

# GENE REGULATION

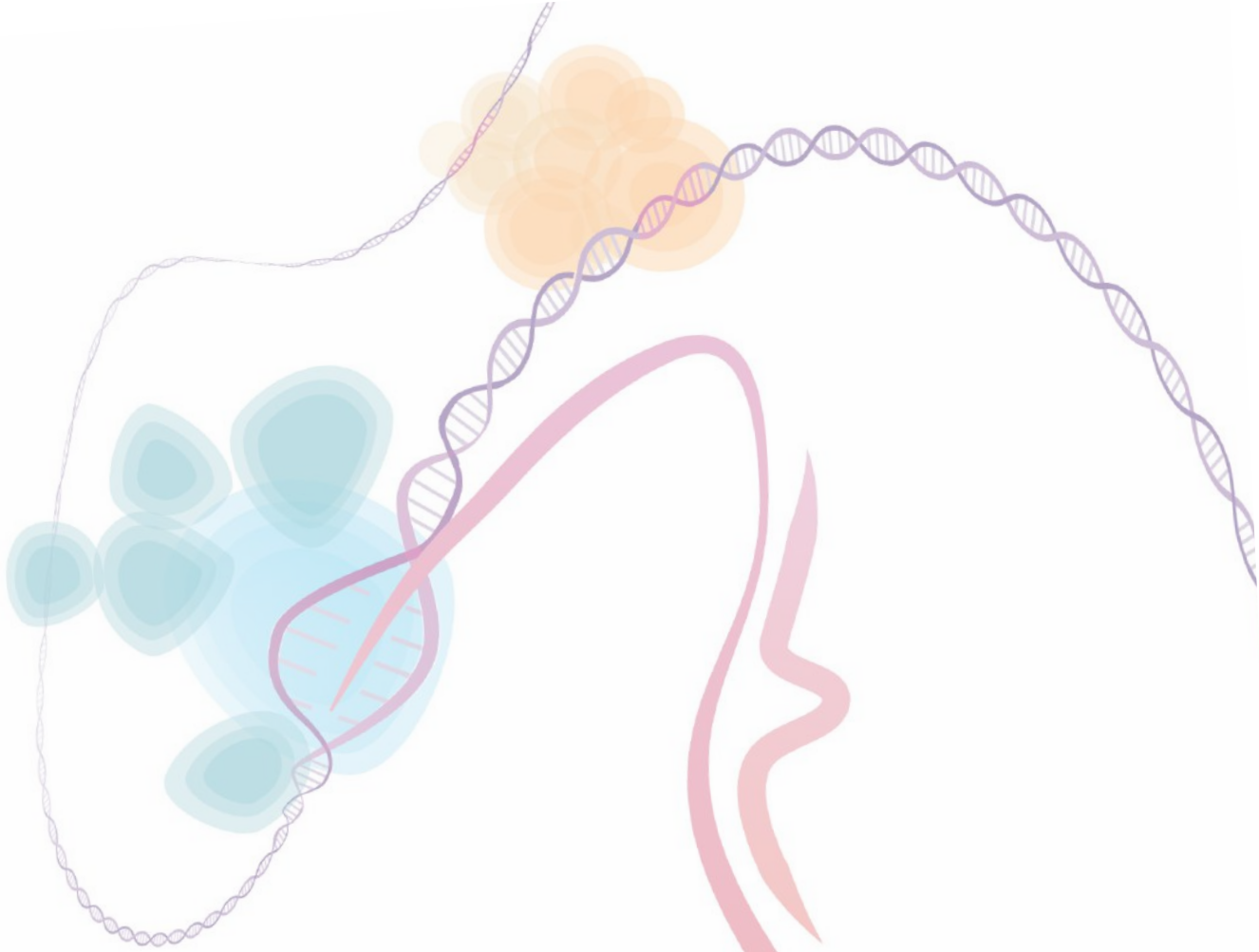
products & services

- functional genomics
- transcription factor analysis
- RNA analysis



ACTIVE  MOTIF®

Enabling Epigenetics Research



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# GENE REGULATION OVERVIEW

## The Dynamic Process of Gene Regulation

Gene regulation is a highly dynamic process that involves not only transcription factor (TF) and effector protein interactions with DNA at promoters and enhancer regulatory regions, but also chromatin remodeling events driven by epigenetic mechanisms. These mechanisms include histone modifications, DNA methylation, and non-coding RNAs that influence accessibility of transcriptional machinery to underlying DNA.

All of these mechanisms work in concert to regulate gene expression. Furthermore, once genes are transcribed, microRNAs and other mechanisms play critical roles in modulating gene expression at the post-transcriptional level, adding yet another layer of complexity to the process.

## Tools to Study Gene Regulation

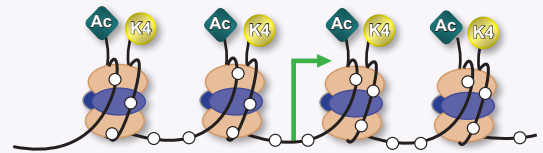
Active Motif is the industry leader in providing innovative tools to enable epigenetics and gene regulation research. Our superior products, services, and support serve our life science, clinical, pharmaceutical, and drug discovery partners. Our selection of products to study gene regulation includes the LightSwitch™ luciferase reporter assay system, TransAM® transcription factor binding assays, and RNA analysis kits and services.

Whether you are already an expert in gene regulation or are just looking to get started, our comprehensive portfolio of products offers end-to-end solutions to meet the specific needs of your research.

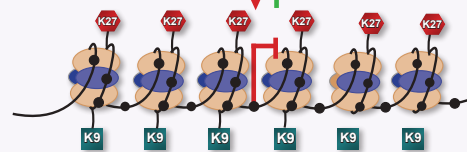
To learn more, visit us at [www.activemotif.com](http://www.activemotif.com).

## Epigenetic Modifications

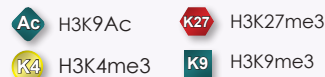
### Active Chromatin



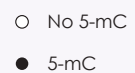
### Silent Chromatin



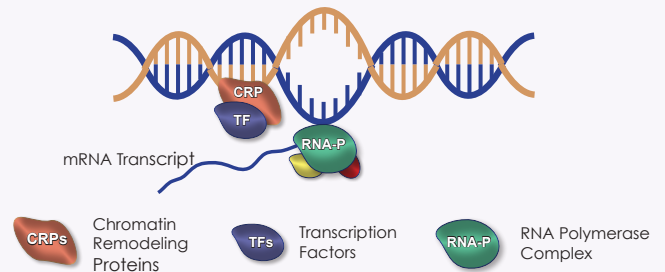
#### Histone Modifications



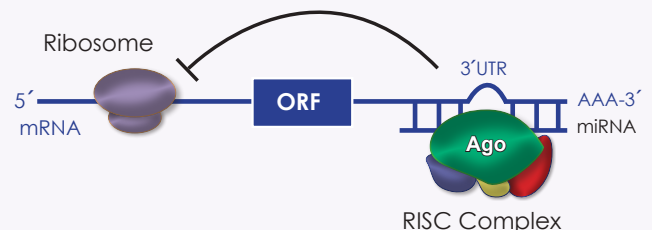
#### DNA Methylation



## Transcriptional Regulation



## Post-transcriptional Regulation

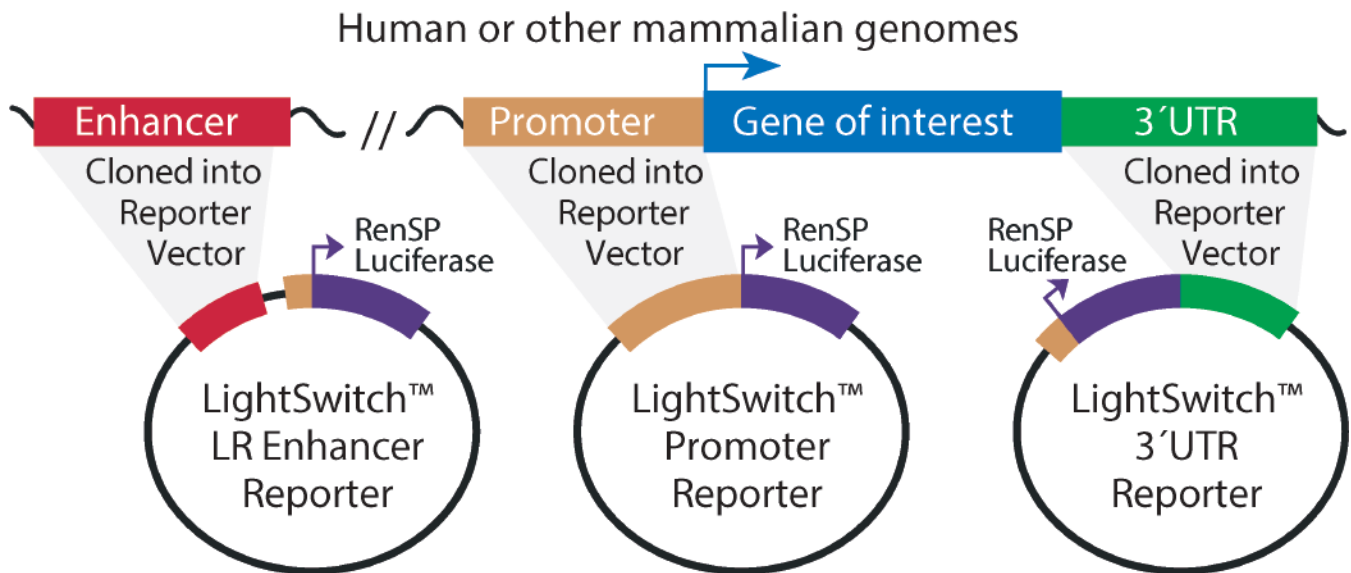


## LIGHTSWITCH™ LUCIFERASE REPORTER ASSAY SYSTEM

Functional genomics is a research area that uses genomic data to study gene expression and function. Cell-based screening using luciferase reporter assays is a powerful approach for high-throughput gene function discovery as well as for drug discovery. Active Motif has taken advantage of the power of reporter assays to develop the LightSwitch luciferase reporter assay system specifically for gene regulation experiments and functional genomics screens.

### Pre-cloned Library of Human Promoters, 3'UTRs, and Other Regulatory Elements

With a library of over 30,000 cloned regulatory elements, custom cloning and mutagenesis services, and a highly optimized Renilla luciferase (RenSP) vector system and assay reagents, the LightSwitch Luciferase Assay System is the complete solution for your gene regulation studies.



Choose a genomic target and vector of interest from our collection of over 30,000 ready-to-transfect constructs.



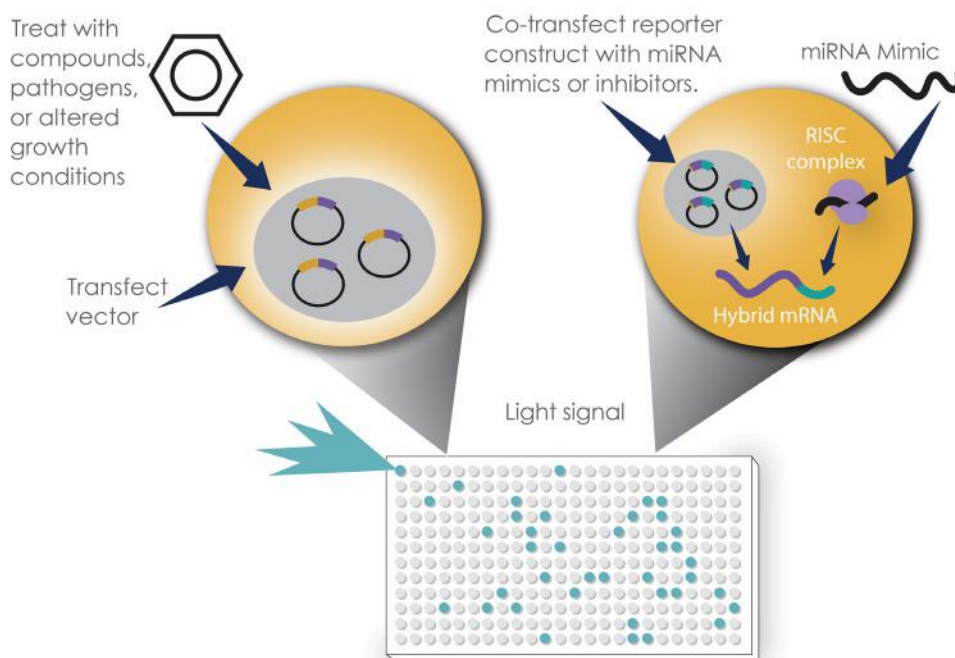
Due to all LightSwitch reporter constructs containing the optimized RenSP luciferase gene, you must use our LightSwitch Luciferase Assay Reagents to obtain optimal results. (Other luciferase assay reagents are not compatible with RenSP.)

# FUNCTIONAL GENOMICS

The LightSwitch Reporter Assay System is a complete solution for performing gene regulation studies and/or high-throughput screening. All LightSwitch vectors utilize the RenSP luciferase, which was engineered specifically for gene regulation experiments to produce higher levels of luminescence with a shorter half-life than other Renilla luciferases.

These optimizations result in RenSP having a higher signal and being more responsive to conditions that induce or repress gene expression, making it ideal for regulatory assays.

LightSwitch reporter vectors provide extremely high sensitivity and dynamic range which not only enables you to assess the activity of regulatory elements, but also map functional motifs or characterize the effects of sequence variation on gene expression.



Measure luciferase activity to determine the functional effects of TF binding, treatments, sequence variations and/or translation of miRNA binding to the hybrid luciferase-3'UTR mRNA

## LightSwitch Custom Cloning & Mutagenesis

If we do not have the genomic sequence you need for your assays in our ready-to-transfect GoClone® library, or if you are working with non-human species, we can generate constructs for you using our custom cloning and mutagenesis services.

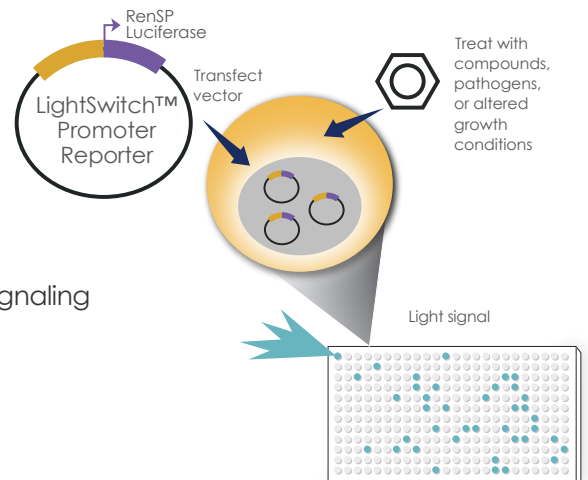
## LIGHTSWITCH™ PROMOTER REPORTER GOCLONE® COLLECTION

The LightSwitch promoter collection includes over 18,000 human promoter sequences cloned into LightSwitch reporter vectors. These transfection-ready LightSwitch GoClones, together with the highly optimized assay reagents, transfection reagents, controls, and detailed protocols allow you to perform your gene regulation reporter assay experiments immediately and ensure you generate the highest quality data possible.

| Key Features                         | Advantages  |
|--------------------------------------|---|
| Pre-cloned human regulatory elements | Over 18,000 transfection-ready promoters, all in the same vector system.  |
| Simple, fast, complete solution      | Eliminate the need for assay development and screen immediately with the pre-cloned vectors and tightly optimized assay reagents and protocols. |
| Quantitative                         | The novel RenSP luciferase technology allows you to measure gene regulation with unparalleled sensitivity, responsiveness, and dynamic range.   |
| Flexible format options              | Scalable assay platform is compatible with both small numbers of samples and thousands of assays in a large screen                              |

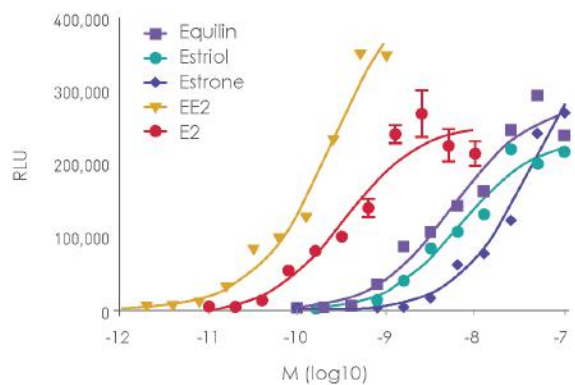
### Is My Promoter Regulated By:

- ✓ Cell Type
- ✓ Environment
- ✓ Compound Treatment
- ✓ cDNA Overexpression
- ✓ siRNA Knock Down of Receptor, Transcription Factor, Signaling Molecule, etc.
- ✓ Functional Consequence of Sequence Variation



### Using the LightSwitch assay system to study Estrogen Receptor activity.

HT1080 cells were co-transfected with a SYT8 Promoter GoClone (Product ID S714388) and an ER cDNA expression plasmid, then treated with five different estrogen compounds at different doses for 24 hours before the luminescence was measured using the LightSwitch Luciferase Assay Kit.





## LIGHTSWITCH™ VALIDATED PATHWAY COLLECTIONS

LightSwitch validated pathway collections contain promoter targets selected from our genome-wide LightSwitch Promoter Reporter GoClone® Collection that have been experimentally validated with pathway-specific inductions to create pathway-specific biomarkers.

These promoter reporter sets of 88 targets and 8 controls in the profiling panel and 8 targets in the biomarker panel, have been selected based on:

- ✓ Published information
- ✓ ChIP-Seq and RNA-Seq data
- ✓ Motif analysis
- ✓ Significant pathway induction response based on our in-house data

### Applications

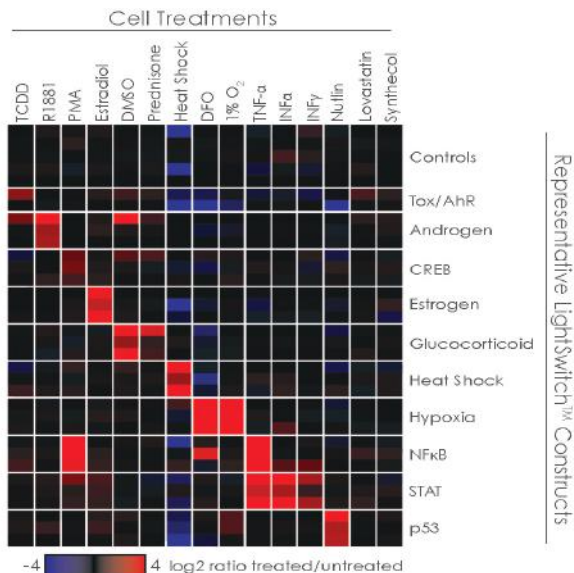
|  |
|--|
| Primary screens (agonist or antagonist mode) |
| Secondary screens, counter screens           |
| Off-target analysis                          |
| Lead compound optimization                   |
| Dose-response analysis                       |

### Advantages

|  |
|--|
| Validated assays eliminate assay development                               |
| Multiple human promoters per pathway controls produce result you can trust |
| Highly scalable and flexible format  |

### Use Validated LightSwitch Pathways Panels for High Throughput Analysis of Regulatory Pathways.

The heat map below shows the inducible activity of a selection of our validated pathway promoters representing 10 different pathways to 15 different treatments.



### Disease & Pathway Profiling Panels

| Pathway activity readout for: |                          |
|-------------------------------|--------------------------|
| HIF-1 $\alpha$                | Hypoxia                  |
| NFkB                          | Inflammation             |
| CREB                          | cyclic AMP               |
| HSF1                          | Heat shock               |
| p53                           | DNA damage, apoptosis    |
| STAT                          | Interferon               |
| SREBP                         | Cholesterol biosynthesis |
| SRF                           | Serum Response           |
| ER                            | Estrogen                 |
| AR                            | Androgen                 |
| GR                            | Glucocorticoid           |
| AhR                           | Toxicity                 |

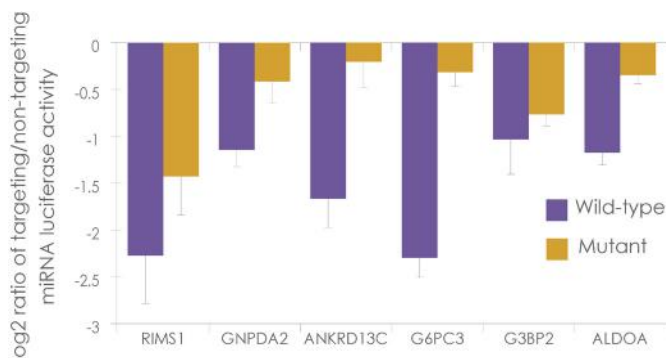
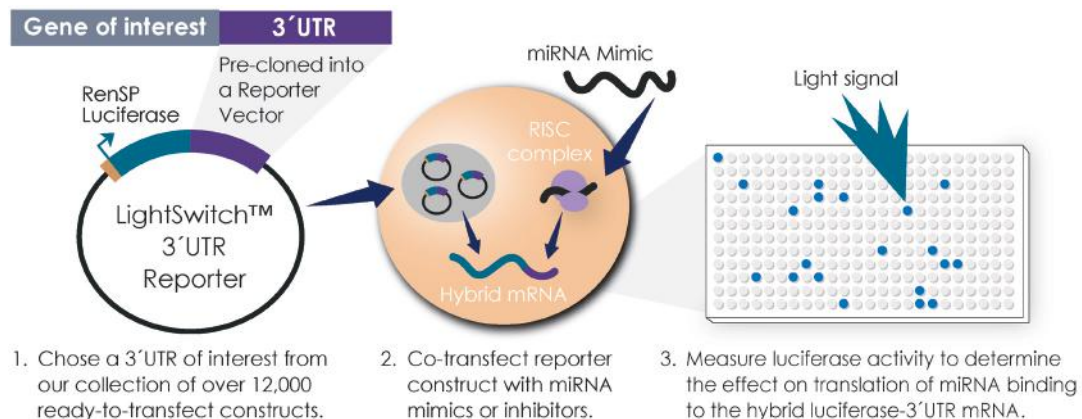
## LIGHTSWITCH™ 3'UTR REPORTER GOCLONE® COLLECTION

The LightSwitch 3'UTR collection includes over 12,000 human 3'UTRs cloned into LightSwitch reporter constructs. The LightSwitch 3'UTR reporter constructs are ideal for performing miRNA target validation, as well as assessing the functional impact of miRNA-3'UTR interactions.

In combination with our miRNA mimics and inhibitors, which can be co-transfected with the 3'UTR reporter constructs, you have everything needed to validate miRNA targets, measure RNA stability, determine translation efficiency, and evaluate the functional impact of miRNAs on a gene-by-gene basis.

### The LightSwitch 3'UTR collection can be used to quantitatively characterize:

- ✓ The impact of the 3'UTR on post-transcriptional gene regulation
- ✓ The impact of a miRNA or siRNA on the regulation of transcript stability or translation efficiency
- ✓ The targets of a miRNA or siRNA
- ✓ The effect of sequence variants on 3'UTR or miRNA function
- ✓ The impact of miRNA inhibitors or stimuli



|          | WT seed seq | Mutant seed seq |
|----------|-------------|-----------------|
| RIMS1    | ACACTCC     | AGICTCC         |
| GNPDA2   | ACACTCCA    | AGICTCCA        |
| ANKRD13C | ACACTCC     | ACAGACC         |
| G6PC3    | ACACTCCA    | ACAGACCA        |
| G3BP2    | ACACTCC     | GGICTCC         |
| ALDOA    | ACACTCCA    | AGGICTCCA       |

### Study miRNA regulation with LightSwitch 3'UTR vectors

LightSwitch 3'UTR Reporter constructs containing the 3'UTRs of 6 genes known to be miR-122 target sites were subjected to site-directed mutagenesis. The wild-type and mutant constructs were co-transfected in HT1080 cells with a miR-122 miRNA mimic or a non-targeting control in triplicate and then assayed using the LightSwitch Assay Kit.



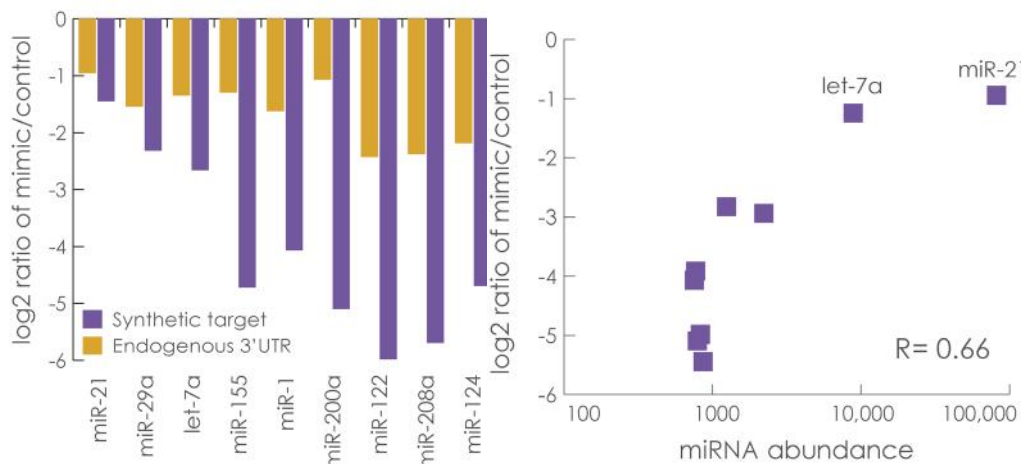
## LIGHTSWITCH™ SYNTHETIC miRNA TARGET REPORTER COLLECTION

The LightSwitch synthetic miRNA target reporter collection is ideal for performing miRNA target validation, as well as assessing the functional impact of miRNA-3'UTR interactions. LightSwitch synthetic miRNA target reporter constructs contain repeats of known 3'UTR sequences that have been optimized for increased miRNA binding. This enables them to provide stronger responses than the endogenous sequences, making them well suited for use as biosensors or positive controls in miRNA target validation experiments. They can also be used to help determine the levels of natural miRNA expression in your cell line and to screen for which cell lines will work best for your miRNA experiments.

### Key Advantages

|                                |  |
|--------------------------------|--|
| <b>miRNA Target Validation</b> | Use as a positive control in miRNA target validation studies   |
| <b>Measure miRNA Levels</b>    | Use as a biosensor to measure relative levels of endogenous miRNA in your cell line  |
| <b>Pre-Screen Cell Lines</b>   | Use to determine which cell lines will be most appropriate for experiments involving the specific miRNA mimics or inhibitors you wish to study |

### Synthetic miRNA Targets Available For >1000 miRNAs



#### Synthetic miRNA target reporters respond more strongly than endogenous 3'UTR target sequences.

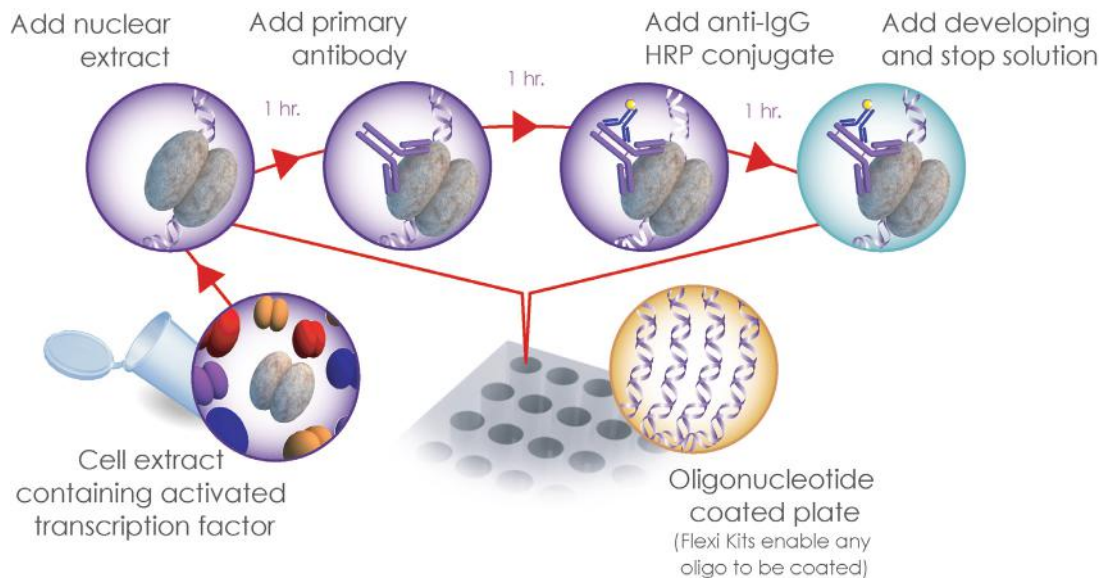
The response of a synthetic element to the appropriate miRNA was measured in HT1080 or HeLa cells for a number of popular miRNAs. Briefly, 100 ng of an individual reporter construct was co-transfected in triplicate with either a microRNA mimic or a non-targeting miRNA control at a final concentration ranging from 20 nM - 50 nM. The average luminescence was calculated for mimic replicates and was then divided by the average for non-targeting control replicates. For a particular cell-line, a strongly-repressed human 3'UTR target is displayed alongside the repression observed for the synthetic target as the log<sub>2</sub> ratio of mimic to non-targeting control signal.

## TRANSAM<sup>®</sup> TRANSCRIPTION FACTOR ACTIVATION ASSAYS

TransAM transcription factor assays are fast, user-friendly, and highly sensitive non-radioactive ELISA-based assays that facilitate the study of transcription factor-DNA binding activity in mammalian tissue and cell extracts. TransAM assays are up to 100-fold more sensitive than gel shift assays and also yield more quantitative results.

### Advantages of TransAM Transcription Factor Assays

- ✓ Up to 100-fold more sensitive than gel shifts
- ✓ Results in fewer than 5 hours
- ✓ Non-radioactive, colorimetric readout
- ✓ No cloning or transfection required
- ✓ 96-stripwell format for scalability



#### Flow chart of the TransAM process.

Activated transcription factor in cell extract binds to the consensus-binding site on the oligo immobilized in the well. Incubation with the supplied primary and secondary antibodies specifically quantifies the amount of activated transcription factor.

# TRANSCRIPTION FACTOR ANALYSIS

**Our TransAM Assay Kits have been cited in more than 1,000 publications**

Select from the list of available transcription factors below and upgrade your transcription factor activation assays.

## Available Transcription Factor Assays

|                       |                 |
|-----------------------|-----------------|
| AP - 1                | ATF - 2         |
| c-Myc                 | C/EPB           |
| CREB & pCREB          | Elk - 1         |
| ER                    | FKHR (FOXO1)    |
| GATA                  | GR              |
| HIF - 1               | HNF             |
| IRF - 3               | MAPK Family     |
| MEF2                  | MyoD            |
| NF - YA               | NFATc1          |
| NFkB                  | Nrf2            |
| 4-Oct                 | p53             |
| PPAR $\gamma$         | Sp1 and Sp1/Sp3 |
| STAT3 and STAT Family | T-bet           |

Active Motif offers many different TransAM assays and formats, along with our Nuclear Extract Kit (cat. nos. 40010 & 40410) for sample preparation and recombinant transcription factors for standard curve generation.

For a complete up-to-date list of available TransAM products and detailed information, visit us online at [www.activemotif.com/transam](http://www.activemotif.com/transam).

# TRANSCRIPTION FACTOR ANALYSIS

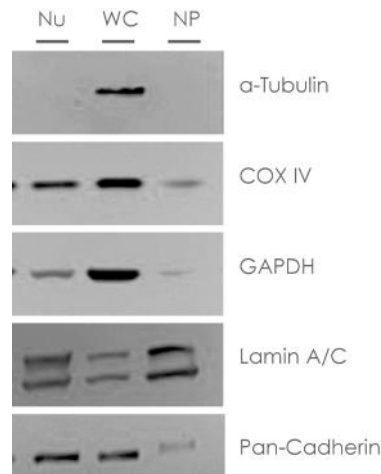
## NUCLEAR EXTRACT KIT

Active Motif's Nuclear Extract Kit offers a simple method for the preparation of highly pure nuclear, cytoplasmic, and whole-cell extracts from mammalian cells and tissues.

Use of the Nuclear Extract Kit eliminates the need to optimize reagents and ensures consistent sample preparation. The nuclear extracts generated with this product are of high yield, concentration, and purity, providing you with the most optimal purified protein fractions for analysis by various methods including Active Motif's TransAM® kits, electrophoretic mobility shift assays (EMSAs), and western blots.

### Use the Nuclear Extract Kit to improve assay results

| Key Highlights       |   |
|----------------------|---|
| <b>Fast and Easy</b> | Streamlined 2-hour protocol that requires only standard laboratory equipment        |
| <b>Scalable</b>      | 100 and 400 reaction kit formats available  |
| <b>Flexible</b>      | Prepare nuclear, cytoplasmic, or whole-cell extracts starting from cells or tissues |
| <b>Reliable</b>      | Quality-controlled reagents to ensure reproducibility                               |
| <b>Robust</b>        | Achieve consistently high nuclear protein yields                                    |



### Easy and efficient sub-cellular fractionation with the Nuclear Extract Kit

Efficiency of cellular fractionation was determined by collecting 20 µg of nuclear (Nu), and whole-cell (WC) extracts, along with nuclear pellet (NP) using the Nuclear Extract Kit and performing Western blot using antibodies against various specific proteins.

| Product             | Format   | Cat. No. |
|---------------------|----------|----------|
| Nuclear Extract Kit | 100 rxns | 40010    |
|                     | 400 rxns | 40410    |

## UNIVERSAL MAGNETIC CO-IP KIT

The Universal Magnetic Co-IP Kit enables greater flexibility for analysis by Co-IP by providing reagents for isolation of native protein complexes from either nuclear or whole-cell lysates prepared from cells or tissue.

The co-immunoprecipitation procedure utilizes protein G-coated magnetic beads for more rapid and efficient IP and wash steps. The beads also greatly reduce background and minimize sample loss for better recovery and improved results from your Co-IP experiments.

### Improve your Co-IP assays with the Universal Magnetic Co-IP Kit

| Key Highlights  |   |
|-----------------|---|
| <b>Flexible</b> | Perform Co-IP of nuclear or cytoplasmic protein complexes |
| <b>Gentle</b>   | Preserves protein interactions & modifications            |
| <b>Fast</b>     | Magnetic beads streamline procedure & reduce background   |
| <b>Complete</b> | Includes reagents for both extraction & IP procedure      |

| Product                      | Format  | Cat. No. |
|------------------------------|---------|----------|
| Universal Magnetic Co-IP Kit | 25 rxns | 54002    |

## ADDITIONAL PRODUCTS

Active Motif offers a broad selection of transcription factor assays, antibodies, and recombinant proteins for studies of development and differentiation, stem cells, inflammation, cancer, and various other areas of research.

Our antibodies are validated in-house for numerous applications, including ChIP-Seq, IP, EMSA, Western blot, IHC, and IF, and our recombinant proteins are suitable for many biochemical assays and high-throughput screens.

### KITS

| Product                            | Format   | Cat. No. |
|------------------------------------|----------|----------|
| Gelshift Chemiluminescent EMSA kit | 100 rxns | 37341    |
| Nuclear Complex Co-IP Kit          | 100 rxns | 54001    |

### ANTIBODIES

| Product                     | Validated Applications      | Available Formats | Cat. No.    |
|-----------------------------|-----------------------------|-------------------|-------------|
| p53 antibody (mAb)          | ChIP, IP, WB                | 200 µg/10 µg      | 39553/39554 |
| YY1 antibody (pAb)          | ChIP-Seq, IHC, IP, WB IP, W | 100 µl/10 µl      | 61779/61780 |
| AbFlex® MAZ antibody (rAb)  | WB                          | 100 µg/10 µg      | 91205/91206 |
| AbFlex® MITF antibody (rAb) | ChIP, ChIP-Seq              | 100 µg/10 µg      | 91201/91202 |
| AML-1/Runx1 antibody (pAb)  | ChIP, ICC, IF, IHC, IP, WB  | 100 µl            | 39000       |
| AML-2/Runx3 antibody (pAb)  | ChIP, EMSA, ICC, IHC, WB    | 100 µl            | 39301       |
| HIF-1 alpha antibody (pAb)  | ChIP, ChIP-Seq, WB          | 100 µl/10 µl      | 39665/39666 |

### RECOMBINANT PROTEINS

| Product                                | Available Formats | Cat. No.    |
|--|-------------------|-------------|
| Recombinant NFκB1 p50 (1-434) protein  | 50 µg/1 mg        | 81032/81732 |
| Recombinant NFκB1 p105 protein         | 20 µg/1 mg        | 81143/81843 |
| Recombinant NFκB3 (RELA / p65) protein | 20 µg/1 mg        | 81086/81786 |
| Recombinant p53 (TP53) protein         | 20 µg/1 mg        | 81091/81797 |
| Recombinant YY1 protein                | 20 µg/1 mg        | 81119/81819 |
| Recombinant MAX protein                | 20 µg/1 mg        | 81017/81717 |
| Recombinant c-Myc / MAX complex        | 20 µg/1 mg        | 81087/81787 |
| Recombinant IKKβ protien               | 20 µg/1 mg        | 81066/81766 |



## RNA ANALYTICAL SERVICES

Historically the function of RNA in the cell was thought to be primarily a messenger that translated information from DNA into protein. We now know that RNA is involved in a diverse array of biological processes including direct catalysis and a multitude of regulatory mechanisms.

### Active Motif offers two main gene expression services

Our comprehensive end-to-end RNA analysis services makes it easy and efficient to incorporate genome-wide transcriptomic studies into your research.

We offer two main services to investigate gene expression on a genome-wide scale; RNA-Seq for identification and quantitation of RNA transcripts, and RNA Pol II ChIP-Seq for quantitation of transcription rates to enable rapid profiling of changes in gene expression associated with transcription factor (TF) and histone modification occupancy.

## RNA-SEQ SERVICES

Simply submit RNA, cells, or tissue samples. Order RNA-Seq alone or combine with ChIP-Seq data to uncover contextual information about:

- ✓ Differential gene expression
- ✓ Changes in gene structure or splicing patterns
- ✓ Effects of TF binding on gene expression
- ✓ Effect of sequence variants on 3' UTR or miRNA function
- ✓ The impact of miRNA inhibitors or stimuli

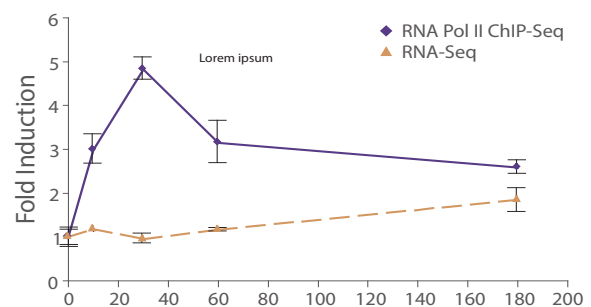
| Key Features   |
|--|
| PolyA Enrichment   |
| Directional library preparation                                |
| Paired-end sequencing on Illumina® sequencing platform         |
| QC performed using Bioanalyzer                                 |
| Data analysis pipelines include differential analysis and GSEA |

## RNA POL II CHIP-SEQ SERVICES

Analysis of RNA Pol II occupancy as a proxy measurement of transcription rates offers the advantage of enabling you to:

- ✓ Measure transcription without the influence of RNA half-life
- ✓ Identify genes poised for transcriptional activation
- ✓ Generate gene expression data from cells used for ChIP-Seq
- ✓ Measure changes at early time points post-treatment
- ✓ The impact of miRNA inhibitors or stimuli

RNA Pol II Changes Are Detected Earlier Than mRNA Changes



### Gene expression profiles vary depending on the analysis method.

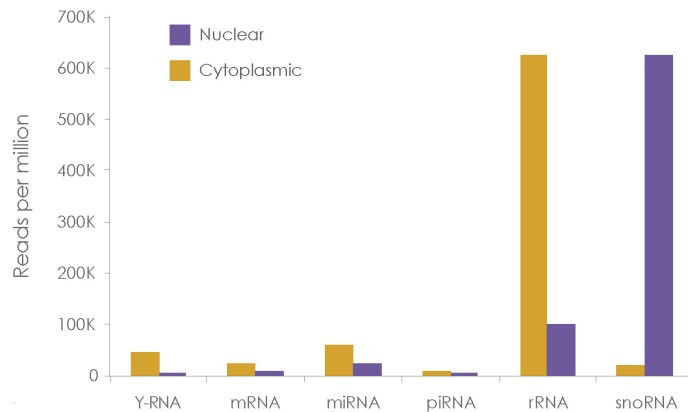
Data for *Igf1r* expression was extracted from RNA-Seq and RNA Pol II ChIP-Seq data sets. Cell treatment resulted in induced gene expression that was measured at various time points. The data show that transcription, as measured by RNA Pol II ChIP-Seq, is induced immediately, while mRNA levels only accumulate over time.

## RNA SUBCELLULAR ISOLATION KIT

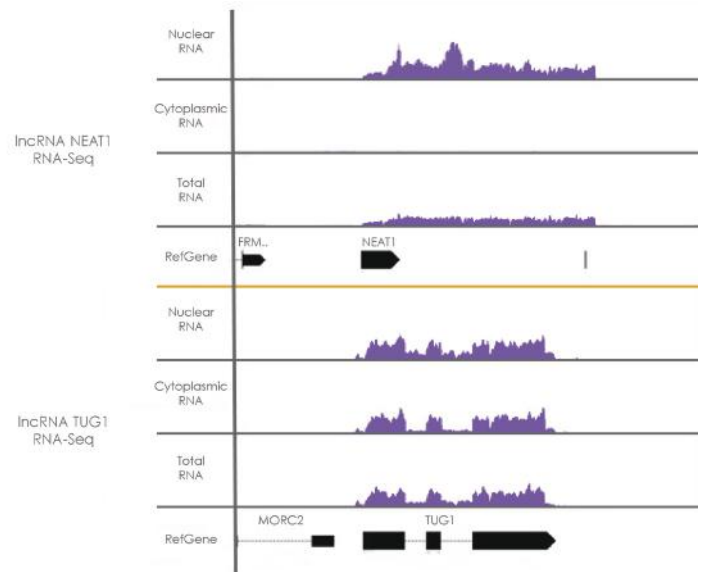
Active Motif's RNA Subcellular Isolation Kit is designed to efficiently isolate separate nuclear and cytoplasmic RNA fractions for downstream analysis. This method can be used to isolate RNA molecules larger than 75 nucleotides, including long non-coding RNAs (lncRNAs), ribosomal RNAs, messenger RNAs, and small nucleolar RNAs (snoRNAs) from cells or tissue without cross-contamination or the use of phenolic compounds.

### Advantages of the RNA subcellular isolation kit:

- ✓ Works with cells and tissues
- ✓ Method avoids the use of phenol
- ✓ Isolates RNA greater than 75 nt, including lncRNAs, snoRNAs, rRNAs, mRNAs, and more
- ✓ Enhanced detection of low abundance transcripts
- ✓ Reduced background from other intronic or mature RNAs
- ✓ Purified RNA is validated for use in RT-qPCR and RNA-Seq



**Use the RNA Subcellular Isolation Kit to easily identify small RNA distributions.** Cytoplasmic and nuclear RNA were isolated from HeLa cells. RNA was processed and size selected to remove large RNA. 75 bp single read sequencing was performed and the data was mapped to miRBase. A frequency of the small RNA (sRNA) distribution for each fraction shows the specificity of subcellular isolation.



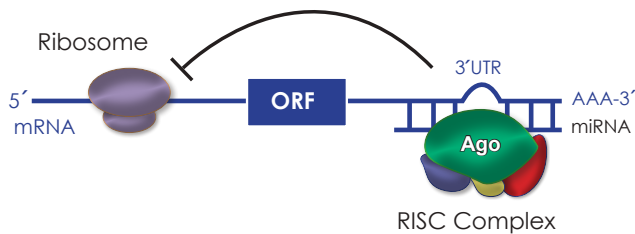
### Focus your RNA-Seq on the relevant sub-cellular fractions

Nuclear, cytoplasmic and total RNA were isolated from HeLa cells. RNA was subjected to ribosomal RNA depletion during NGS library preparation. Samples were then sequenced using the Illumina® HiSeq and 100 bp paired end reads with 50 M reads per sample. NEAT1 is a lncRNA that is primarily located in the nucleus, while TUG1 is a lncRNA known to have both nuclear and cytoplasmic localizations.

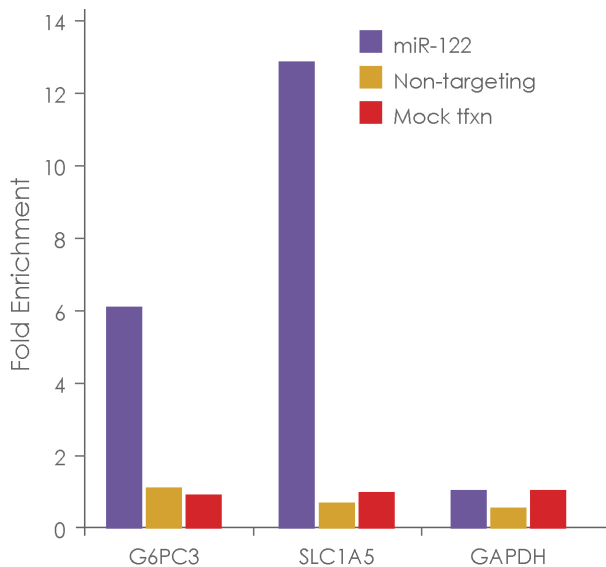
| Product                       | Format  | Cat. No. |
|-------------------------------|---------|----------|
| RNA Subcellular Isolation Kit | 30 rxns | 25501    |

## miRNA TARGET IP KIT

Active Motif's miRNA Target IP Kit was designed to capture and identify the physical interactions of miRNAs with endogenous mRNA transcripts for validation of the binding targets of specific miRNAs. The targeting of a microRNA (miRNA) to a specific mRNA is mediated through the formation of an RNA Induced Silencing Complex (RISC), containing a combination of various RNA-binding proteins along with the Argonaute (Ago) protein and miRNA. The Active Motif miRNA Target IP Kit utilizes a pan-Ago antibody that recognizes Ago1, Ago2 and Ago3 proteins for immunoprecipitation (IP) of miRNA/mRNA interactions as part of the RISC complex.



**The miRNA within a RISC complex enables precise silencing of specific mRNA transcripts.** The key components in a RISC complex are an Ago protein and a miRNA. The Ago protein binds the miRNA, positioning it in a conformation that enables the RISC to base-pair in a Watson-Crick manner with a mRNA transcript. This leads to either inhibition of translation (shown) or increased degradation of the targeted transcript.



**Identify miRNA target genes.** The miRNA Target IP Kit was used on HT1080 cells transfected with either a miR-122 mimic, a non-targeting miRNA control, or a mock plasmid control. Following IP using the Ago1/2/3 antibody or Negative Control IgG included in the kit, qRT-PCR was performed using primers for G6PC3 and SLC1A5, which are known targets of miR-122, and GAPDH, a common housekeeping gene that is not known to be targeted by miR-122.

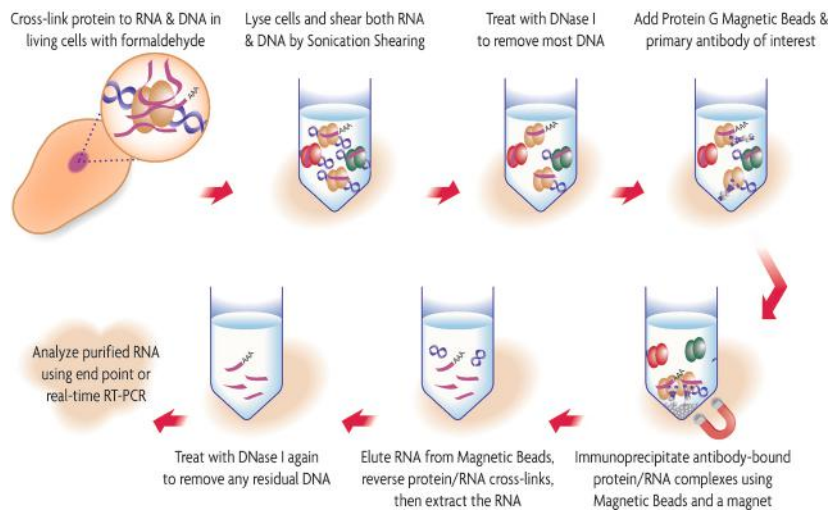
| Product             | Format  | Cat. No. |
|---------------------|---------|----------|
| miRNA Target IP Kit | 10 rxns | 25500    |

## RNA CHIP-IT<sup>®</sup> KIT

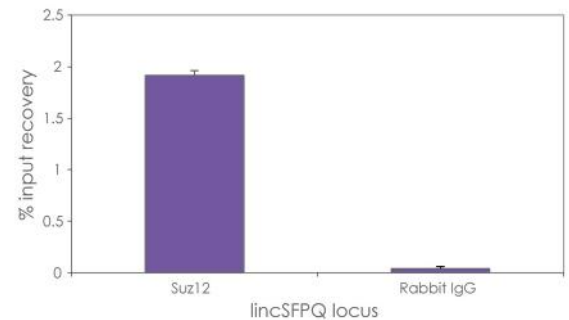
### Isolate and study RNA-protein complexes specifically from chromatin

To facilitate the characterization of the role of RNA in genome regulation and organization, Active Motif has applied the efficient magnetic bead-based protocol used to develop our ChIP-IT Express Kits to develop the first of its kind kit for RNA ChIP. The RNA ChIP-IT Kit was designed to specifically enable investigation of RNA-protein interactions in a chromatin context and has been optimized for RNA.

- ✓ Specifically tailored to study chromatin-associated RNA
- ✓ Designed to remove DNA while maintaining RNA integrity
- ✓ Step-by-step protocols for fixation of chromatin, sonication and immunoprecipitation, all optimized for RNA preservation
- ✓ Separate control kit available with control antibody and primers



Flow chart of the RNA CHIP-IT method



**Study chromatin-associated RNAs.** The RNA ChIP-IT Kit was used on 10 µg samples of DNase I-treated HeLa chromatin with 10 µl of Suz12 antibody (Cat. No. 39357) and 2 µg of Normal rabbit IgG. The RNA-IP was performed overnight at 4°C. Real-time RT-PCR was performed using primers for the lincRNA SFPQ locus.

| Product                      | Format  | Cat. No. |
|------------------------------|---------|----------|
| RNA CHIP-IT <sup>®</sup> Kit | 10 rxns | 53024    |

## ADDITIONAL PRODUCTS

Active Motif has many products to enable RNA analysis and transcription research. Our antibodies are validated in-house for numerous applications, including ChIP-Seq, IP, EMSA, Western blot, IHC, and IF, and our recombinant proteins are suitable for many biochemical assays and high-throughput screens.

### KITS

| Product      | Format   | Cat. No. |
|--------------|----------|----------|
| mTRAP™ Total | 12 rxns  | 23012    |
| mTRAP™ Midi  | 100 rxns | 23024    |
| mTRAP™ Maxi  | 6 rxns   | 23006    |

### ANTIBODIES

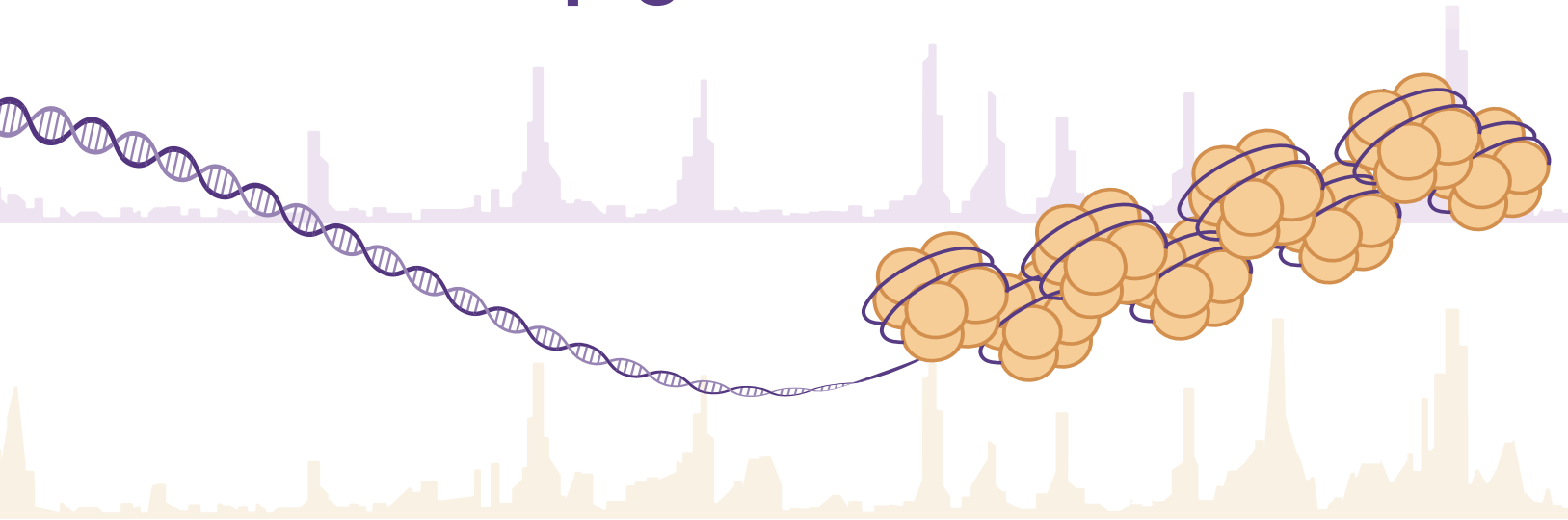
| Target  | Validated Applications                 | Available Formats | Cat. No.    |
|---|--|-------------------|-------------|
| N6-Methyladenosine (m6A) antibody (mAb)         | DB, IP                                 | 100 µg/10 µg      | 61755/61756 |
| AbFlex® N6-Methyladenosine (m6A) antibody (rAb) | DB, IP, W                              | 100 µg/10 µg      | 91261/91262 |
| CTCF antibody (pAb)                             | ChIP, ChIP-Seq, EMSA, ICC, IF, IHC, WB | 100 µg/10 µg      | 61311/61312 |
| DNMT1 antibody (mAb)                            | ChIP, IHC, IP, WB                      | 100 µg            | 61467       |
| p53 antibody (mAb)                              | ChIP, IP, WB                           | 200 µg/10 µg      | 39553/39554 |
| AbFlex® RNA Pol II antibody (rAb)               | ChIP-Seq, IF, WB                       | 100 µg/10 µg      | 91151/91152 |
| RNA pol II antibody (mAb)                       | ChIP, ChIP-Seq, ICC, IF, WB            | 200 µl/50 µl      | 39091/39497 |
| RNA pol II CTD phospho Ser2 antibody (mAb)      | ChIP, ChIP-Seq, ICC, IF, IP WB         | 100 µg/10 µg      | 61083/61084 |

### RECOMBINANT PROTEINS

| Product                              | Available Formats | Cat. No.    |
|--------------------------------------|-------------------|-------------|
| Recombinant ALKBH5 protein           | 50 µg/1 mg        | 31589       |
| Recombinant FTO protein              | 20 µg/1 mg        | 31572/31972 |
| Recombinant METTL3/METTL14 complex   | 20 µg             | 31570       |
| Recombinant RNA Pol II - CTD protein | 10 µg             | 81036       |



# Advance Your Research with End-to-End Epigenetic Services



## Popular Services:

ChIP-Seq

ATAC-Seq

RIME (ChIP-mass spec)

Histone PTM Mass Spec

DNA Methylation Assays

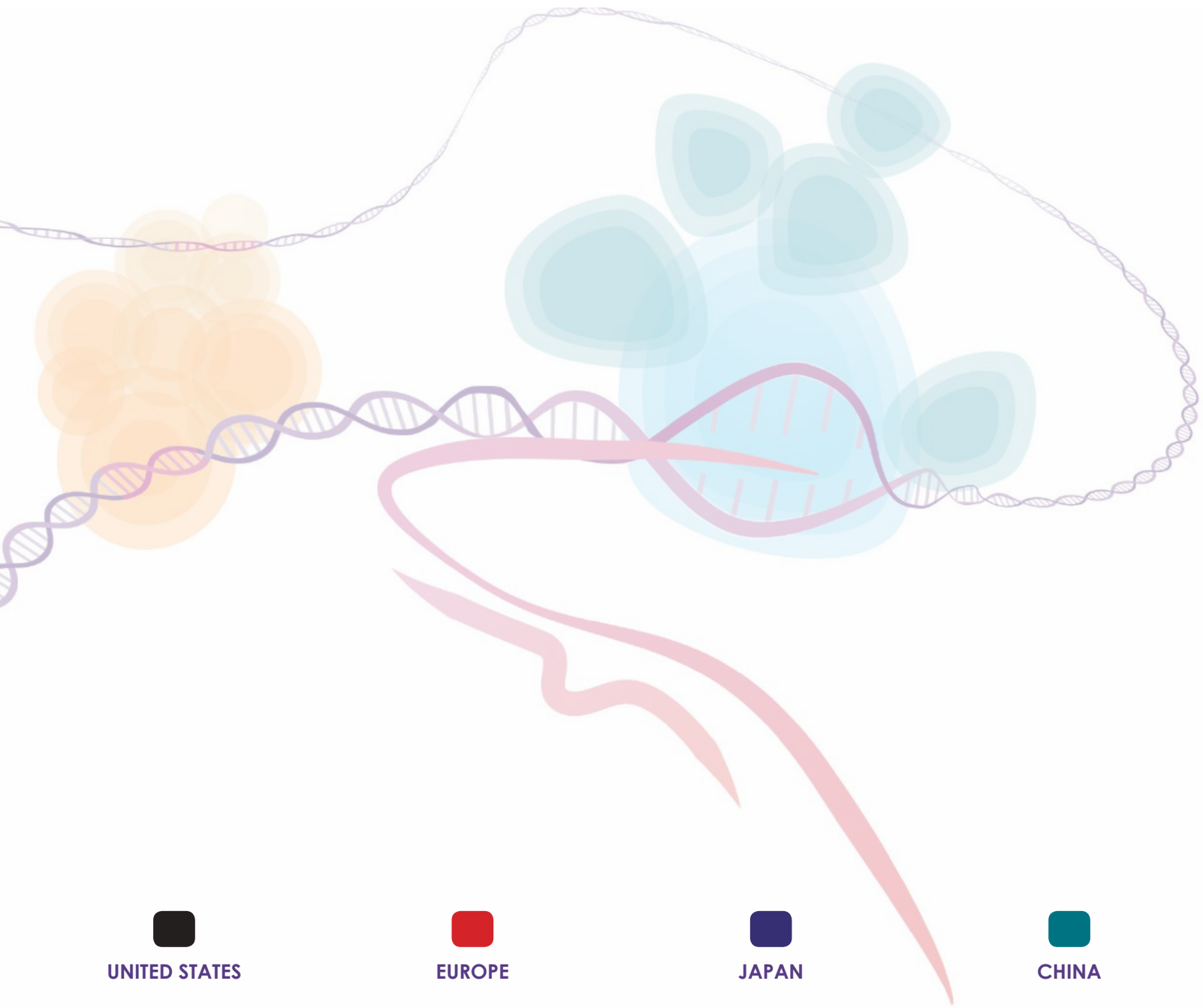
RNA-Seq

**Our Epigenetic Services have been cited in >250 peer reviewed publications.**

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