Instituto de Salud Carlos III

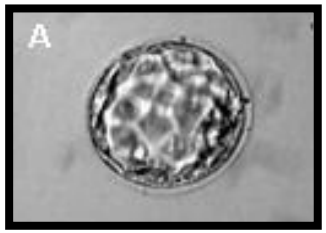
PRINCIPE FELIPE
CENTRO DE INVESTIGACION

New hESC derivation strategies maintaining embryo viability

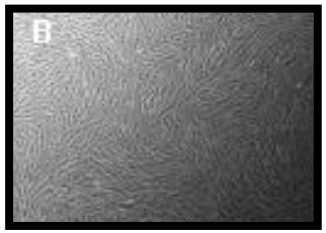
Carlos Simón

Spanish Stem Cell Bank-Valencia Node

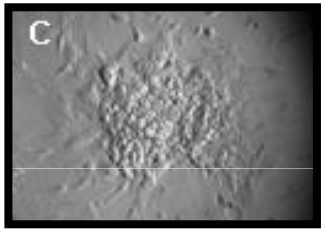
Human Embryonic Stem Cells



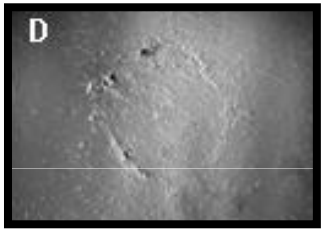
Human Blastocyst



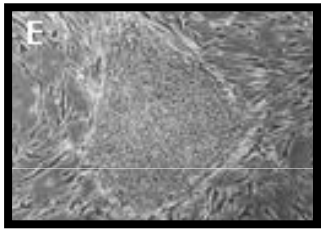
Human foreskin fibroblasts (FSK)



Inner Cell Mass attached on FSK



hESC primary colony



hESC indifferentiated colony

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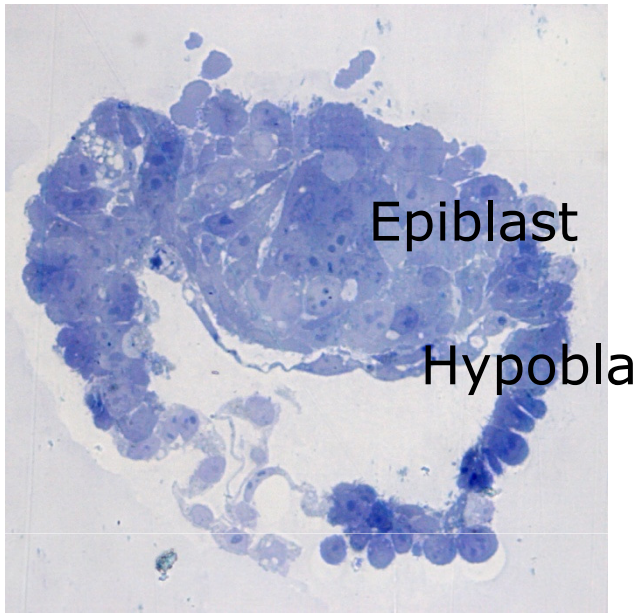
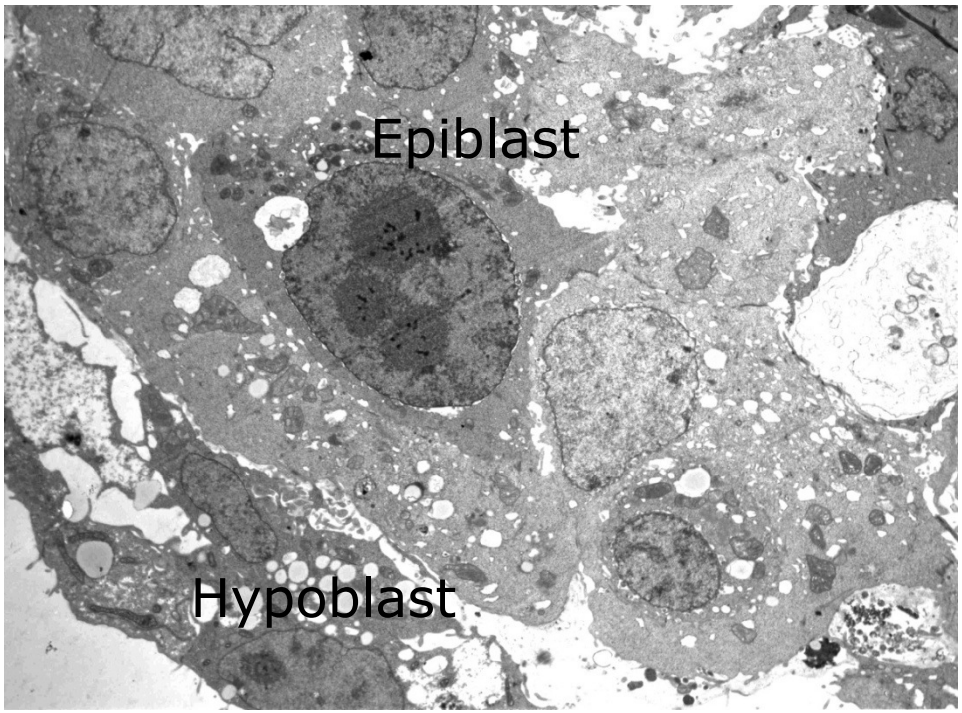
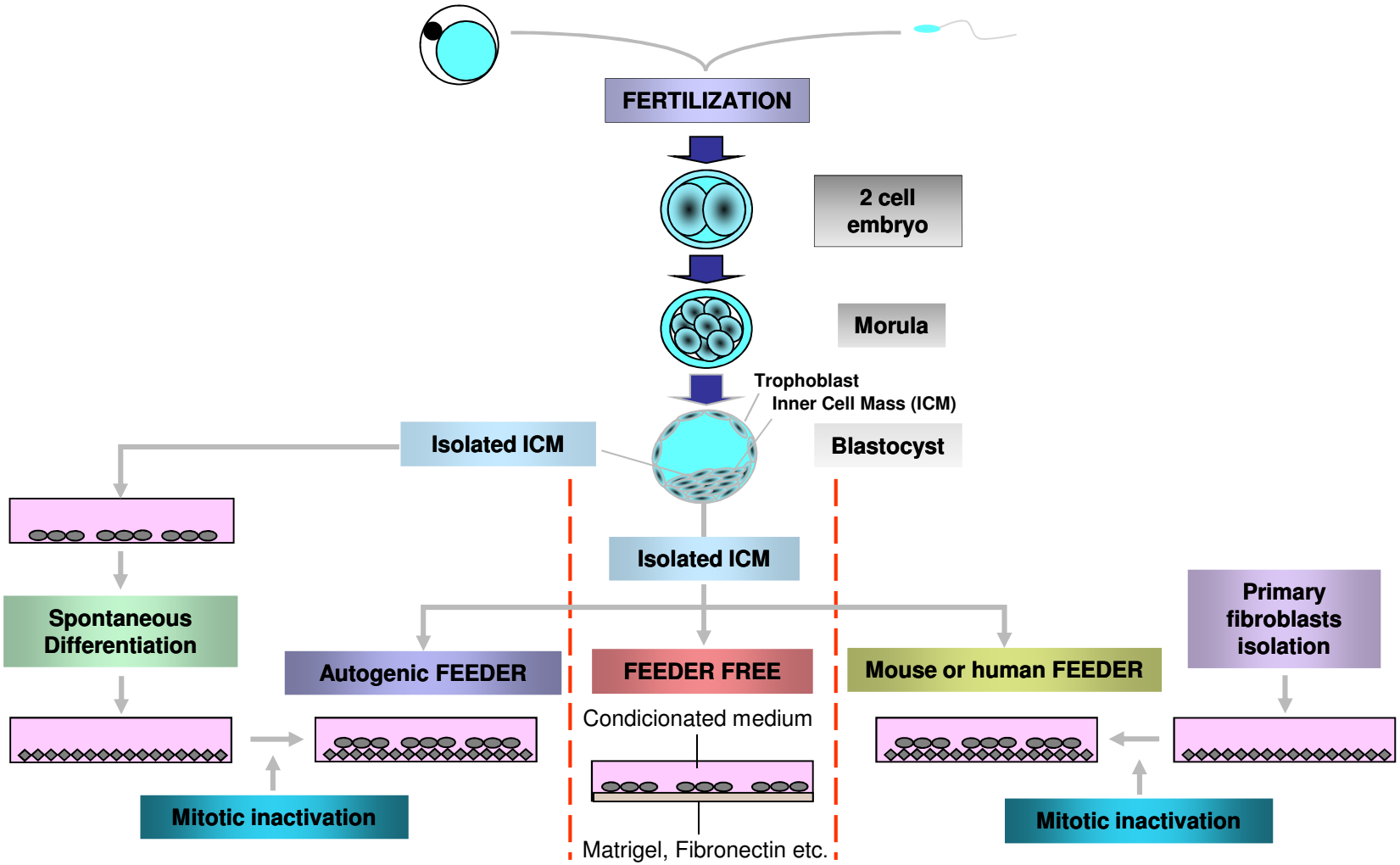
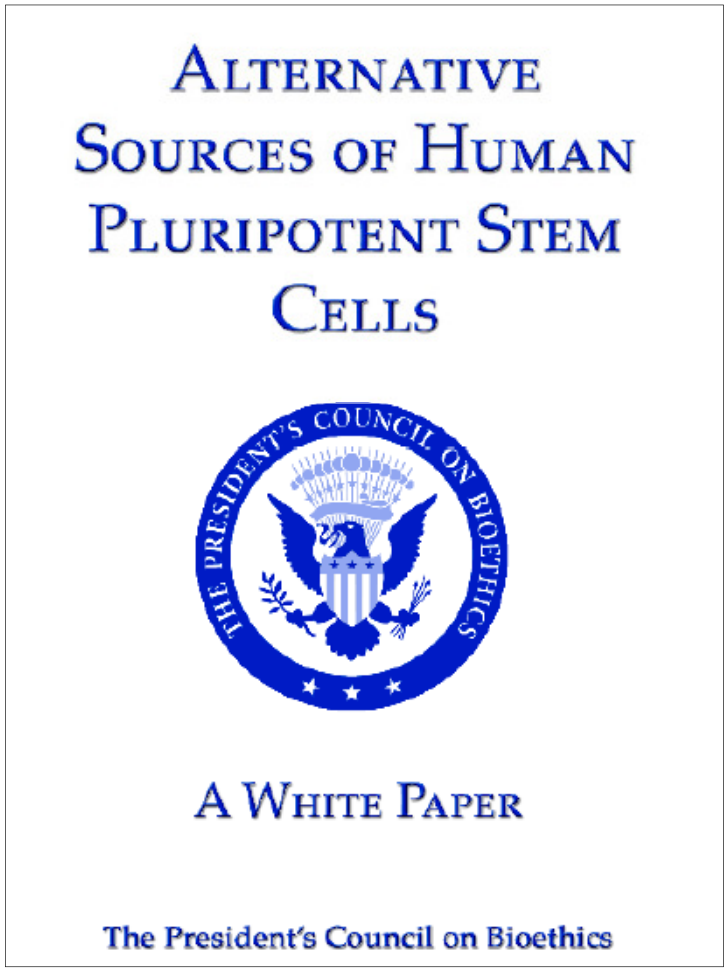

Epiblast
Hypoblast


Epiblast
Hypoblast

Human Embryonic Stem Cells





http://bioethics.gov/reports/white_paper

Alternative sources of human Pluripotent Stem Cells

Biological artifacts

Embryofree strategies → Somatic Cell Reprograming

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Alternative sources of human Pluripotent Stem Cells

Embryofriendly strategies

Organismically died embryos

Blastomere Biopsy

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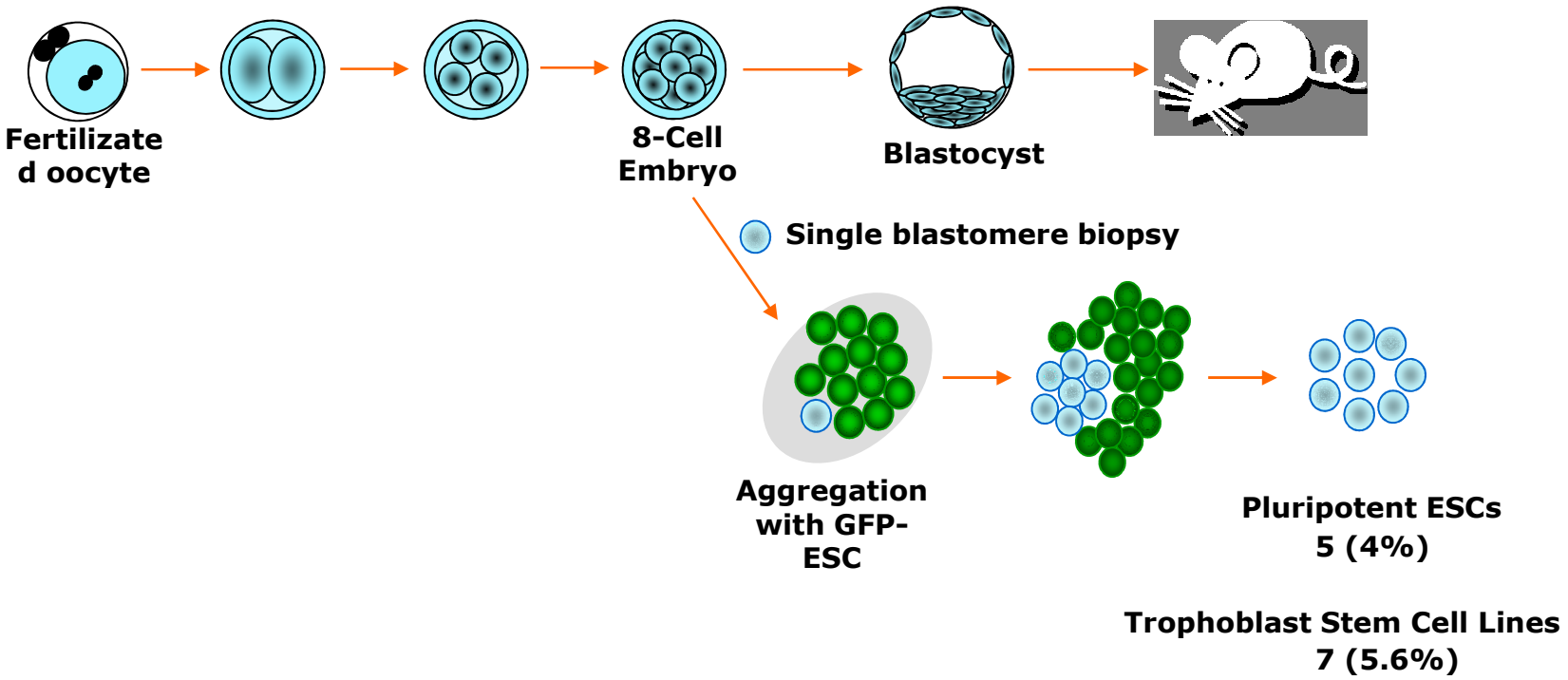
Embryonic and extraembryonic stem cell lines derived from single mouse blastomere



Chung Y *et al*. Nature 2006; 439: 216

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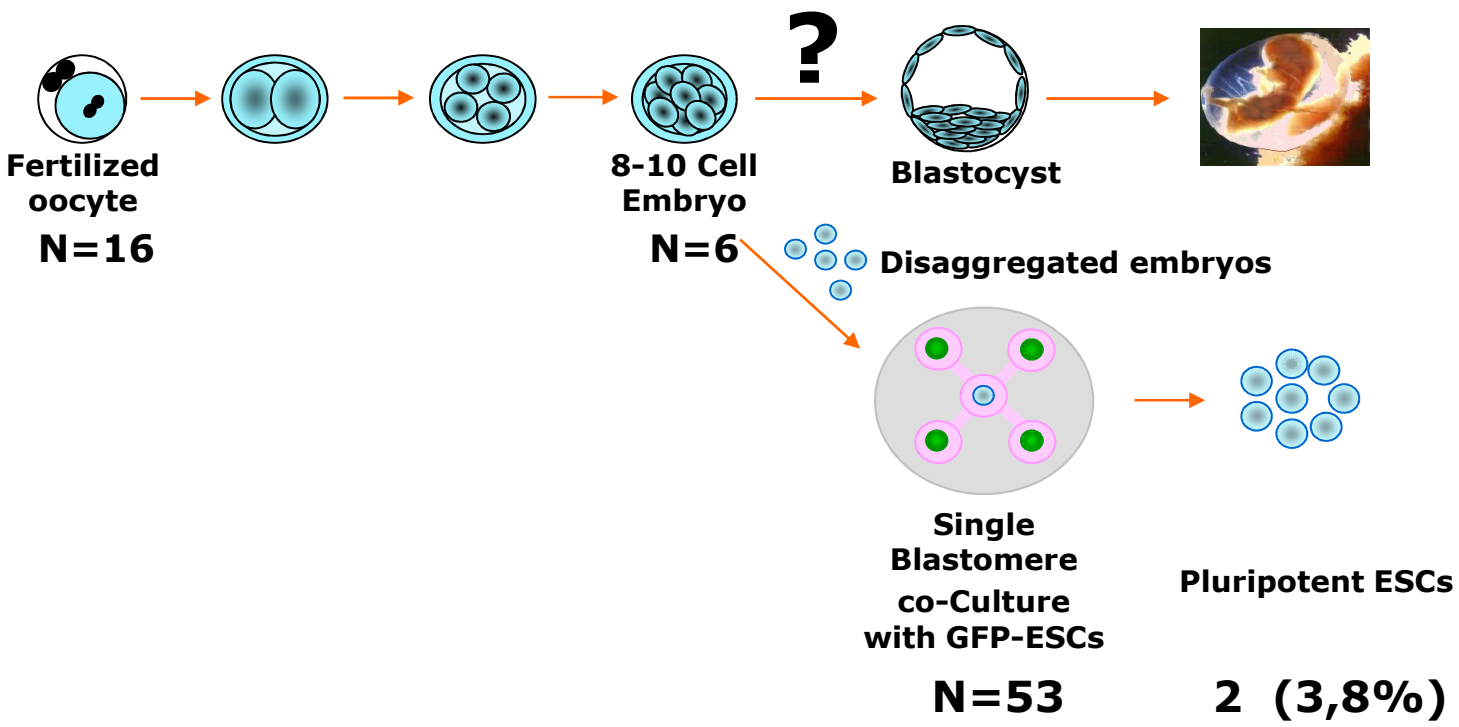
Human Embryonic Stem Cells derived from Single Blastomeres



Klimanskaya I *et al*. Nature 2006; 444:481

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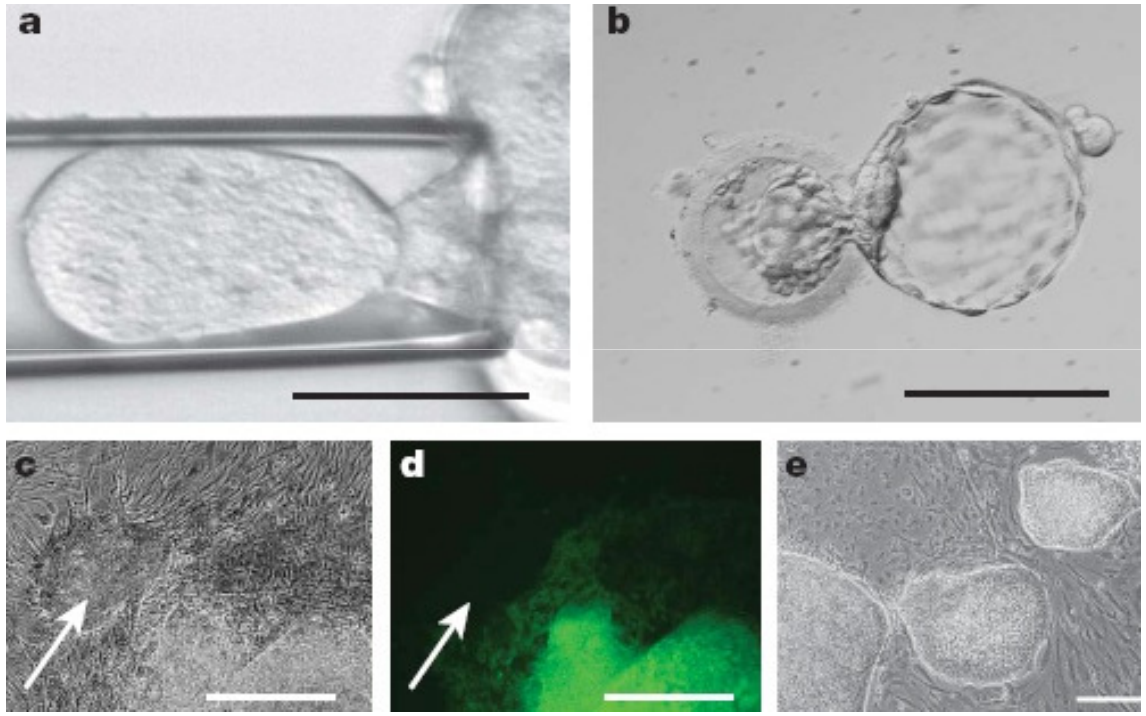
Human Embryonic Stem Cells derived from Single Blastomeres



Blastomere-derived outgrowth close to a colony of GFP-positive hESCs

Morphology of blastomere-derived hESC colonies

Klimanskaya I *et al*. Nature 2006; 444:481

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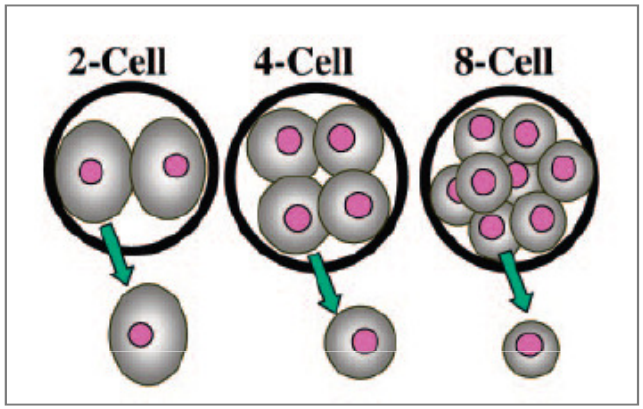
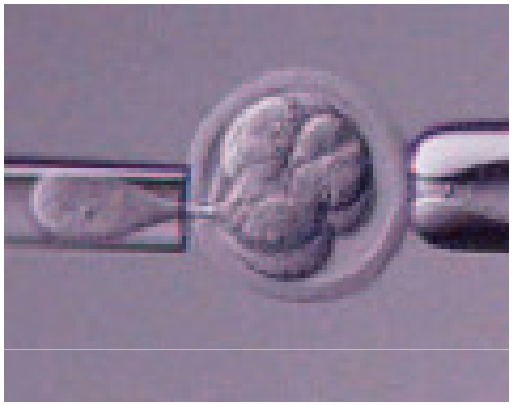
Efficient establishment of mouse embryonic stem cells from single blastomeres and polar bodies





MEFs

HES medium (KoSR+ACTH)

Wakayama S *et al*. STEM CELLS 2007;25:986

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Efficient establishment of mouse embryonic stem cells from single blastomeres and polar bodies

Table 1. Effect of the developmental stage of the embryo on embryonic stem cell establishment from blastomeres

Origin of cells	Genotype of embryo	Stage of blastomere	No. of blastomeres used	Established ES cell lines	
				No.	%
Blastomere	F2	Two-cell	23	16	69[a]
		Early four-cell	110	45	40[b]
		Late four-cell	60	13	22[c]
		Eight-cell	85	12	14[c]
	GFP	Two-cell	20	10	50
		Early four-cell	7	2	28
		Eight-cell	30	5	16
Control	F2	Blastocyst	16	14	88

Wakayama S *et al*. STEM CELLS 2007;25:986

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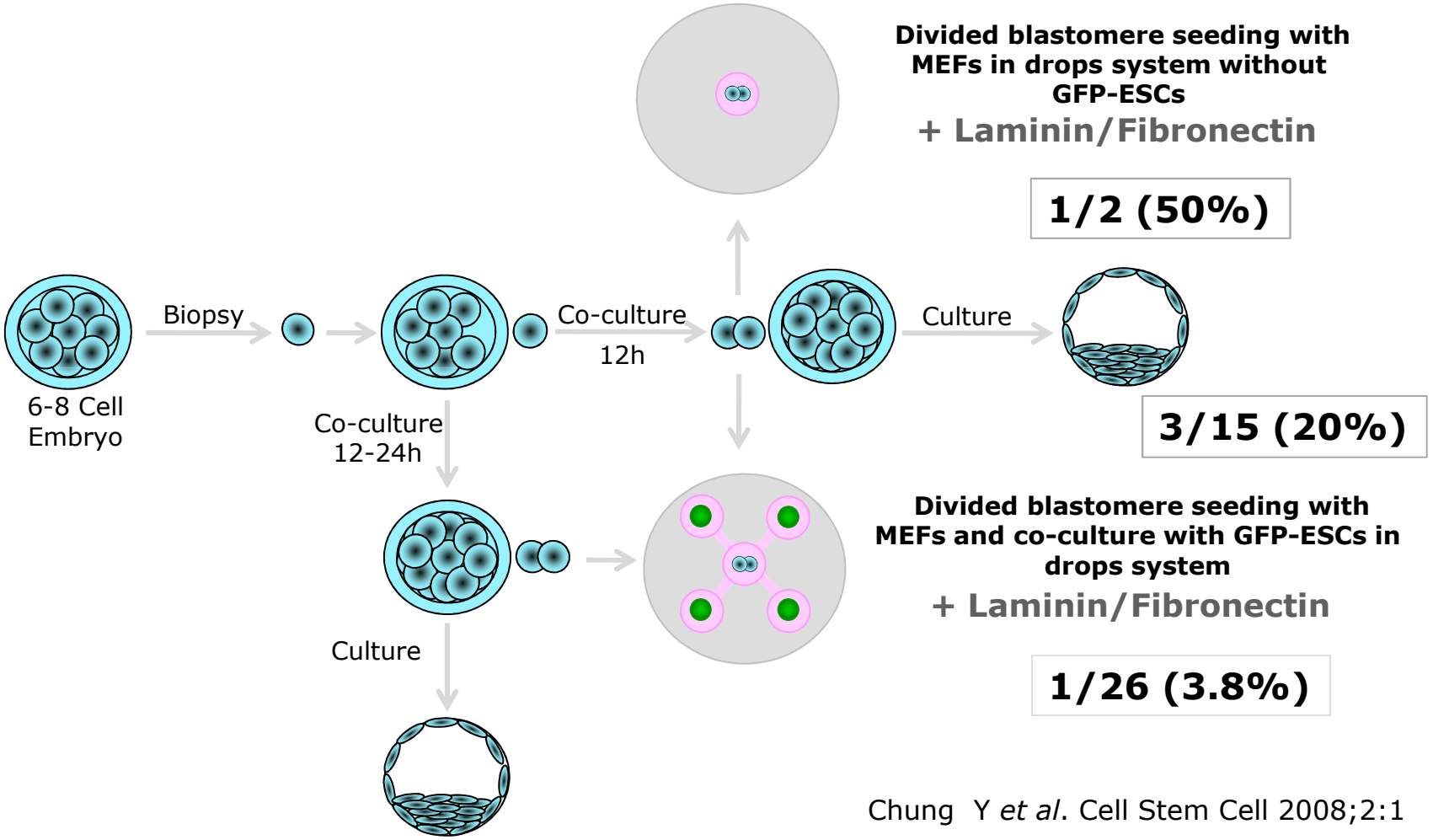
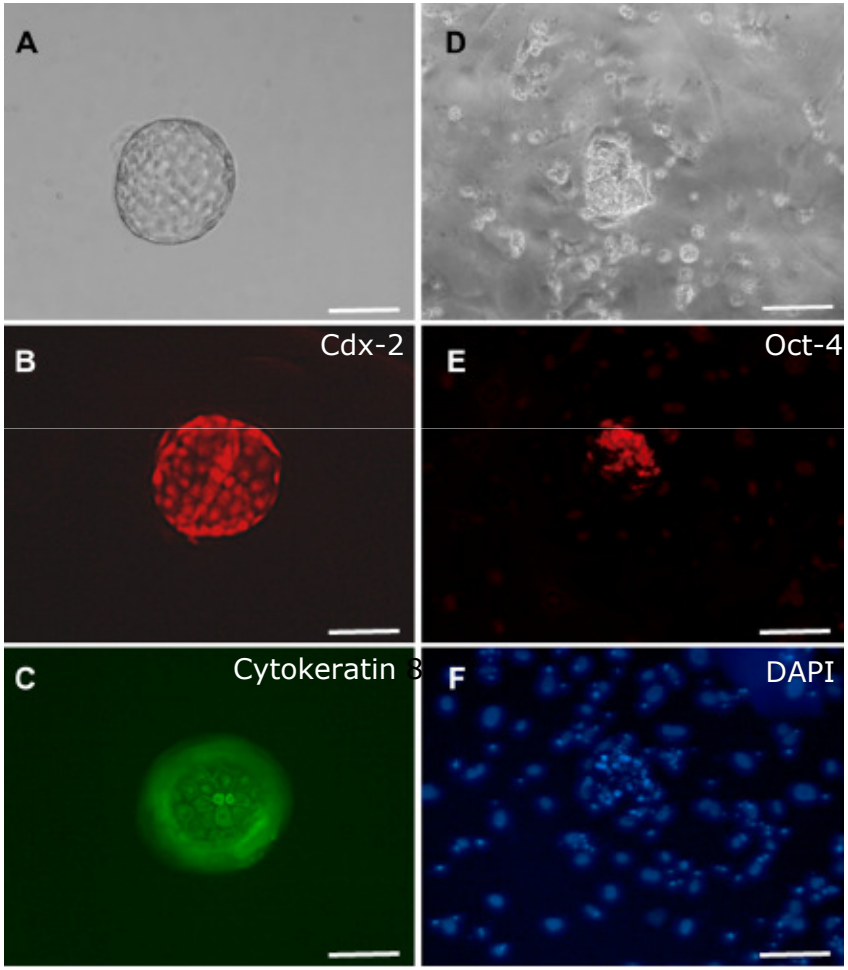
Human Embryonic Stem Cell lines generated without embryo destruction



Chung Y *et al*. Cell Stem Cell 2008;2:1

Human Embryonic Stem Cell lines generated without embryo destruction



Chung Y *et al*. Cell Stem Cell 2008;2:1

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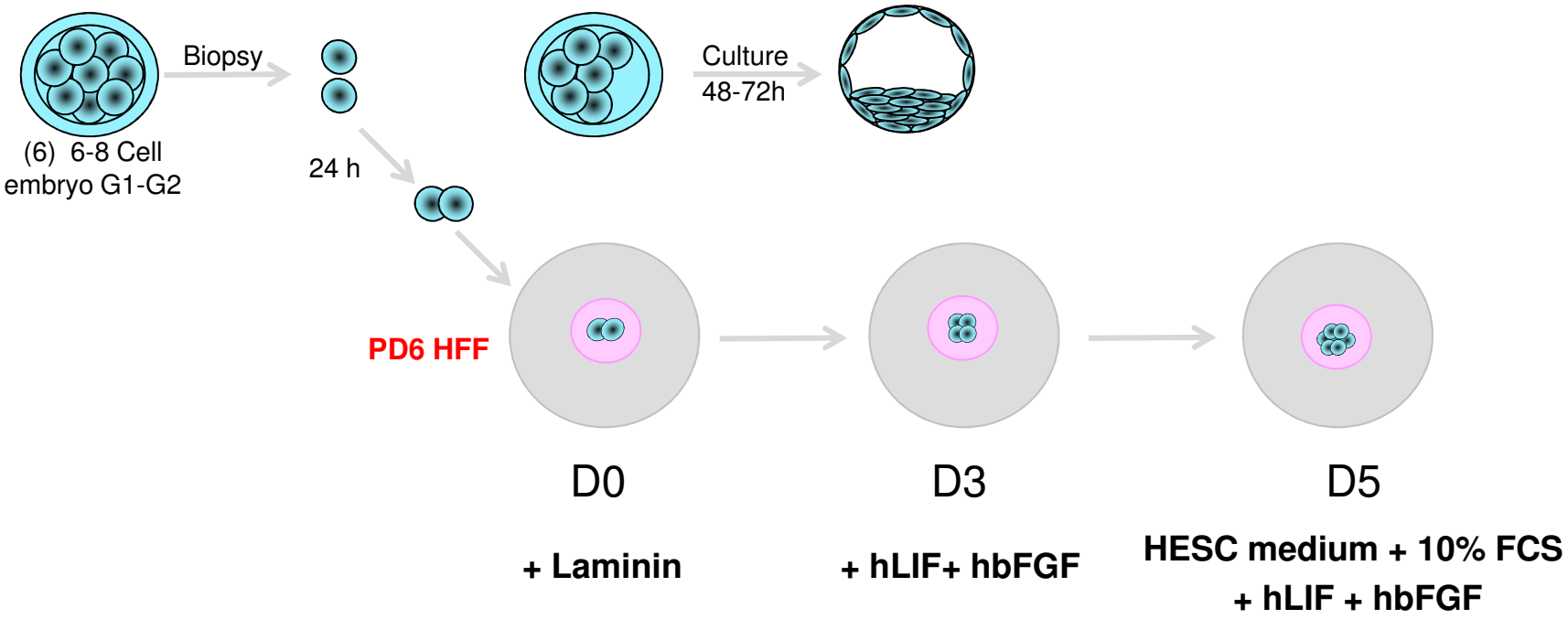
Derivation of human embryonic stem cell lines from biopsied blastomere on human feeders with a minimal exposure to xenomaterials



Dusko I *et al.* Stem Cell Dev 2009; 18: 1343

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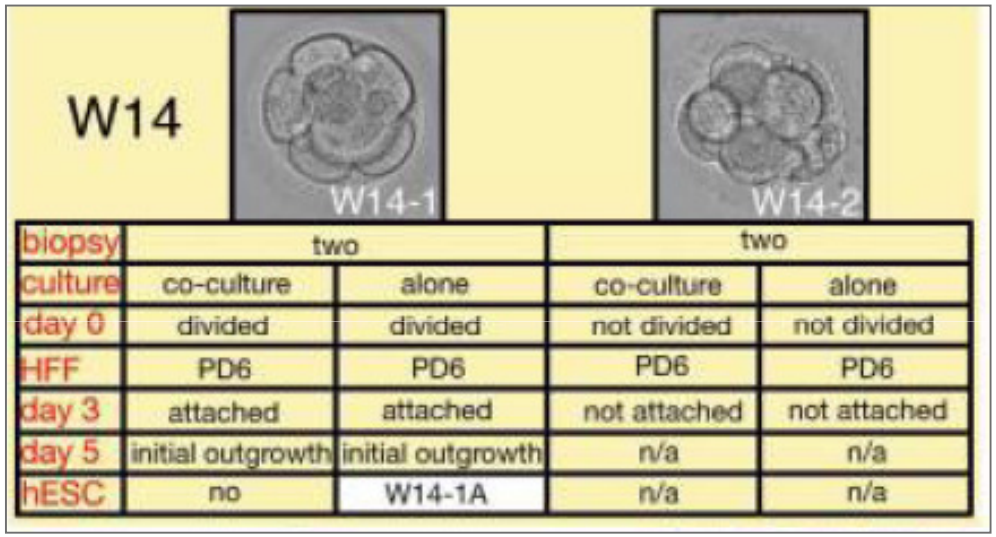
Derivation of human embryonic stem cell lines from biopsied blastomere on human feeders with a minimal exposure to xenomaterials



	BLASTOMERES	DIVIDED	ATTACHED	OUTGROWTH	LINES
PD6 HFF	4	2 (50%)	2 (100%)	2 (100%)	1 (50%)

Dusko I *et al*. Stem Cell Dev 2009; 18: 1343

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Derivation of human embryonic stem cell line from single biopsied blastomere at Valencia Stem Cell Bank

Project 3:
Derivation of hESCs maintaining embryo viability

Approved by:

Commission of Follow-up and Control
of the Donation of Cells and Human Tissues
of the Institute of Health Carlos III

December 13th, 2006,
Permission to use 180 embryos

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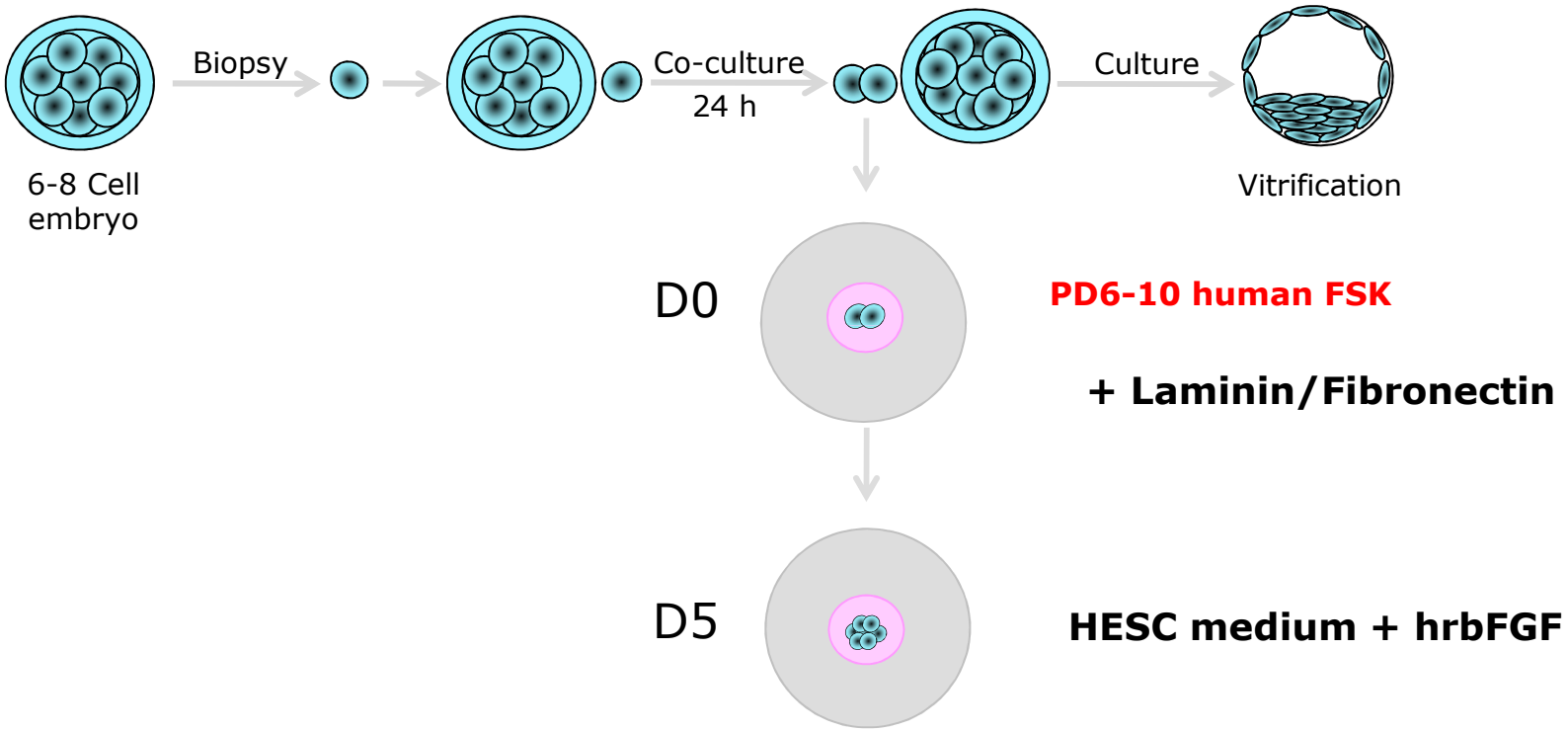
VAL-10B


Biopsy
Co-culture
24 h
Culture
6-8 Cell
embryo
Vitrification
D0
PD6-10 human FSK
+ Laminin/Fibronectin
D5
HESC medium + hrbFGF

Derivation of human embryonic stem cell line from single biopsied blastomere at Valencia Stem Cell Bank. VAL-10B

EMBRYO MORPHOLOGY	SINGLE BIOPSIED BLASTOMERE	DIVIDED BLASTOMERE	ATTACHED	OUTGROWTH	CELL LINE
G II	8	6 (75%)	8 (100%)	6 (75%)	1 (17%)
G III	13	7 (54%)	13 (100%)	1 (8%)	0
G IV	7	2 (29%)	4 (57%)	1 (14%)	0
TOTAL	28	15 (54%)	25 (89%)	8 (32%)	1 (6%)

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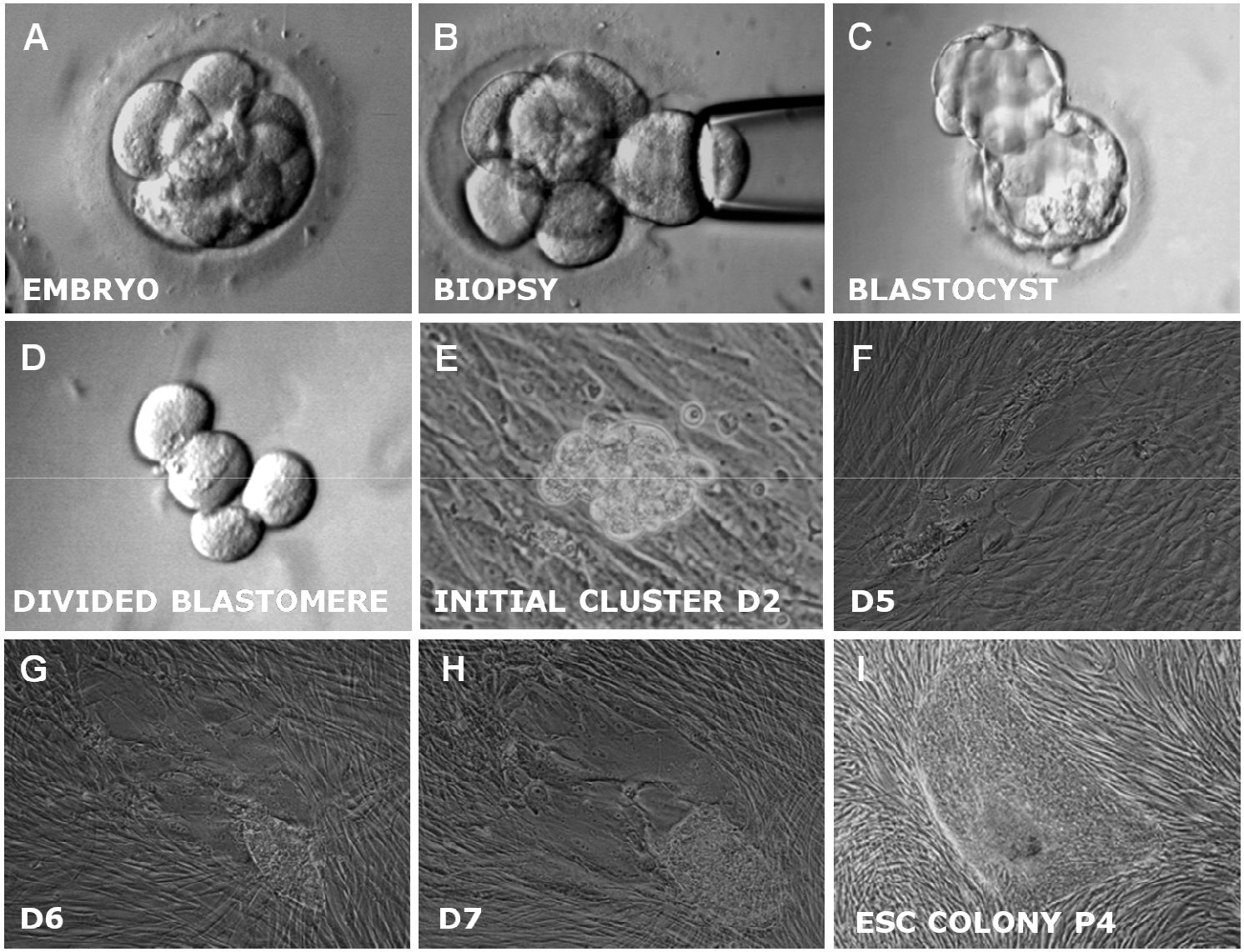
VAL-10B



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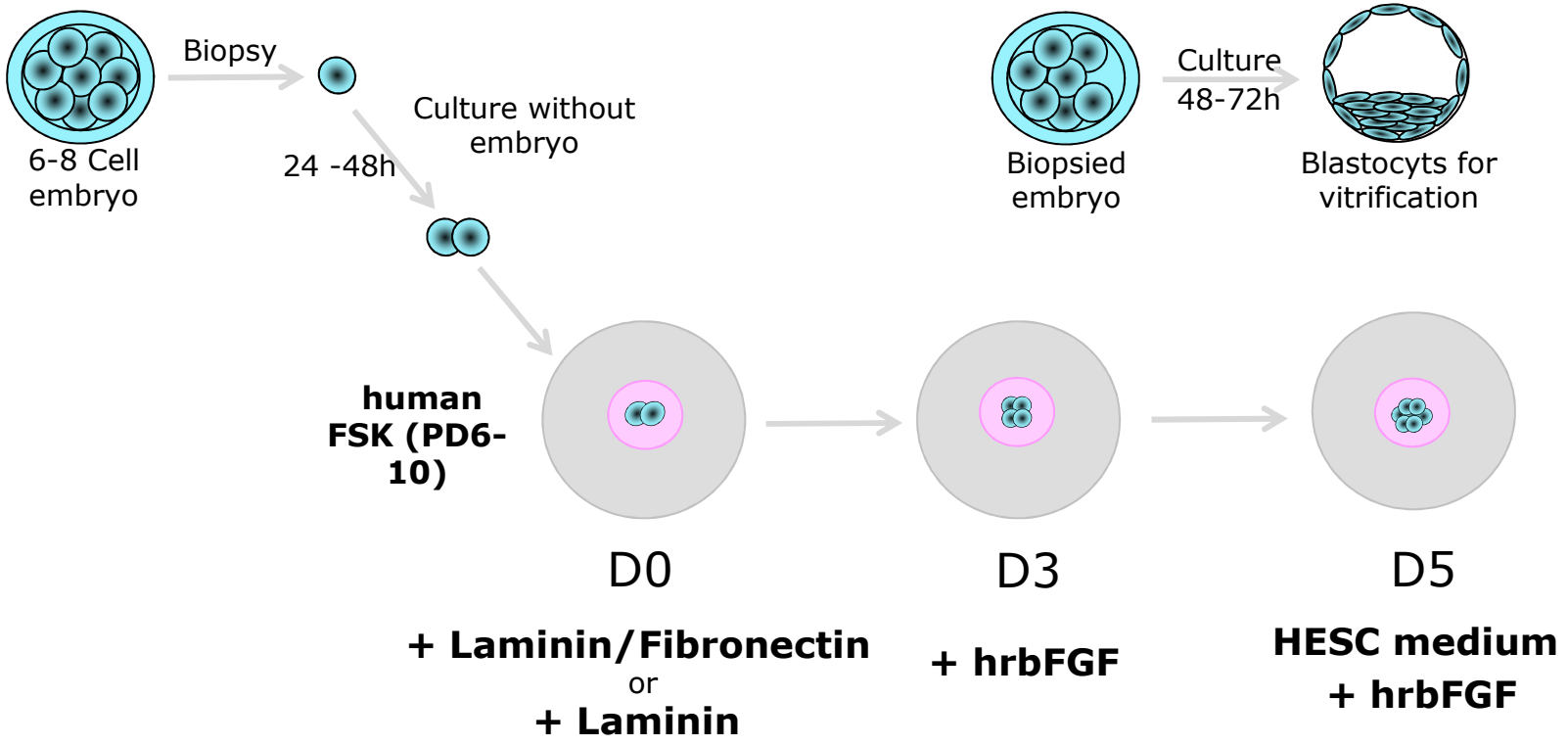
VAL-11B



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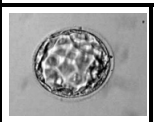
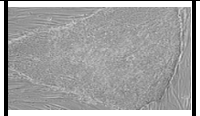
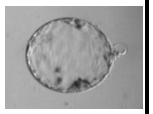
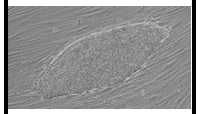
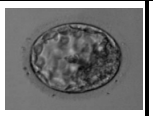
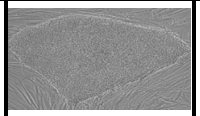
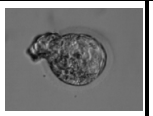
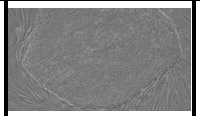
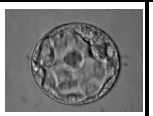
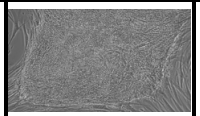
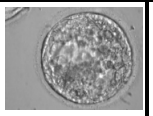
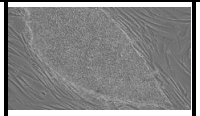
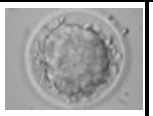
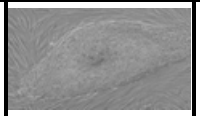
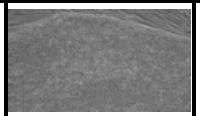
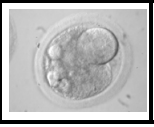
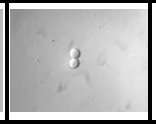
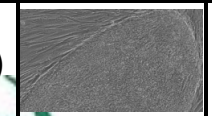
VAL-11B

# EMBRYO	SINGLE BIOPSIED BLASTOMERE	CULTURE	DIVIDED BLASTOMERE	FACTOR		ATTACHED	OUTGROWTH	LINE
				Laminin Fibronectin	Laminin			
3	3	A(*)	1	•		0	n/a	
3	3	A	3		◊	0	n/a	
			66,7%					
3	3	A-B	1	•		3	3	0
3	3	A-B	0		◊	0	n/a	
			16.7%					
3	3	B (*)	0	•		1	1	0
3	3	B	1		◊	2 (66,7%)	1(50%)	1 (50%)
			16,7%					

A: Cleavage Embryo Medium
B: Blastocyst Medium

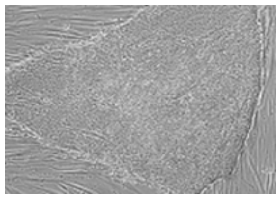
Registered Cell Lines at Spanish Stem Cell Bank (http://www.isciii.es/htdocs/terapia/terapia_lineas.jsp)

Cell Line	Embryo		Colony Morphology		Last passage Criovials
VAL-3		Whole normal blastocyst stage embryo (46,XY) derivation in human foreskin		10X, P42	80 29 CV
VAL-4		Whole normal blastocyst stage embryo (46,XX) derivation in human foreskin		10X, P40	54 38 CV
VAL-5		Whole normal blastocyst stage embryo (46,XX) derivation in human foreskin		10X, P43	49 37 CV
VAL-6M		Whole monogenetic affected embryo derivation (DM Type1) (46,XY) in human foreskin		10X, P45	49 29 CV
VAL-7		Derivation from ICM isolated by laser (46,XY) in human foreskin		10X, P22	65 18 CV
VAL-8		Whole normal blastocyst stage embryo (46,XX) derivation in human foreskin		10X, P6	22 30 CV
VAL-9		Whole normal morula stage embryo (46,XY) derivation in human foreskin		10X, P6	55 43 CV
VAL-10B		 Derivation from single biopsied blastomere (46,XY) in human foreskin		10x, P13	25 39 CV
VAL-11B		 Derivation from single biopsied blastomere (46,XX) in human foreskin		10x, P15	25 46 CV

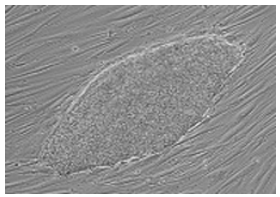
Characterization

1. Morphology

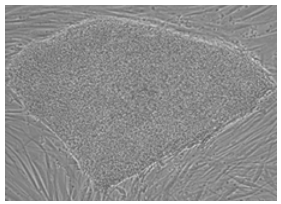
VAL-3



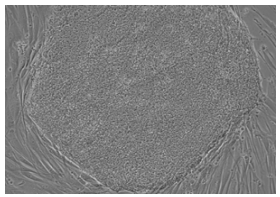
VAL-4



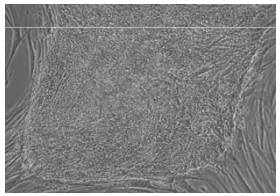
VAL-5



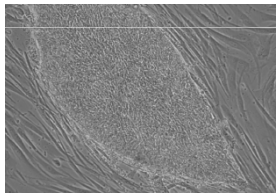
VAL-6M



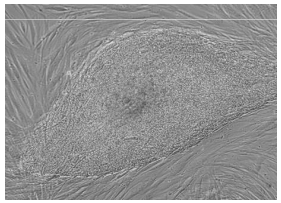
VAL-7



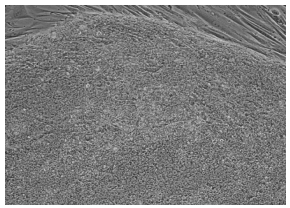
VAL-8



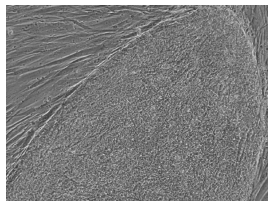
VAL-9



VAL-10B



VAL-11B



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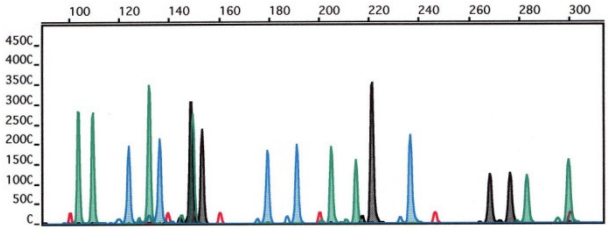
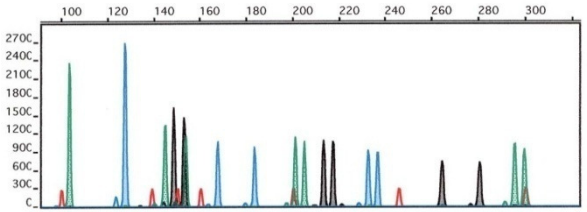
Characterization

3. Imprinting

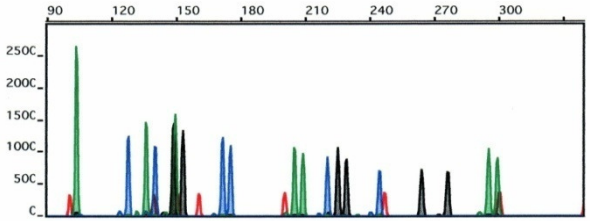
VAL-3



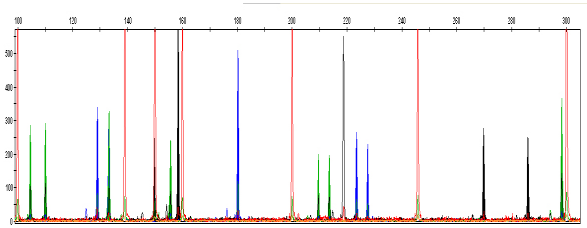
VAL-4



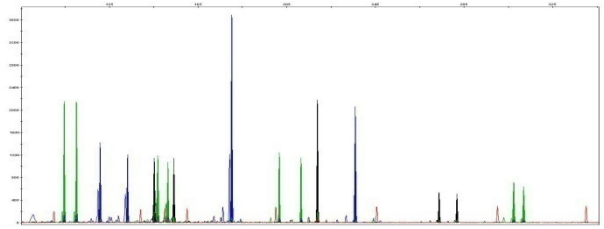
VAL-5



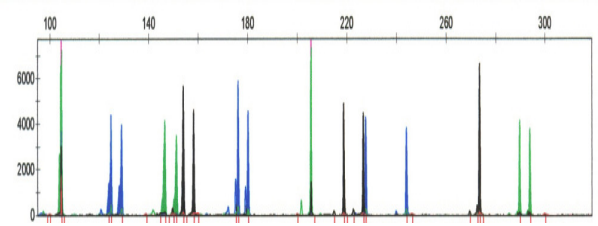
VAL-6M



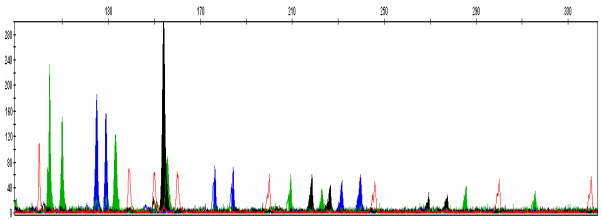
VAL-7



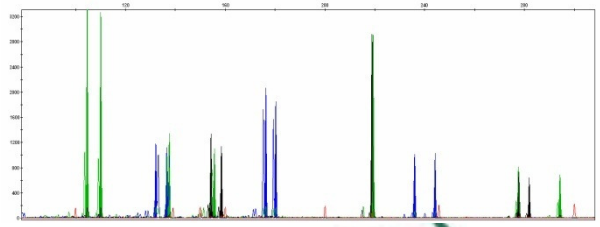
VAL-8



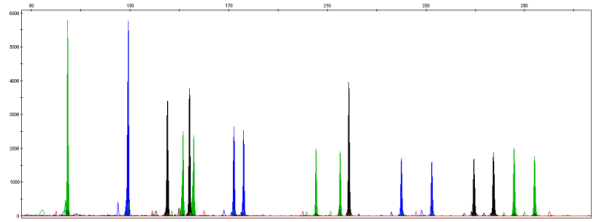
VAL-9



VAL-10b



VAL-11b



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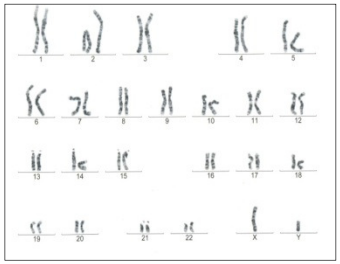
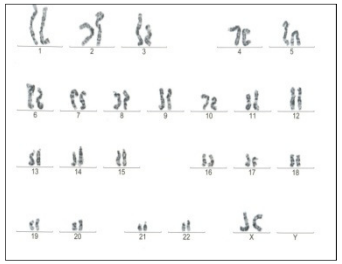
Characterization

4. Karyotype

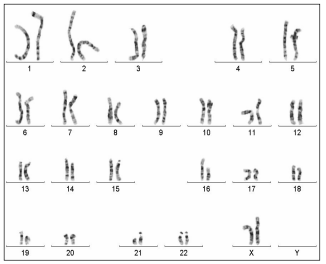
VAL-3
(46,XY)



VAL-4
(46,XX)



VAL-5
(46,XX)



VAL-6M
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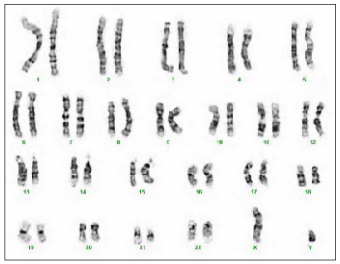
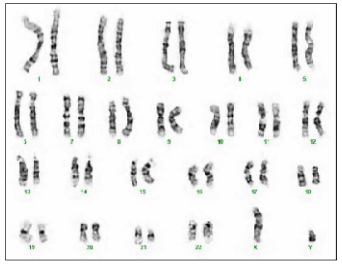
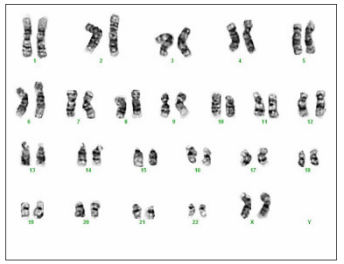


VAL-7
(46,XY)



VAL-8
(46,XX)



VAL-9
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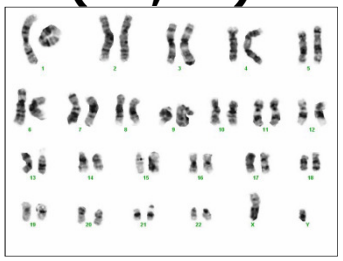
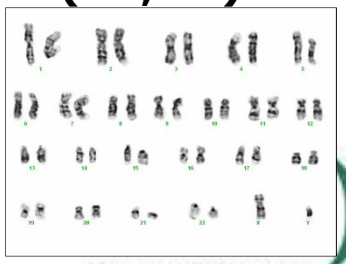
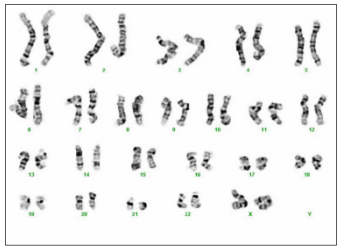


VAL-10B
(46,XY)

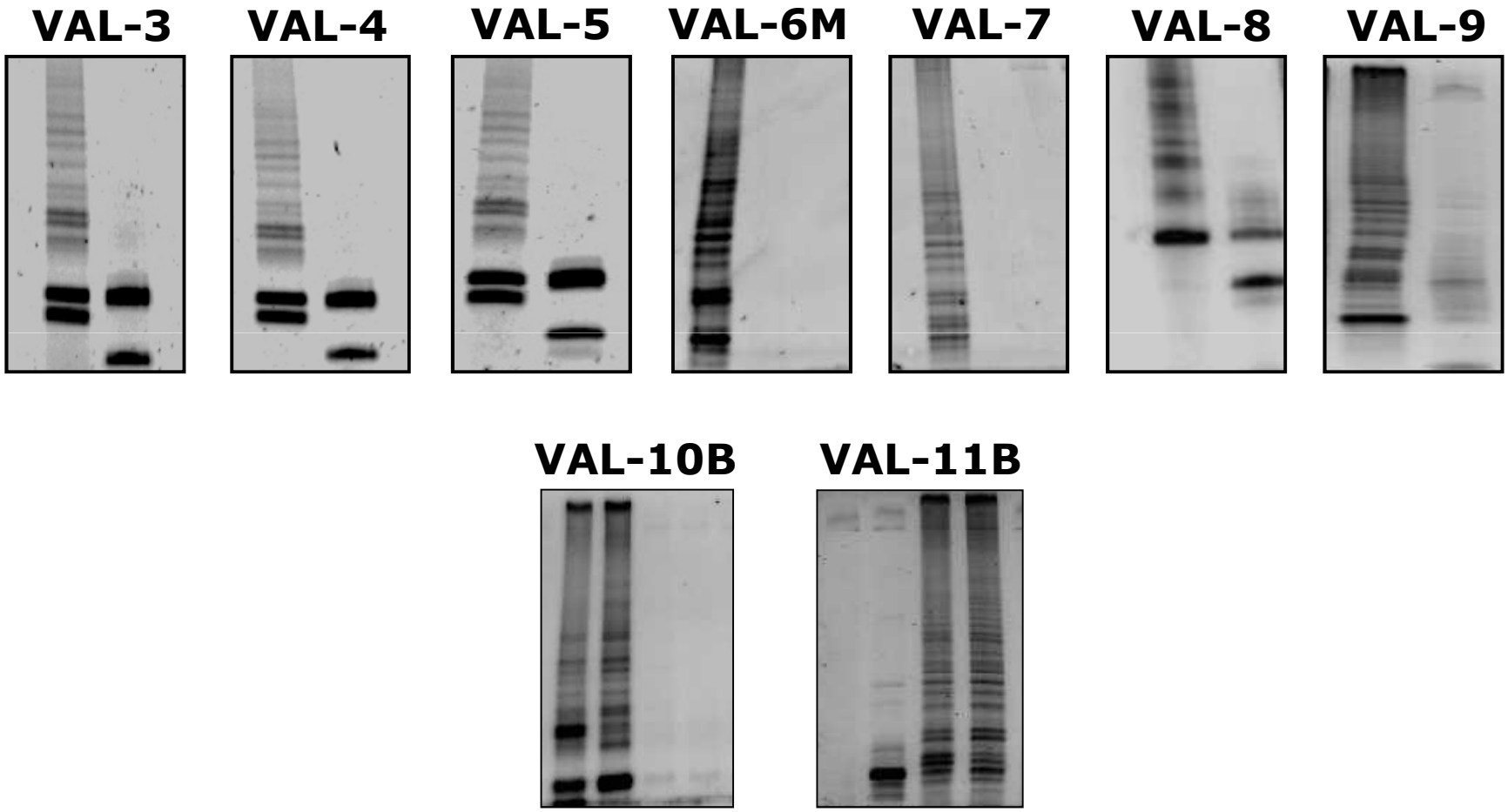


VAL-11B
(46,XX)



Characterization

5. Telomerase activity



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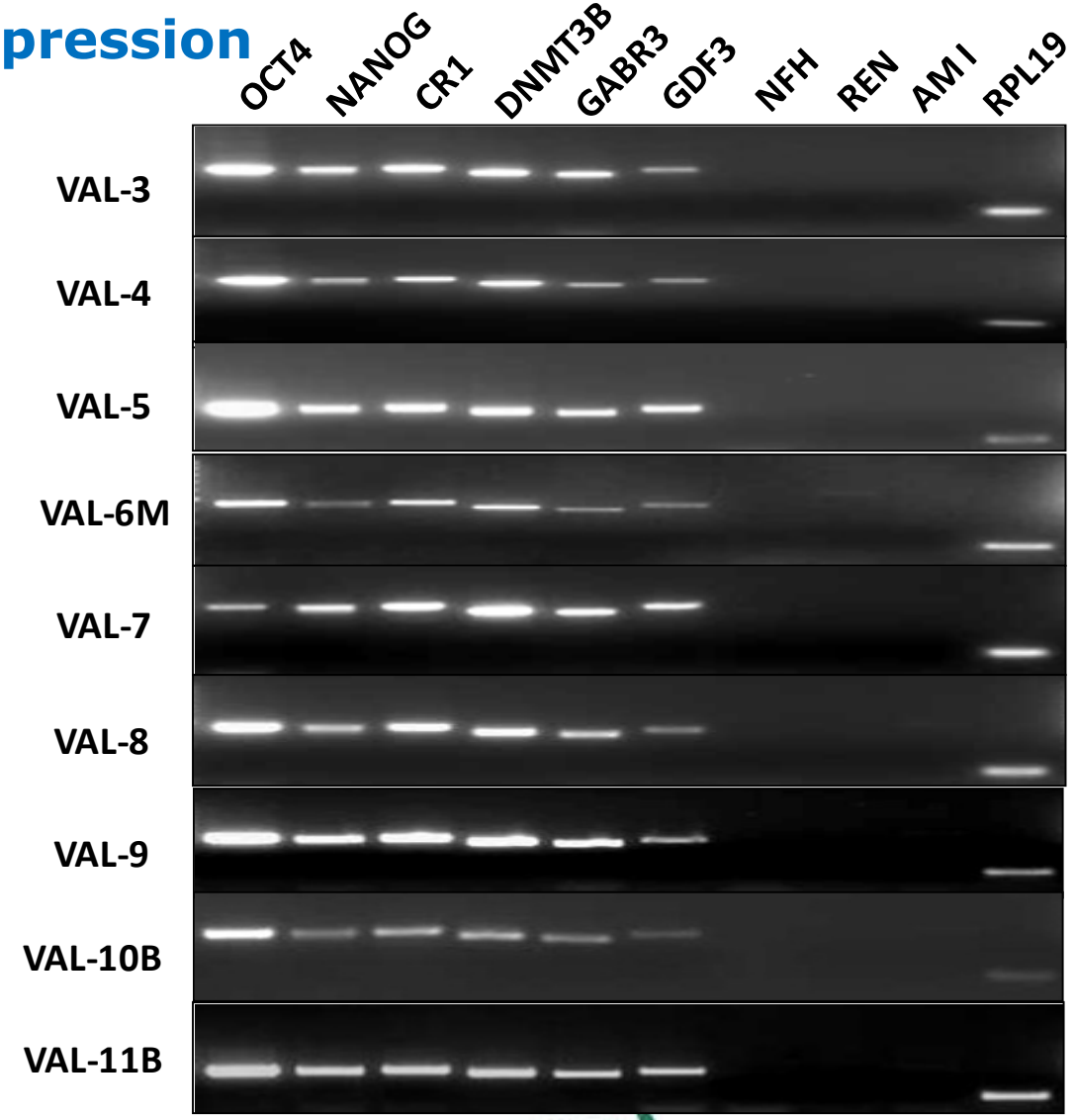
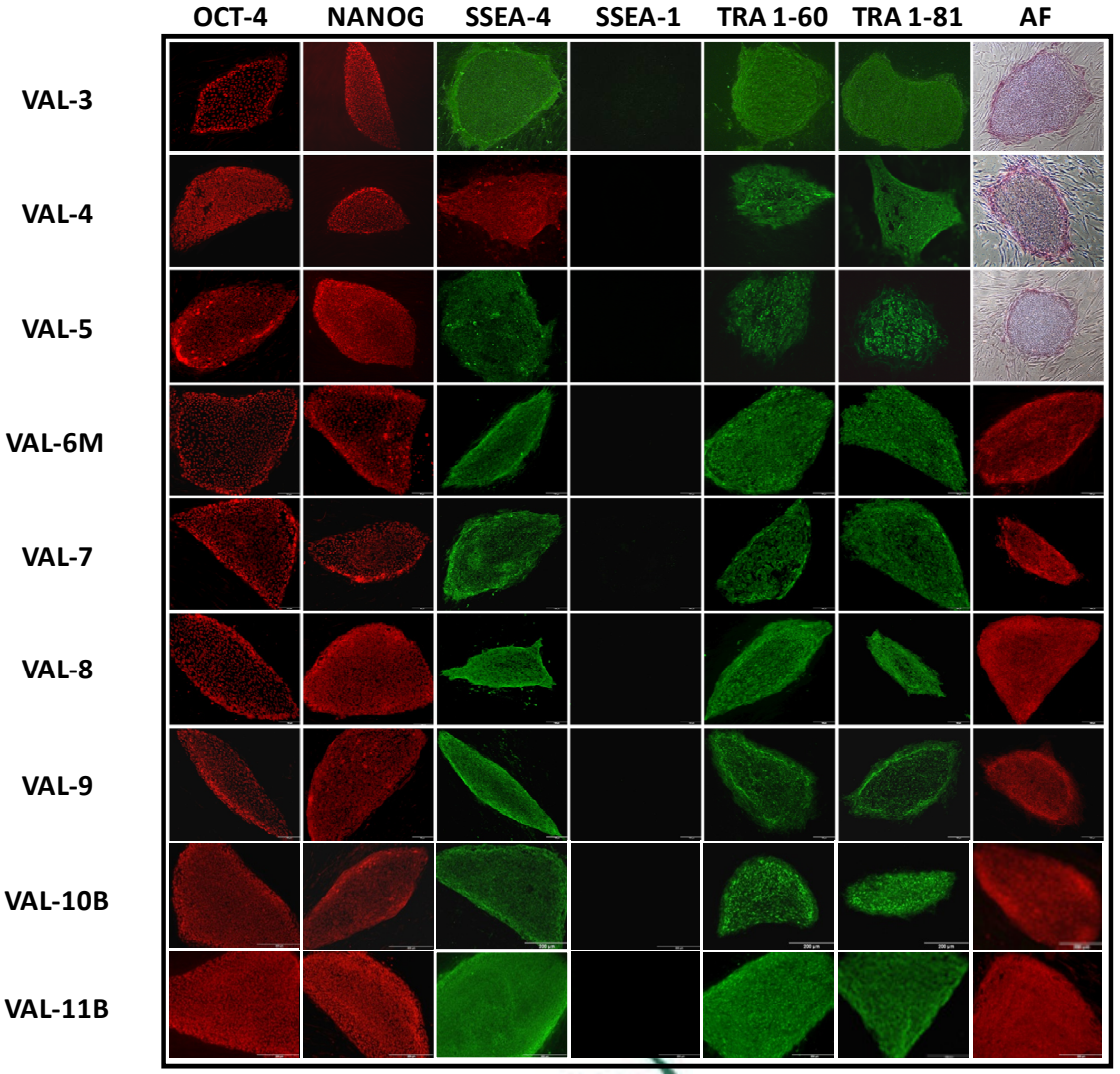
Characterization

6. Gene expression



Characterization

7. Immunohistochemistry



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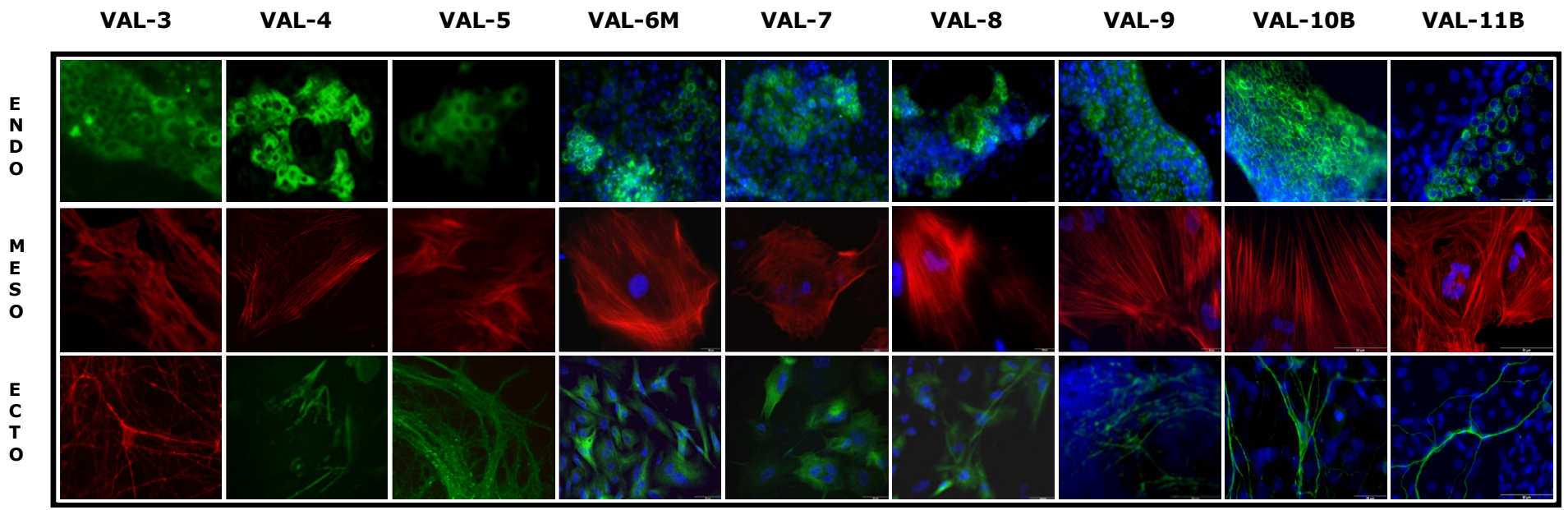
Characterization

8. In *vitro* differentiation



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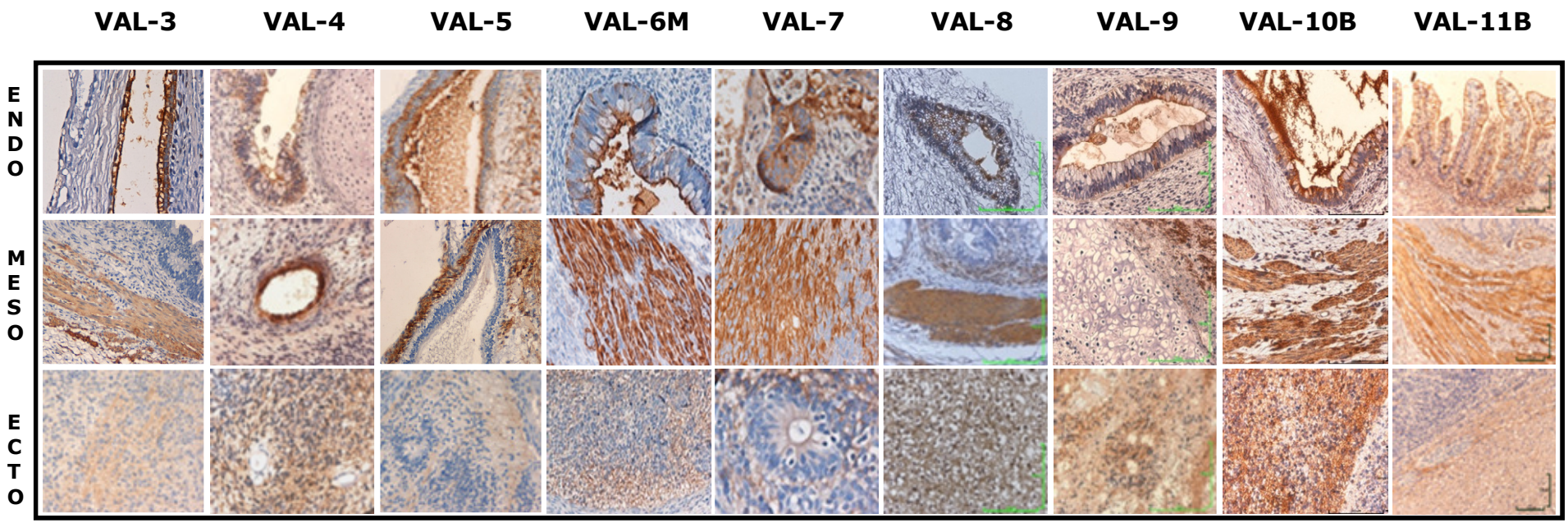
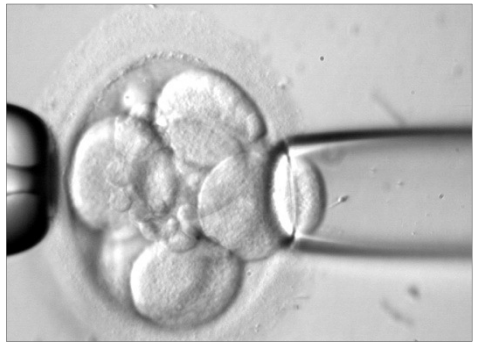
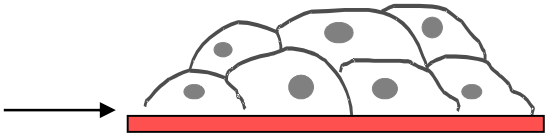
Characterization

9. *In vivo* differentiation

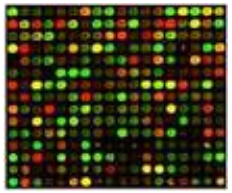


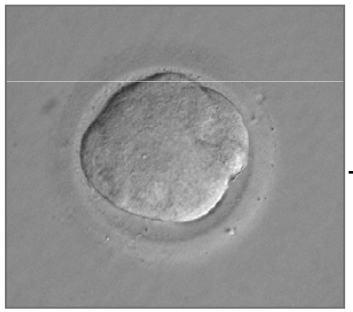
Molecular Analysis

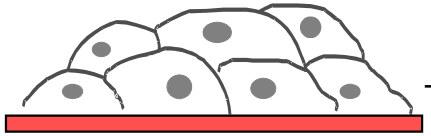




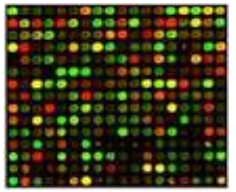
VAL-10B, VAL-11B

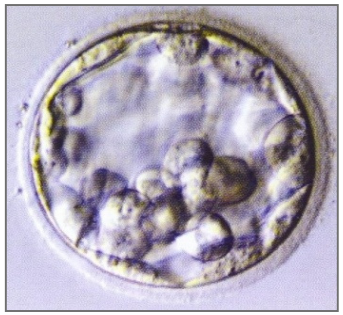


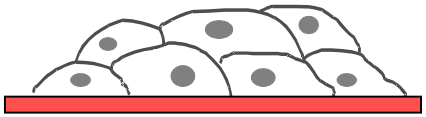




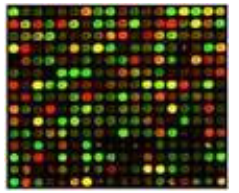
VAL-9







VAL-5, VAL-7, VAL-8



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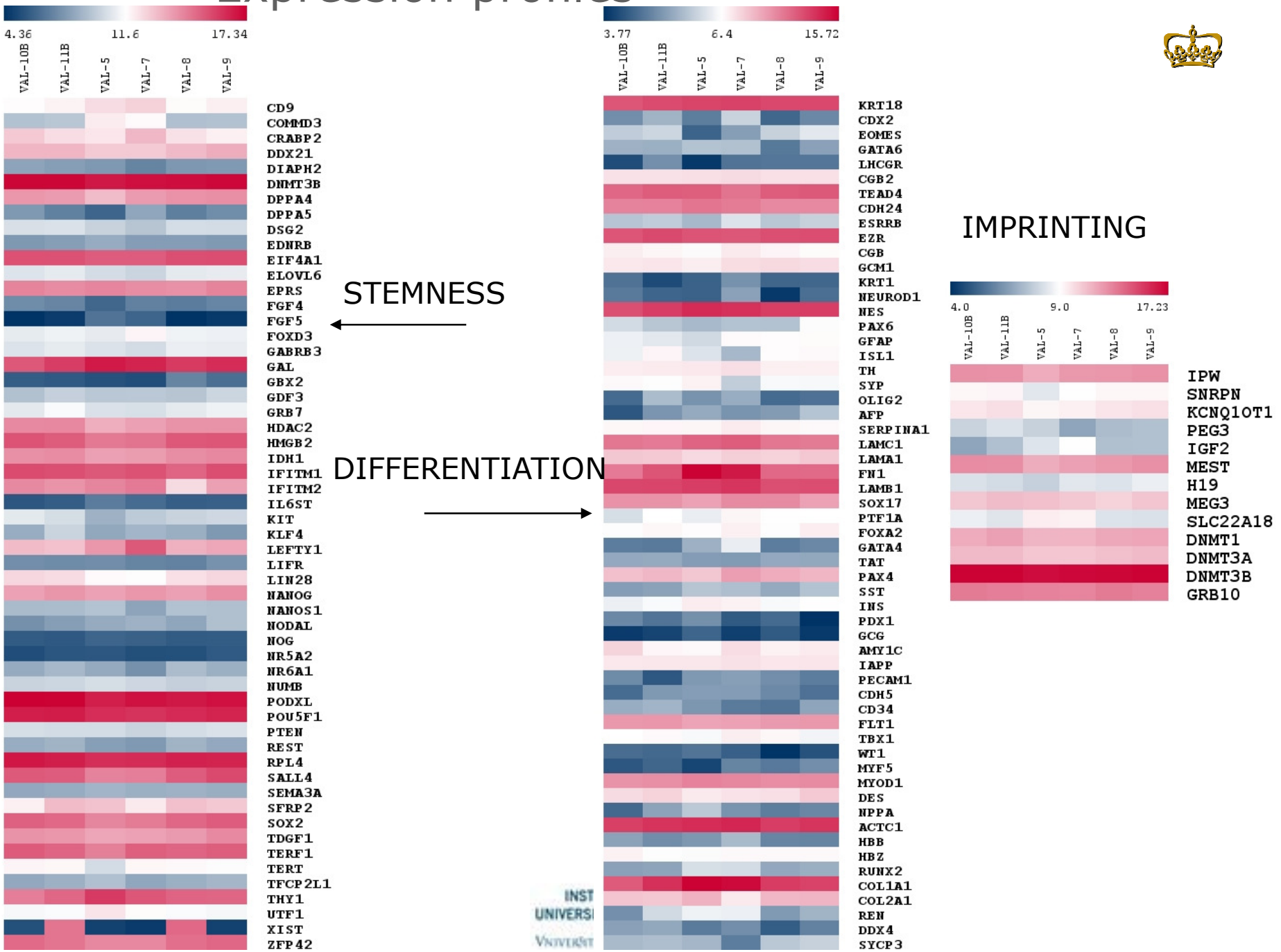
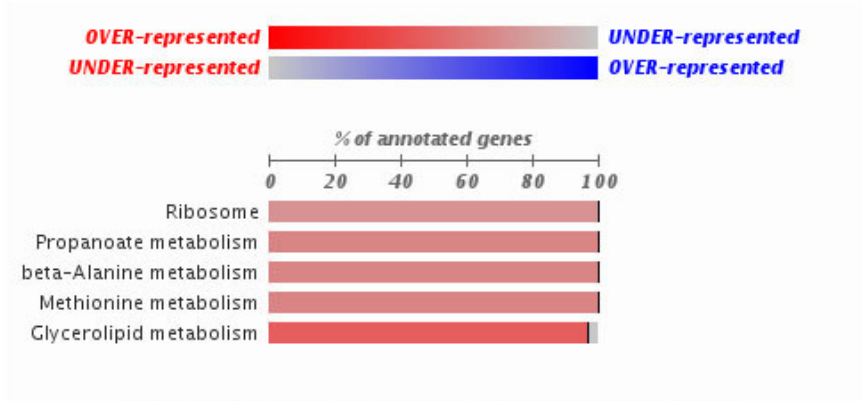
Expression profiles



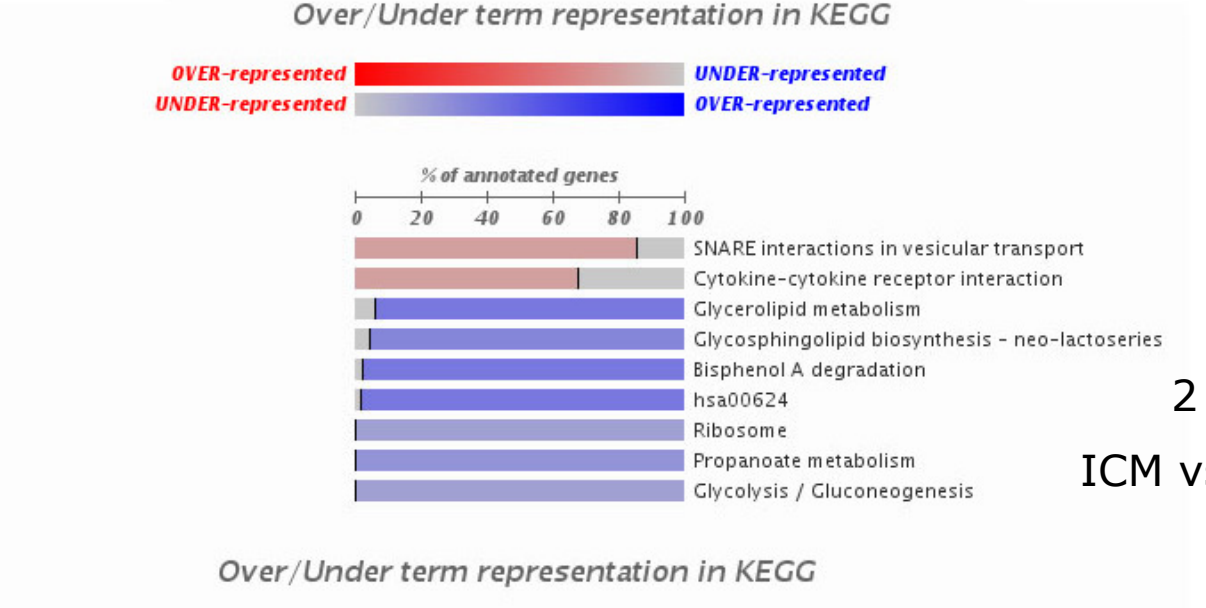
Functional analysis

KEGG ANALYSIS



3 vs 2

Morula vs ICM origin



2 vs 1

ICM vs Blastomere origin

Differences between Stem Cell Types

	Blastomere-derived hESC	Whole embryo-derived hESC	Partenogenic hESC	iPSC	Adult SC	Cord blood SC
UNLIMITED PROLIFERATION	yes	yes	yes	yes	no	no
NATURAL GENE REGULATION (IMPRINTING)	yes	yes	no	n/d	yes	yes
PLURIPOTENCY	yes	yes	yes	yes	no	no
UNMODIFIED	yes	yes	yes	no	yes	yes
PRESERVE EMBRYO	yes	no	yes	yes	yes	yes
IMMUNOCOMPATIBLE	yes	sometimes	yes	yes	yes	yes

Ideal for use in:
- cell therapy
- embryo toxicity screening
- drug discovery

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Derivation of hESC lines mantaining embryo viability

CONCLUSIONS

•Derivation from single blastomere constitute an alternative method to overcome controversies that involves the original procedure concerning the destruction of human embryos.

•Currently this strategy has been prooven efficient in rodents and humans.

•We have derived two hESC from single biopsied blastomere maintaining embryo viability (N Engl J Med 2009).

•hESC derivated from single blastomere have similar phenotype and genotype as those derived from morula and blastocysts

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