# Factorial™30: Evaluate 30 Transcription Factors in single experiment

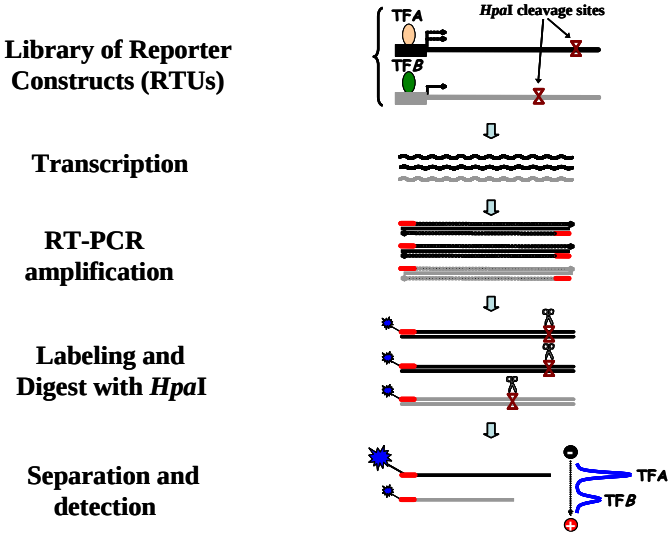
Attagene Inc. proudly introduces FACTORIAL™30, the founding product line that is based on our proprietary technology[1] and enables profiling activities of thirty most researched TF families within cell, all simultaneously and quantitatively.

Signals that control genome expression are executed through coordinated changes in the activities of transcription factors (TFs) - proteins that bind gene regulatory elements and modulate transcription. Mammalian genomes encode about 2000 TFs that, according to DNA binding similarities, can be classified into few hundred families. By analyzing activities of multiple TFs one should be able to obtain an informative snapshot of gene regulatory network. However, high-content analysis of TFs requires adequate tools.

Of the few tools currently available to researchers who seek to study cell regulation at the level of transcription factor activities, none is geared towards quantitative high-content assessments. Assays that make use of reporter gene constructs (e.g., luciferase reporters) can be used to analyze only one or two transcription factors at a time. In contrast, DNA-binding assays can potentially be used to assess multiple transcription factors, but provide limited biological information because DNA binding is only one of many factors in the regulation of transcription factor activity.

Attagene has conceptualized and developed a simple homogeneous assay, termed Factorial™, enabling assessment of multiple transcription factors' activities in a single experiment.

**❑ A key aspect of the FACTORIAL™ technology is that it employs highly homogeneous detection protocol providing highly uniform conditions for detecting multiple transcription factors.**




**Principle of the FACTORIAL™ technology**

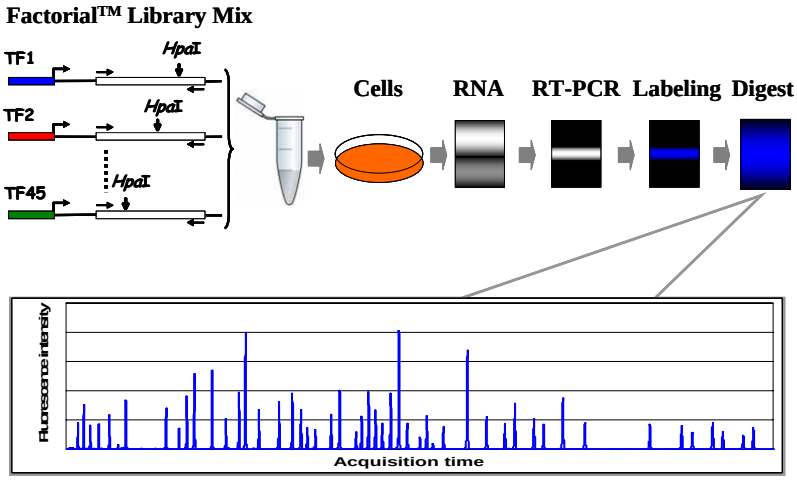
The crux of the FACTORIAL™ technology is a library of uniformly constructed Reporter Transcription Units (RTUs). Each FACTORIAL™ RTU is made in a common plasmid backbone and contains a unique TF-inducible promoter. When co-introduced into cell of interest, the RTUs produce reporter RNAs in amounts commensurable with the activities of the corresponding TFs present in a cell. To provide equal detection opportunities for different transcription factors, all FACTORIAL™ RTUs are supplied with essentially identical reporter sequences. To distinguish reporter sequences produced by different RTUs, each sequence is tagged with *Hpa*I restriction cleavage site, position of which varies among the RTUs. Thus, reporter sequences can be discriminated upon cleavage with the corresponding processing enzyme. The cleaved reporter species are separated by high resolution capillary electrophoresis (sequencing) and quantified.

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

**With our FACTORIAL™ TF profiling system, you can…**

- ❑ **analyze basal and induced transcription factor activity profiles in a variety of commonly used cell lines;**
- ❑ **assess the function of a gene of interest in the context of its effects on activities of multiple transcription factors;**
- ❑ **investigate mechanisms of action of biologically active agents, including chemical compounds, peptides, siRNAs, and dominant-negative variants of proteins;**
- ❑ **explore signal transduction pathways underlying alterations in gene expression profiles generated by microarray hybridization.**

## Workflow of FACTORIAL™ assay



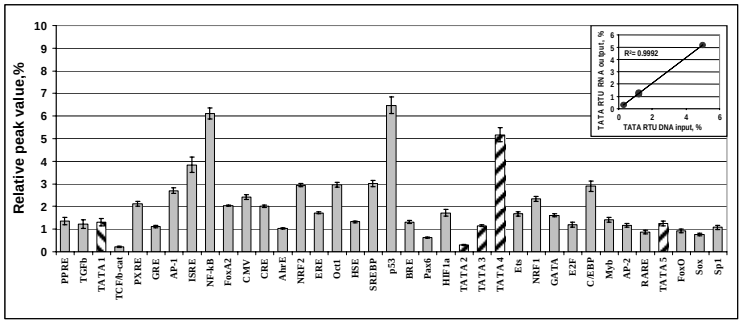

**Capillary Electrophoregram**

For multiplexed detection of transcription factors the Factorial™ TF reporter library is introduced into cells of interest by using standard transfection procedure. Some time after transfection, total cellular RNA is isolated and reversely transcribed. The reporter cDNAs are amplified by PCR using a pair of primers that are common for all reporters. The PCR products are labeled with a fluorescent dye and digested by the *Hpa*I. The digest produces a spectrum of fluorescently labeled DNA fragments of different lengths that are resolved by capillary electrophoresis (CE) and detected as separate fluorescent peaks.

To perform the Factorial™ detection protocol you will need standard molecular biology equipment, including PCR thermocycler and capillary electrophoresis instrument (available from your nearest sequencing facility). Alternatively, you may submit your RNA samples to a designated Attagene's Factorial™ Detection facility. More information on Factorialtm related services is forthcoming and will be available on Attagene's website.

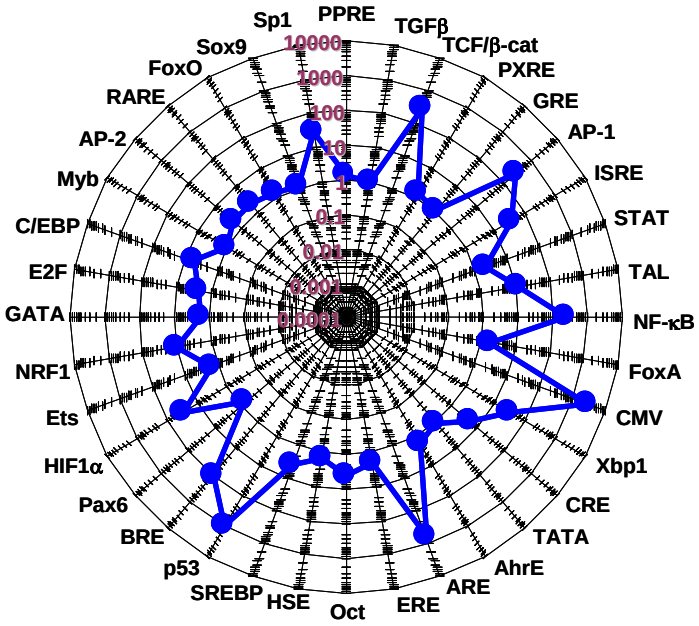
## FACTORIAL™ assay data analysis

The capillary electrophoresis data are processed by using ATTAGRAPH™ software. The software algorithms allow for subtraction of background fluorescence, precise sizing of the reporter peaks, and noise/reporter peaks discrimination. The relative fluorescent values of the individual reporter peaks as well as specific RTU activities values can be exported into Microsoft Office Excel for further analysis.




## Factorial30™ product selection guide

All our current products feature Factorial30™ TF profiling library. We offer several Factorial30™ products to choose from. Since activities of individual TFs may vary substantially in different cell types, we provide six different configurations of the Factorial30™ library, each assigned a set of particular cell types. The table below will help you to determine which one of Factorial30™ configurations will suit the best cell line of your choice. If you do not see the name of your cell line in the table, we suggest that you buy Factorial30™ Explorer Kit which contains trial samples of all six Factorial30™ configurations. To accommodate researchers with specific needs we may create a unique, custom made Factorial30™ configuration which will be specific for the cells of your interest. Please contact us for more information and pricing on the custom made Factorial™ libraries.




Basal profile of TF activities generated by Factorial™ system in human hepatocellular carcinoma cells, HepG2. Data represent mean values +/- SD of three independent experiments.

| Factorial30™ configuration | Cell types tested |
|---|---|
| Factorial30™ A | NIH 3T3, CHO-K1, U87MG, HCT116, COS-7, ZR-75-1, SH-Sy5y |
| Factorial30™ B | 3T3 L1, C3H/10T1/2, MDCK, MCF-7, C2C12, K 562, RAW264.7, INS-1 |
| Factorial30™ C | HeLa |
| Factorial30™ D | Caco-2, HepG2, SW480, VERO |
| Factorial30™ E | NHDF |
| Factorial30™ F | HEK 293 |

## Factorial30™ product line

The following reagent packages are currently available:

**Factorial30™ TF profiling systems.**
Each Factorial30™ TF profiling system is supplied with one particular configuration of the Factorial30™ Reporter Library. The package also includes set of essential Factorial™-specific reagents. Each system is sufficient to perform 20 experiments.

**Factorial30XL™ TF profiling systems.**
These packages contain all the components of Factorial30™ system but also include additional reagents that are required for performing detection steps of the assay, including DNase I enzyme, RT and PCR enzymes with corresponding reaction buffers, and dNTPs. Alternatively, these additional reagents can be obtained from other suppliers.

Both Factorial30™ and Factorial30XL™ TF profiling systems also available in **Explorer format** that includes aliquots of all six configurations of the Factorial30™ reporter library.

**ATTAGRAPH Reader™ Software** is required for analysis of data generated by the Factorial30™ system and is made available to our customers thru Attagene's website.

## Factorial30™ Reporter library

| | | |
|---|---|---|
| AhrE | FOXO | PAX6 |
| AP-1 | GATA | p53 |
| AP-2 | GRE | PPRE |
| ARE | HIF1α | RARE |
| BRE | HSE | SOX9 |
| CRE | ISRE | SP1 |
| C/EBP | Myb | SREBP |
| ERE | NF-κB | TCF/β-cat |
| ETS | NRF1 | TGFβRE |
| FOXA | OCT | XBP1 |

The table above lists transcription factors and cis-regulatory elements that can be evaluated by the Factorial30™ TF profiling systems

## How to Order

Orders can be placed 24 hours a day by fax or e-mail, Customer Service and Sales Representatives are available via telephone between 9:00 am and 5:00 pm Eastern Time, Monday through Friday, to take your order.


**Telephone:** **Toll-free 1-888-721-2121**
**919-313-0473**
**Fax:** **919-313-0172**
**E-mail:** **info@attagene.com**
**Mail: Attagene Inc., 7030 Kit Creek Rd., Morrisville, NC, 27560**


• **Pricing : Please inquire about our introductory prices**

| Available kits | Cat. Number |
|---|---|
| **Factorial™ 30.A** | FPS 03020A |
| **Factorial™ 30.B** | FPS 03020B |
| **Factorial™ 30.C** | FPS 03020C |
| **Factorial™ 30.D** | FPS 03020D |
| **Factorial™ 30.E** | FPS 03020E |
| **Factorial™ 30.F** | FPS 03020F |
| **Factorial™ 30.Explorer** | FPS 03020Ex |
| **Factorial™ 30.A-XL** | FPS 03020A-XL |
| **Factorial™ 30.B-XL** | FPS 03020B-XL |
| **Factorial™ 30.C-XL** | FPS 03020C-XL |
| **Factorial™ 30.D-XL** | FPS 03020D-XL |
| **Factorial™ 30.E-XL** | FPS 03020E-XL |
| **Factorial™ 30.F-XL** | FPS 03020F-XL |
| **Factorial™ 30-XL Explorer** | FPS 03020-XLEx |