

<https://medicinaprecisionandalucia.iavante.es> | #PANMEP

PANMEP

PROGRAMA ANDALUZ DE FORMACIÓN EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

EXPERTO UNIVERSITARIO EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

TÉCNICAS

FORMACIÓN
IIVANTE
Fundación
Progreso y Salud



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COLABORA



ORGANIZAN



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

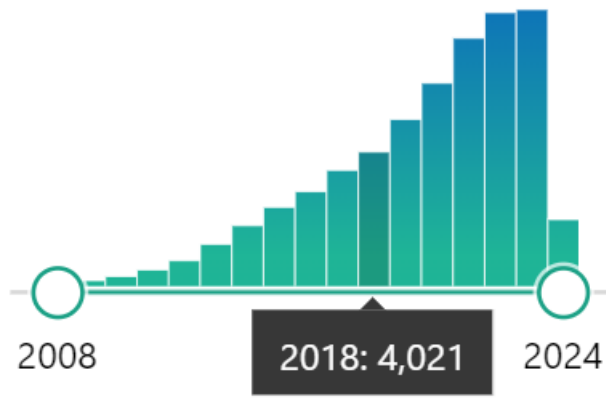
Secuenciación

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.



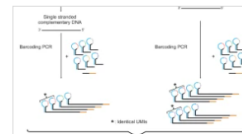
INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS



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

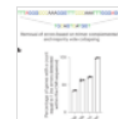
Digital RNA sequencing using unique molecular identifiers and well-performing reverse transcription allows detection of mutant allele frequencies < 0.1%.

Manuel Luna Santamaría, Daniel Andersson ... Anders Ståhlberg

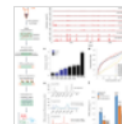


Homotrimer barcodes enable accurate counting of RNA molecules during high-throughput RNA sequencing

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



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

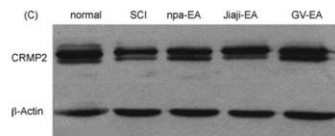
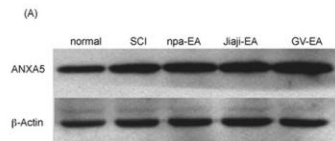
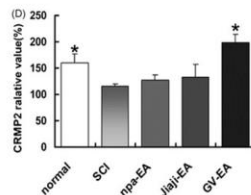
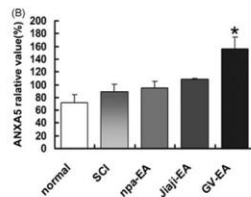
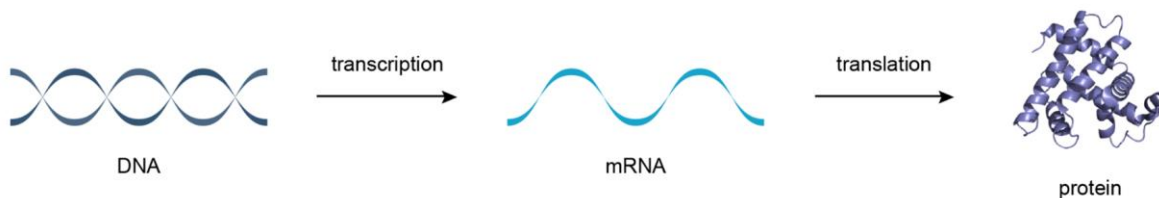


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lowing new insights into

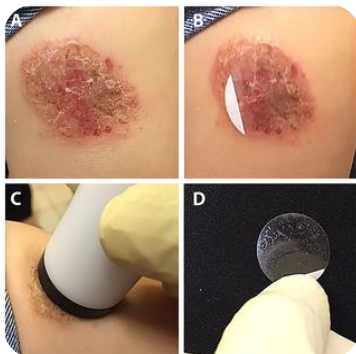
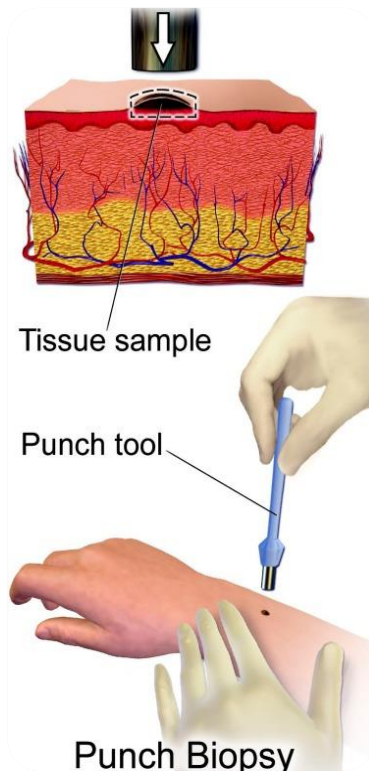
INTRODUCCIÓN AL ANÁLISIS DE ARN MEDIANTE SECUENCIACIÓN POR NGS

Dogma central de la biología molecular:

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



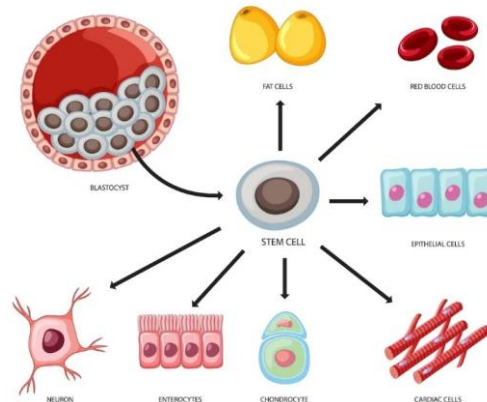
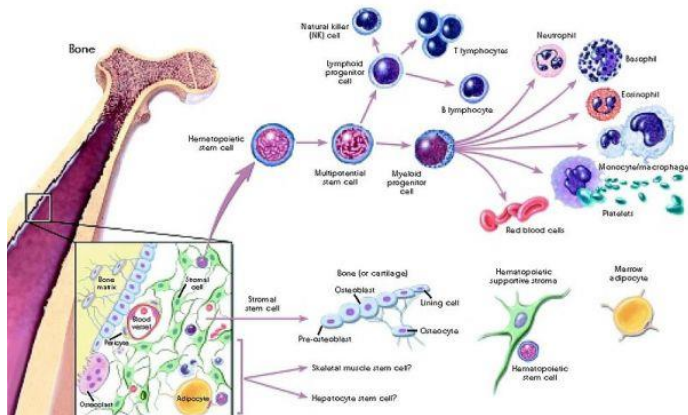
Recogida de muestras



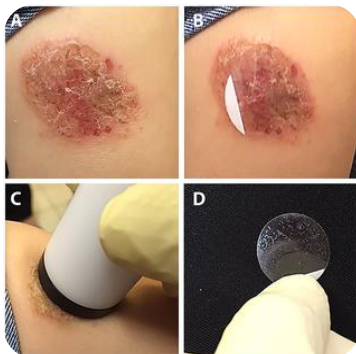
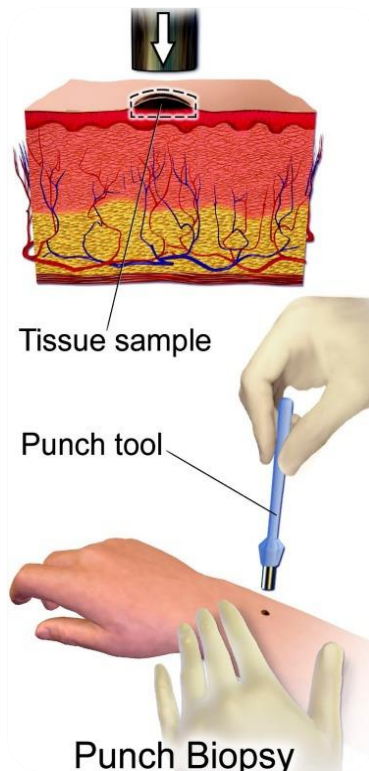
The diagram illustrates the differentiation of stem cells. It is divided into two main horizontal sections: a light blue top section for 'Totipotent' and a light green bottom section for 'Pluripotent'.

Totipotent: This section shows the initial stages of development. It starts with an 'Oocyte' (a large grey sphere with an orange nucleus) and a 'Sperm' (a small, tadpole-like cell). An arrow points to a 'Morula' (a green sphere containing several orange nuclei). Another arrow points to a 'Blastocyst' (a green sphere with a cluster of orange nuclei on one side and a layer of smaller green cells on the other).

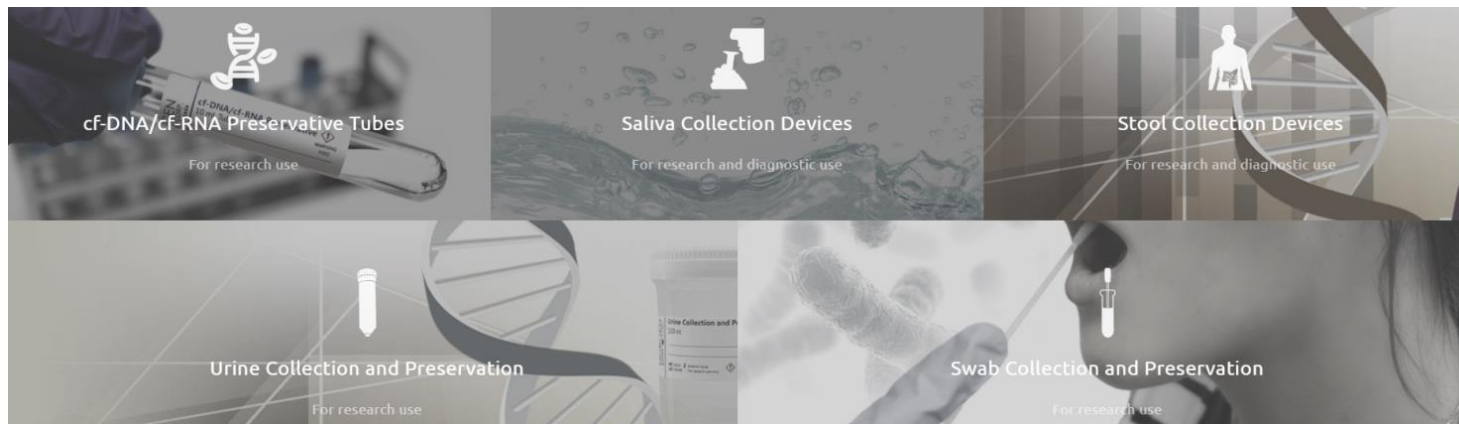
Pluripotent: This section shows the differentiation of the inner mass cells. An arrow points from the 'Inner Mass Cells' (a cluster of orange nuclei) to three specialized tissues, each represented by an illustration and a label: 'Digestive Tissue' (stomach and intestines), 'Nervous Tissue' (brain), and 'Cardiac Tissue' (heart). A red bracket groups these three tissues under the label 'Multipotent / Unipotent'.



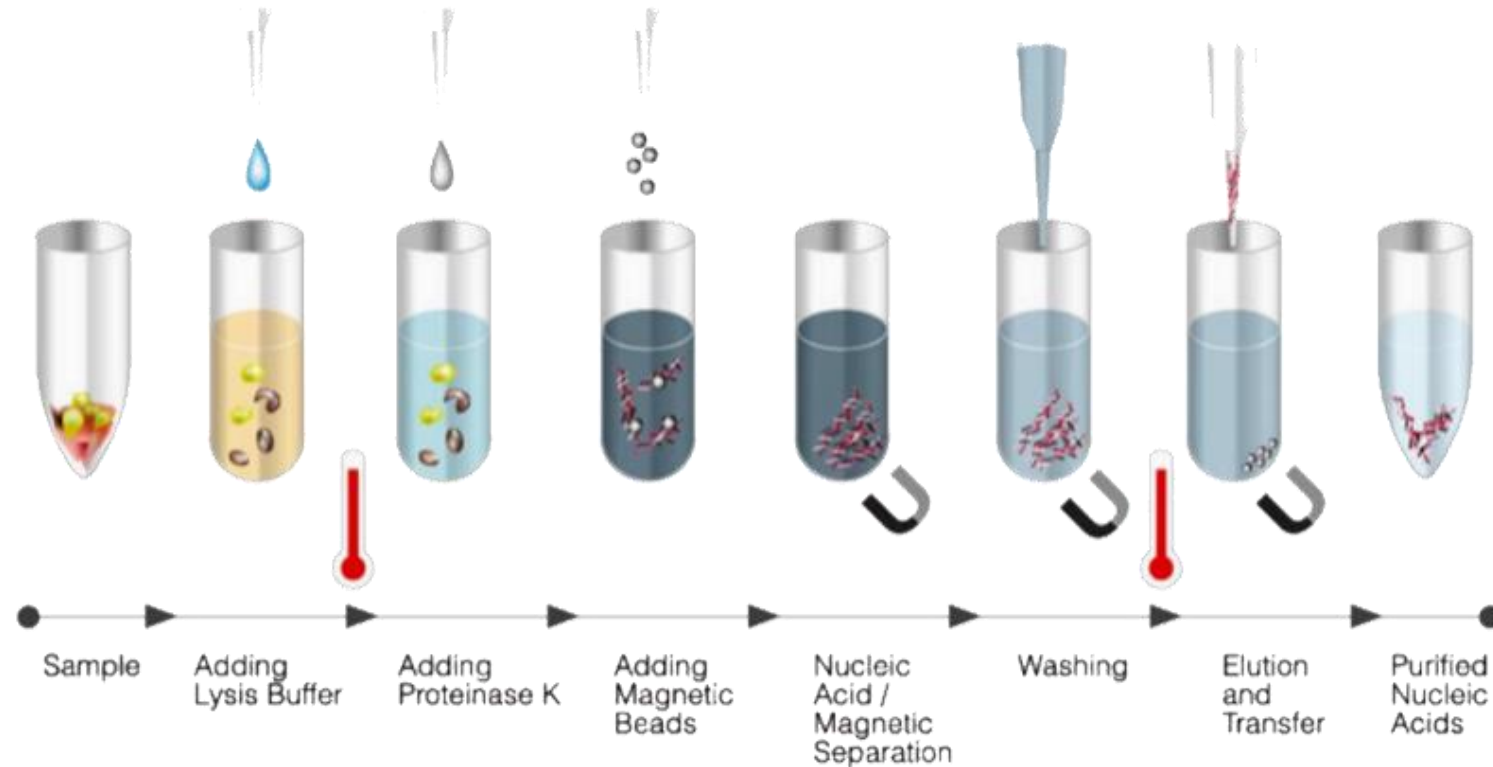
Recogida de muestras



Estabilización del ARN



Extracción de ARN



Extracción de ARN



Cuantificación y calidad del ARN: RIN



TapeStation



Nanodrop



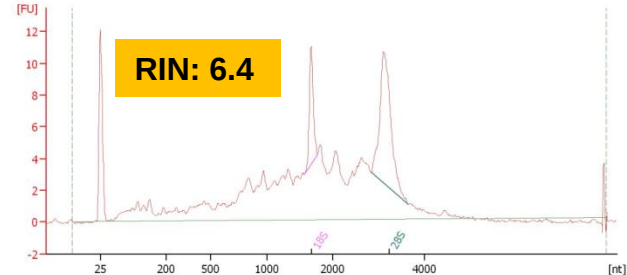
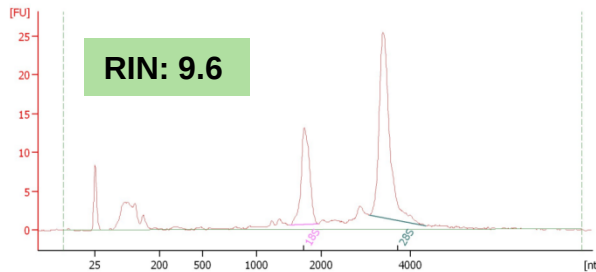
NanoQuant

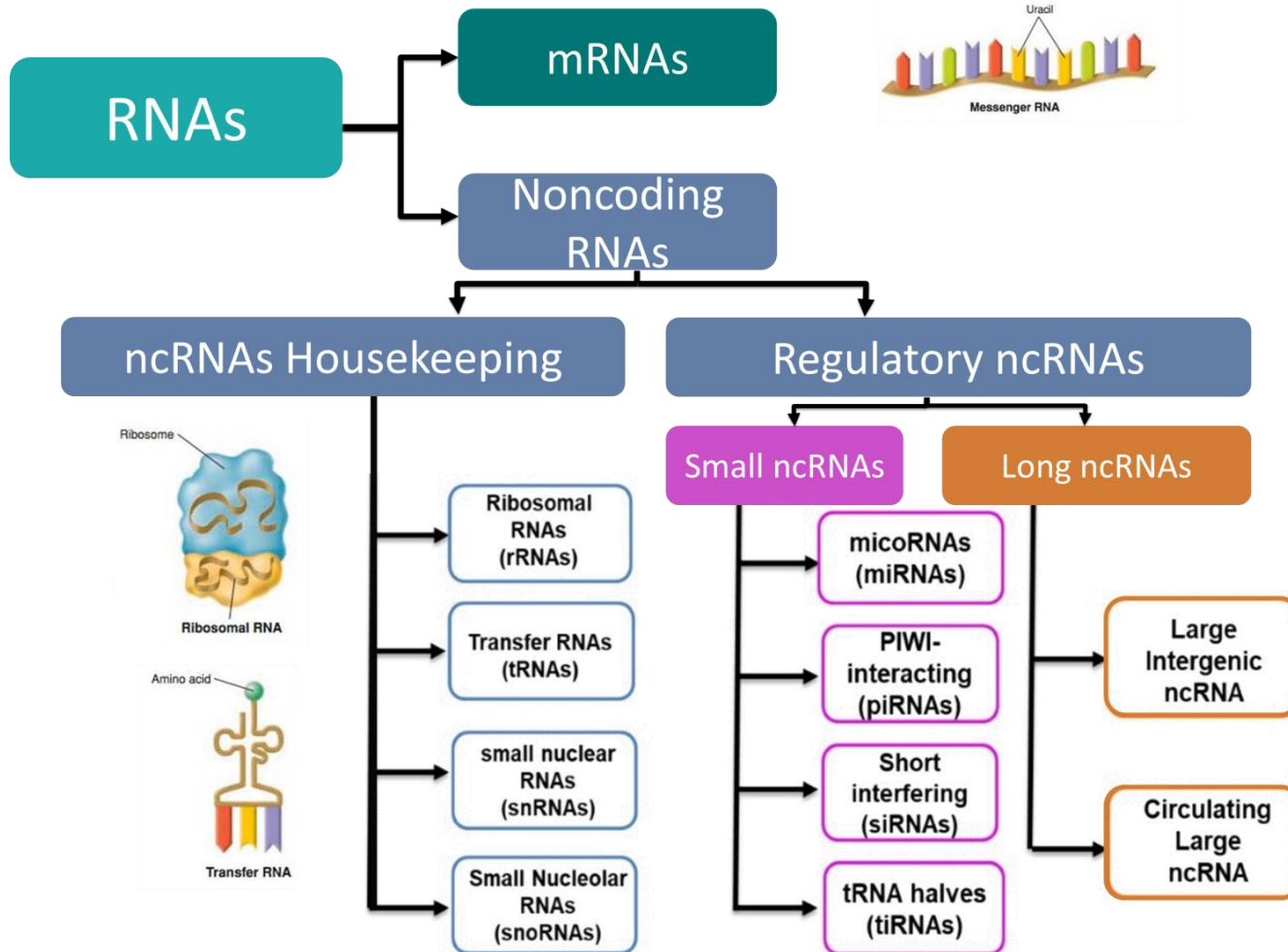
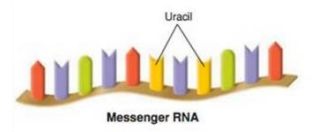


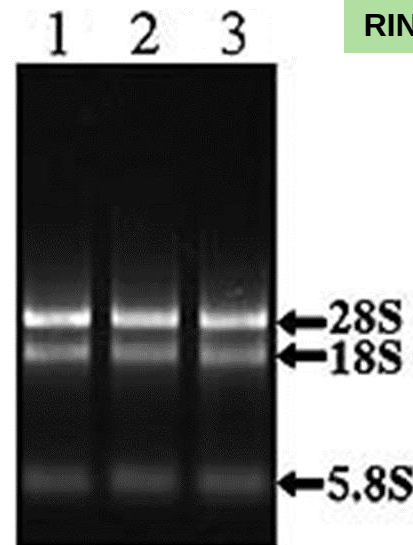
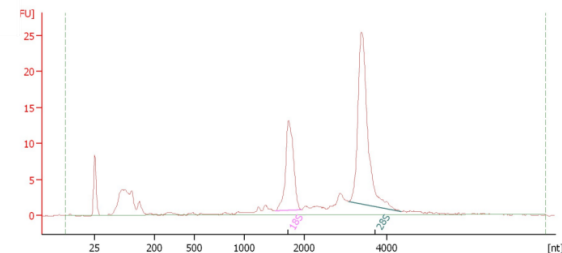
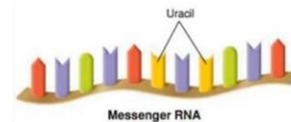
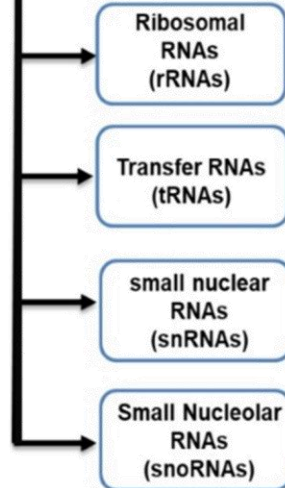
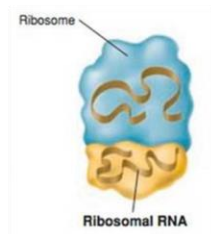
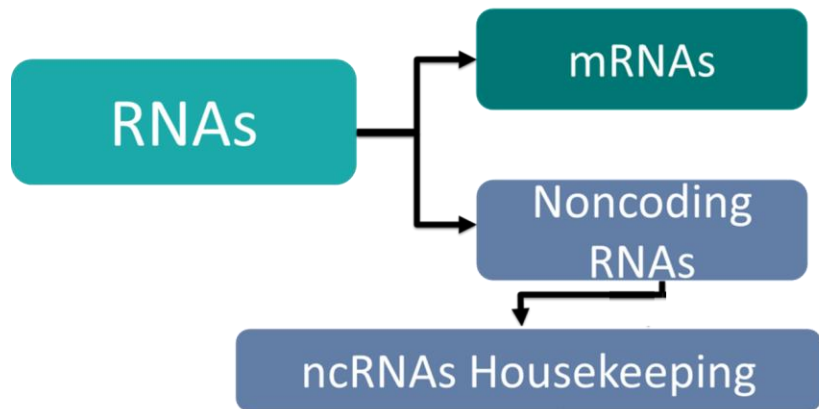
Qubit



Bioanalyzer

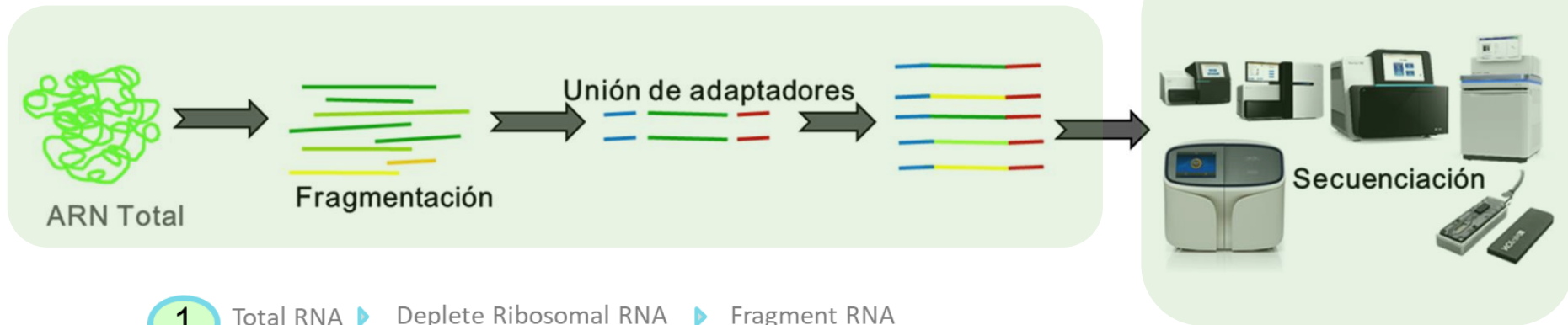






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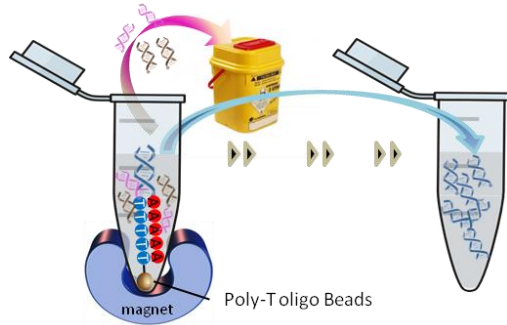
Librería de RNA



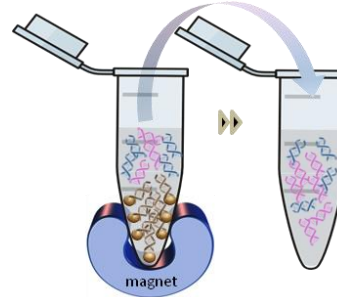
- 1 Total RNA ▶ Deplete Ribosomal RNA ▶ Fragment RNA mRNA ▶ Purify polyA mRNA ▶ Fragment mRNA
- 2 First Strand cDNA and Second Strand cDNA
- 3 Adenilate 3' Ends
- 4 Ligate adaptors and index
- 5 Enrich DNA fragment
- 6 Validate, Normalize and Pool Libraries

- 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
mRNA ▶ Purify polyA mRNA ▶ Fragment mRNA

2

First Strand and Second Strand cDNA



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

RNA Libraries: Total RNA and mRNA



1 Total RNA ▶ Deplete Ribosomal RNA ▶ Fragment RNA
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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



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** RNA Libraries: Total RNA and mRNA

Well	orden en bande	Set	Set index	[P7]	[P8]	PCR final	Volumen (µL) elución final	Observaciones	qubit BR
A01	1	C	A03	UDR0009	GCATAGATAT	AATAGGAGAT	13	27 y reseqo 25	
B01	2	C	B03	UDR0109	AGATAGTATA	GTATATGAGC	13	27 y reseqo 25	
C01	3	C	C03	UDR0211	GCACCGAGAT	GGCGGCGATG	13	27 y reseqo 25	
D01	4	C	D03	UDR0112	CTTACCGGAC	TAGAGGCTAT	13	27 y reseqo 25	
E01	5	C	E03	UDR0213	TGAGGATATC	TATGCAATGG	13	27 y reseqo 25	
F01	6	C	F03	UDR0214	TTATAGCGGA	GGCGTCTGTT	13	27 y reseqo 25	
G01	7	C	G03	UDR0215	CGGTAGAGAT	CAGGAGATTA	13	27 y reseqo 25	
A02	8	C	H03	UDR0216	CCGAGAGCGT	TGCTAGCTAT	13	27 y reseqo 25	
B02	9	C	A04	UDR0217	GCATATGAGC	TGCTATGATG	13	27 y reseqo 25	
C02	10	C	B04	UDR0218	TTCCTCTATT	TGTGACCTTGA	13	27 y reseqo 25	
D02	11	C	C04	UDR0219	TTACATTTAG	ACATACATAT	13	27 y reseqo 25	
E02	12	C	D04	UDR0220	CTAGCTACCG	ACATACATAT	13	27 y reseqo 25	
F02	13	C	E04	UDR0221	TAGTTCGGTA	CCATGTGTAG	13	27 y reseqo 25	
G02	14	C	F04	UDR0222	CTATATATAG	TAGTGTCTCG	13	27 y reseqo 25	
A03	15	C	G04	UDR0223	TAGCATAGCG	GCATAGCGCA	13	27 y reseqo 25	
B03	16	C	H04	UDR0224	AGCTGTATGT	ATTCGATATG	13	27 y reseqo 25	
C03	17	C	A05	UDR0225	TAGTGGAGAG	AGTACCTATA	13	27 y reseqo 25	
D03	18	C	B05	UDR0226	CGCGATATCT	GACCGGAGAT	13	27 y reseqo 25	
E03	19	C	C05	UDR0227	GCATCATATY	CGTTGAGGCT	13	27 y reseqo 25	
F03	20	C	D05	UDR0228	ACATAGCGTA	TTACTTCTCT	13	27 y reseqo 25	
G03	21	C	E05	UDR0229	GCCTTTAGCT	CACGTCGAGC	13	27 y reseqo 25	
A04	22	C	F05	UDR0230	GTGTATATCT	GCCTATATCT	13	27 y reseqo 25	
B04	23	C	G05	UDR0231	TGACGGGCGT	AGTCAACCAT	13	27 y reseqo 25	
C04	24	C	H05	UDR0232	CAGTATATAG	TGAGGCGGCTA	13	27 y reseqo 25	
D04	25	C	A06	UDR0233	TACAGAGCTY	CAGGTGTGCA	13	27 y reseqo 25	
E04	26	C	B06	UDR0234	CTGTGTGTAC	GACAGAGAGG	13	27 y reseqo 25	
F04	27	C	C06	UDR0235	GTCCACTAT	TGTATCTGTT	13	27 y reseqo 25	
G04	28	C	D06	UDR0236	ATAGTTAGCA	GTCTAAGTAG	13	27 y reseqo 25	
A05	29	B	A11	UDR0177	GTGAAATATA	GAGATGTGGA	13	27 y reseqo 25	
B05	30	B	B11	UDR0178	GATCGTGGCG	GTGATATGTT	13	27 y reseqo 25	
C05	31	B	C11	UDR0179	TATCGGAGGG	GGCGAATAAG	13	27 y reseqo 25	
D05	32	B	D11	UDR0180	CGCTGTCTCA	ATTACACAGC	13	27 y reseqo 25	
E05	33	B	E11	UDR0181	AATCGGAGCA	AATTGGGCGGA	13	27 y reseqo 25	
F05	34	B	F11	UDR0182	AATTGTGGA	TGTCAAGTTT	13	27 y reseqo 25	
G05	35	B	G11	UDR0183	TTCTAGAGGG	GCGGATATCT	13	27 y reseqo 25	
A06	36	B	H11	UDR0184	ATCGAGGTAT	CAACGTCTAGC	13	27 y reseqo 25	
B06	37	B	I09	UDR0185	ACTGTATTATC	CGCGGCTAGA	13	27 y reseqo 25	

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**

RNA Libraries: Total RNA and mRNA



1 Total RNA ▶ Deplete Ribosomal RNA ▶ Fragment RNA
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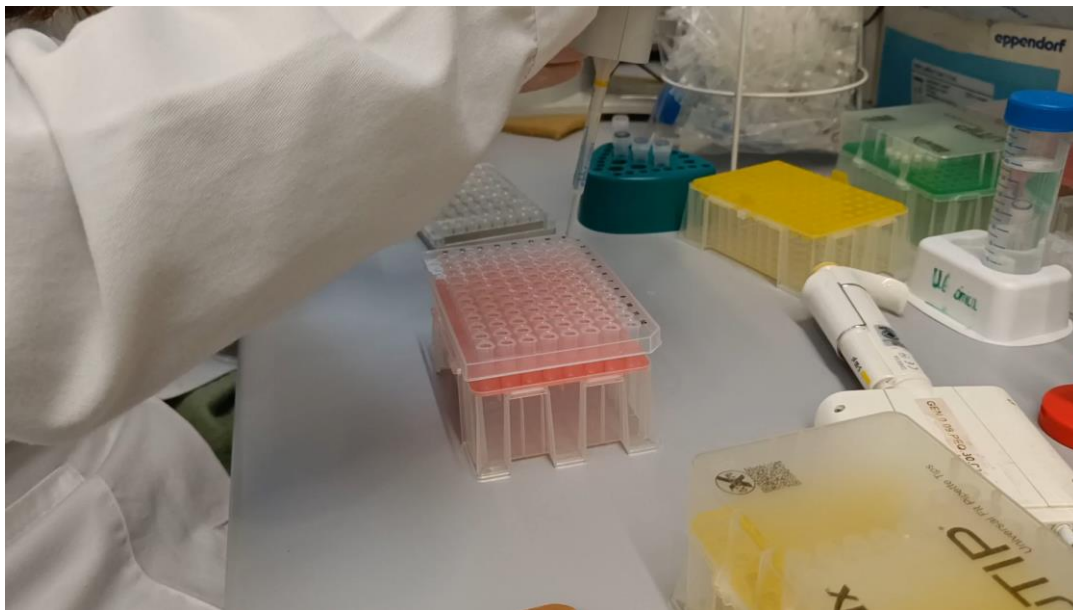
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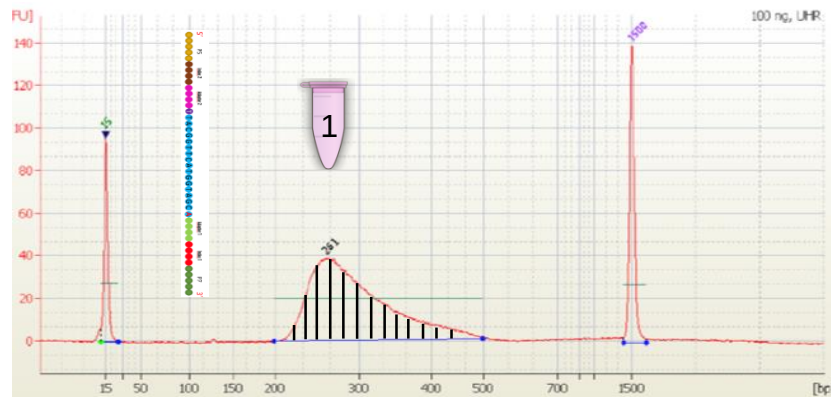
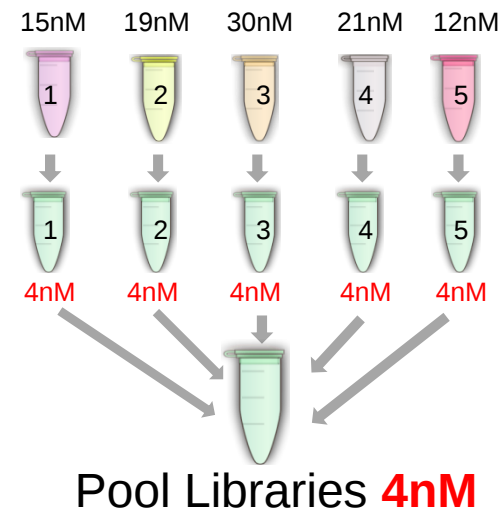
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6 **Validate, Normalize and Pool Libraries**



RNA Libraries: Total RNA and mRNA



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7 Sequencing

Introduction to Sequencing by Synthesis

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2 First Strand cDNA and Second Strand cl

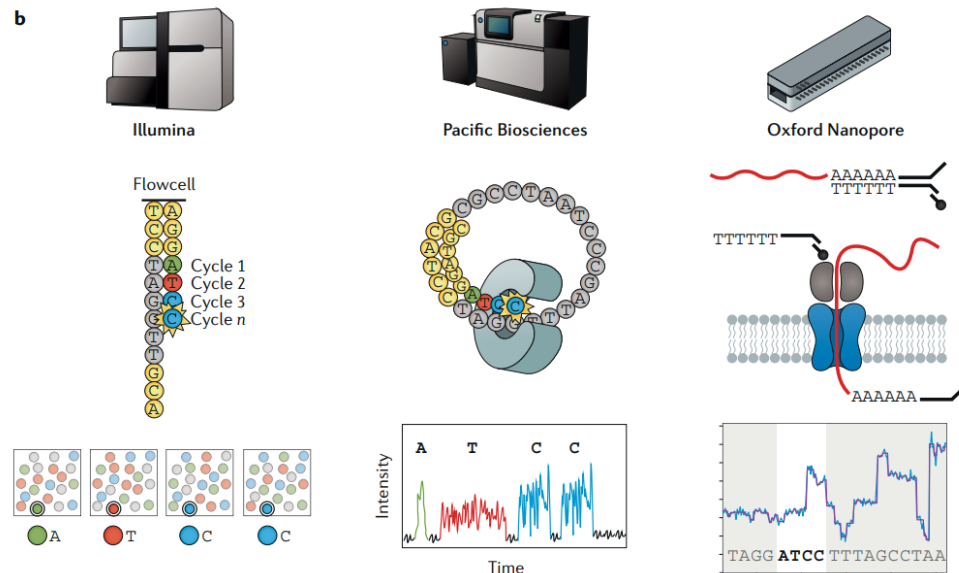
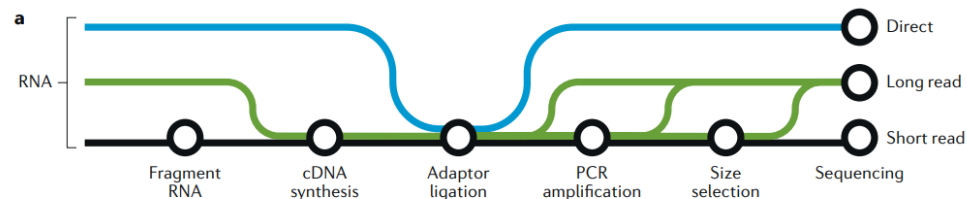
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