

SUPPLEMENTARY MATERIAL

Table S1: Experimental conditions tested in this study. All the skin samples were generated from the abdomen (unexposed) of healthy Caucasian women.

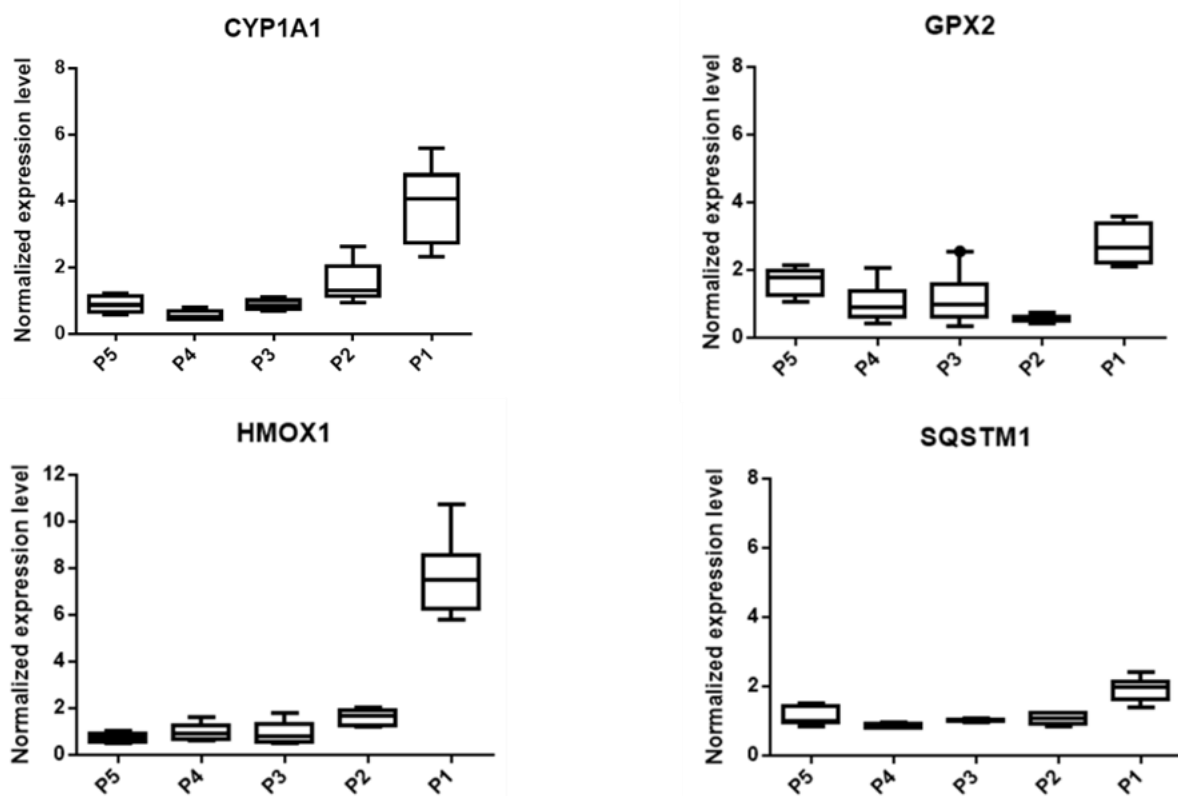
VOLUNTEER ANNOTATIONS	AGE (YEARS)	PHOTO-TYPES	GENERAL MORPHOLOGY EVALUATION	DNA MICRO ARRAYS	LMD & qPCR	IMMUNOHISTOCHEMISTRY	ULTRASTRUCTURE ANALYSIS
V1	47	II	X	X		X	
V2	19	III-IV	X	X	X	X	
V3	41	II	X	X	X	X	
V4	39	II	X	X	X	X	X (skin explants)
V5	50	II	X			X	
V6	31	III	X			X	
V7	50	II	X			X	
V8	35	III	X			X	
V9	65	II-III	X		X	X	
V10	44	II	X			-	X (stripping from skin explants)

Table S2. List of pollutants used in the present work.

POLLUTANT	PROVIDER	REFERENCE	ORGANIC SOLVENT
BENZENE	Fluka	12550	/
BENZO(A)PYRENE	Sigma	B1760	DMSO
ARSENIC	Sigma	202657	Nitric acid
NICKEL	Sigma	577995	Nitric acid
LEAD	Sigma	391352	Nitric acid
CADMIUM	Sigma	265365	Nitric acid
DIESEL PARTICLES	Nist	1650b	Ethanol

A

	P1	P2	P3	P4	P5
mg/m ³	100.000	1.000	200	100	50
µg/ml	100	1	0.2	0.1	0.05

B**Figure S1: Composition of the pollutant mix and determination of the optimal dose.**

Each compound of pollutant mix (P) was solubilized in appropriate organic solvent (OS) and mixed at the same concentration. The OS control corresponds to a mixture of solvents at the same concentration present in P. A: 5 doses (expressed in mg/m³ or µg/ml) were evaluated. B: Quantifications of gene expression by RT-qPCR. Values for each dose are represented in box-plots. They correspond to the threshold of the induction ratios calculated from the quantifications performed for each condition (3 explants/condition). The middle line corresponds to the median and the standard deviation bars observed above and below the box plots indicate the maximum and minimum values.

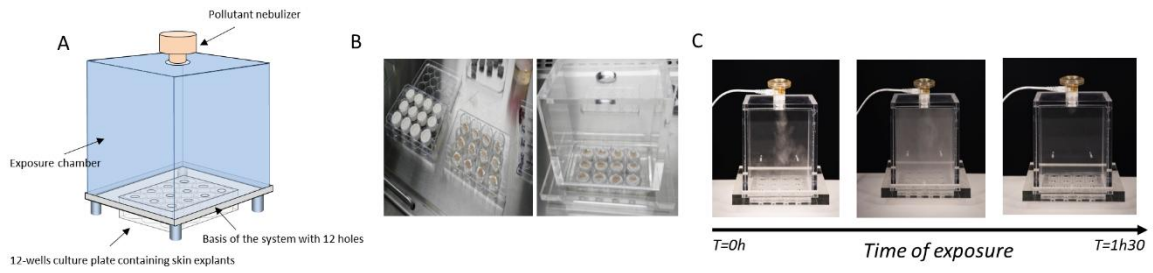
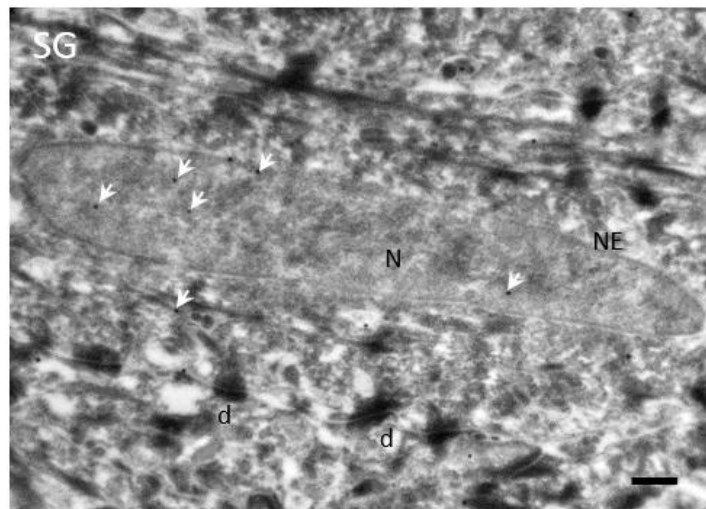


Figure S2: The Pollubox® device.

A: Schematic representation of the PolluBox® device; B: Explants were placed in a 12-wells culture plate which was inserted at the bottom of the box. C: Nebulization of the pollutant mix (3ml) was performed for 1h30.

A



B

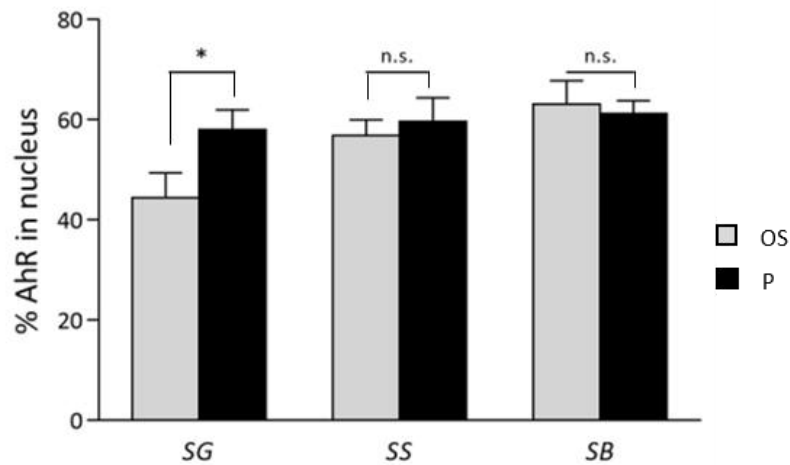


Figure S3: AhR localization in keratinocytes 48h after P exposure.

Ultrathin sections of skin explants were immunostained and observed by TEM. 10 cells of each epidermal layer were considered to study the nuclear localization of the receptor.

A, Electron micrograph of granular keratinocyte 48h after P exposure. Gold particles are marked by white arrows. **B**, % AhR in nucleus for 10 cells of *stratum granulosum* (SG), *stratum spinosum* (SP) and *stratum basale* (SB) after organic solvent (OS) or pollutant mix (P) exposure. Proportion of AhR in nucleus is calculated as follow: % AhR in nucleus = gold particle number in nucleus/total gold particle number per cell x 100. Data are expressed as the mean \pm SEM; * $P < 0.05$; n.s., not significant; d: desmosome; NE: nuclear envelope; N, nucleus; scale bar = 250nm.

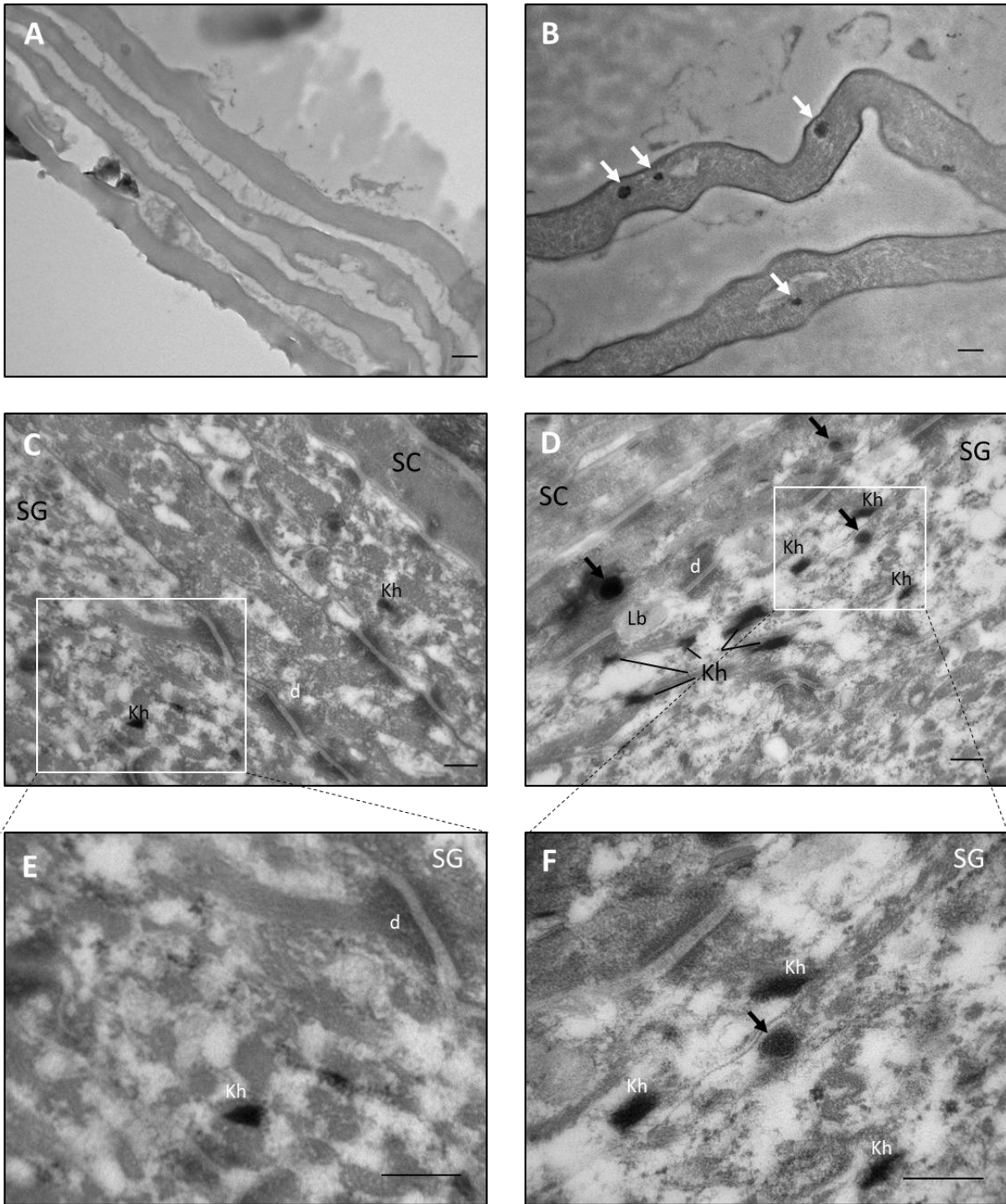


Figure S4: Observation of upper epidermal layers of skin explants by transmission electron microscopy

Micrograph of corneocytes collected by tape-stripping of skin explants un-exposed (A) or exposed to a diesel particle-enriched solution during 24h (B).

(C-F), Micrographs of ultrathin sections of human explants 48h after OS (C, E), or P (D, F) exposure. **Electron dense elements** smaller than 300 nm are indicated by white (B) and black (D, F) arrows.

SC: stratum corneum; SG: stratum granulosum; d: desmosome; Kh, keratohyalin granule; Lb: lamellar body. Scale bars: 250 nm

Table S3. List of the primary antibodies used in the present work.
Abbreviations: HIER (Heat Induced Epitope Retrieval).

ANTIBODY	Nature	SUPPLIER	Cat#	ANTIGEN RETRIEVAL	SECONDARY ANTIBODY LABEL
AhR	Mouse monoclonal	Thermo Fisher Scientific	MA1-514	-	GOLD PARTICLE
CYP1B1	Rabbit polyclonal	Abcam	ab326459	HIER	PEROXYDASE
UGT1A	Mouse monoclonal	Santa Cruz	sc-271268	-	PEROXYDASE
MafG	Rabbit polyclonal	Novus Biologicals	NBP2-15019	-	PEROXYDASE
MT-1H	Mouse monoclonal	Dako	M0639	HIER	PEROXYDASE
HO-1	Mouse monoclonal	Novus Biologicals	NBP1-97507	-	PEROXYDASE
AKR1C3	Rabbit polyclonal	Novus Biologicals	NBP1-31378	HIER	PEROXYDASE

Table S4: List of modulated genes in response to P which correlated in functional networks. Fold changes (FC) were calculated from the mean values of 3 explants per condition (PvsOS) generated from each donor (V1, V2, V3 and V4) and |FC| were calculated after evaluation of a two way ANOVA using a design based on each probe including treatment factor (PvsOS) and volunteer factors in order to take into account the heterogeneity between volunteers.

	GeneName	V1	V2	V3	V4	Two -way ANOVA with removeBatchEffect of volunteers			
		Fold change(FC) (P versus OS)				FC_PvsOS	Pval_PvsOS	Pval_Treatment	Pval_Volunteers
AHR-TARGET GENES	CYP1A1	2,3	2,4	4,3	16,8	4,0	4,18E-06	4,45E-07	1
	CYP1B1	1,7	0,9	3,1	2,8	1,8	3,78E-04	8,75E-07	1
	CYP4F11	4,8	7,3	2,5	1,7	3,8	1,32E-07	6,85E-08	1
	EPHX1	1,9	1,9	1,2	1,9	1,7	8,07E-06	2,29E-05	1
	UGT1A6	1,9	1,3	1,8	1,8	1,9	6,37E-05	4,57E-05	1
	UGT1A8	2,1	1,4	1,9	1,9	1,7	4,68E-05	2,56E-05	1
	ABCC3	2,7	2,9	2	1,9	2,3	1,50E-07	1,43E-06	1
	NRF2-TARGET GENES	SQSTM1	1,6	3	1,3	2,1	2,0	2,47E-06	6,73E-06
MAFG		2	3,1	1,3	2,1	2,0	1,58E-08	9,57E-07	1
GPX2		3,8	4,3	2	1	2,3	1,47E-03	2,95E-03	1
GCLC		2,2	2,8	2,2	1,9	2,3	1,36E-09	7,21E-10	1
AKR1C1		9,4	18,7	4,3	5,5	8,9	1,80E-12	8,85E-12	1
AKR1C3		5,2	8,3	3,4	3	4,5	1,45E-12	1,53E-12	1
AKR1C8P		10,4	18,7	4,2	5,1	8,1	4,12E-12	9,36E-12	1
HMOX1		9,7	10,5	2,8	2,3	4,5	1,61E-06	1,23E-05	1
PIR		1,7	1,5	1,7	1,1	1,4	1,29E-03	2,48E-04	1
OSGIN1		2,3	3,8	1,5	3	2,5	1,96E-07	4,63E-07	1
GLRX		2,7	3,8	2,1	0,8	2,1	5,46E-04	1,67E-03	1
SRXN1		4,6	5,1	2,4	2,5	3,5	1,44E-11	4,49E-11	1
PRDX1		1,6	1,6	1,4	1,8	1,7	1,72E-09	6,27E-09	1
TXNRD1		7,9	18,1	2,6	3,8	6,0	5,44E-09	2,85E-08	1
MT1E		7,1	8,1	3,4	3,9	4,8	2,08E-08	5,50E-08	1
MT1H		81,1	109,1	3,6	11,5	18,3	1,14E-06	2,97E-07	1
MT1G		31	86,4	12,8	31,2	22,1	2,30E-09	1,41E-09	1
MT1M	6,3	8,8	1,5	2,6	3,6	4,60E-06	1,01E-05	1	
SKIN BARRIER FUNCTION ASSOCIATED GENES	FLG	3,5	1,8	1,4	0,4	1,4	1,16E-01	5,71E-02	1
	FLG2	1,6	0,8	1,5	0,3	-1,1	6,05E-01	3,20E-01	1
	IVL	1,4	1,9	1,2	0,8	1,3	1,08E-02	2,72E-02	1
	LOR	3,1	1,2	1,6	0,3	1,2	4,26E-01	6,12E-01	1
	CRNN	1,6	0,4	1,2	0,3	-1,4	1,46E-01	6,19E-02	1
	LCE1A	3,1	2,4	1,5	0,6	1,6	1,41E-01	3,68E-01	1
	LCE1B	4,6	2,5	1,4	0,6	1,6	3,59E-01	6,79E-01	1
	LCE1C	4,3	1,8	1,6	0,5	1,7	1,25E-01	3,39E-01	1
	LCE1E	5,3	2,5	1,7	0,6	1,7	2,21E-01	4,57E-01	1
	LCE2A	2,1	2	1	0,5	-1,0	9,63E-01	9,76E-01	1
	LCE2C	2,5	2,2	1,3	0,4	1,1	8,37E-01	9,28E-01	1
	LCE2D	2,8	2,6	1,3	0,5	1,2	6,70E-01	8,80E-01	1
	LCE3B	3,9	3,6	1,5	0,6	1,7	1,49E-01	3,81E-01	1
	LCE3C	7,9	8	1,1	1	2,6	6,12E-03	2,15E-02	1
	LCE3D	3,6	3,6	1,1	0,9	1,8	3,11E-02	8,10E-02	1
	LCE3E	7,1	6,8	0,9	0,9	2,1	3,14E-02	8,76E-02	1
	LCE4A	4,6	2,1	0,9	0,7	1,7	1,60E-01	3,33E-01	1
	LCE5A	3,5	1,5	0,9	0,9	1,2	7,40E-01	7,13E-01	1
	LCE6A	3,8	2,6	1,9	0,5	1,6	1,94E-01	4,63E-01	1
	SPRR2A	1,8	2,1	0,7	0,6	1,0	9,41E-01	9,22E-01	1
	SPRR2B	3,5	2,5	0,7	0,6	1,3	4,86E-01	8,23E-01	1
	SPRR2D	1,9	1,9	0,7	0,6	-1,1	5,18E-01	7,24E-01	1
	SPRR2G	1,8	1,7	0,4	0,5	-1,3	6,98E-01	8,08E-01	1
	SPRR3	3,8	3,1	0,5	1,4	1,6	8,31E-01	1,22E-01	1
	S100A7	1,6	1	0,4	0,2	-1,7	6,01E-01	1,84E-01	1
	S100A7A	5,4	1,3	0,9	0,4	-1,0	8,92E-02	9,41E-01	1
	AQP1	0,3	0,3	0,5	0,4	-2,68	7,06E-09	7,74E-09	1
CD44	0,5	0,3	0,6	0,5	-2,17	6,09E-09	4,38E-08	1	
KRT31	0,5	0,4	0,4	0,6	-1,99	2,76E-08	2,84E-08	1	
METHYLATION	DNMT3B	3,1	5,6	1,5	0,9	2,22	6,11E-04	9,99E-05	1

Table S5: List of downregulated genes in response to P. Fold changes (FC) were calculated from the mean values of 3 explants per condition (PvsOS) generated from each donor (V1, V2, V3 and V4) and |FC| were calculated after evaluation of a two way ANOVA using a design based on each probe including treatment factor (PvsOS) and volunteer factors in order to take into account the heterogeneity between volunteers.

(cf. table S5.doc)

Table S6: List of primers designed for the study

GENE	SEQUENCE 5' → 3'		EFFICIENCY	GENE	SEQUENCE 5' → 3'		EFFICIENCY
AKR1C3	F	CTCACTGAAGAAAGCTCAATTGGA	102,30%	HMOX1	F	CTTCTCCGATGGGTCCTTACACT	90%
	R	AAAGTTCCTCACCTGGCTTAGAG			R	TCACATGGCATAAAGCCCTACA	
UGT1A6	F	CATCGAATCTTGCGAACAACA	94,34%	MAFG	F	GTTTTTCTGACACAGCGAATCG	100,50%
	R	GGGTGACCAAGCAGATCGTT			R	AAAGTTAAGGTGGAAGGGAAGACA	
UGT1A8	F	TTGGGCAAAATCCCTCAGA	97,08%	B2M	F	GGTCACCAGGGCTGCTTTT	93,60%
	R	GGGTGACCAAGCAGATCGTT			R	GTAACCATGTAGTTGAGGTAATGAA	
MT1E	F	CCTGATGTGGGAACAGCTCTTC	102,70%	GAPDH	F	AATTGCTATGTGTCTGGGTTTCATC	89%
	R	CAGCAAATGGCTCAGTGTGTATT			R	CCACTTTTTCAATTCTCTCCATTC	
CYP1B1	F	TCCAGCTTTGTGCCTGCTACTAT	87,70%	AHR	F	AACGGAGGCCAGGATAAAGTGTAGAG	84,80%
	R	GGGAATGTGGTAGCCAAGA			R	CATCAGACTGCTGAAACCCTAGATAAT	