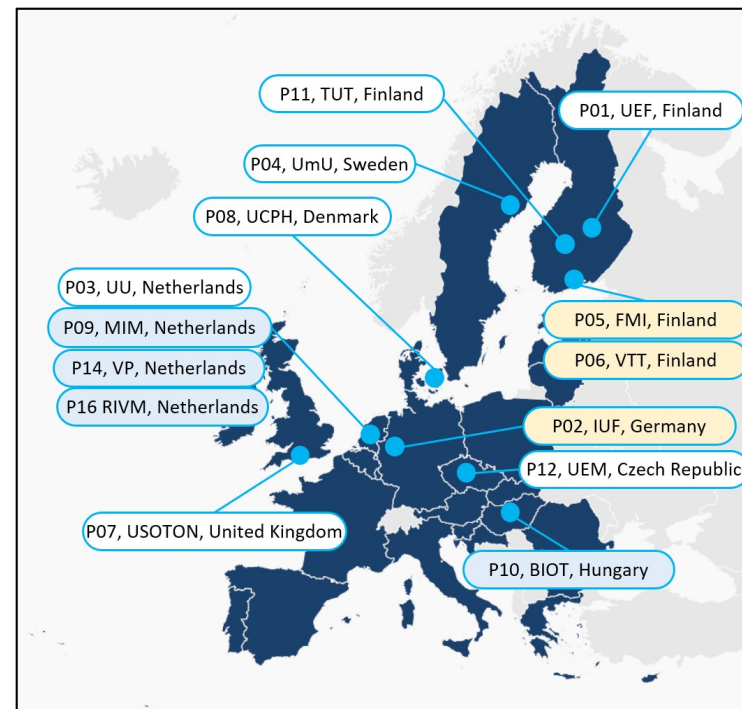# Introduction to mRNA-seq Analysis

Rosalba Giugno

Simone Avesani

Manuel Tognon

Transport derived Ultrafines and the Brain Effects (TUBE)
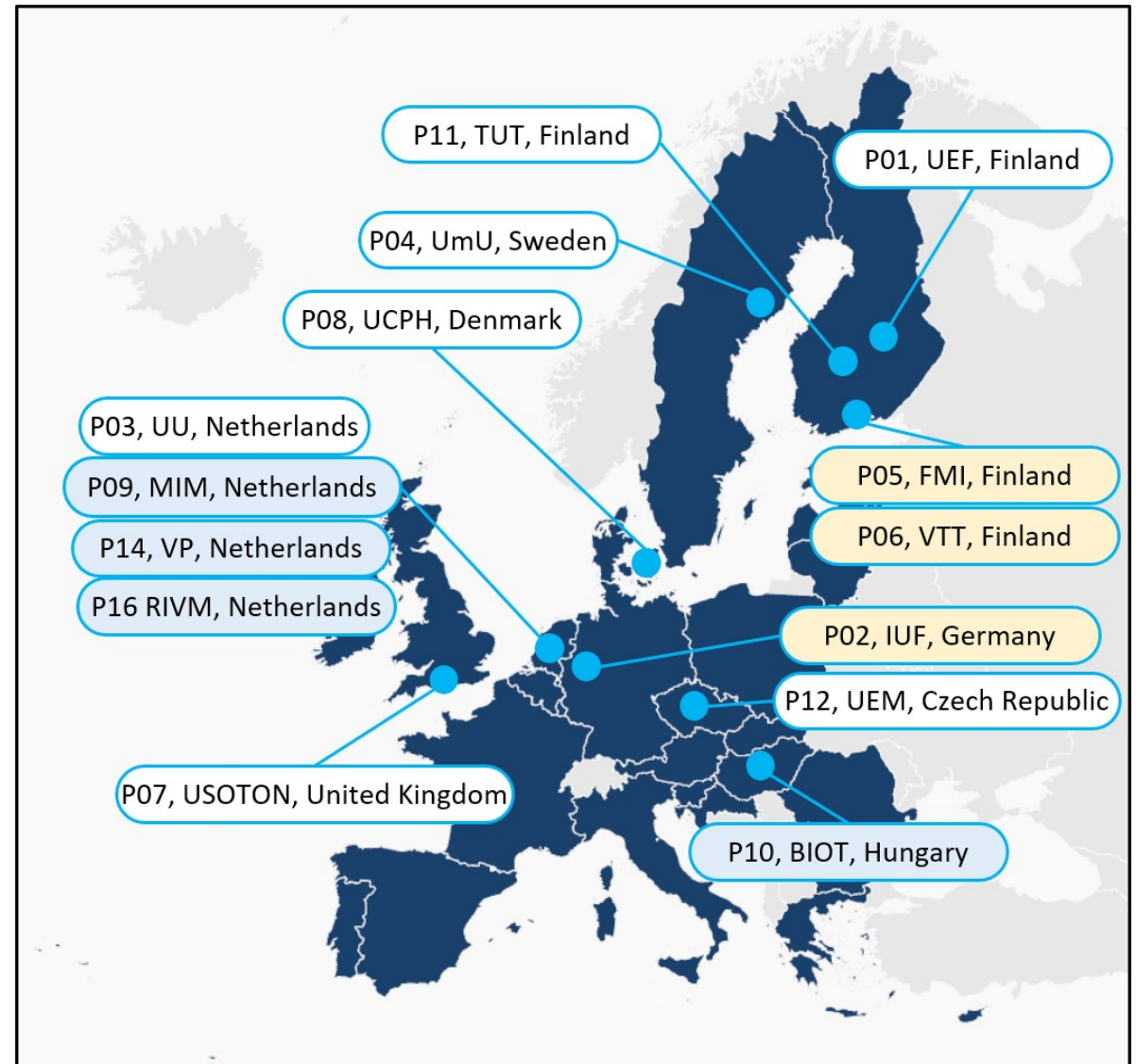
https://www.tube-project.eu/

TUBE's Partners

Many partners each of one specialized in a different experimental phase:

- **Finland**: sample collection and library preparation

- **Czech Republic**: sequencing, epigenetics

- **Italy (Verona)**: data analysis

P11, TUT, Finland
P01, UEF, Finland
P04, UmU, Sweden
P08, UCPH, Denmark
P03, UU, Netherlands
P09, MIM, Netherlands
P14, VP, Netherlands
P16 RIVM, Netherlands
P05, FMI, Finland
P06, VTT, Finland
P02, IUF, Germany
P12, UEM, Czech Republic
P07, USOTON, United Kingdom
P10, BIOT, Hungary

Alzheimer's Disease

Alzheimer's Disease

- Neurodegenerative disease

- Irreversible disease

- Average life expectancy is 10 years

Alzheimer's Disease

- Neurodegenerative disease

- Irreversible disease

- life expectancy is 10 years

Main symptoms:

- loss of memory
- repetitive or impulsive behavior
- loss of physical abilities
- loss of memory smell

Challenges

- There is no cure

- There is no a diagnostic test ➡️ exclusion of all other pathologies

- It is very difficult to study the evolution of the disease

Goal of the experiment

★ Try to find an innovative way to study the Alzheimer's disease evolution

★ Discover a potential way to diagnose the disease

Goal of the experiment

★ Try to find an innovative way to study the Alzheimer's disease evolution
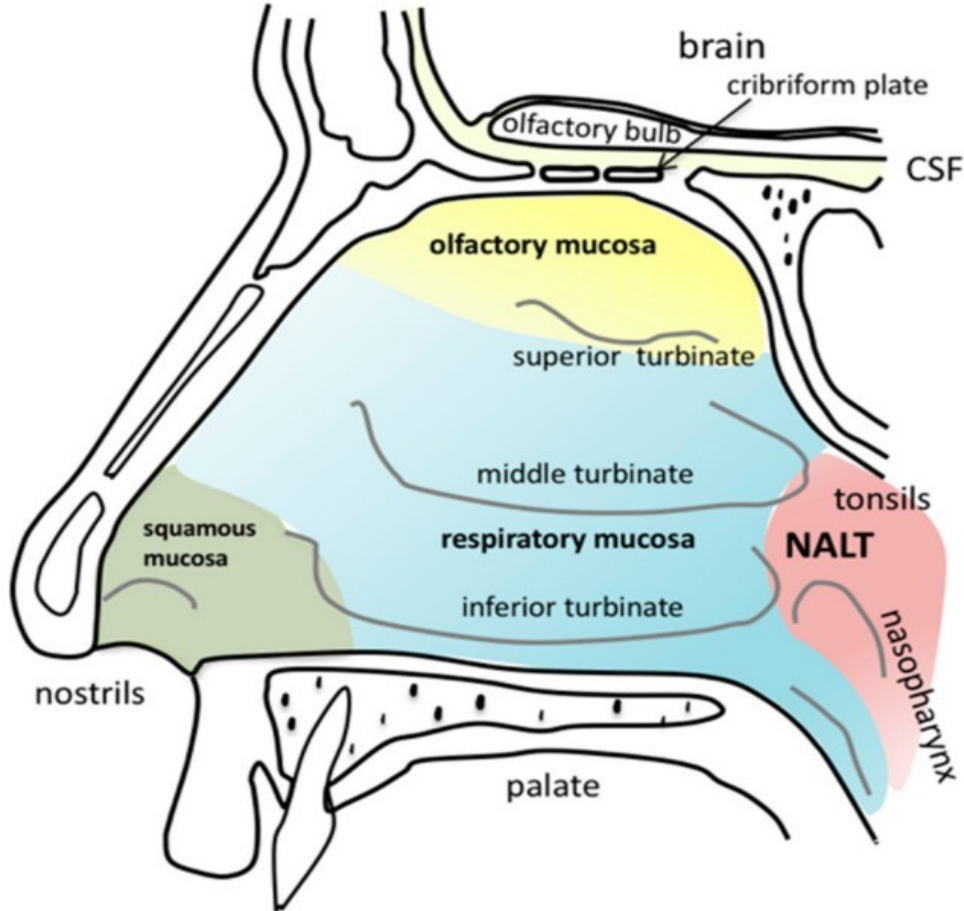
★ Discover a potential way to diagnose the disease

↓

Starting from the Olfactory Mucosa

Olfactory Mucosa

- Divide nose cavity from the brain
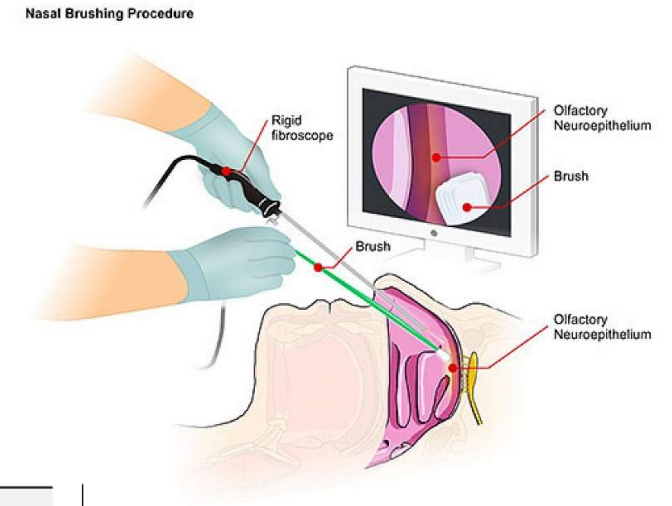
- The odors perception take place

→ loss of memory smell

What to do?

What to do?

- **STEP 1**: take OM samples with biopsy from healthy, sick and mild cognitive subjects

Group	ID	gender	age when biopsied	ApoE genotype
control (10)	102	female	78	34
	105	female	73	33
	106	male	67	34
	108	female	73	34
	109	male	64	34
	110	female	72	33
	111	female	72	33
	112	female	73	33
	113	female	70	33
	115	male	68	33

Group	ID	gender	age when biopsied	ApoE genotype
MCI (11)	201	male	82	34
	205	male	77	34
	207	male	68	33
	208	male	63	34
	209	male	72	23
	210	female	72	33
	212	female	73	22
	213	female	72	33
	214	female	76	33
	215	female	62	34
	216	male	63	44

Group	ID	gender	age when biopsied	ApoE genotype
AD (12)	301	female	64	33
	302	male	80	33
	303	female	67	44
	304	female	58	44
	305	female	80	33
	306	female	75	44
	307	male	71	44
	308	male	61	34
	309	female	67	44
	311	male	60	33
	313	male	65	44
	314	male	71	34

What to do?

- **STEP 2**: sequence the genomic content of the cells sampled

NB: different type of sequencing!

Bulk vs Single Cell sequencing
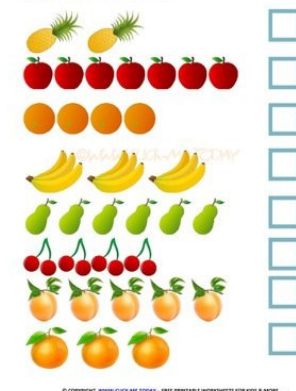
Bulk vs Single Cell sequencing
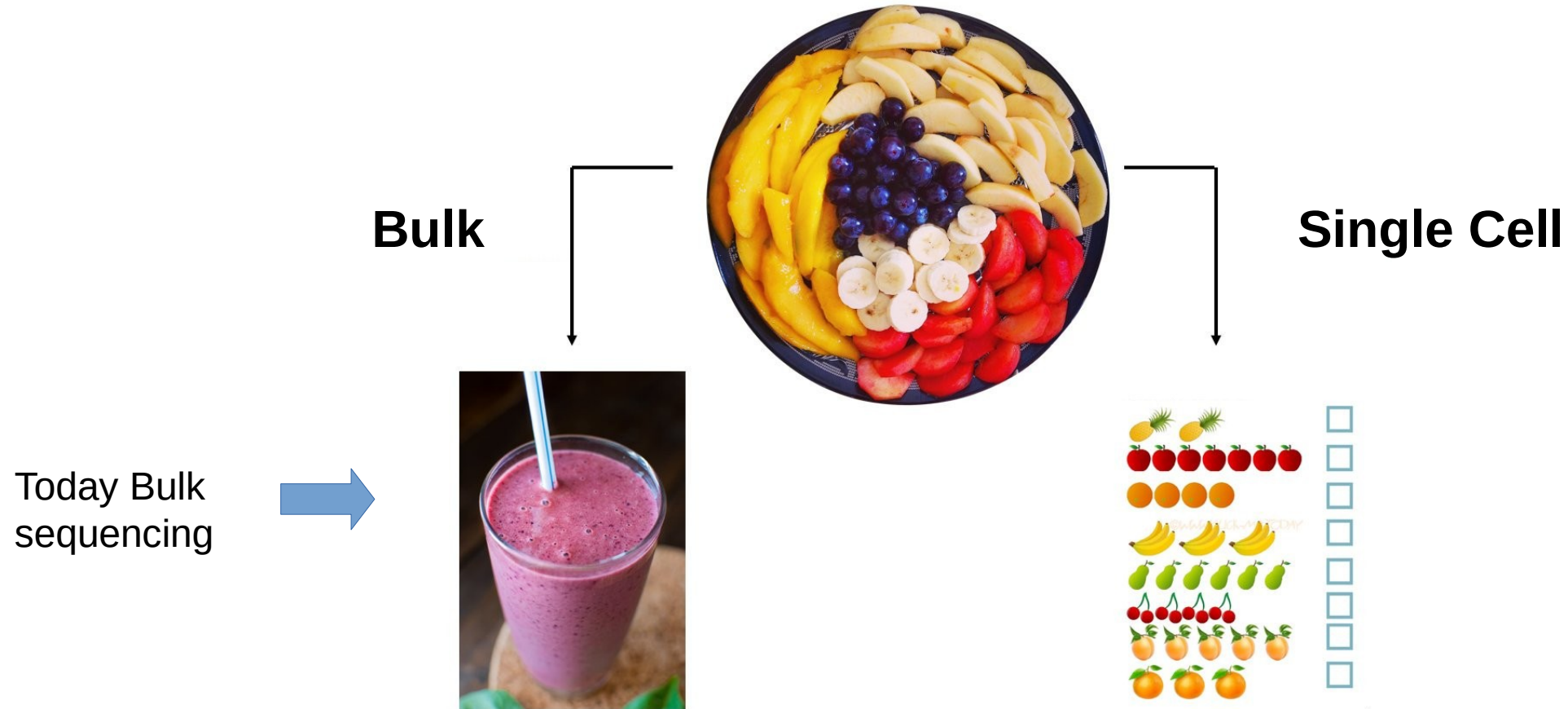
Bulk

Single Cell

Bulk vs Single Cell sequencing

Bulk

Single Cell

Today Bulk
sequencing

What to do?

STEP 3: search if there are genomic differences between healthy and sick samples

Genes more or less expressed in sick subjects respect to healthy subjects!
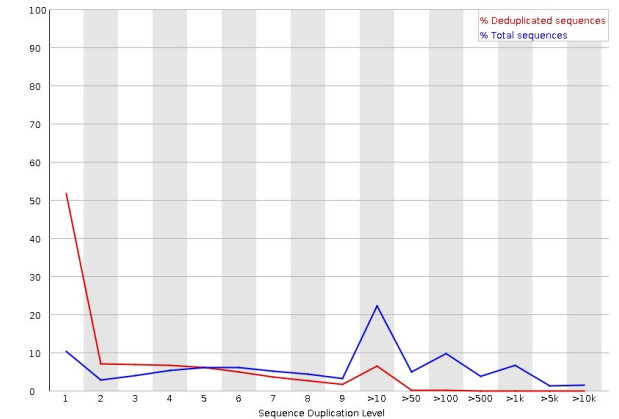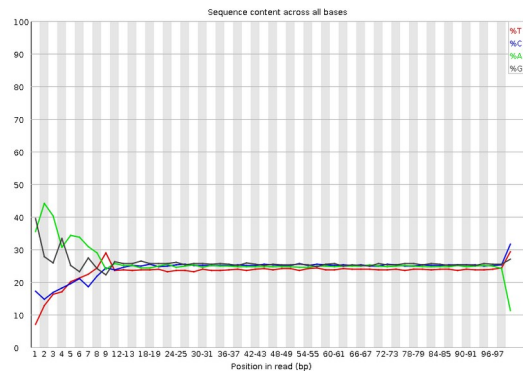
Pipeline

Sequencing
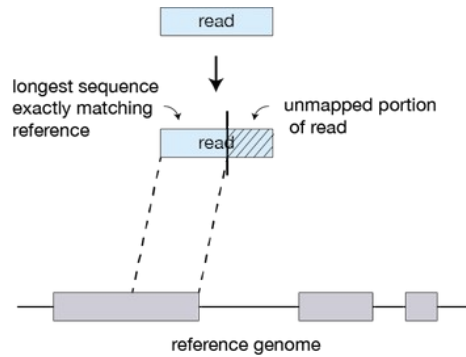
Quality Control

Pre-processing

Quality Control after pre-processing

Pipeline

Alignment

Quantification

R analysis

Differential expression analysis