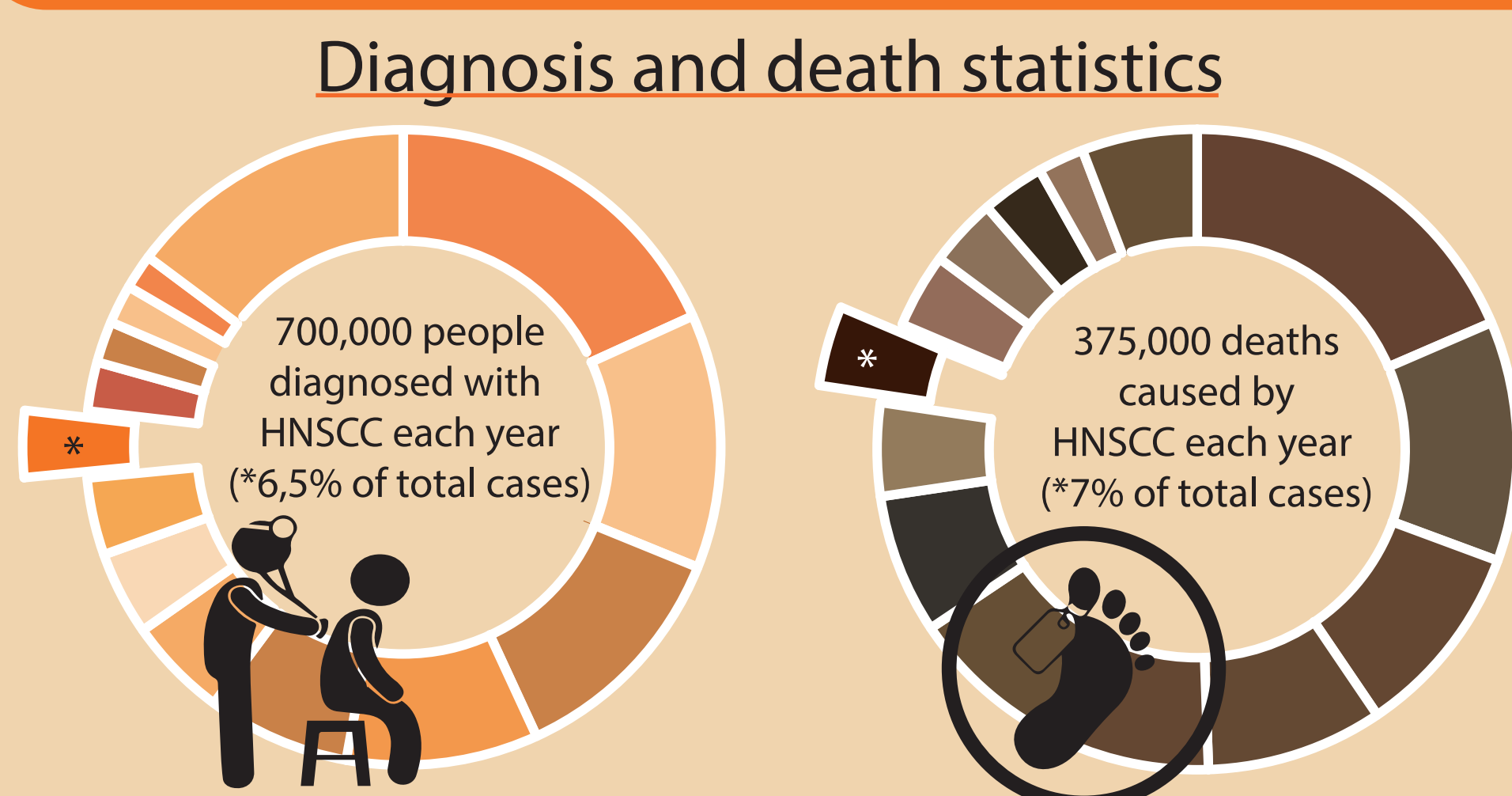# Designing a novel methylation array for head and neck squamous cell carcinoma (HNSCC) detection
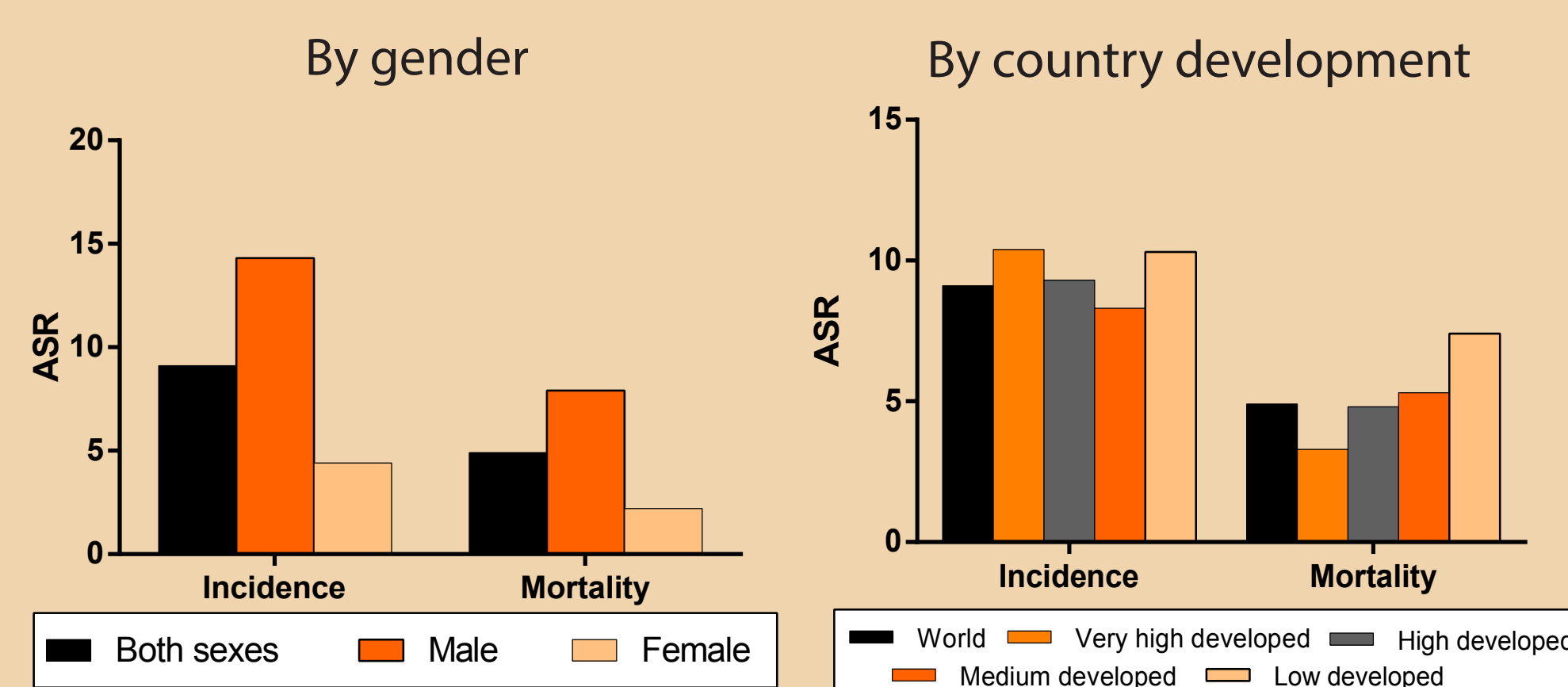
UAB

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HNSCC general facts and statistics

Diagnosis and death statistics

700,000 people diagnosed with HNSCC each year (*6,5% of total cases)

375,000 deaths caused by HNSCC each year (*7% of total cases)

Diagnosis and death statistics, outty pie piece represent the percentage of HNSCC in comparison to the whole cancer population.

Incidence and mortality rates of HNSCC

By gender

By country development

Legend: Both sexes, Male, Female
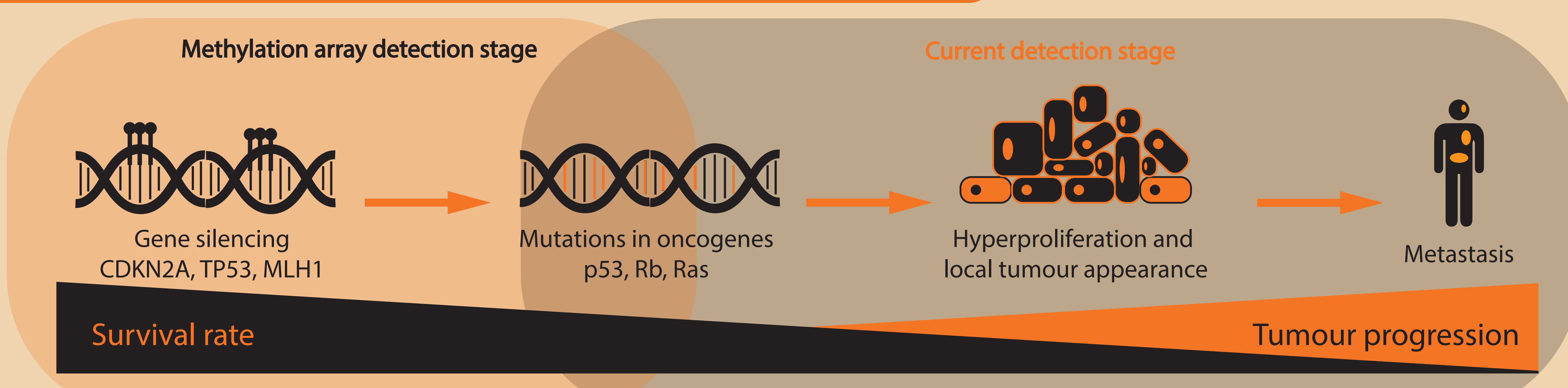Legend: World, Very high developed, High developed, Medium developed, Low developed

Incidence and mortality rates of HNSCC: ASR represent number of sick people per 100,000 healthy people in a age-standardize population.

5-years prognosis depending on detection stage

Local tumour detection

Detection with nodal affectance

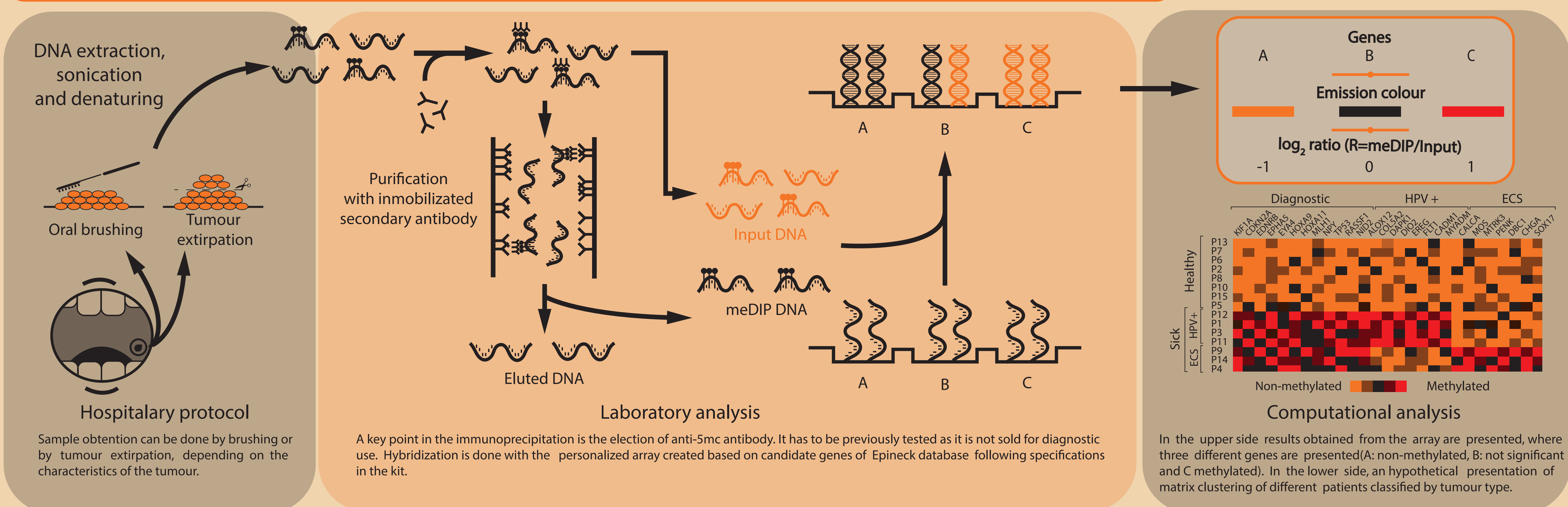Detection with metastasis

: Alive   : Dead

5-years prognosis depending on detection stage, mortality rate is sketched as ten people, where alive is represented as orange person and death as black o

HNSCC developement and diagnosis strategy

Methylation array detection stage

Current detection stage

Gene silencing CDKN2A, TP53, MLH1

Mutations in oncogenes p53, Rb, Ras

Hyperproliferation and local tumour appearance

Metastasis

Survival rate

Tumour progression

HNSCC development and diagnosis strategy: HNSCC development is inversely related to survival rate, at first stage CDKN2A, TP53 and MLH1 are silenced, later these silenced gene turns into mutations in oncogenes that start neoplasic process which can spread around the body at the last stage.
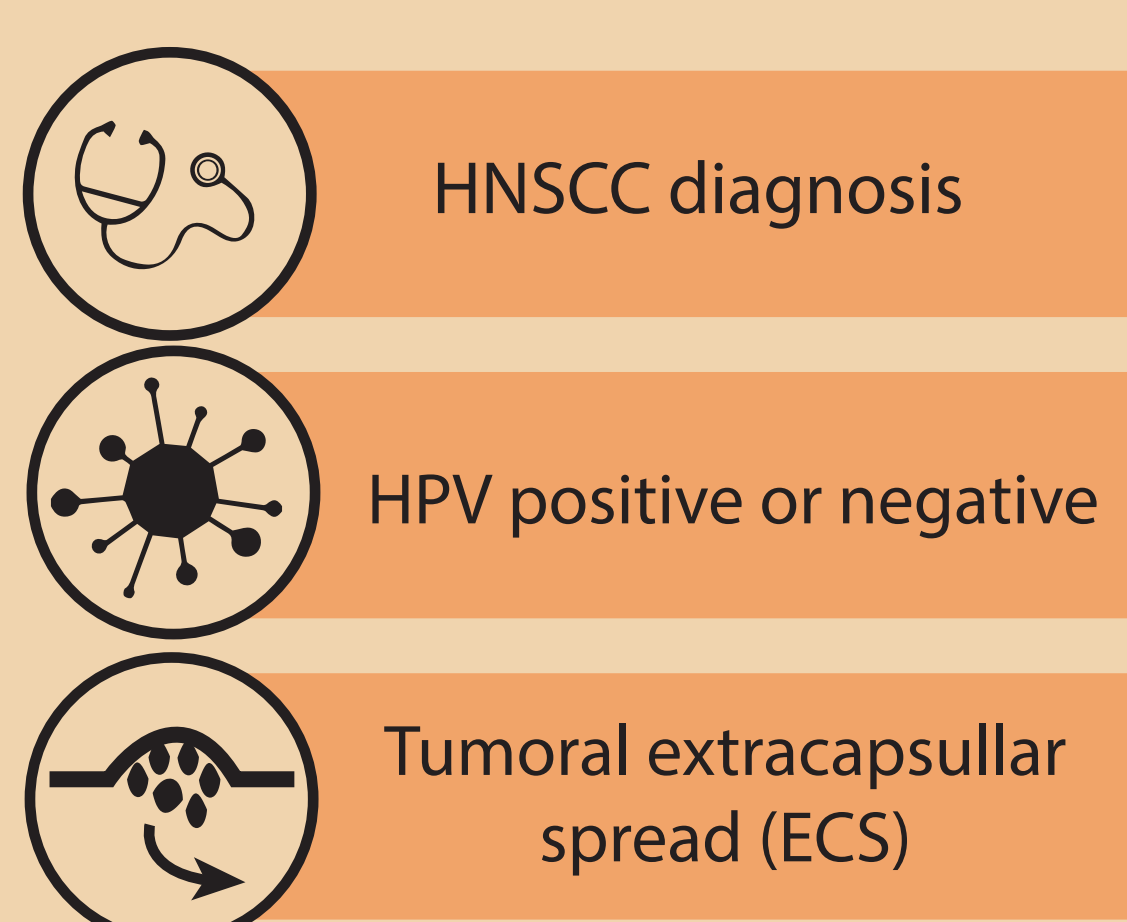
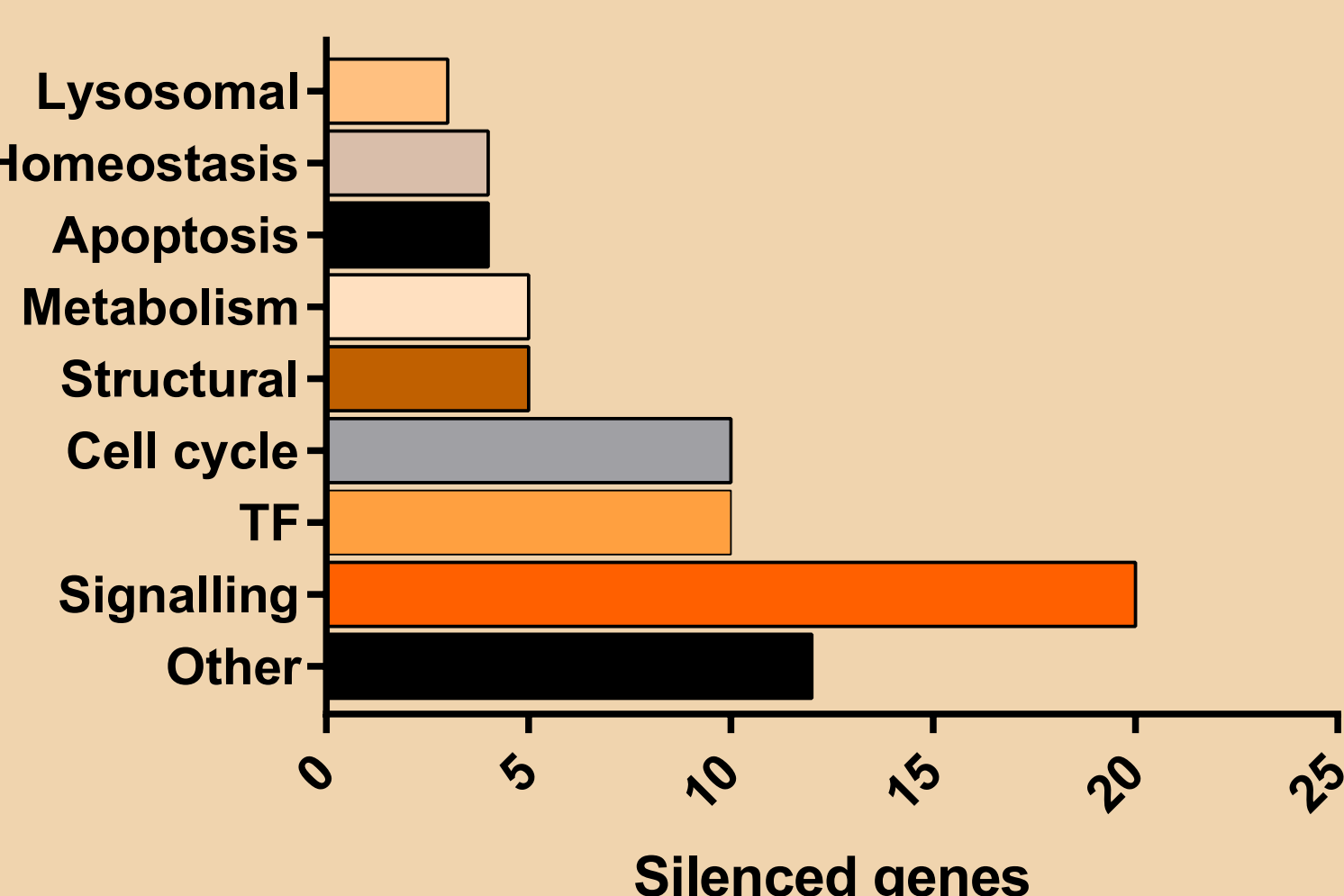## Roadmap to epigenetic diagnosis: From patient's mouth to bedside

DNA extraction, sonication and denaturing

Oral brushing

Tumour extirpation

Hospitalary protocol

Sample obtention can be done by brushing or by tumour extirpation, depending on the characteristics of the tumour.

Purification with inmobilizated secondary antibody

Input DNA

meDIP DNA

Eluted DNA

Laboratory analysis

A key point in the immunoprecipitation is the election of anti-5mc antibody. It has to be previously tested as it is not sold for diagnostic use. Hybridization is done with the personalized array created based on candidate genes of Epineck database following specifications in the kit.

Genes
A   B   C
Emission colour
log_2 ratio (R=meDIP/Input)
-1   0   1

Diagnostic   HPV+   ECS

Non-methylated   Methylated

Computational analysis

In the upper side results obtained from the array are presented, where three different genes are presented(A: non-methylated, B: not significant and C methylated). In the lower side, an hypothetical presentation of matrix clustering of different patients classified by tumour type.

MeDIP (Methylated DNA ImmunoPrecipitation)-chip technique: This figure shows the protocol for HNSCC diagnosis by using MeDIP-chip from the tumour extraction to the computational analysis.

HNSCC diagnostic methylation array: Objectives and discussion

Methylation array objectives

HNSCC diagnosis

HPV positive or negative

Tumoral extracapsullar spread (ECS)

Functional classification of silenced genes

Silenced genes

Epineck methylation array objectives and gene classification: From left to right. The main objective of this array is to allow early stage diagnosis of HNSCC. In order to accomplish this goal, array genes have been classified into HNSCC diagnosis, HPV positive or negative and extracapsullar spread (ECS). Candidate genes have been functionally classified and this has been represented in a column diagram.

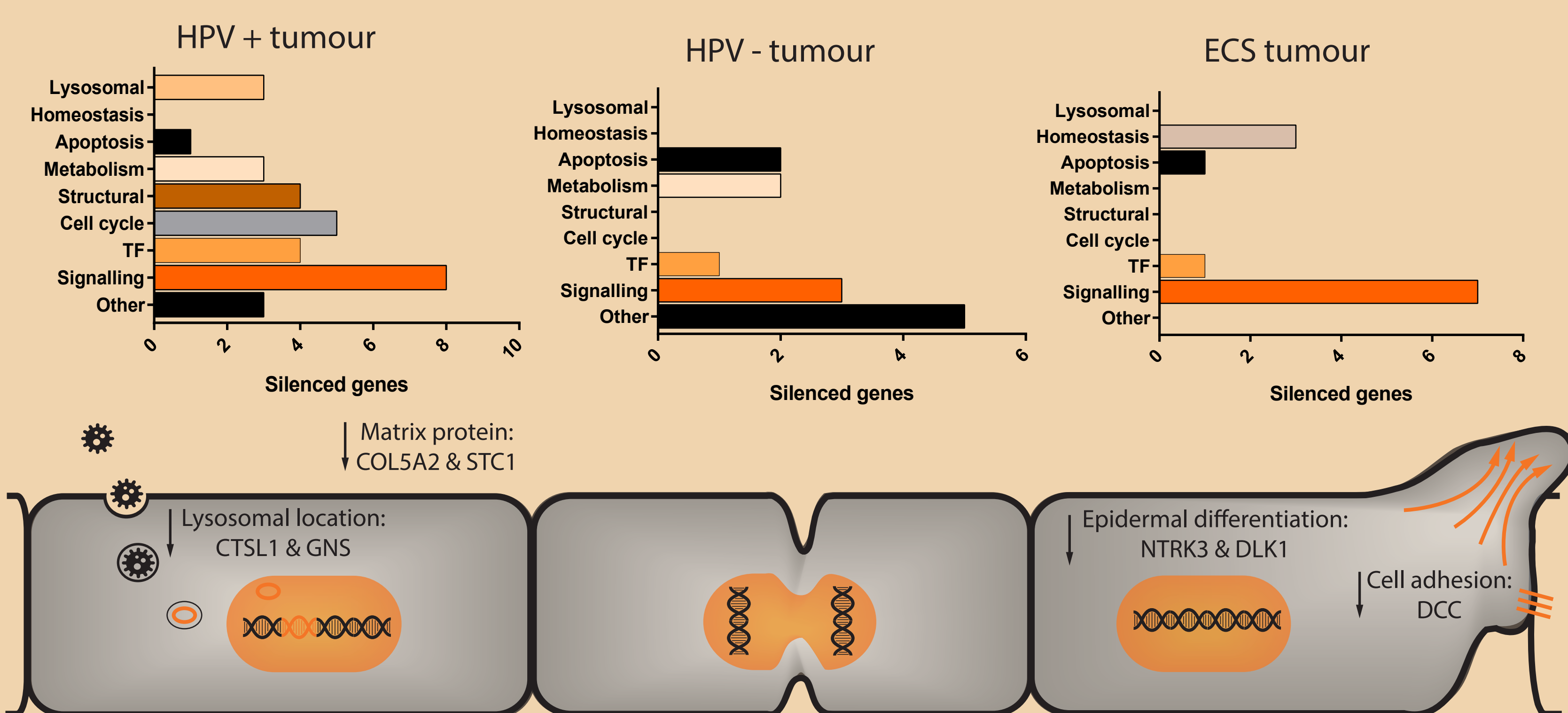### Does gene silencing follow a rational pattern?

HPV + tumour

Silenced genes

HPV - tumour

Silenced genes

ECS tumour

Silenced genes

↓ Matrix protein: COL5A2 & STC1

Lysosomal location: CTSL1 & GNS

Epidermal differentiation: NTRK3 & DLK1

Cell adhesion: ↓ DCC

Fig.5: Does gene silencing follow a rational pattern? From left to right HPV+, HPV- and ECS. The upper side of this image sequence represents column diagrams where the classification is shown by tumour type. The lower side shows thre cells and the rational pattern the gene silencing follows.
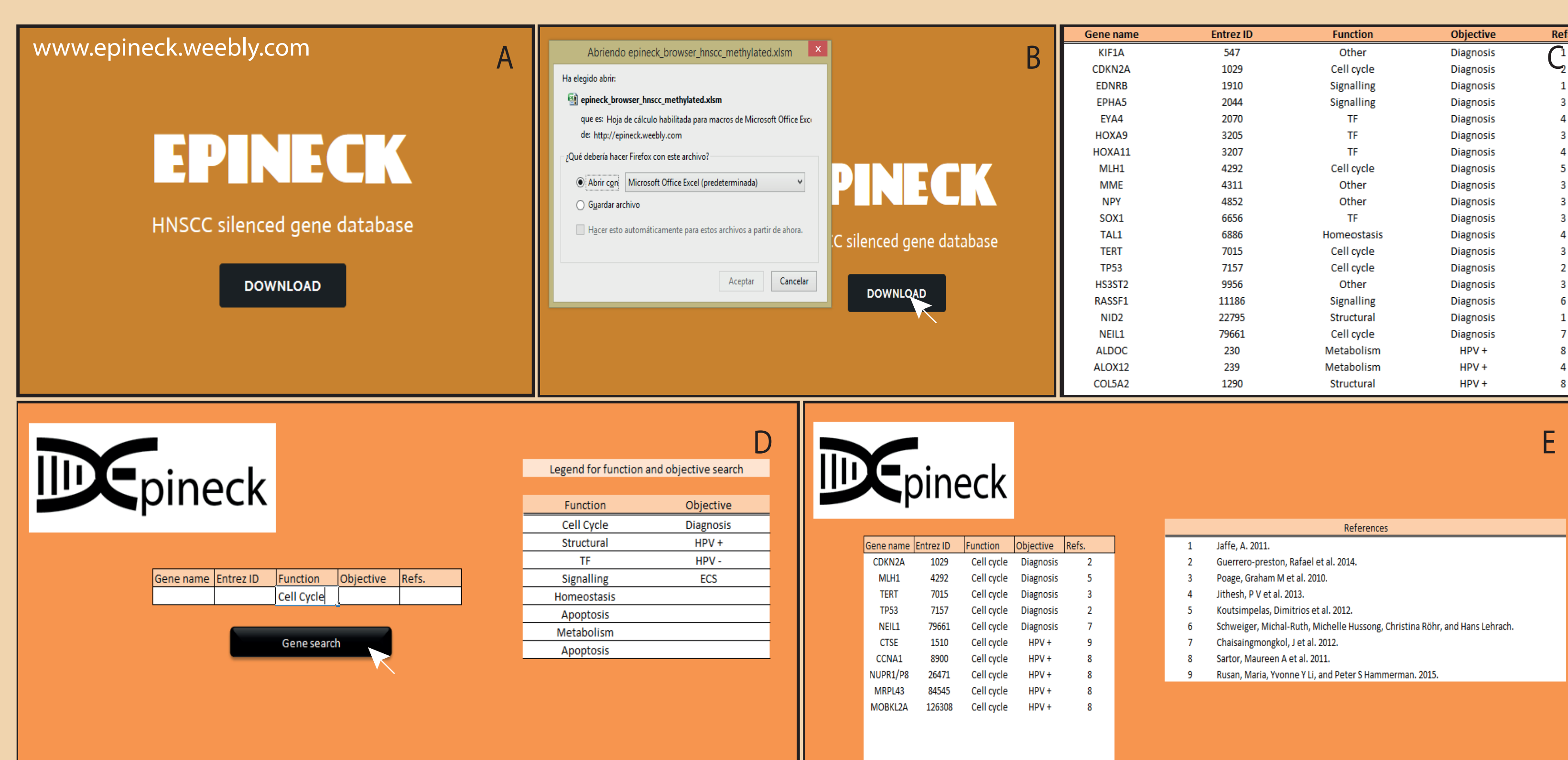
Epineck: HNSCC silenced gene database and browser

www.epineck.weebly.com

EPINECK
HNSCC silenced gene database
DOWNLOAD

Gene name	Entrez ID	Function	Objective	Refs
KIF1A	547	Other	Diagnosis	1
CDKN2A	1029	Cell cycle	Diagnosis	1
EDNRB	1910	Signalling	Diagnosis	2
EPHA5	2044	Signalling	Diagnosis	3
EYA4	2070	TF	Diagnosis	3
HOXA9	3205	TF	Diagnosis	4
HDNA11L	3207	TF	Diagnosis	4
MLH1	4292	Cell cycle	Diagnosis	5
MME	4311	Other	Diagnosis	1
NPY	4852	Other	Diagnosis	3
SOX1	6656	TF	Diagnosis	3
TAL1	6886	Homeostasis	Diagnosis	3
TERT	7015	Cell cycle	Diagnosis	6
TP53	7157	Cell cycle	Diagnosis	2
HUST72	9956	Other	Diagnosis	1
RASSF1	11186	Signalling	Diagnosis	6
NID2	22795	Structural	Diagnosis	3
NELL1	79661	Cell cycle	Diagnosis	7
ALDOC	230	Metabolism	HPV +	8
ALOX12	239	Metabolism	HPV +	8
COL5A2	1290	Structural	HPV +	8

Legend for function and objective search

Function	Objective
Cell Cycle	Diagnosis
Structural	HPV +
TF	HPV -
Signalling	ECS
Homeostasis	
Metabolism	
Apoptosis	

Gene search

Gene name	Entrez ID	Function	Objective	Refs
CDKN2A	1029	Cell cycle	Diagnosis	2
MLH1	4292	Cell cycle	Diagnosis	4
TERT	7015	Cell cycle	Diagnosis	2
TP53	7157	Cell cycle	Diagnosis	1
NELL1	79661	Cell cycle	Diagnosis	2
CTSI	1514	Cell cycle	HPV +	1
CCNA1	8900	Cell cycle	HPV +	1
hUPELPB	28475	Cell cycle	HPV +	4
MPFLAD	84065	Cell cycle	HPV +	3
MOBKL2A	126308	Cell cycle	HPV +	1

References
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4. Hfrhert, P V et al. 2013.
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7. Choranfronged L, et al. 2011.
8. Sartor, Maureen A et al. 2011.
9. Rusan, Maria, Yvonne Y Li, and Peter S Hammerman. 2015.

Fig.6: Epineck: HNSCC silenced gene database & browser. A: Epineck webpage has been created "www.epineck.weebly.com" where database and browser document and project information is available. B: When download button is clicked, a Excel datasheet download starts, this excel datasheet contains both browser and database. C: Search can be done by introducing several keywords in the browser, these keywords are written on the right part of the image. D: Results appear in "Results" page with all the genes that fulfill your query.

Conclusions

- Epigenetic mutations have a great relevance in early stages of HNSCC
- Epimutations can be used to develop HNSCC early diagnosis protocols
- Gene silencing pattern follows a rational sequence based on their molecular biology.
- All data compiled during this bachelor thesis is publicly available in the following web: www.epineck.weebly.com

References
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