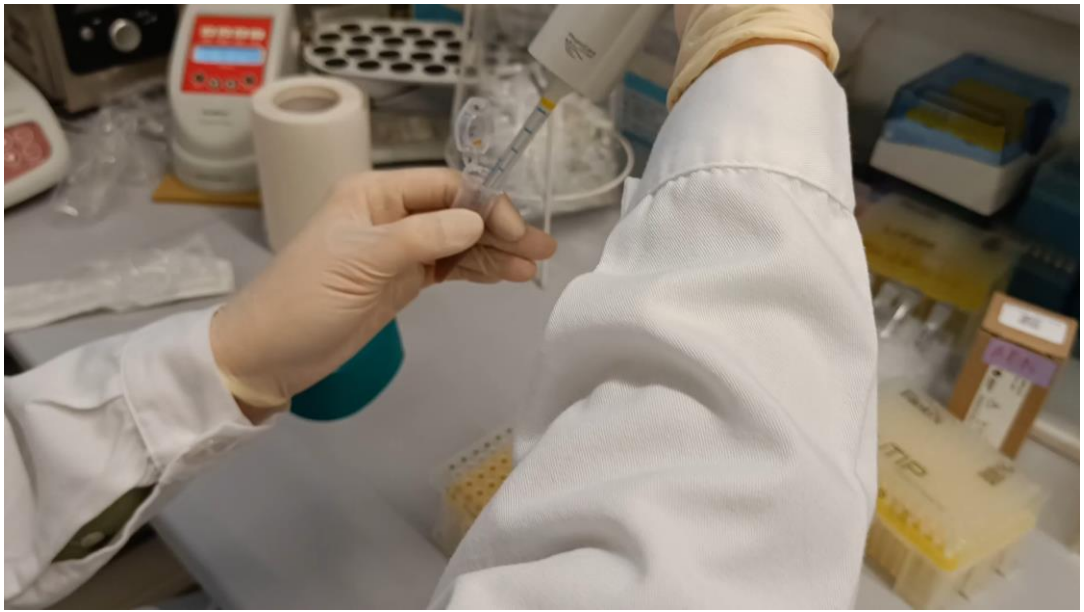
2

First Strand and Second Strand cDNA



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2 First Strand cDNA and Second Strand cDNA

3 **Adenilate 3´Ends**

RNA Libraries: Total RNA and mRNA

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2. First Strand cDNA and Second Strand cDNA
3. Adenilate 3´Ends
4. **Ligate adaptors and index**

RNA Libraries: Total RNA and mRNA

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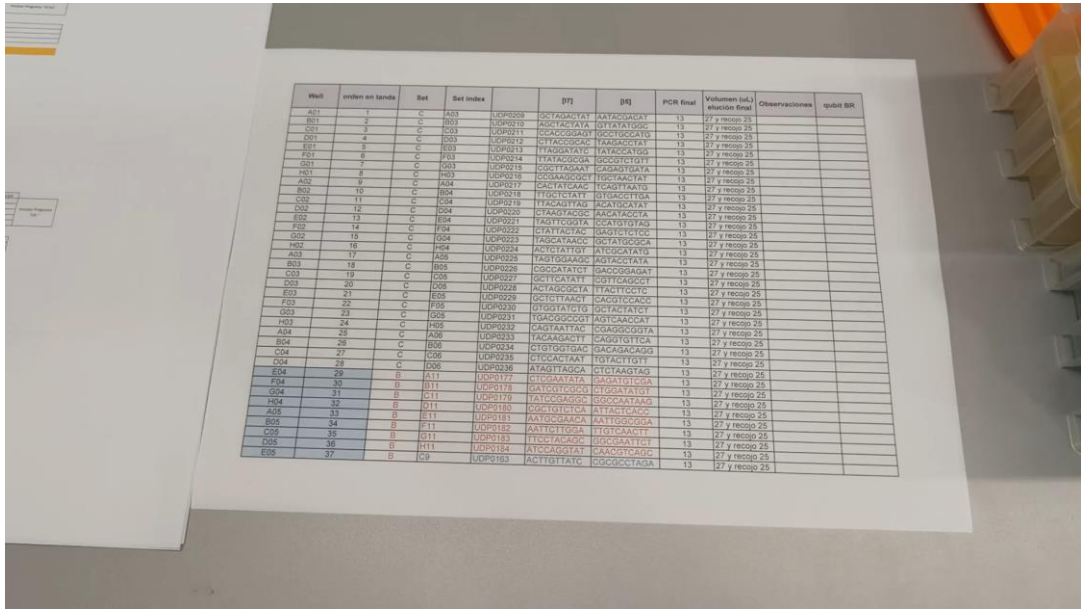
mRNA ▸ Purify polyA mRNA ▸ Fragment mRNA

2 First Strand cDNA and Second Strand cDNA

3 Adenilate 3´Ends

4 **Ligate adaptors and index**

RNA Libraries: Total RNA and mRNA



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5. **Enrich DNA fragment**

RNA Libraries: Total RNA and mRNA

RNA Libraries: Total RNA and mRNA

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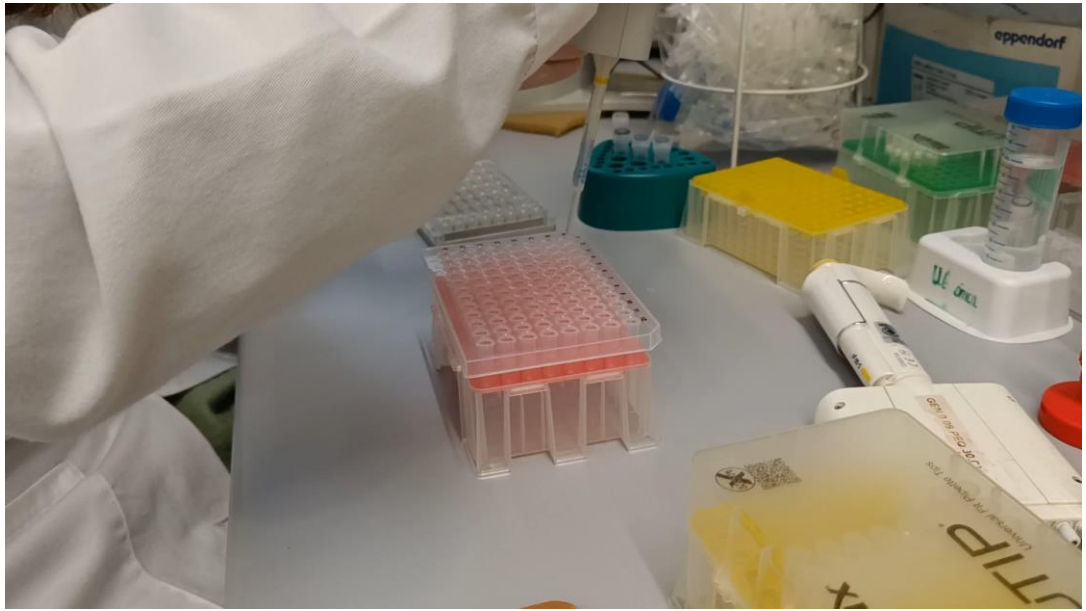

RNA Libraries: Total RNA and mRNA

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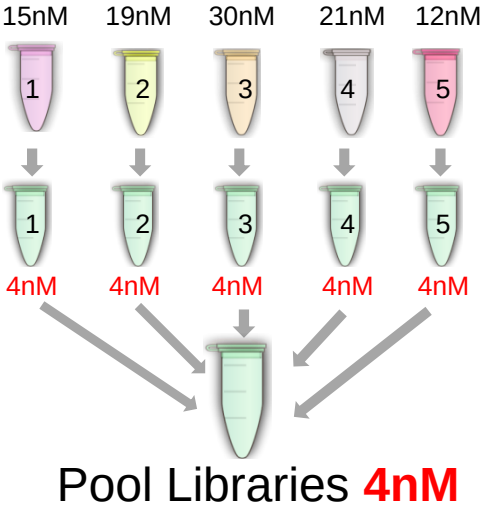
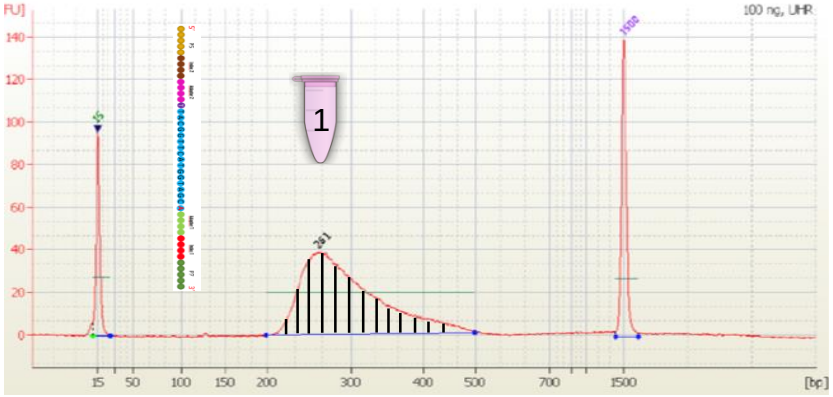
6. **Validate, Normalize and Pool Libraries**





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5. Enrich DNA fragment
6. Validate, Normalize and Pool
7. **Sequencing**

1. Total RNA ▸ Deplete Ribosomal RNA ▸ Fragment RNA
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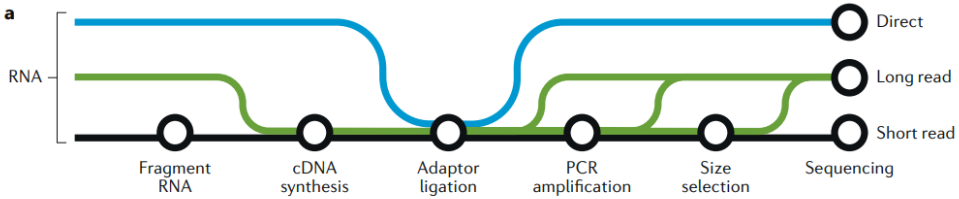
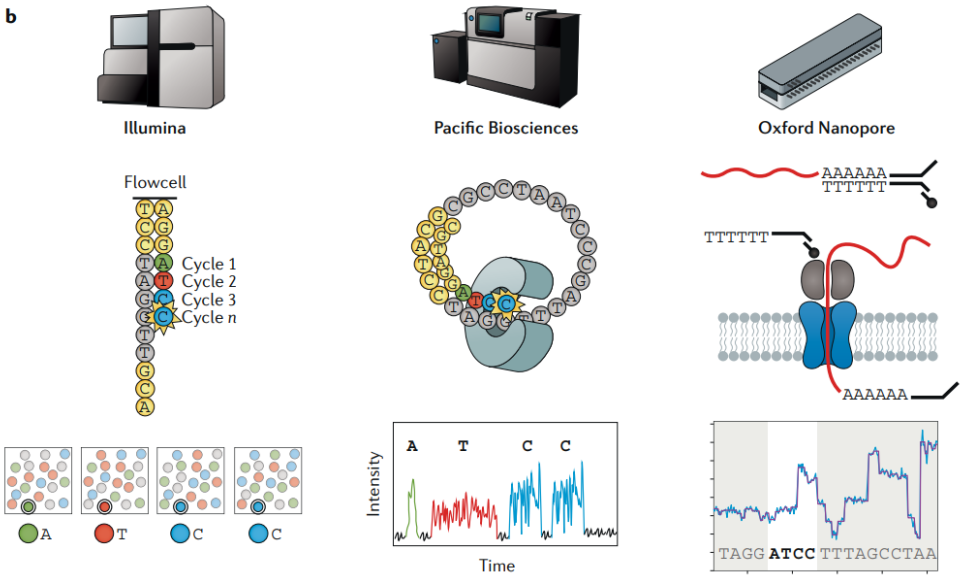
6. Validate, Normalize and Pool Libraries
7. **Sequencing**





Stark R, Grzelak M, Hadfield J. RNA sequencing: the teenage years. *Nat Rev Genet*. 2019

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