

Skin Science: The Use of Genomics Tools in Skin Care

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What is “Skin Science?”

- The use of “hard science” techniques and principles in guiding cosmeceutical product development
 - Genomics - analysis of genes
 - Proteomics – analysis of proteins
 - Histological techniques – visual (microscopic) analysis of skin cells
- Based on the fact that changes in the skin’s appearance (wrinkles, fine lines, dullness, age spots) result from changes in basic biological and physiological processes
 - Cellular aging (cell cycle, growth factors, aging factors – sirtuins)
 - Anti-oxidant production/removal
 - Inflammation
 - DNA repair
 - Extracellular matrix (i.e., collagens, elastins)

The Biological Basis of Skin Characteristics

Physical Characteristics

Fine lines, wrinkles, age spots, dullness



Changes in Biological/Physiological Processes

Inflammation, cell cycle/regeneration,
oxidant formation/anti-oxidant production, aging molecules,
extracellular matrix integrity



Changes in gene expression and protein expression

(sirtuins, collagens, keratins, growth factors,
metalloproteinases, interleukins)

Changes in Gene Expression Are Biomarkers

- Easily measurable (using high tech genomics methods)
- Indicative of biological events that underlie skin's appearance

For cosmeceuticals:

- Faster and less subjective than clinical testing
- Identify underlying biological mechanisms
 - For example, multiple different biological pathways involved in inflammation and anti-aging in skin

Genomics: History and Current Applications

- Field was borne from the human genome project
 - identified and sequenced 30,000 human genes
- Produced high throughput technologies that measure/analyze:
 - *Gene expression (which genes are turned on or off in a given condition)*
 - Gene sequence (determines if the DNA code is altered)
 - SNP's – single nucleotide polymorphisms
- Technologies are being used in all areas of human health (personalized medicine), animal health, environmental and agricultural industries
- Just beginning to emerge in the cosmeceutical industry

Benefits of Genomics to the Cosmeceutical Industry

- Improve product formulations to achieve desired effects
- Validate product efficacy at biological level
 - Faster and less subjective than clinical studies
- Cost-savings
 - May find that a small amount of expensive ingredient produces same effect as higher concentration
- Ensure product safety
- Each of these things will *improve marketability*

Current Uses in Skin Care Industry

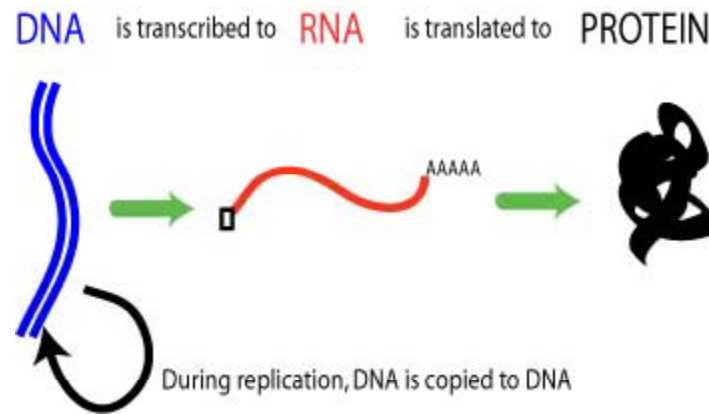
Most common:

- Determine how specific ingredients or products regulate gene expression
- Create “custom” products based on an individual’s gene sequence

Additional uses:

- Compare different skin conditions to understand how they differ at the molecular level (i.e., aging vs young skin, hyperpigmentation vs normal pigmentation)
- Identify biomarkers of efficacy for quicker analysis of product safety and efficacy
 - p53 a gene that regulates DNA repair was recently identified as a biomarker for testing sunscreen efficacy
 - Authors suggest this is a better measure than SPF
 - Lens et al., International Journal of Dermatology, 2008, Vol. 47, pp. 1234-1239

Gene Expression is Based on the Central Dogma of Biology



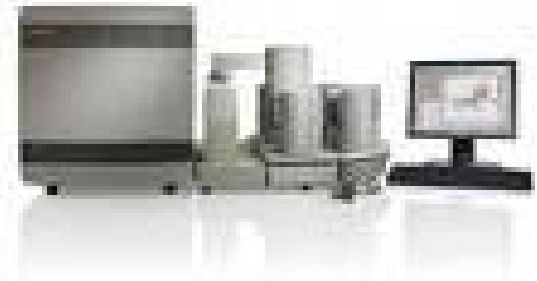
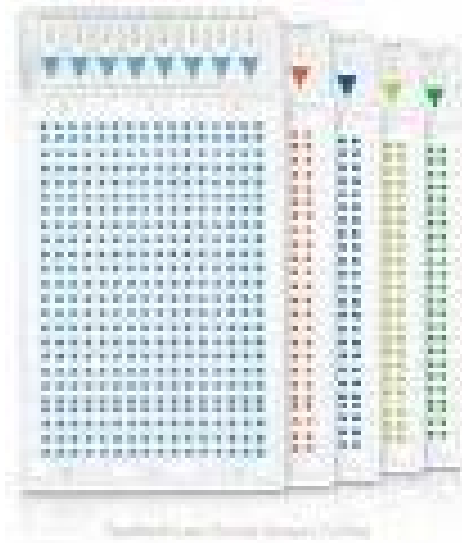
- All cells in a person's body have the same **DNA** or “genes”
- When the gene is activated it is turned into **RNA**
- Different cell types are produced by activation of unique sets of genes
- Specific RNAs assemble specific proteins such as collagen and keratin in skin cells
- Aging, disease and other conditions will influence the regulation of specific genes
- Gene expression technologies measure the amount of **RNA** in a given cell or tissue

Genomics Technologies: Affymetrix Microarrays (Gene Chips)



- Measure up to 30,000 genes at a time
- Chips available for over 30 organisms
- Ideal for discovery
 - What does my product/ingredient do?
 - Identifying novel mechanisms of action
 - Comparing biological activities of different products

Taqman Real Time PCR (Polymerase Chain Reaction)



- Taqman Low Density Array (TLDA)
- Less expensive than microarrays; very sensitive method – highly quantifiable
- Can measure up to 384 genes at a time
- Arrays are custom-designed with your genes of interest
- More focused experimental approach

Genomics Study for *Elina* Organic Skin Care

- Used quantitative real time PCR to understand how *Elina Ambra-Lift* acts at the biological level; which genes were turned “on or off” by the product
 - we tested the final product rather than individual ingredients
- For the study, *Elina Ambra-Lift* was applied to a 3D *in vitro* skin culture model that contains both epidermal and dermal skin cell layers
 - The test product was applied for 48 hrs.
 - The control cultures received no cream
- Expression of 91 custom-selected genes that regulate biological functions that underlie skin cell aging and anti-aging processes were measured (plus 5 control genes)
- Assessed skin cell integrity using a standard histological stain (hematoxylin & eosin)

In Vitro 3D Skin Culture System

- Using a 3D human *in vitro* skin model (EpiDerm FT; purchased from MatTek)
- 3d model contains epidermis (keratinocytes, stratified corneum layer) and dermis (viable fibroblasts)
- *Model allows application and testing of final cosmetic product, rather than single ingredients*

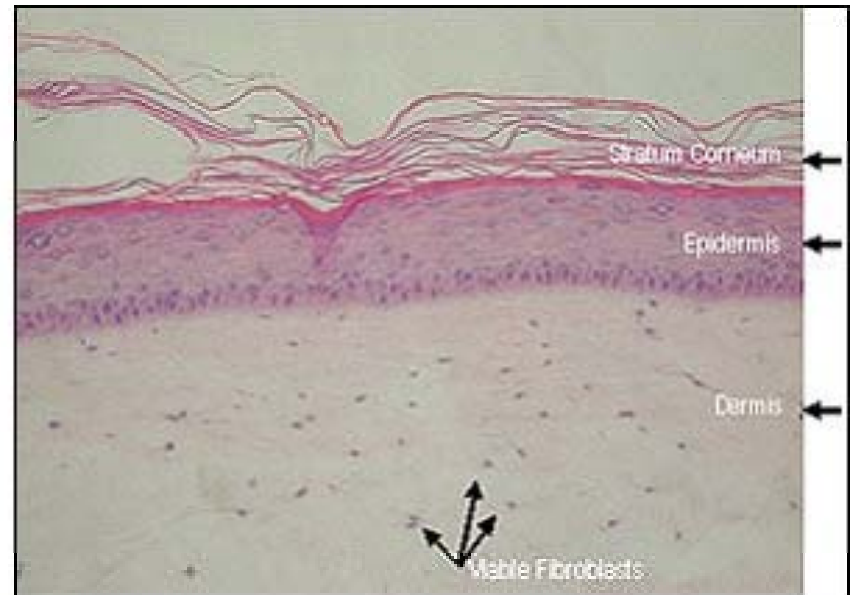
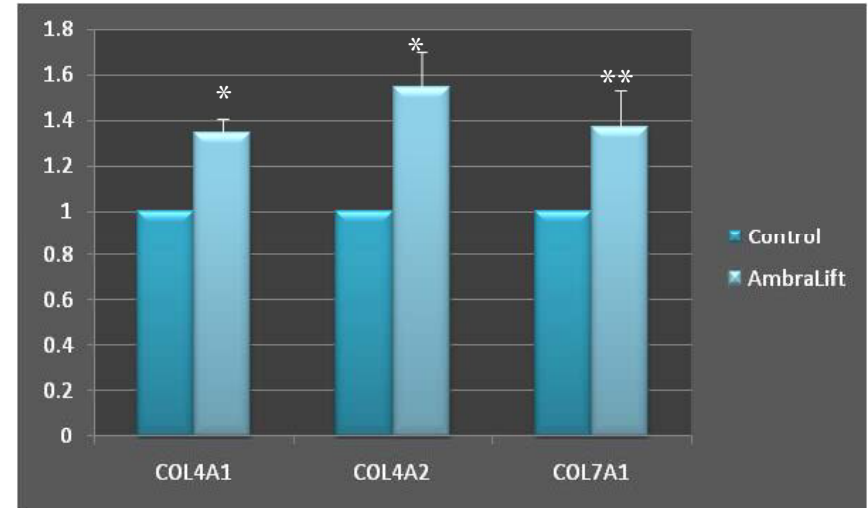
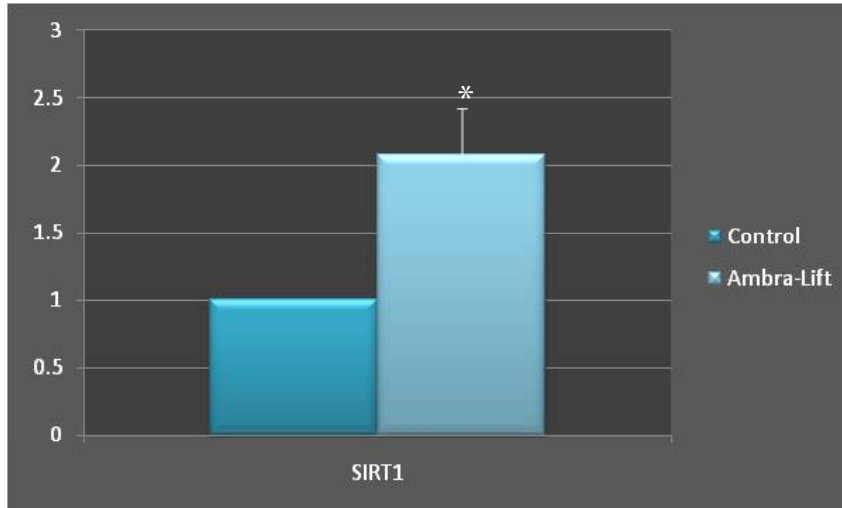


Image from MatTek

Elina Ambra-Lift Regulated Expression of Specific Genes Important for Maintaining Healthy Skin

- 48 hr exposure to *Elina Ambra-Lift* produced statistically significant changes in 47 out of 91 genes tested
 - T-tests were performed to determine statistical significance
 - All of the 47 genes were statistically significant at $p < 0.05$ or less
- 30 genes were downregulated; their levels were decreased after exposure to *Ambra-Lift*
- 17 genes were upregulated; their levels were increased after exposure to *Ambra-Lift*
- Detailed results can be obtained at www.elinaskincare.com

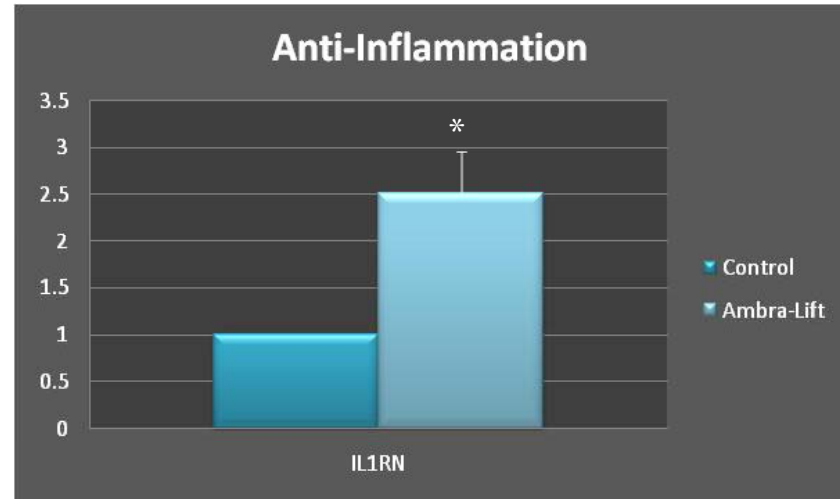
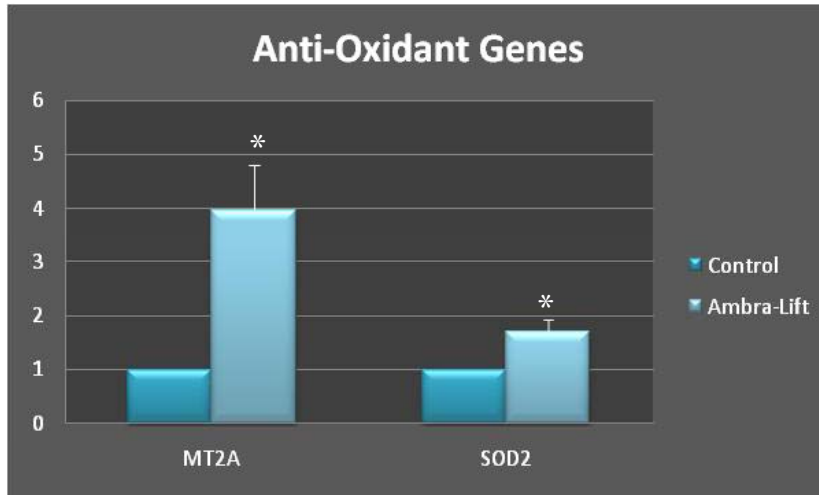
Elina *Ambra-Lift* Increases Expression of Anti-Aging and Anti-Wrinkle Genes



* Statistically significant at $p < 0.02$; ** statistically significant at $p < 0.06$

- Sirtuin 1 (SIRT1) is a well characterized anti-aging gene; *Ambra-Lift* increased expression
- Collagen 7A1 (COL7A1) is considered a “biochemical marker of wrinkles,” whereas decreased expression is associated with wrinkles; *Ambra-Lift* increased expression
- Collagen 4A1 and 4A2 are important for maintaining skin firmness; *Ambra-Lift* increased expression

Elina Ambra-Lift Induces Anti-Oxidant and Anti-Inflammatory Gene Expression



* Statistically significant at $p < 0.01$

- *Elina Ambra-Lift* increased expression of 2 important anti-oxidant genes which help protect cells from damage (metallothionein 2, superoxide dismutase 2)
- *Elina Ambra-Lift* increased expression of the interleukin 1 receptor antagonist (IL1RN), which decreases activity of a powerful inflammatory molecule, interleukin 1 beta (IL-1 β)
- These results demonstrate that *Elina Ambra-Lift* activates genes involved in protective cellular mechanisms

Summary: Elina *Ambra-Lift* Activates Genes that Promote Anti-Aging and Anti-Wrinkle Effects

- The use of genomics to validate the biological effects is an innovative approach
- The experimental study design allowed for testing of the final product rather than single ingredients
- *Elina Ambra-Lift*, an organic product, naturally induced expression of genes that regulate anti-wrinkle and anti-aging effects in the skin
 - Collagens
 - Anti-Oxidants
 - **Sirtuin- 1**
- Elina will use these data to increase her presence from a localized, midwest market to a more prominent, national product leader

Future Studies/Capabilities

- Test additional products
- Experimental design can be modified to test other conditions or endpoints. For example:
 - Protection from UV exposure or other types of damage
 - Repair from damage caused by UV exposure or other conditions

Summary and Market Projection

- Genomics testing methods will play a more prominent role in cosmeceutical product development and testing
- This will be driven by:
 - Changes in Federal guidelines for more stringent regulations on marketing claims and safety guidelines
 - Consumer awareness and their demand for products with proven efficacy
 - Competition; keeping up with companies/products that are using “hard science” approaches in their product development