

# Non-Invasive Gene Expression Analysis Assay for Psoriasis

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## Introduction

Significant progress has been made in the treatment of moderate to severe psoriasis through blocking key cytokines such as TNF- $\alpha$ , IL-17A and IL-23 involved in the pathogenesis and progression of the disease. However neither the marketed drugs nor the drug candidates in development show complete response rates. Stratifying patients into potential responders and non-responders to a given targeted therapeutic prior to or within a few weeks after initiation of treatment and the ability to effectively monitor psoriasis would certainly benefit patients, physicians and insurance providers. Previous studies have suggested the presence of altered cytokine gene expression patterns in non-responders, but current serum or surgical biopsy-based assays have failed to generate information suitable to reliably identify such a patient group. In this study, we introduce a new non-invasive molecular assay that more accurately detects skin cytokine changes in psoriasis patients and can be useful to monitor the disease as well as to potentially also predict the treatment responses.

## Materials and Methods

An adhesive patch-based non-invasive skin biopsy device (DermTech, La Jolla, USA) was used to collect epidermal skin tissue samples from healthy volunteers and psoriasis patients in IRB approved studies.<sup>1</sup> In psoriasis patients, both lesional and non-lesional skin samples were obtained. Total RNA was extracted from these samples and used for cytokine gene expression analysis with TaqMan RT-qPCR. A panel of 13 key cytokines, mainly in the Th17 pathway, involved in psoriasis was studied and expression levels were calculated through the qPCR threshold cycle counts (Ct).

## Results and Discussion

With the adhesive patch-based skin biopsy device, epidermal tissue was successfully collected non-invasively from all test subjects and total RNA was isolated (Figure 1). Psoriatic lesions yielded more skin tissue and higher amounts of RNA than non-lesional skins or skins from healthy volunteers.

Figure 1 depicts RNA yield data from non-lesional and lesional skin in psoriasis patients.

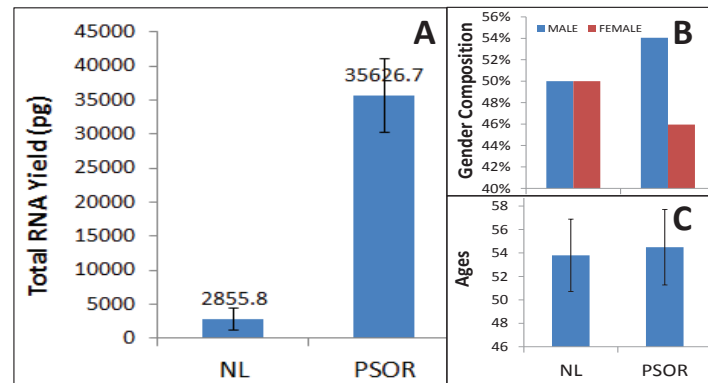


Figure 1. Total RNA yields (pg) from lesional (PSOR) and non-lesional (NL) skin in psoriasis patients.

The RNA isolated was sufficient in amount and quality to enable qPCR detection of gene expression changes. Figure 2 shows qPCR analyses on several key genes (IL-17, IL-23, DEFB4 and S100A9) and compares their expression levels in both PSOR and NS (normal skin) with dilutions of RNA input. Elevated gene expressions (shown as downward shifts of Ct values) are clearly seen in PSOR skin samples for most targets. Linear changes and parallel curves for Ct values of both target gene and housekeeping gene (ACTB) with changing RNA input confirms both the quality of the isolated RNA for gene expression and the accuracy of the qPCR analysis adopted to the current assay.

Psoriasis is characterized by a complex pathological process affected by a number of cytokines and their interactions. A thorough understanding of the disease requires a thorough understanding of these cytokines within the target organ. Figure 3 shows a heatmap constructed from the Ct values of 13 cytokine genes from 53 RNA samples (14 NML, 15 NL and 24 PSOR skin samples). A darker red on the heatmap shows a lower Ct or an increased gene expression while a darker blue-grey shows a higher Ct value or a lower gene expression. The psoriatic lesional skin samples show a distinctly different heatmap pattern from that seen with non-lesional or normal skin samples.

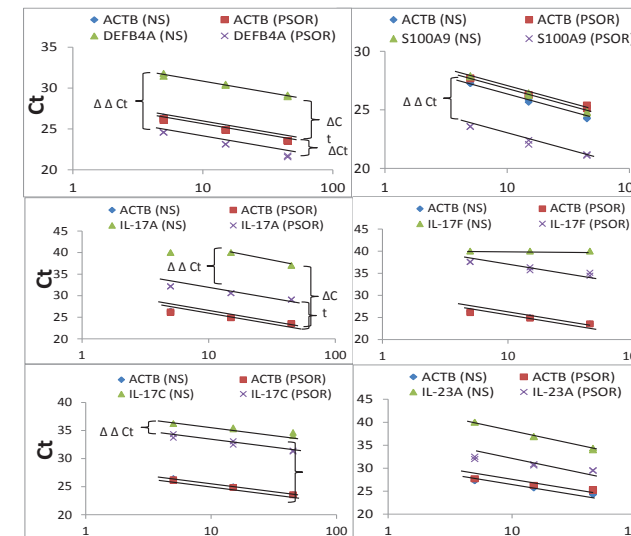


Figure 2. Ct measurements of genes in lesional (PSOR) and normal skin (NS) samples at different RNA input levels.

Notably, gene expression patterns of psoriasis samples displayed a high degree of uniformity and consistency, which might be attributed to the novel non-invasive skin biopsy method with adhesive patches that collect layers of homogenous target cells directly from the most impactful layers of the target organ yielding strong gene expression signals of high uniformity across patients. This is a clear advantage compared to the serum (liquid biopsy)

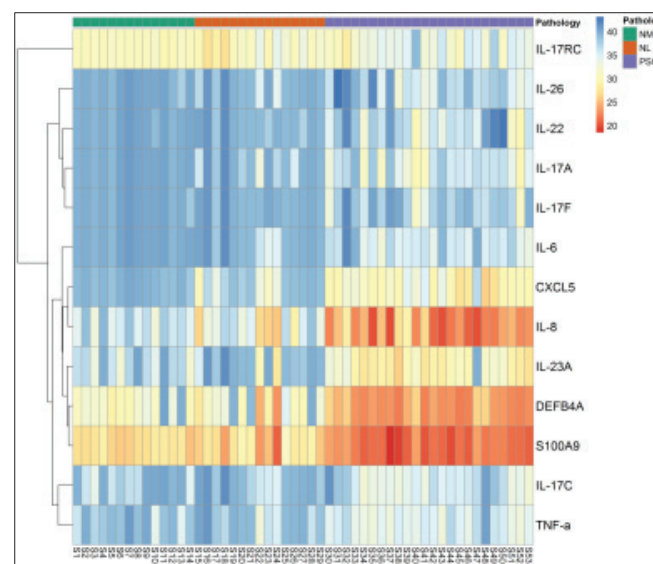


Figure 3. Heatmap of Ct values of 13 key genes from normal (NML), and psoriatic lesional (PSOR) and non-lesional (NL) skin samples collected with adhesive patch-based devices.

or surgical skin biopsy-based molecular assays where the changed molecular signals in lesional target cells can become too weak (diluted) or too noisy (contaminated by other cells) to detect or to provide meaningful information. This uniformity advantage makes our non-invasive gene expression analysis particularly useful for psoriasis studies, that use both disease monitoring and prediction of flare-ups.

In addition, our non-invasive gene expression analysis assay also detected subgroups of patients, where the expression levels of key genes (IL-17, 22, 26) in the Th17 pathway varied (Figure 4). This may be associated with patient groups who respond or fail to respond to select targeted therapeutics allowing to differentiate between responders and non-responders.

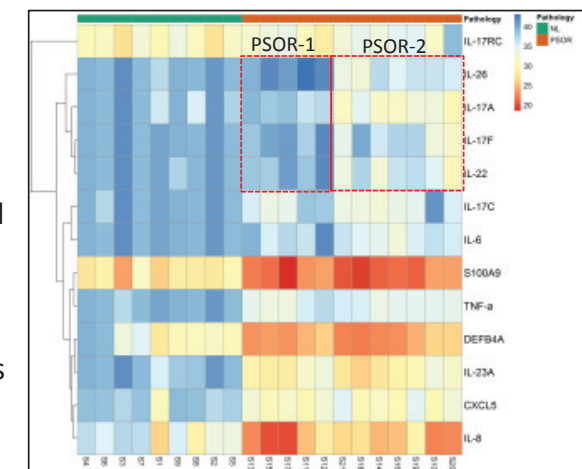


Figure 4. Heatmap of Ct values of 13 genes from paired lesional (PSOR) and non-lesional (NL) skin samples collected from treatment naïve psoriatic patients, with 2 distinct subgroups of gene expressions in lesional tissues (PSOR-1 and -2).

## Conclusion

The adhesive patch-based non-invasive biopsy device described reliably collects epidermal skin tissue samples from lesional and non-lesional skin of psoriasis patients in sufficient quantity and quality for molecular analysis. The gene expression analysis described is a robust assay for psoriasis and useful for disease monitoring, prediction of flare-ups and also potentially be able to differentiate between responders and non-responders to aid physicians in their treatment decisions. Studies to test this notion are ongoing. The assay has distinct advantages over traditional serum or surgical-biopsy-based molecular analyses.

## Reference

1. Yao et al., Analytical Characteristics of a Noninvasive Gene Expression Assay for Pigmented Skin Lesions; ASSAY and Drug Development Technologies, Vol.14 No.6, 2016.

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