Factorial™ Biosensors
for Drug Discovery and Toxicology

Attagene offers functional characterization of compounds according to their effect on regulatory and toxicity pathways of mammalian cells. Our services utilize patented multiplex profiling technology termed Factorial™.

Principle

Activity of transcription factors (TF) is regulated by multiple mechanisms such as phosphorylation, nuclear translocation, ligand binding or co-factor recruitment. To directly measure pathways regulating TF activity, Attagene has developed a novel pathway profiling technology, Factorial™ enabling multiplex assessment of hundreds of reporters in a single tissue-culture well. Factorial™ assay utilizes an unique library of TF-specific reporters. All reporters are analyzed in a single reaction well followed by quantitative analysis using capillary electrophoresis (1).

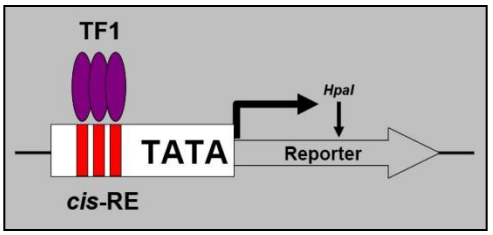
Major advantages of Factorial™ technology over conventional pathway profiling assays

- Multiplexing: Factorial™ analyses more than 40 pathways in a single assay well. The endpoint set is easily expandable and customizable. Relatively small quantities of compound are needed for the assay.
- High uniformity. All Factorial™ reporters produce virtually identical RNA messages with no detectable variability in stability of individual reporter RNA enabling precise comparison of the activities of multiple TFs.
- Fast detection of TF activity changes. Factorial™ detection is performed on the mRNA level, therefore Factorial™ reporters react faster than conventional protein-based reporters to both stimulatory and inhibitory effects. Also, Factorial™ can be used to analyze protein synthesis inhibitors, eliminating reporter protein translation artifacts.
- Superior repeatability and reproducibility of the data. All reporters are detected simultaneously in the same assay well and by single reaction creating highly homogeneous detection conditions. Multiple control endpoints, assaying all reporters in the in the same well and in the same reaction makes Factorial™ assay extremely tolerant to poor sample quality such as RNA degradation, variations in transfection efficiency and sample processing.

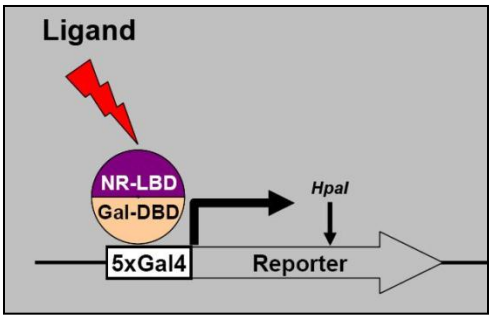
[1]Romanov et al., *Nature Methods* **5**(3):253-60 (2008).

Content-rich Factorial Platforms

Based on the Factorial™ technology, Attagene offers in-house services based on two distinct cell-based bioassays useful for determining the mode of action and evaluation of toxicity of drug candidates:



Factorial-TF™ (cis) platform reports the effects of compounds on the activity of more than *40 transcriptional pathways* in a set of toxicologically relevant human cell lines. For complete list of Factorial-TF™ assay endpoints please see appended *Table 1 and 2*. The main advantage of Factorial-TF™ system is that it analyzes a broad spectrum of endogenous transcriptional pathways.



Factorial-NR™ (trans) platform evaluates the agonistic and antagonistic properties of compounds across *48 human hormone nuclear receptors*. For complete list of Factorial-NR™ assay endpoints please see *Table 3 and 4*. The main advantage of Factorial-NR™ system is that all nuclear receptors are over-expressed in tested cells guaranteeing the response to potential ligands.

Factorial™ Facilities

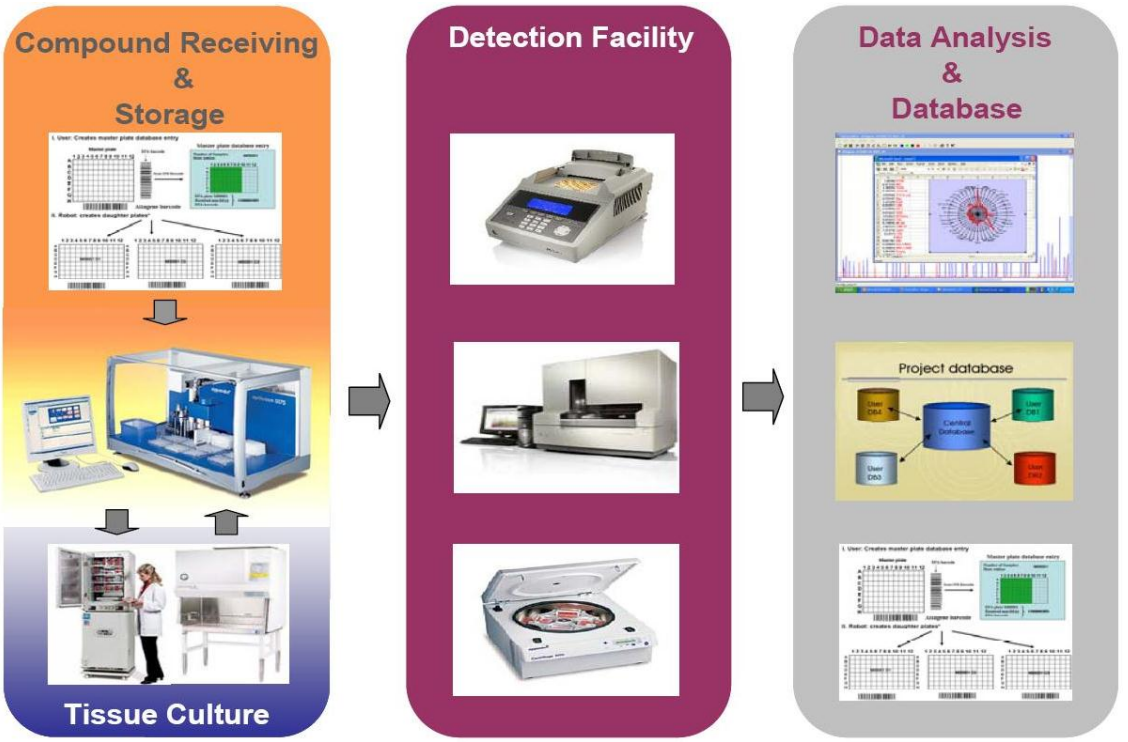


Table 1. List of Factorial-TF1 Endpoints

#	Endpoint Name	Transcription Factor / Pathway
1	TGFb	SMAD Family / TGFb Pathway
2	HNF6	The transcription factor hepatocyte nuclear factor 6
3	TCF/b-cat	TCF-1 Family/ Wnt Pathway
4	E-box	c-Myc, Upstream stimulatory factor 1 (USF-1)
5	PPRE	Peroxisome proliferator activating receptor (PPAR)a, d, g
6	NFI	The nuclear factor 1 (NF1) family proteins
7	PXR	The pregnane X receptor (PXR), Xenobiotic Pathway
8	GRE	The glucocorticoid receptor (GR)
9	AP-1	The activator protein 1 (AP-1: c-fos/c-jun)
10	ISRE	Interferone regulatory factors IRF1, IFR3 / Interferone Pathway
11	MRE	The metal regulatory transcription factor 1 (MTF-1)
12	STAT3	Signal transducer and activator of transcription 3(STAT3) / IL-6 Pathway
13	NF-kB	The nuclear factor kappa B family / TNFa, IL-1b Pathways
14	FoxA	The forkhead box protein A
15	CMV	Cytomegalovirus promoter-enhancer
16	Xbp1	X-Box protein 1, Protein misfolding, EPR stress Pathway
17	CRE	cAMP-responsive DNA-binding protein (CREB) Family / cAMP Pathway
18	AhR	The Aryl hydrocarbon receptor (AhR) / Xenobiotic Response
19	EGR	Early growth response protein 1 (EGR1)
20	ARE	Antioxidant Response Element (ARE)-binding Nuclear factor (erythroid-derived 2)-like 2 (NRF2)
21	ERE	Estrogen Receptor (ER) pathway
22	Oct	The POU domain Family, octamer transcription factor family
23	LXR (DR4)	The liver X receptor family (Direct repeat 4-binding proteins)
24	HSE	The heat shock factor -1 (HSF-1) / heat shock pathway
25	SREBP	Sterol Regulatory Element-Binding Proteins (SREBPs)
26	p53	The p53 transcription factor / DNA damage Response
27	BRE	SMAD Family / Bone morphogenetic protein pathway
28	Pax	The transcription factor paired box (PAX)
29	HIF1a	The hypoxia-inducible factor-1a (HIF1a) / hypoxia pathway
30	VDRE	The vitamin D receptor (VDR) / vitamin D pathway
31	RORE	Retinoic acid receptor -related orphan receptor proteins (ROR) a,b,g
32	Ets	The ETS (E-twenty six) transcription factor family
33	GLI-1	The Gli-1 transcription factor/ Hedgehog (Hh) signaling pathway
34	NRF1	The nuclear respiratory factor 1
35	GATA	The GATA-binding factor family
36	E2F	The E2F transcription factor family
37	C/EBP	The CCAAT-enhancer-binding proteins transcription factor family
38	Myb	The MYB (myeloblastosis) family of transcription factors
39	PBREM	The phenobarbital responsive enhancer module /constitutive androstane receptor (CAR) pathway
40	FXRE (IR1)	The farnesoid X receptor (FXR), an inverted repeat-1 (IR1) binding protein
41	AP-2	The activating protein 2 (AP-2) family of transcription factors
42	RARE(DR5)	The retinoic acid receptors (RARa),b,g
43	FoxO	Forkhead box proteins FOXO1 and FOXO3
44	SOX	The SOX transcription factor family
45	Sp1	Ubiquitous Sp1 family of transcription factors
46	Myc	The c-Myc transcription factor

Table 2. List of Factorial-TF2 Endpoints

#	Name:	Description:
1	**AhRE**	AhR pathway (same as in Factorial-1)
2	**BAX**	Promoter region (genotoxicity related)
3	**CD95**	Promoter region (genotoxicity related)
4	**cFos**	Promoter region (MAP pathway activated)
5	**cJun**	Promoter region (MAP pathway activated)
6	**DR0**	Nuclear receptor response element (direct repeat 0)
7	**DR1**	Nuclear receptor response element (direct repeat 1)
8	**DR2**	Nuclear receptor response element (direct repeat 2)
9	**GADD34**	Promoter region (genotoxicity related)
10	**GADD-153**	Promoter region (genotoxicity related)
11	**HLTR**	LTR of human HIV virus
12	**Hox1**	Hox1 response element
13	**HSE**	Heatshock element (same as in Factorial-1)
14	**INS-326**	Human insulin promoter (-326 fragment)
15	**INS-A1**	Human insulin promoter (A1 element)
16	**INS-A3**	Human insulin promoter (A3 element)
17	**INS-GG2**	Human insulin promoter (GG2 element)
18	**IR0**	Nuclear receptor response element (inverted repeat 0)
19	**IR1**	Nuclear receptor response element (inverted repeat 1)
20	**IR2**	Nuclear receptor response element (inverted repeat 2)
21	**IR4**	Nuclear receptor response element (inverted repeat 3)
22	**IR5**	Nuclear receptor response element (inverted repeat 4)
23	**IR6**	Nuclear receptor response element (inverted repeat 4)
24	**ISRE-2**	IFN Pathway, IRF2 transcription factor
25	**KLF1**	KLF response element (stem cells)
26	**KLF3**	KLF response element (stem cells)
27	**MEF2**	MEF2A response element
28	**MMP1**	Promoter region (genotoxicity related)
29	**Nanog**	Nanog Promoter region (stem cells)
30	**NFAT**	NFAT response element
31	**NKX22**	NKX2-2 response element
32	**Oct/Sox**	composite element, (stem cells)
33	**p21**	Promoter region (genotoxicity related)
34	**PDX**	PDX1 response element
35	**PEA3**	PEA3 response element (Ets family)
36	**RIP A34**	Rat insulin promoter (A34 element, PDX1 responsive)
37	**Sestrin-1**	Promoter region (genotoxicity related)
38	**Sestrin-2**	Promoter region (genotoxicity related)
39	**SRE**	Serum response element
40	**STAT1**	Stat1 response element
41	**TRE**	AP1/Ets composite element, PMA responsive

Table 3. List of Factorial-NR1 Endpoints

(Nuclear Receptors)

#	Name:	Nomenclature:	Ligands:
1	AR	NR3C4	Testosterone, 6-Fluorotestosterone
2	CAR	NR1I3	Xenobiotics, CITCO
3	ERα	NR3A1	Estradiol-17, 4-OH tamoxifen
4	ERRα	NR3B1	Orphan
5	ERRγ	NR3B3	DES, 4-OH tamoxifen
6	FXR	NR1H4	Bile acids, CDCA
7	GR	NR3C1	Cortisol, dexamethasone
8	HNF4α	NR2A1	Orphan
9	LXRα	NR1H3	Oxysterols, T0901317
10	LXRβ	NR1H2	Oxysterols, T0901317
11	NURR1	NR4A2	Orphan
12	PPARα	NR1C1	Fatty acids, leukotriene B_4, fibrates
13	PPARδ	NR1C2	Fatty acids
14	PPARγ	NR1C3	Fatty acids, thiazolidinediones
15	PXR	NR1I2	Xenobiotics, Rifampicin
16	RARα	NR1B1	Retinoic acid
17	RARβ	NR1B2	Retinoic acid
18	RARγ	NR1B3	Retinoic acid
19	RORβ	NR1F2	Orphan
20	RORγ	NR1F3	Orphan
21	RXRα	NR2B1	9-cis-Retinoic acid
22	RXRβ	NR2B2	9-cis-Retinoic acid
23	TRα	NR1A1	Thyroid hormones
24	VDR	NR1I1	Vitamin D, 1,25-dihydroxyvitamin D_3

Table 4. List of Factorial-NR2 Endpoints

(Nuclear Receptors)

#	Name:	Nomenclature:	Ligands:
1	COUP-TFI	NR2F1	Orphan
2	COUP-TFII	NR2F2	Orphan
3	DAX-1	NR0B1	Orphan
4	EAR2	NR2F6	Orphan
5	ERβ	NR3A2	Estradiol
6	GCNF	NR6A1	Orphan
7	HNF4g	NR2A2	Orphan
8	LRH-1	NR5A2	Orphan
9	MR	NR3C2	Aldosterone, spirolactone
10	NGFI-B	NR4A1	Orphan
11	PNR	NR2E3	Orphan
12	PR	NR3C3	Progesterone
13	Rev-erbα	NR1D1	Orphan
14	Rev-erbβ	NR1D2	Orphan
15	RORα	NR1F1	Orphan
16	RXRγ	NR2B3	9-cis-Retinoic acid
17	SF1	NR5A1	Orphan
18	SHP	NR0B2	Orphan
19	TLX	NR2E2	Orphan
20	TRβ	NR1A2	Thyroid hormones
21	TR2	NR2C1	Orphan
22	TR4	NR2C2	Orphan
23	AR (neg)	NR3C4	antagonist detection mode
24	ERa (neg)	NR3A1	antagonist detection mode
25	GAL4	yeast	negative control

Table 5. List of cell lines verified to be compatible with Factorial assay.

#:	Name	ATCC#	Species:	Tissue / Morphology:
	Cell lines			
1	A549	CCL-185	human	lung, epithelial carcinoma
2	3T3-L1	CL-173	mouse	fibroblast, pre-adipocytes
3	BE(2)-C	CRL-2268	human	epithelial, neuroblastoma
4	C3H/10T1/2	CCL-226	mouse	fibroblast, pluripotent, pre-adipocyte
5	CWR-22R	CRL-2505	human	prostate, androgen receptor positive
6	F-9	CRL-1720	mouse	mouse teratocarcinoma, stem cells
7	HaCat	n/a	human	Human keratinocyte cell line
8	HCT116	CCL-247	human	colorectal, epithelial
9	HEK293	CRL-1573	human	kidney, epithelial
10	HeLa	CCL-2	human	epithelial, adenocarcinoma
11	**HepG2 (HG19, clone)***	HB-8065	**human**	**liver, hepatocarcinoma, in-house clone**
12	HT1080	CCL-121	human	fibroblast, connective tissue
13	INS-1	n/a	rat	insulinoma
14	Jurkat, E6-1	TIB-152	human	lymphoblast
15	MCF-7	HTB-22	human	mammary gland; breast
16	MDA-MB-231	HTB-26	human	mammary gland; breast
17	MIA PaCa-2	CRL-1420	human	pancreatic carcinoma
18	NIH/3T3	CRL-1658	mouse	fibroblast
19	PA-1	CRL-1572	human	ovary, teratocarcinoma
20	RAW264.7	TIB-71	mouse	monocyte/macrophage
21	RIN-5F	CRL-2058	rat	rat insulinoma
22	SH-SY5Y	CRL-2266	human	neuronal, neuroblastoma
23	SW480	CCL-228	human	epithelial, colorectal
24	U-87 MG	HTB-14	human	glia, epithelial glioblastoma
25	VERO	CRL-1586	monkey	kidney epithelial
26	ZR-75-1	CRL-1500	human	mammary gland; breast
	Primary cells			
1	NHDF	n/a	human	human dermal fibroblasts, primary cells
2	Primary rat hepatocytes	n/a	rat	Primary rat hepatocytes

***** Compatible with trans-Factorial assay

Please contact Attagene for custom cell lines

Recommended Cell System (**HG19/HepG2**):

Factorial assay is cell-based analysis and can be performed in any transfectable cells. We recommended to use our in-house developed HepG2-derived cell line HG19 that retains many features of primary human hepatocytes. These cells express human PXR, ERα and exhibit increased metabolic capacity, useful for analysis of compound metabolites.

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