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PANMEP

PROGRAMA ANDALUZ DE FORMACIÓN EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

EXPERTO UNIVERSITARIO EN MEDICINA PERSONALIZADA Y DE PRECISIÓN

TÉCNICAS

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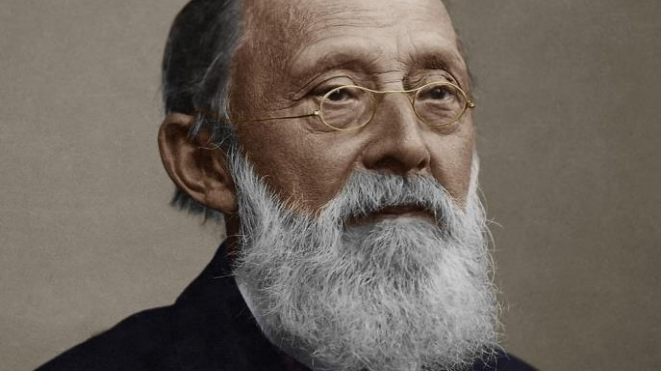
Universidad Internacional de Andalucía

Junta de Andalucía
Consejería de Salud y Consumo

PLATAFORMAS PARA ANÁLISIS DE CÉLULA ÚNICA, ESPECIFICACIONES TÉCNICAS Y METODOLÓGICAS

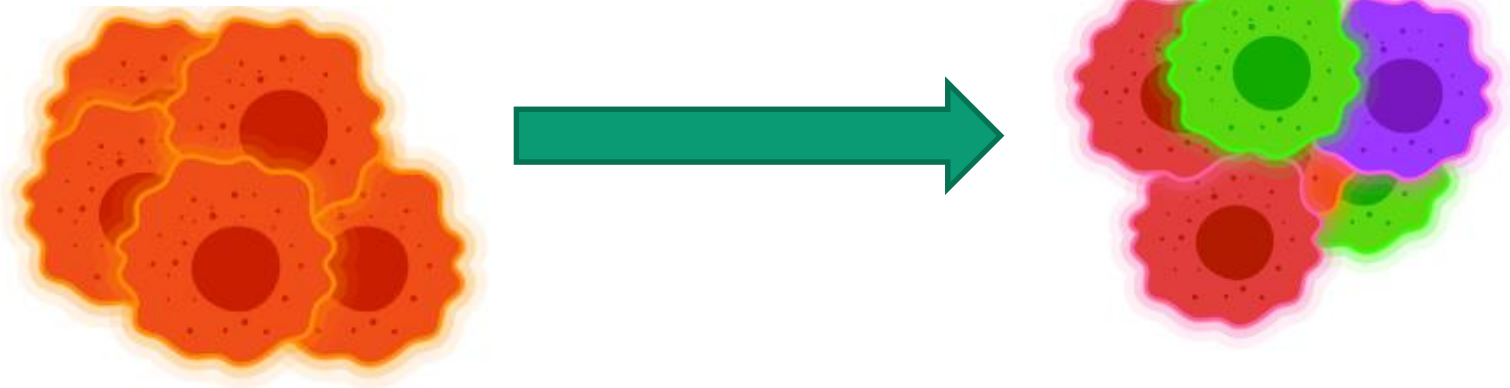
Presentación de las principales herramientas de análisis de célula única

MARIA JOSE SERRANO . PhD
GRUPO BIOPSIA LIQUIDA E INTERCEPCION DEL CANCER
UGC ANATOMIA PATOLÓGICA. HUVN / GENYO . GRANADA



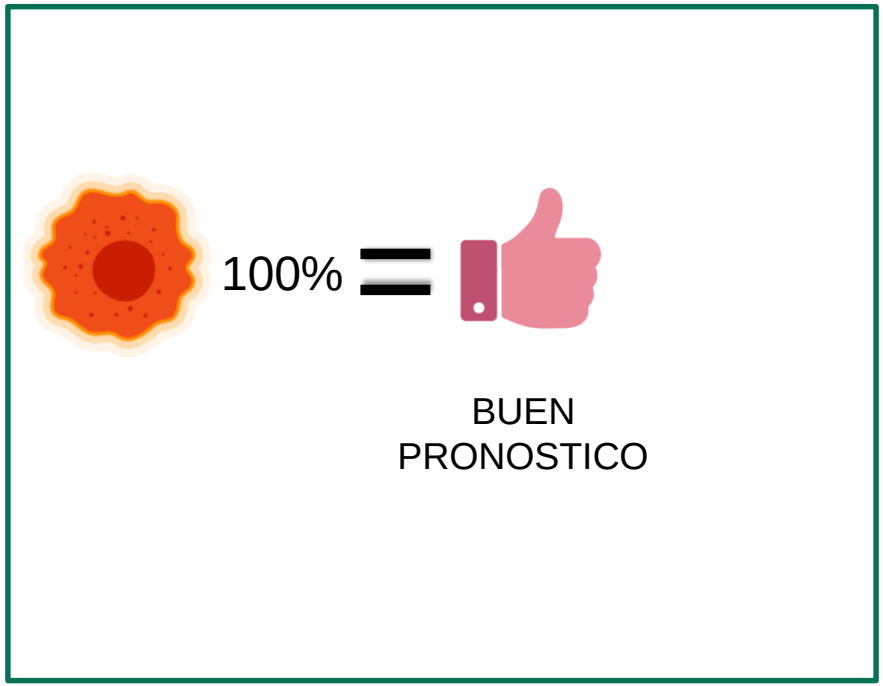
No matter how we twist and turn we shall always come back to the cell

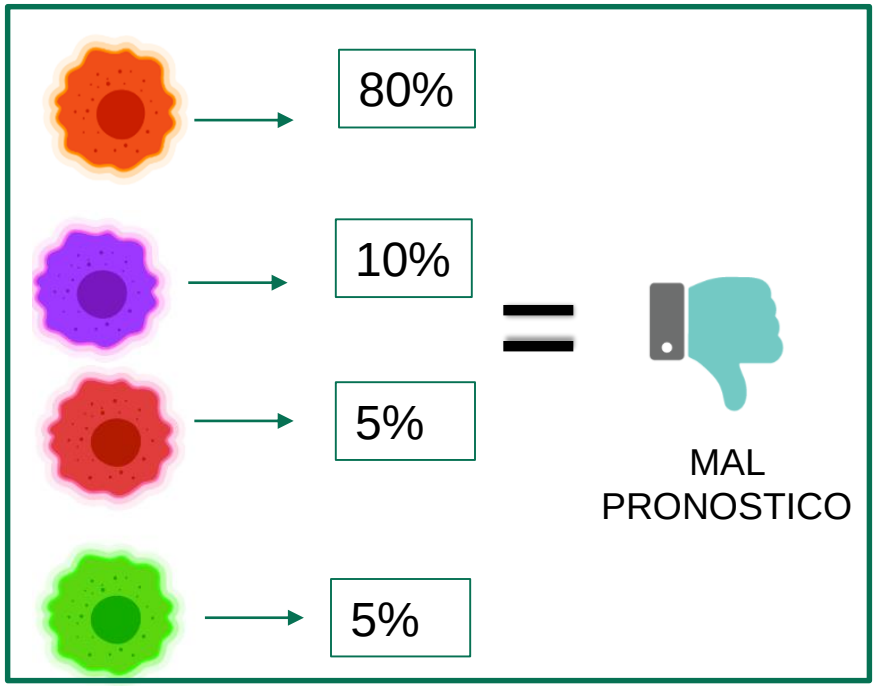
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas



ENFERMEDAD
HETEROGENEIDAD Y
DINAMISMO

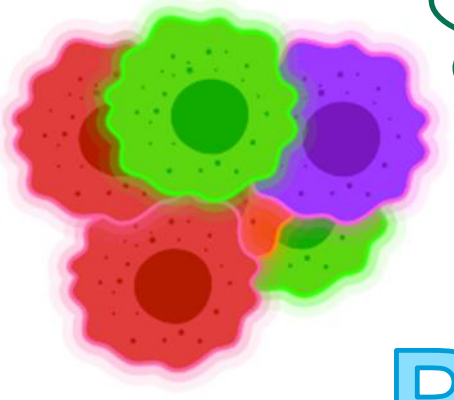
Las características de estas células (aunque se encuentren en fracciones pequeñas pueden condicionar la progresión de la enfermedad, la respuesta al tratamiento y por tanto la SUPERVIVENCIA DEL PACIENTE



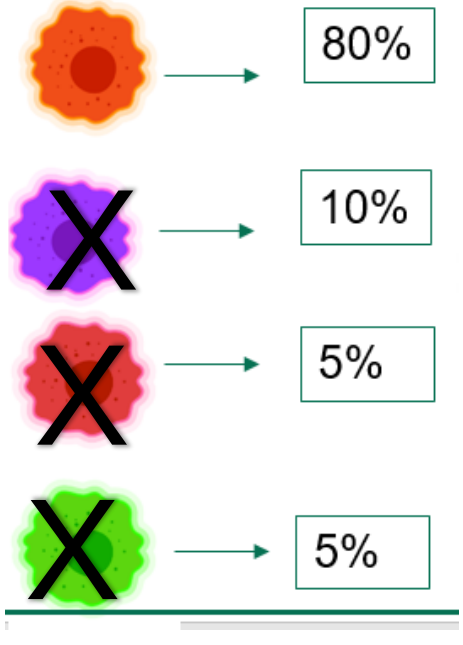


Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

Comprender la diversidad es importante para tratar eficientemente al paciente.

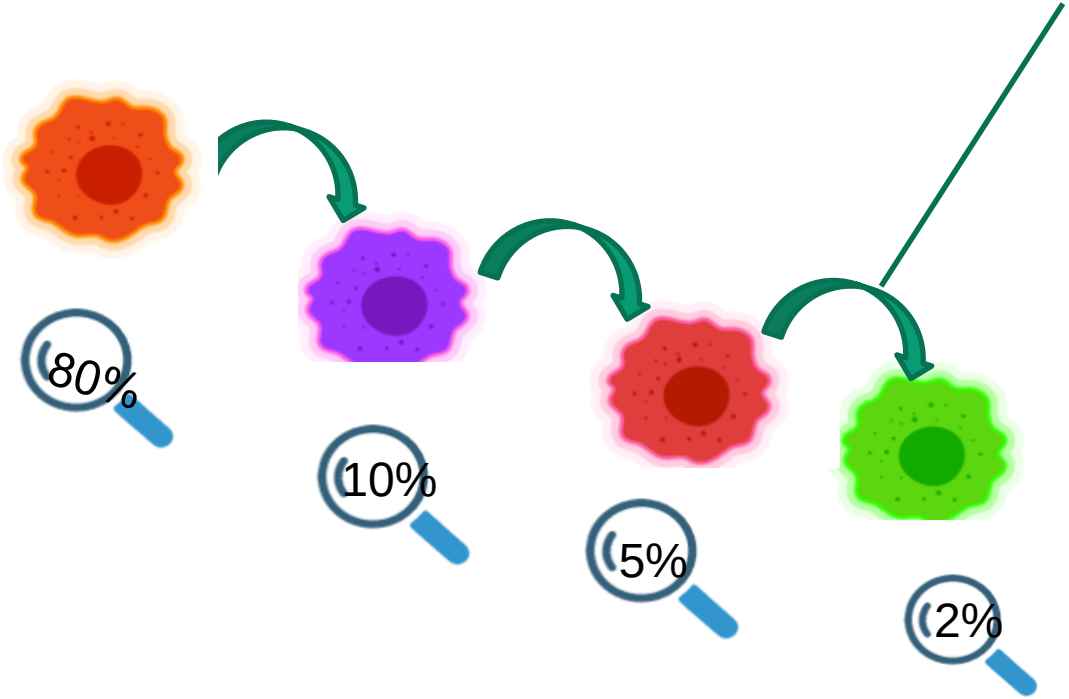


PROBLEMA



Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

Análisis biológico completo

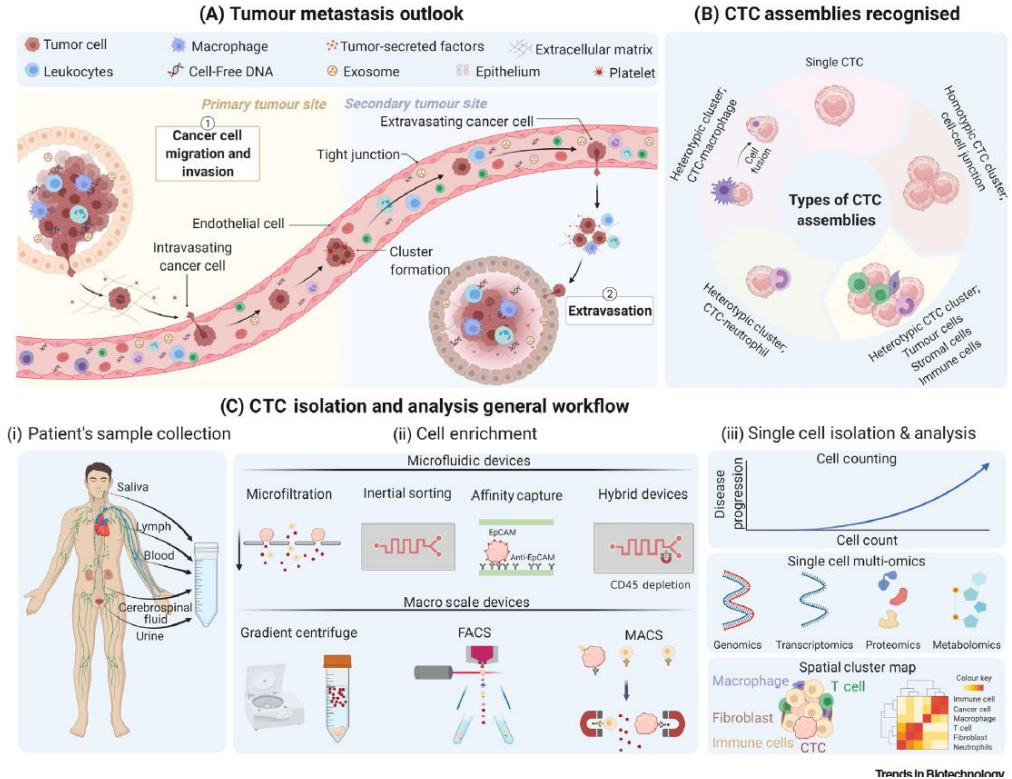


OBTENER Y COMBINAR LA INFORMACION DE TODAS LAS CELULAS

GENOMA
TRANSCRIPTOMA
PROTEOMA
METABOLOMA

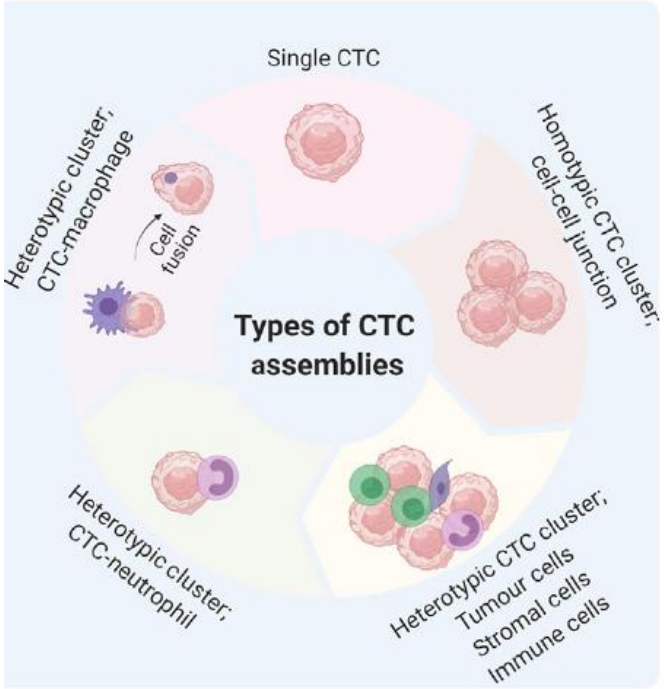
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

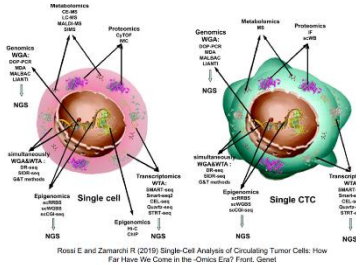
Análisis biológico completo

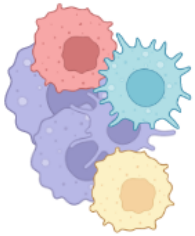


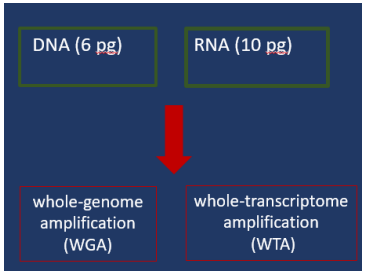
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

EN ANALISIS DE CTC



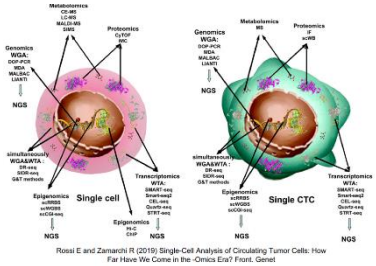




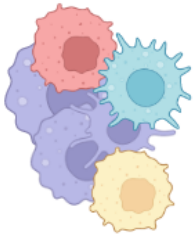


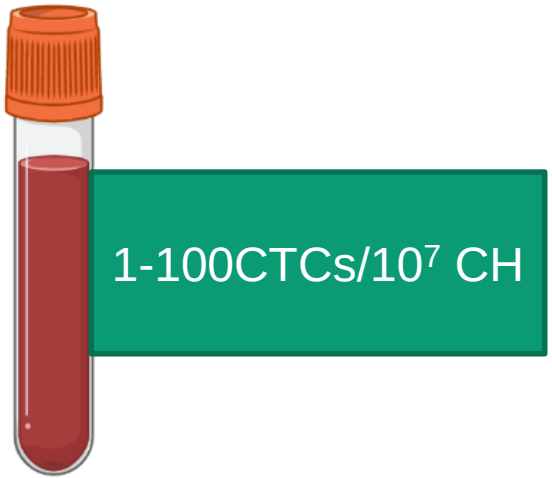
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

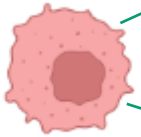
EN ANALISIS DE CTC



Rossi E and Zamarchi R (2019) Single-Cell Analysis of Circulating Tumor Cells: How Far Have We Come in the -Omics Era? Front. Genet



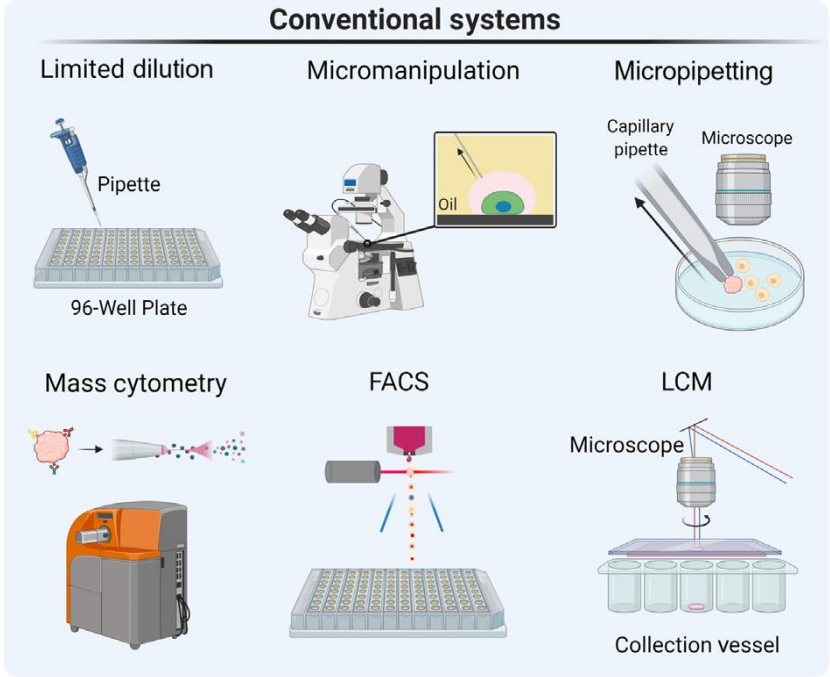




6pg DNA

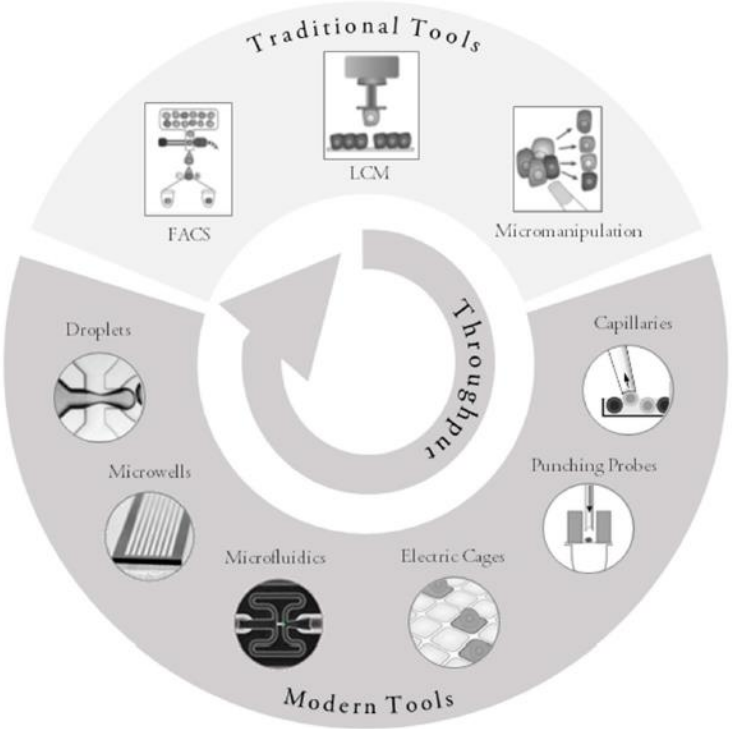
10pg RNA

Como analizamos esta heterogeneidad



Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

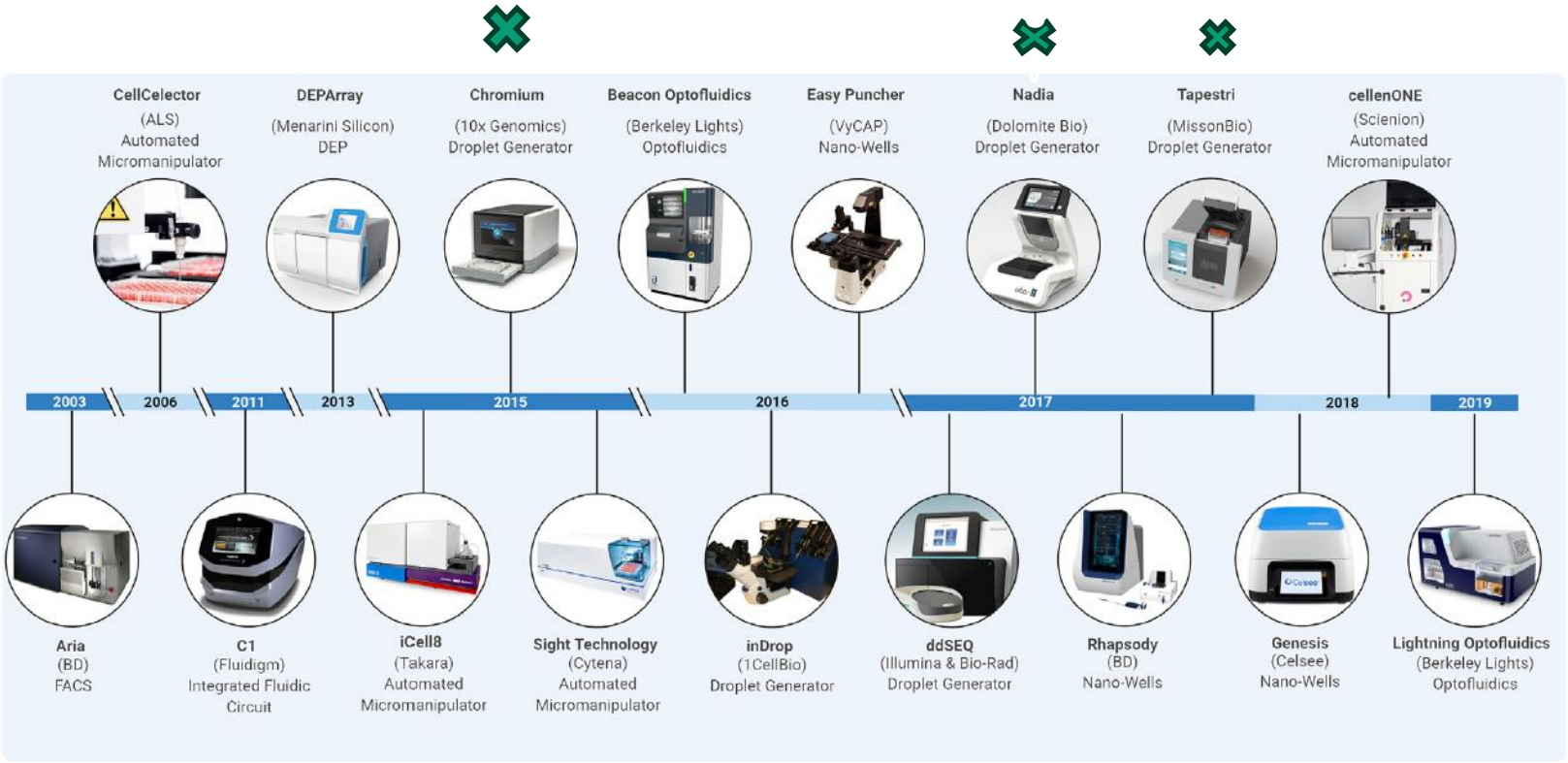
2. Nuevas plataformas



CLASIFICACION DE TECNOLOGIAS

- ✓ **Tecnología/principio para la recolección de una sola célula**
- ✓ **Rendimiento (High-Throughput Devices, LOW-Throughput Devices)**
- ✓ **Aplicación dedicada si la hay**
- ✓ **Posibilidad de inspeccionar visualmente y seleccionar celdas**

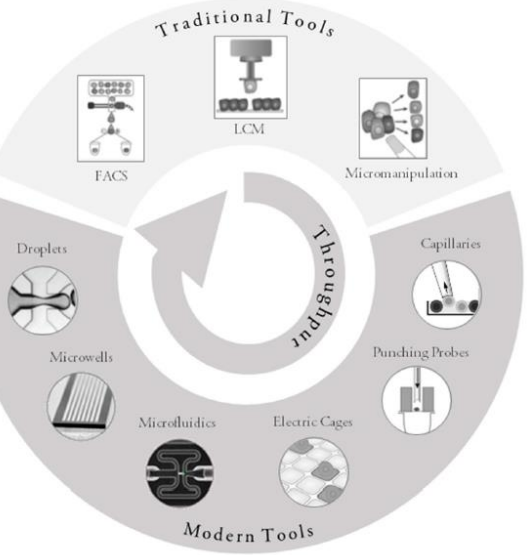
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas



Trends in Biotechnology

Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

2. Nuevas plataformas

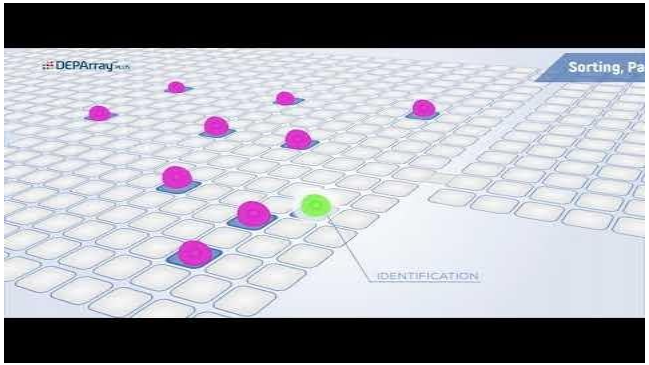


ALTO RENDIMIENTO

- ✓ **Chromium System (10x Genomics)**
- ✓ **Nadia and RNA-Seq System (Dolomite Bio)**
- ✓ **InDrop System (CellBio)**
- ✓ **Single-Cell Sequencing Solution**
- ✓ **Tapestri Platform (MissionBio)**
- ✓ **DEPArray**

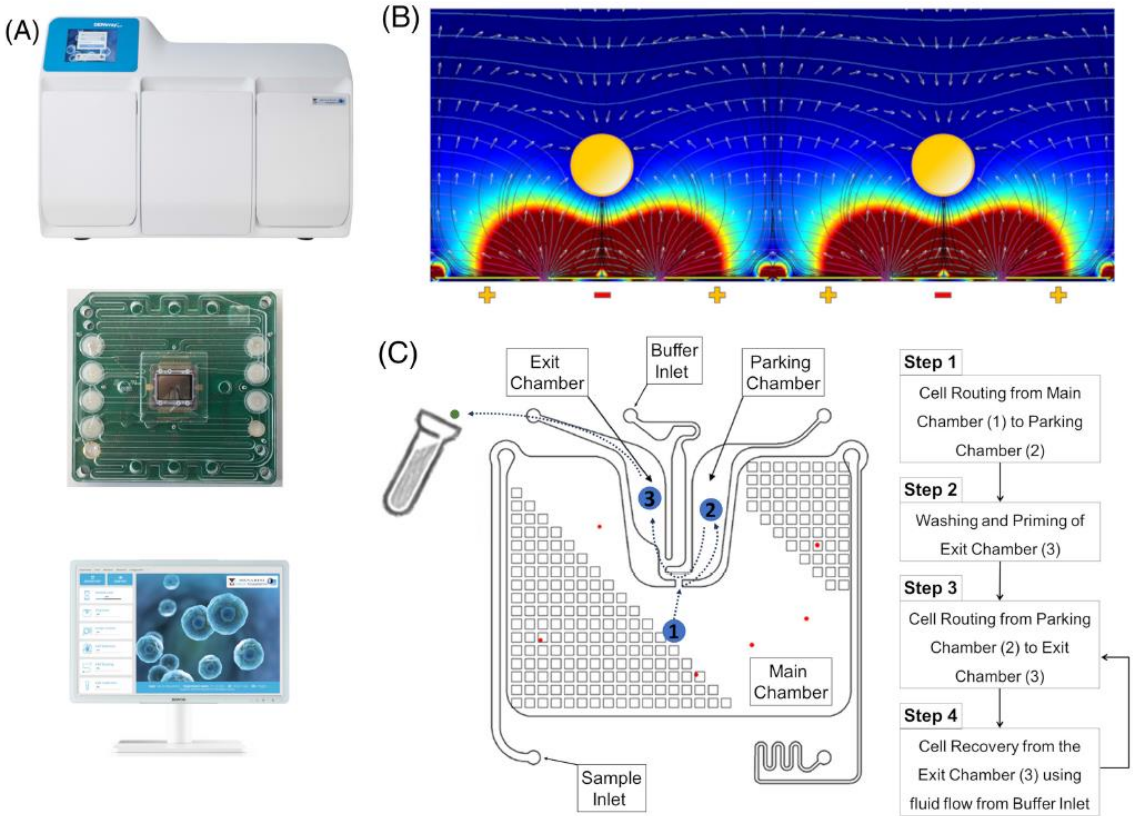
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

DEPArray NxT and DEPArray System (Menarini Silicon Biosystems)



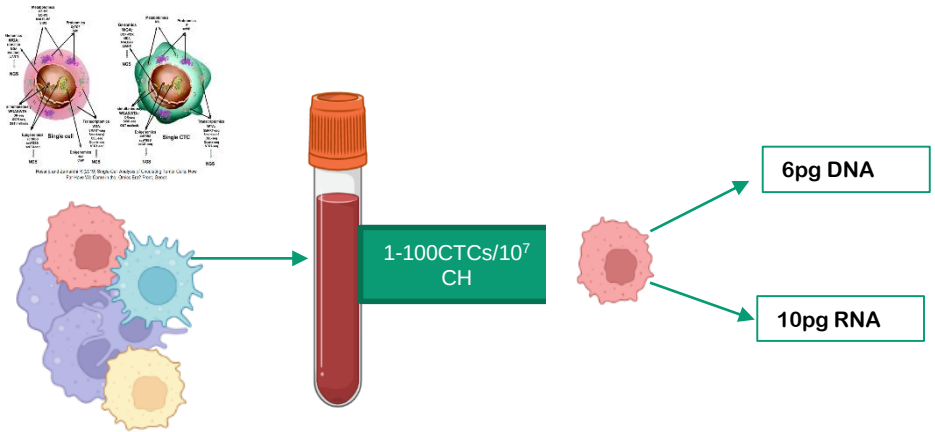
Utiliza la capacidad de un campo eléctrico no uniforme para ejercer fuerzas sobre partículas neutras polarizables, como las células, que están suspendidas en un líquido. Este principio electrocinetico , que permite atrapar las células, recibe el nombre de dielectroforesis (DEP). Las celdas registran los cambios en el campo eléctrico y las células van posicionándose y quedando atrapadas . El cartucho se escanea en varios canales fluorescentes para identificar el objetivo. Ver video

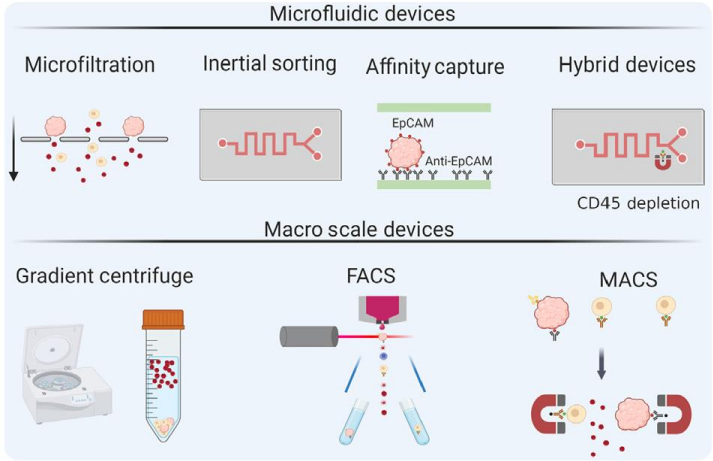
DEPArray NxT and DEPArray System (Menarini Silicon Biosystems)



Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

ANALISIS DE CELULAS TUMORALES CIRCULANTES

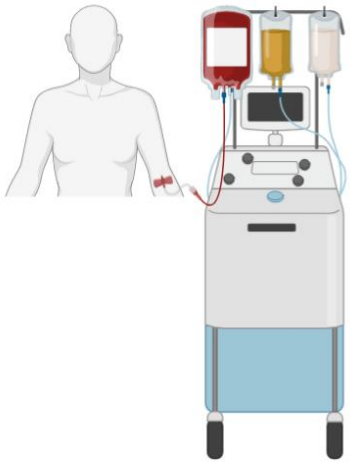




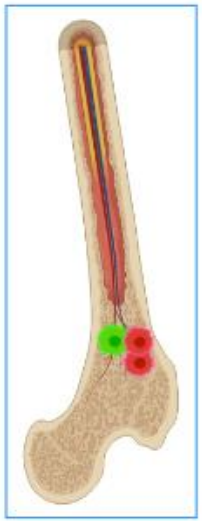
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

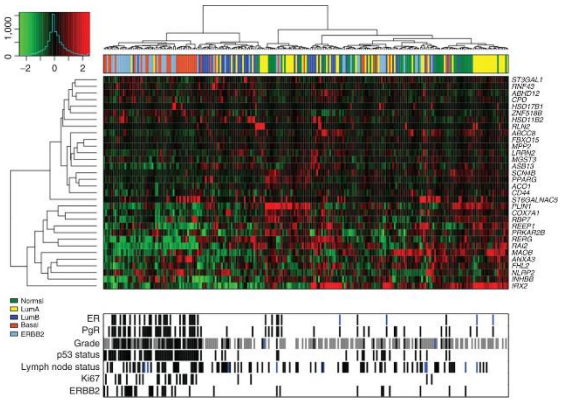
HETEROGENIDAD DE LAS CTC A TRAVES DEL ANALISIS GENOMICO

LIMITACIONES
- Numero de CTCs
- Background



Leucoferesis
CTC



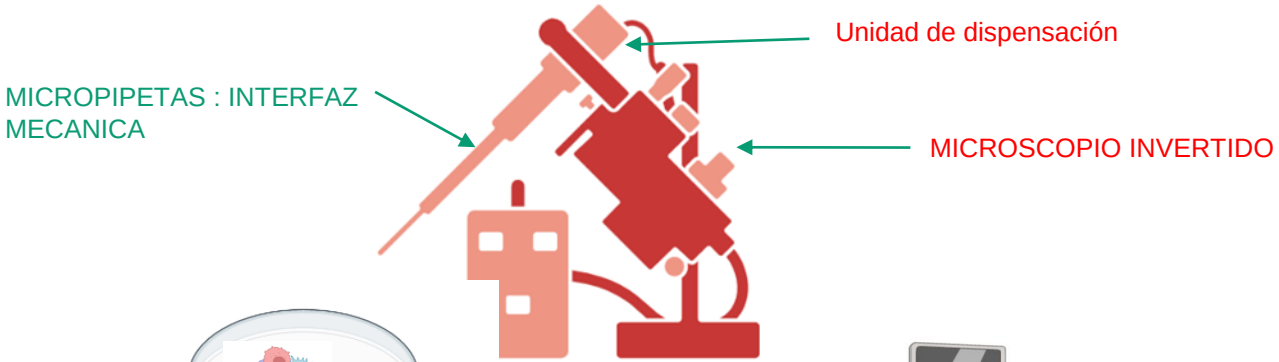


Cancer Discov (2015) 5 (5): 506–519.

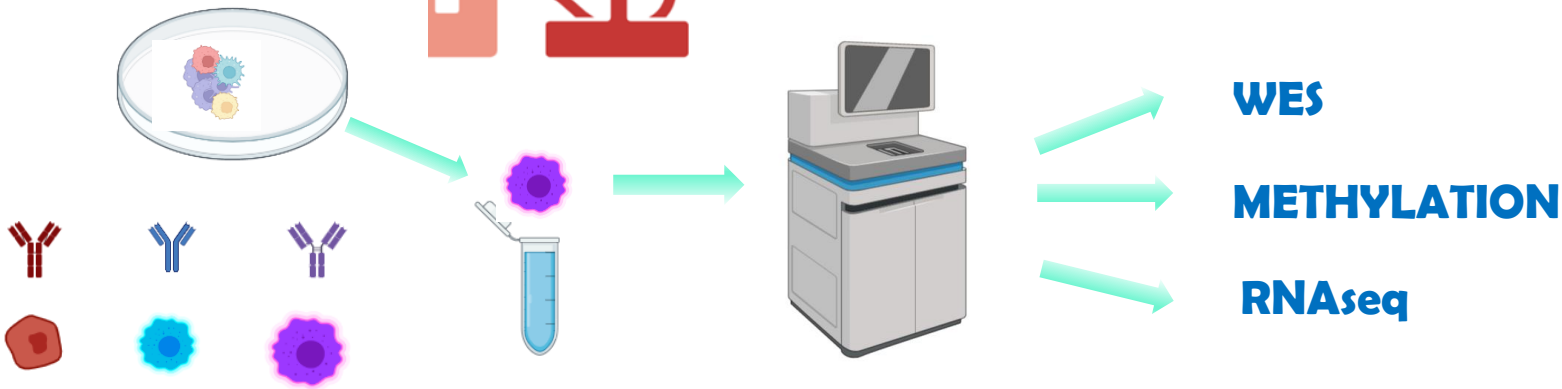
Obtención de MO
DTC

Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

HETEROGENIDAD DE LAS CTC A TRAVES DEL ANALISIS GENOMICO



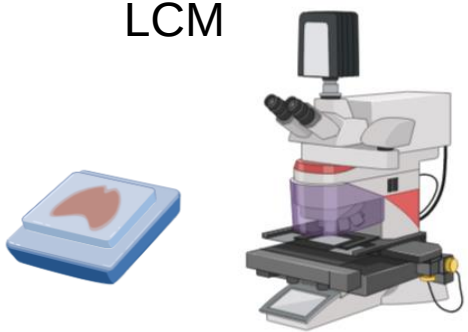
- Laborioso
- Costoso
- Dañar las células



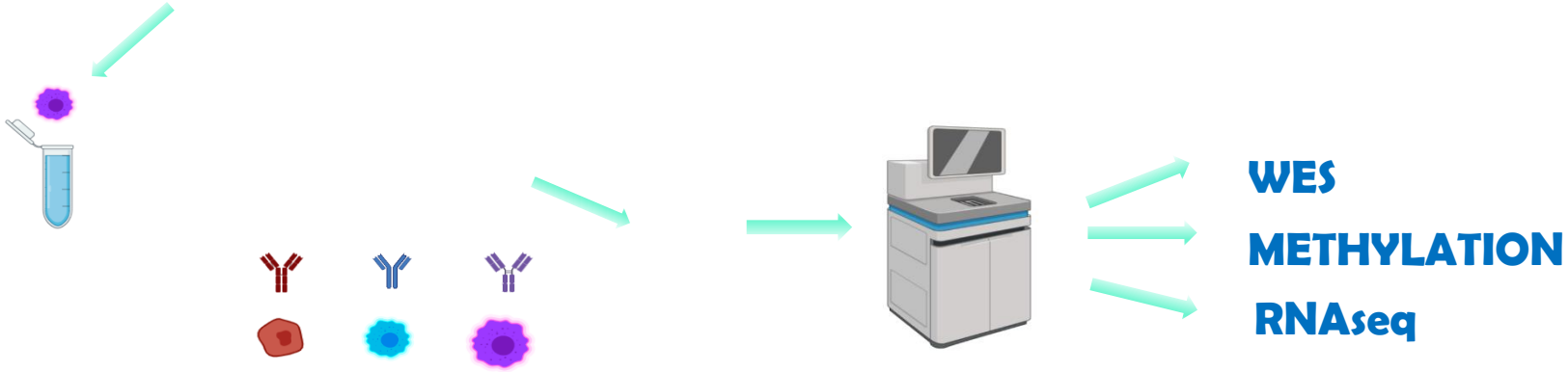
Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

HETEROGENIDAD DE LAS CTC A TRAVES DEL ANALISIS GENOMICO

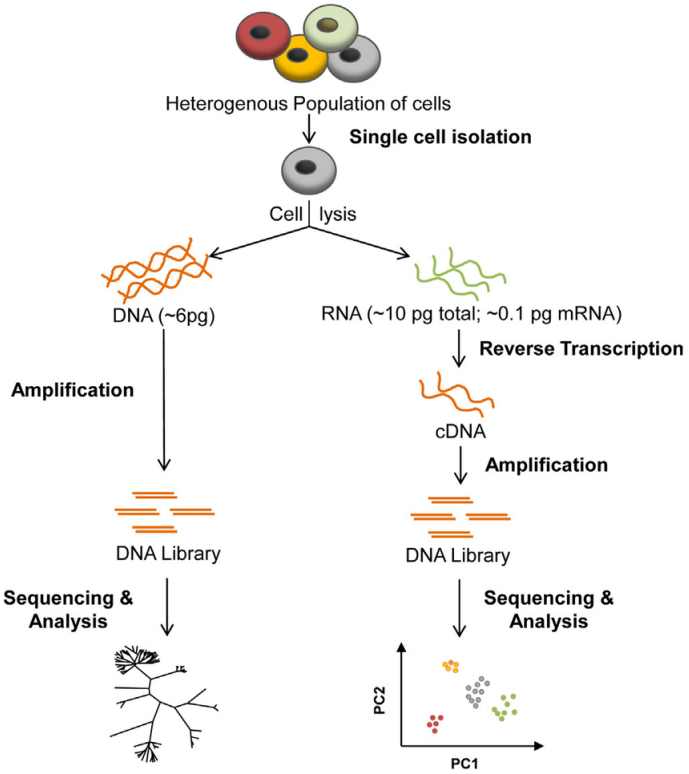
LCM

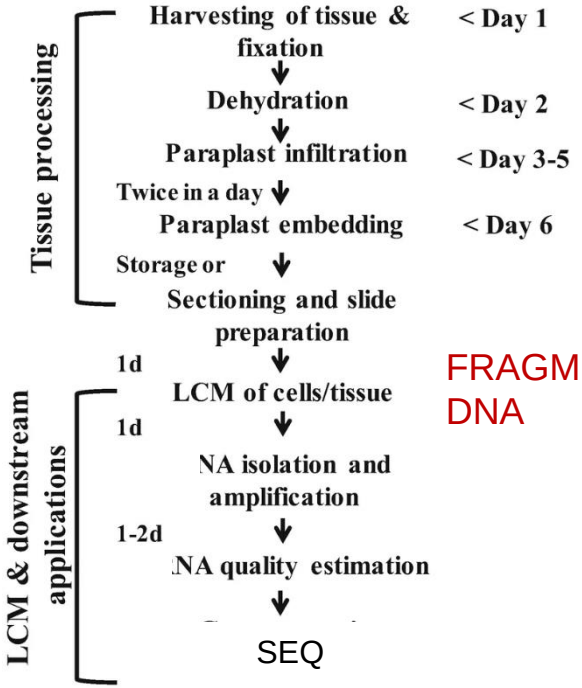


- Laborioso
- Costoso
- Dañar las células



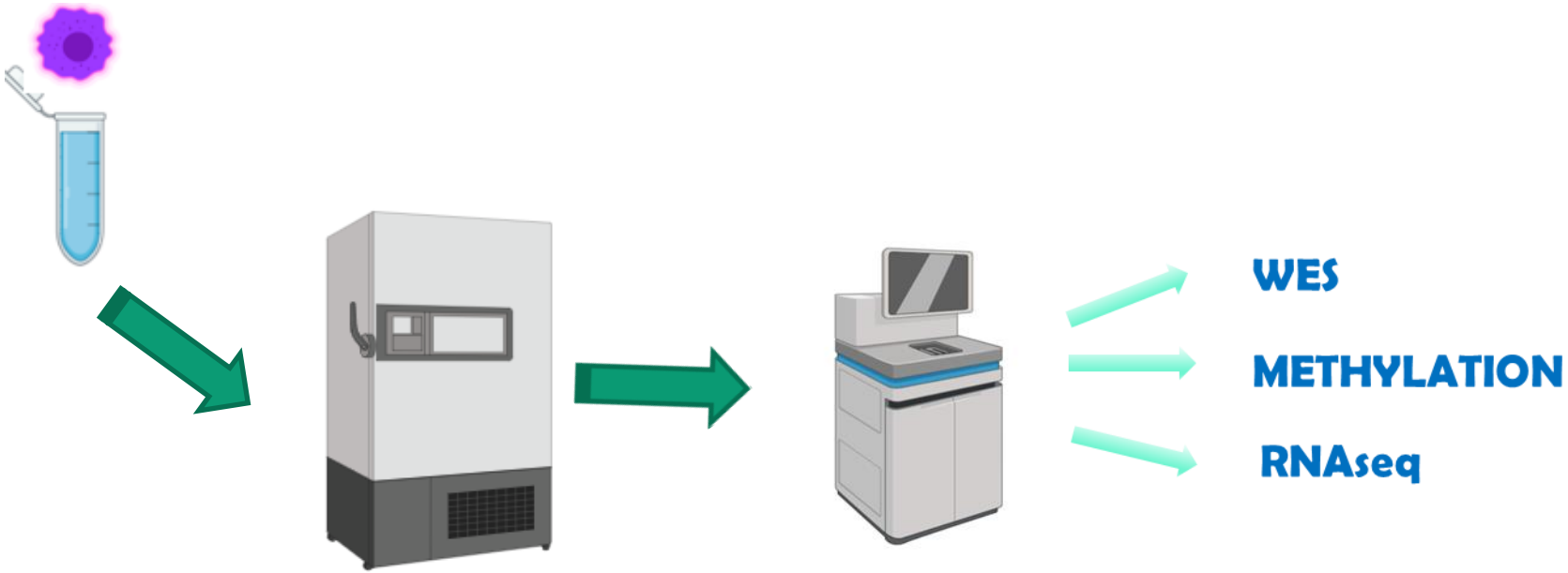
SINGLE CELLS VS TEJIDO CON MICRODISECCION





FRAGMENTACION DNA

SINGLE CELLS VS TEJIDO CON MICRODISECCION

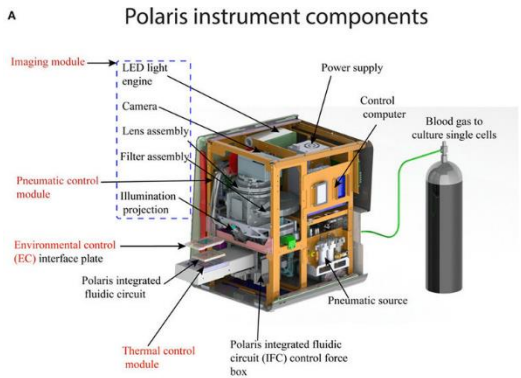


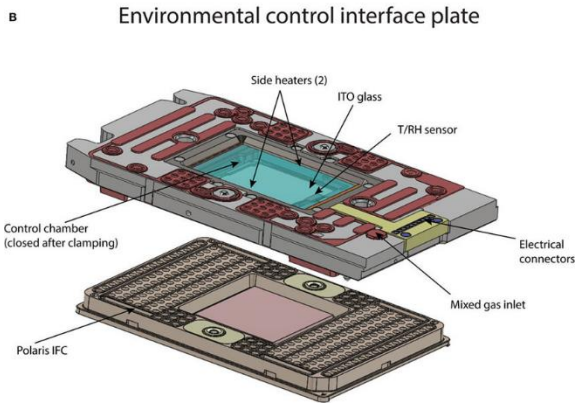
Sistema de análisis genómico y transcriptómico de células individuales

NEXT GEM de 10X Genomics, en la que se encapsulan las células junto con Gel Beads en vesículas denominadas Gel Beads en Emulsión (GEMs). Vesículas de reacción individuales donde la muestra es etiquetada. Este marcaje se consigue gracias a que cada Bead contiene millones de oligonucleótidos marcados con secuencias barcode. La plataforma utiliza la tecnología microfluídica para encapsular, procesar y analizar desde cientos hasta millones de células. Para ello, utiliza un protocolo de encapsulado de célula única en gotas/beads individuales y marcaje de cada célula individual con un código de barras molecular y con los reactivos necesarios para proceder a la identificación de dianas y amplificación. El equipo es capaz de encapsular y procesar, en cada carrera, hasta 80.000 células individuales con una eficiencia de captura hasta el 65%. La tecnología de captura de células únicas tiene una tasa de captura de dobletes (2 células encapsuladas a la vez) inferior al 1% por cada 1000 células.

Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

HETEROGENIDAD DE LAS CTC A TRAVES DEL ANALISIS GENOMICO





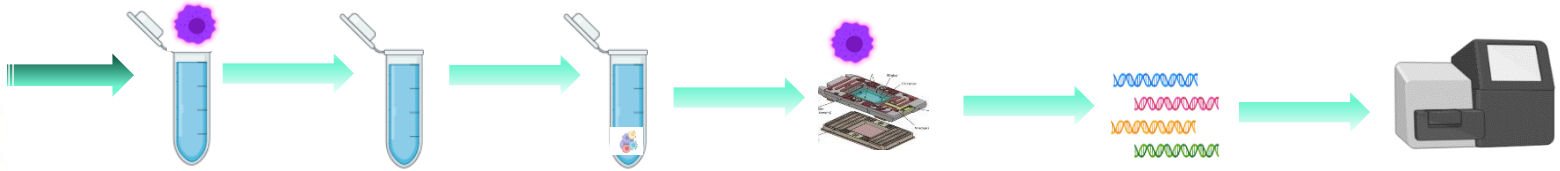
883 CELULAS

21$/C----------10$/C

REACTIVOS MICROFLUIDICA Y SECUNAICIONJ

Plataformas para análisis de célula única, especificaciones técnicas y metodológicas

HETEROGENIDAD DE LAS CTC A TRAVES DEL ANALISIS GENOMICO



RPMI
10%+
iRNAse

PBS

45 µL CTCs
+
30 µL
Reactivos POLARIS

48 sitios

Preamplificación

Preparación librerías

Secuenciacion

IAVANTE

NOMBRE

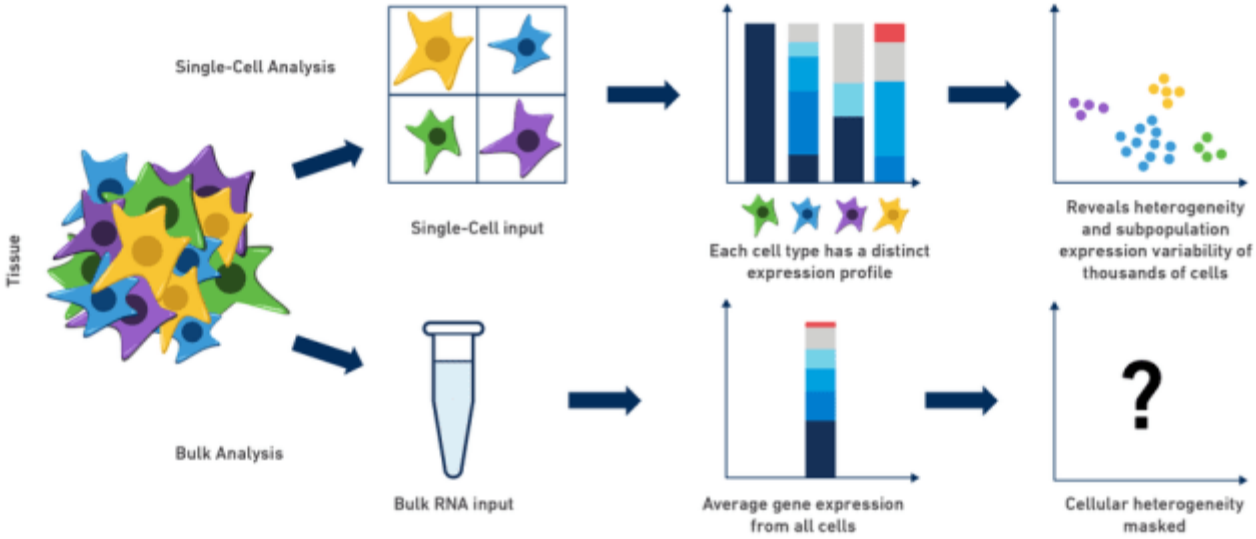
VENTAJAS Y LIMITACIONES

VEN

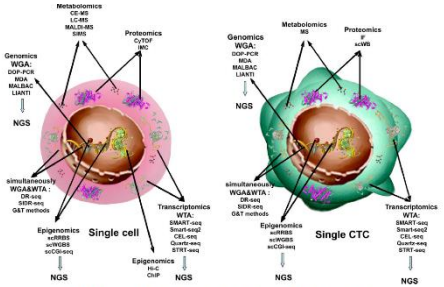
Table 1. Detailed comparison between most-used single-cell isolation techniques and how they relate when dealing with CTC analysis

	Micromanipulation	FACS	Droplet generators	Nanowells	DEP and optofluidics	Limited dilution	LCM
Capture efficiency	High	Moderate–high	Moderate	Moderate–high	High	Low	High
Doublet rate	Low Dependent on operator's skills and/or concentration of cells on the imaging slide	Low Related to sort mask	Low–moderate Related to the loading concentration	Low–moderate Related to the loading concentration	Low Related to the loading concentration	Low Related to the loading concentration	Low–moderate Related to the loading concentration
Throughput	Low	High	Moderate	Moderate–high	Moderate	Extremely low	Low
Upfront cell selection	Yes	Yes	No	No	Yes	No	Yes
Starting amount	Hundreds–thousands	Tens of thousands–millions	Five hundred–tens of thousands	Five hundred–tens of thousands	Up to tens of thousands	Hundreds	Hundreds
Laboratory skills	Moderate	High	Moderate–high	Moderate–high	Moderate–high	Low	Moderate–high
Cell stress	Low	Moderate–high	Moderate	Low	Moderate	Low	High
Equipment costs	Moderate–high	High	Moderate–high	Low	Extremely high	Extremely low	High
Commercial products	CellCelector (ALS) Eppendorf micromanipulators SIGHT – Families (Cytena) cellenONE (Scienion) iCell8 (Takara)	FACSAria (BD Sciences)	GEM technology (10xGenomics) ddSEQ (Illumina & Bio-Rad) Tapestri Platform (Mission Bio) Nadia (Dolomite Bio) InDrop (1CellBio)	Rhapsody (BD Sciences) C1 (Fluidigm) Easy Puncher (VyCAP) Celsee	DEPArray (Menarini Silicon Biosystems) Lightning Optofluidic (Berkeley Lights) Beacon Optofluidic (Berkeley Lights)	Standard laboratory pipettes	Arcturus XT (Thermo Fisher) LMD6&7 (Leica Microsystems) CellCut (MMI)
Recommendations	Often suitable after an initial enrichment with a great flexibility for different downstream analysis. Higher throughput is achieved via automated systems. Ability to select individual cells that can significantly lower the analysis costs.	Suitable for second purification and samples with high contamination. Using FACS for single-cell isolation often becomes challenging when dealing with low sample input such as CTCs.	Not recommended for pure low load CTC samples. Not flexible with different analysis types. For CTC analysis, sample pooling is required which will increase the analysis costs.	Vary in range from simple to complex systems and are mostly cost-effective. They are more flexible with downstream analyses. Each nanowell can be used for isolation and/or reaction chamber for different analysis.	High control in cell handling and great choice for single CTC isolation in an automated way. However, they are complex and have an extremely high setup and operational costs.	Limited dilution and LCM approaches are less commonly used for CTC isolation due to their technological limitations including, low throughput and labour intensiveness.	

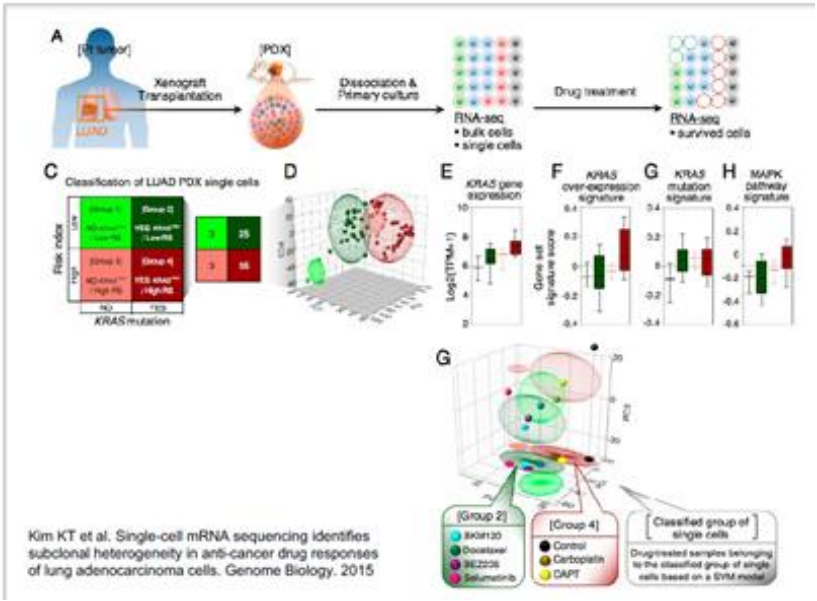
Análisis de célula única vs análisis de tejido



Análisis de célula única vs análisis de tejido

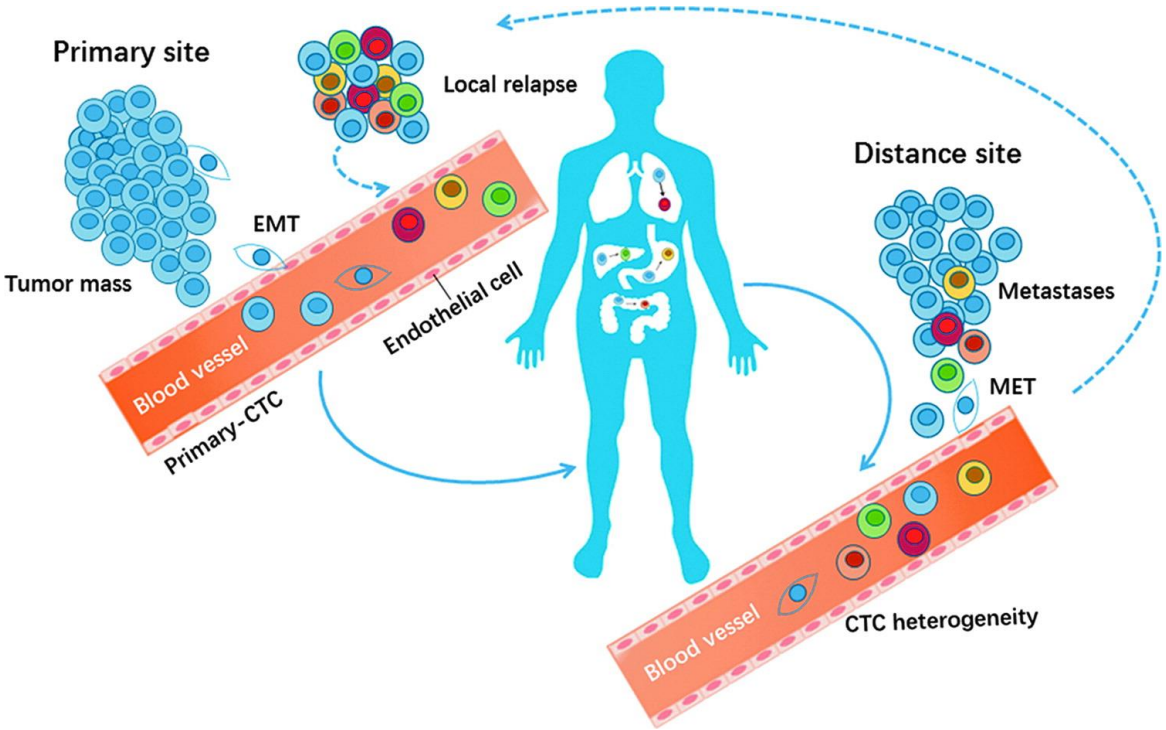


Rossi E and Zamarchi R (2019) Single-Cell Analysis of Circulating Tumor Cells: How Far Have We Come in the -Omics Era? Front. Genet



Kim KT et al. Single-cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells. Genome Biology. 2015

Aplicabilidad clínica



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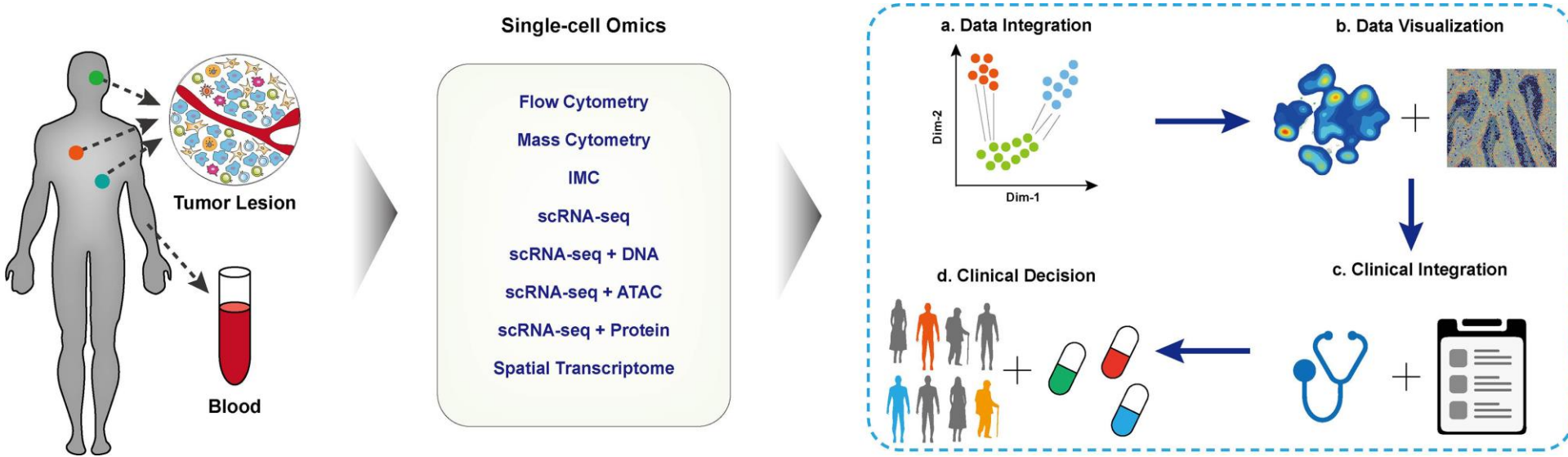

Aplicabilidad clínica

Cancer type	Single-cell methods	Highlights	Ref
Early lung adenocarcinoma	CyTOF, scRNA-seq	Comparing the paired immune signatures across tumor lesion, normal lung tissue, and blood, Lavin et al. Identified the tumor lesion-specific immune regulations, especially the modifications of innate immune cells	(74)
Breast cancer	Imaging mass cytometry	The high-dimensional pathology images of breast cancers characterized the disease-related spatial resolved cellular signatures.	(77)
Hepatocellular carcinoma	scRNA-seq	In-depth integration of single-cell data with bioinformatic methods, Zhang et al. identified the migration of immune cells, especially the LAMP3+ dendritic cells, potentially contributing to lymphocyte activation.	(87)
Myeloproliferative neoplasms	scRNA-seq + genotyping	Integrating the cellular mutation genotypes and transcriptomic data, Nam et al. revealed the upregulation of NF-κB and IRE1-XBP1 pathways in mutated cells. And the modifications of mutations in transcriptomic outputs.	(82)
Mixed-phenotype acute leukemias	scRNA-seq + protein, scATAC-seq	By comparing the transcriptomic and epigenetic blood development maps between healthy and MPAL patients, Granja et al. uncovered the patient-specific regulatory networks, such as the RUNX1 regulation of CD69 in tumor patients.	(47)
Primary pancreatic tumors	scRNA-seq, spatial transcriptomics	With intersection analyses of scRNA-seq data and spatial transcriptomic data, Moncada et al. revealed the interactions of different cells in tumor microenvironments, especially the colocalization of inflammatory fibroblasts and cancer cells.	(86)
Basal or squamous cell carcinoma	scRNA-seq + TCR	Comparisons between the tissue TCR repertoires before and after immunotherapies, Yost et al. uncovered the new entered T cell clonotypes rather than the exhausted T cell clonotypes that may respond to immunotherapy.	(105)
Hepatocellular carcinoma	scRNA-seq	By comparing the immune landscape between primary and early-relapse HCC patients, Sun et al. indicated the innate-like CD8 T cells might contribute to an early relapse of HCC.	(119)
Pancreatic ductal adenocarcinoma	snRNA-seq, spatial transcriptomics	Comparisons of the PDAC samples before and after chemoradiotherapy, Hwang et al. revealed the basal rather than the classical phenotype of malignant cells might benefit the therapy efficiency with single-cell and spatial transcriptomic inspections.	(121)

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CENTRO DE SIMULACIÓN

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