Identification and verification of changes in gene and protein expression from limited samples

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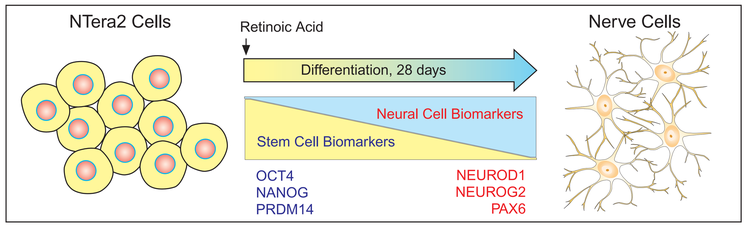
Abstract

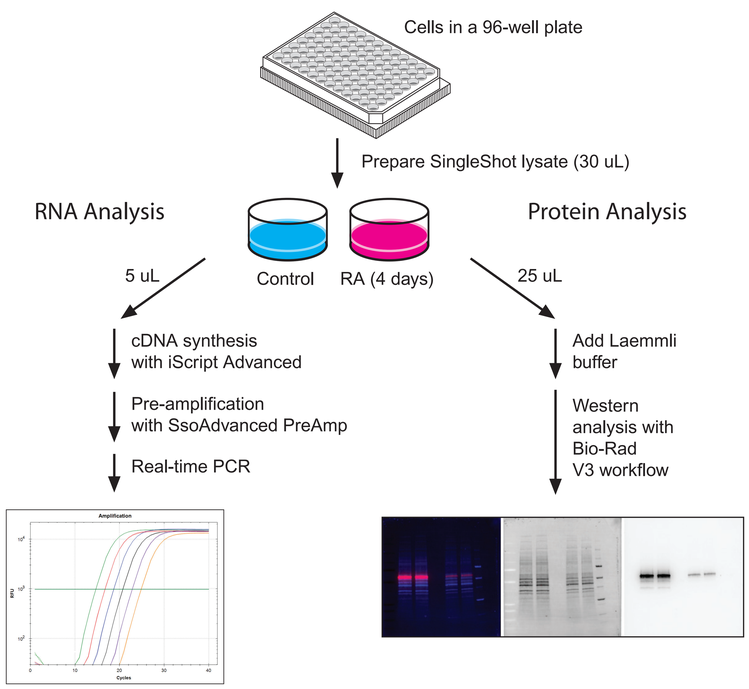
Identification and validation of regulatory changes is challenging when analyzing small samples, especially when both gene and protein expression analysis is necessary. We developed a novel workflow that allows for screening up to 96 genes for differential mRNA expression, and validation that the protein expression level of selected targets are indeed changed. To validate our workflow we assessed changes that occur in NTera2 cells, a human stem cell model system, after four days of differentiation initiated by retinoic acid. NTera2 were grown in a 96-well plate under control or differentiated conditions. One well each of control and differentiated cells were harvested to generate a cell lysate. A small portion of each lysate was analyzed for mRNA expression; we incorporated a pre-amplification step to allow the analysis of a large panel of genes associated with pluripotency and differentiation. We observe differential expression of several genes, including NANOG and OCT4 that are down-regulated in the differentiated cells. We then performed Western blot analysis, using the remaining cell lysate, to assess NANOG and OCT4 protein levels. We find that the level of protein for these targets is significantly decreased in the differentiated cells, consistent with the mRNA results. These findings demonstrate that (i) mRNA and protein analyses can be conducted from the same cell sample, and (ii) a large panel of gene targets can be screened to identify candidates for subsequent verification by protein analysis. We envision that this workflow can enable streamlined analysis and verification of regulatory changes at both the mRNA and protein level in samples that are typically refractory to such analysis.

Model system: Human stem cell differentiation



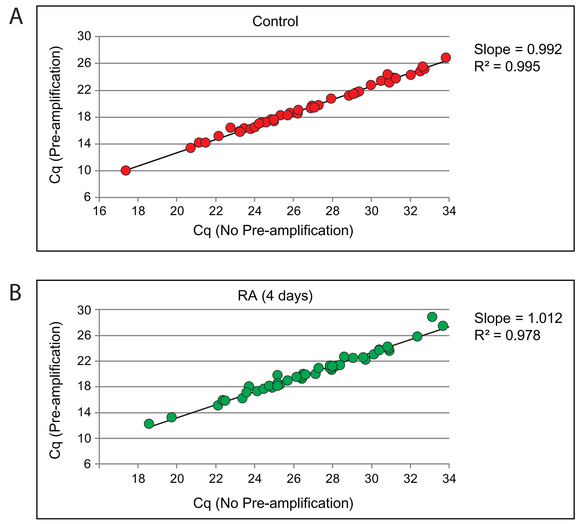
NTera2 cells (NT2) are a well established model system of human stem cell behavior. When treated with retinoic acid (RA), NT2 differentiate into neurons. During differentiation the expression of stem cell biomarkers decrease and the expression of neural biomarkers increase (1, 2).

Workflow: Isolation of RNA and protein from small samples



Analysis of RNA

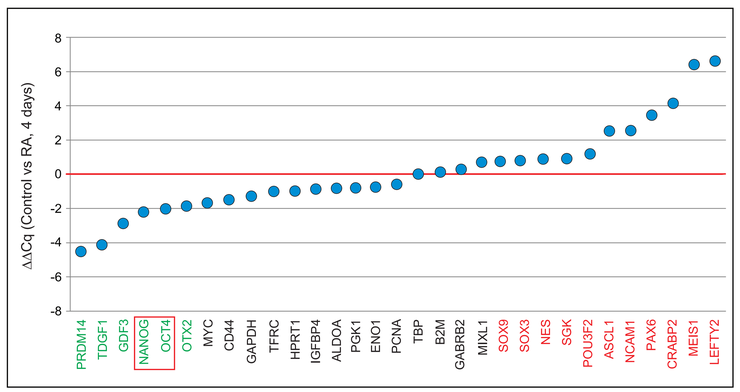
Pre-amplification of SingleShot lysates maintains target gene expression levels



cDNA was prepared from SingleShot lysates using iScript Advanced. A portion of the cDNA samples were pre-amplified with SsoAdvanced PreAmp Supermix using a panel of pluripotency and neural differentiation gene targets. Real-time PCR was performed with the PreAmp and no PreAmp (standard cDNA) samples and the Cq values were plotted. (A) Analysis of control NT2 cells; (B) analysis of NT2 cells treated with RA for 4 days. We observe an excellent correlation between the pre-amplification and no pre-amplification Cq values. This demonstrates that PreAmp works with SingleShot lysates and that the target gene expression levels are faithfully maintained.

Identification of genes regulated during early differentiation

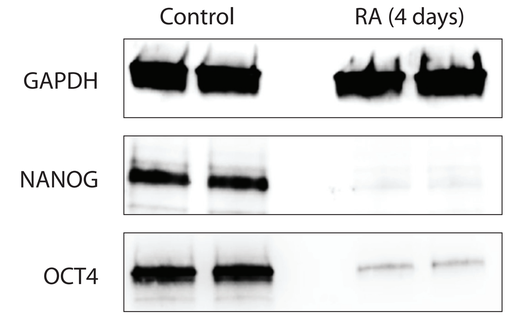
Genes involved in pluripotency and neural differentiation show regulated expression



Target gene expression was normalized with TBP and analyzed to identify genes regulated during stem cell differentiation. ΔΔCq values comparing the control and 4 day RA NT2 samples for selected target genes are shown. Green type indicates known pluripotency genes that are suppressed during differentiation. Red type indicates known neural genes that are induced during differentiation. OCT4 and NANOG are key pluripotency genes whose RNA is significantly down-regulated. We chose to investigate if the protein level of these biomarkers is also suppressed.

Analysis of protein

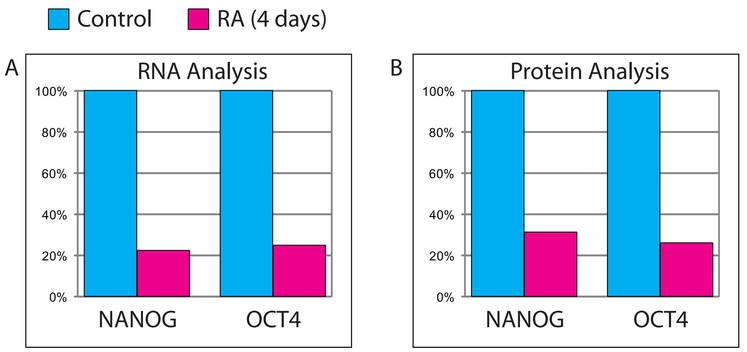
Western analysis of SingleShot lysates confirms protein regulation in early differentiation



The NT2 SingleShot samples were analyzed using Bio-Rad's V3 Western Workflow to quantify the amount of GAPDH, NANOG and OCT4 protein. We observe that the level of GAPDH protein is relatively constant between the samples. However, the level of NANOG and OCT4 protein is significantly decreased in NT2 cells treated with RA for 4 days. This data is expected and consistent with the results of our RNA analysis.

Correlation between RNA and protein expression

NANOG & OCT4 RNA and protein have similar levels of suppression in early differentiation



We quantified the suppression of NANOG and OCT4 RNA and protein, and present the results as bar graphs. TBP was used to normalize target RNA expression; total protein was used to normalize target protein expression. We observe an excellent correlation between the RNA and protein results, this indicates that post-transcriptional mechanisms are likely not involved in NANOG and OCT4 regulation by RA.

Summary and conclusions

We have developed a new workflow that allows for quantification of both RNA and protein from the same small sample. We envision that this workflow can enable streamlined analysis and verification of transcriptional and post-transcriptional regulatory changes at both the mRNA and protein level in samples that are typically refractory to such analysis.

References

1. Andrews, P.W. 1984. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev. Biol. 103:285–293.

2. Pleasure, S.J., and V.M. Lee. 1993. NTera2 cells: a human cell line which displays characteristics expected of a human committed neuronal progenitor cell. J. Neurosci. Res. 35:585–602.