

Comparison of 1 cycle and 2 cycle RNA Labelling Methods for Microarray-based Transcription Profiling Analysis with Laser Capture Microdissected Samples.

Simon Plummer¹, Sheona Smith¹, Tara Hill², Steve Kain³, Ulrich Sauer⁴.

¹CXR Biosciences Ltd, James Lindsay Place, Dundee UK; ²Agilent Technologies UK Ltd, Stockport, UK; ³Agilent Technologies Inc, Santa Clara, USA; ⁴PALM Microlaser Technologies, Bernried, Germany.

Introduction

Understanding molecular mechanisms of *in vivo* toxicity as reflected by parallel changes in gene expression requires techniques that are capable of measuring alterations in cell-types that are relevant to the toxicity. Collection of RNA from tissue sections to perform microarray-based transcription profiling analysis is time-consuming due to the amounts of RNA required to perform this technique. To determine the feasibility of applying whole genome microarray-based transcription profiling analysis to laser microdissected tissue sub-regions for mechanistic studies of rat fetal testes toxicity we have compared data generated using 1 cycle (1R) and 2 cycle (2R) RNA labelling protocols. Evaluation of the biological significance of the data generated by a 2R protocol in relation to the conventional 1R protocol has enabled the assessment of the utility of the more time-efficient 2R protocol for investigating toxicological mechanisms.

Experimental outline

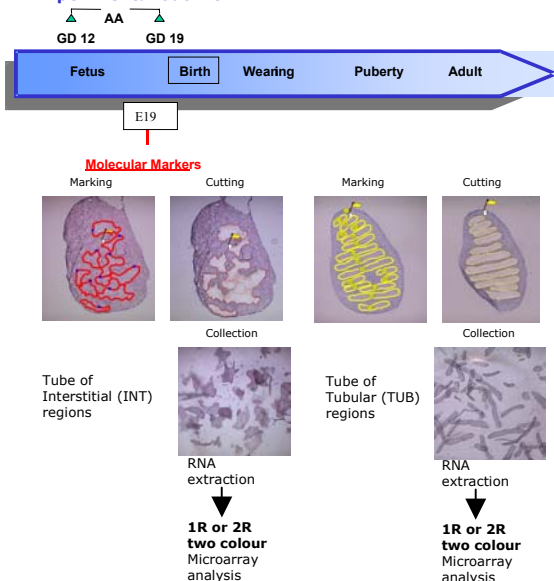


Figure 1: Structure of experiment. Male rats were exposed *in-utero* to anti-androgen (AA) on gestational days (GD)12-19. Transcription profiling analysis was performed on RNA extracted from laser microdissected foetal testes INT and TUB regions at GD 19. Laser microdissection was performed on a PALM microbeam (PALM, Bernried, Germany)

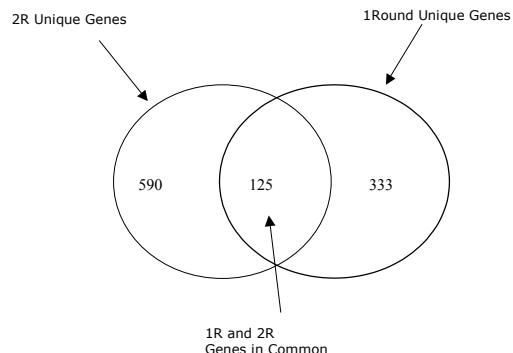
25ng or 500pg total RNA isolated from INT was amplified to aRNA through 1 or 2 cycles (1R or 2R), respectively, with the EpicentreTargetAmp 2-Round aRNA Amplification Kit 2.0. aRNA was directly labelled with Cy3 or Cy5 using a Kretech ULS aRNA Fluorescent Labelling Kit and hybridised on a Whole Rat Genome 60mer oligo microarray (Agilent #G4131A). Separate hybridisations were performed with RNA from foetal testes of 3 different *in utero* DBP-exposed litters against a pool of RNA isolated from foetal testes of 3 different control (vehicle exposed) litters. We included 'dye' swap replicates giving a total of 5 microarrays (5 data points per gene) for each labelling method.

ANOVA analysis (Rosetta Luminator[®] software) on data from each group of 5 arrays was used to select 'Signature genes' from each protocol ($P < 0.001$). The compare biosets function of Rosetta Luminator software was used to compare the two signature gene lists. This analysis identified genes that were **uniquely** or **commonly** identified in the 1R and 2R signature lists, Figure 2.

Ingenuity Pathways Analysis[®] software was used to assess the biological significance of the gene expression changes in the 1R and 2R signature lists.

Transcription profiling results

ANOVA analysis of microarray data (5 data points per gene) from the INT region identified 458 and 715 genes that were significantly regulated ($P < 0.001$) after one and two rounds of amplification, respectively (Figure 2). Compare biosets analysis (Luminator) shown in the Venn diagram below (Figure 2) indicate that only ~25% of signature genes were in common between one and two round protocols.



Inter-array variability assessed using Luminator compare plots.

Correlation coefficients in signature genes identified with replicate arrays were 0.73 and 0.88 with the 1R and 2R protocols, respectively. Figure 3

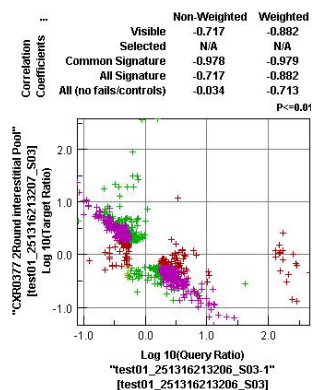


Figure 3. Luminator compare plots showing correlation between signature genes identified in replicate arrays (dye swaps) hybridised with RNA labelled using the 2R protocol. Correlated genes are highlighted in PURPLE.

Ingenuity Pathways Analysis results

A comparison between the 1R and 2R signature genes for the interstitial the region showed the same biological functions were identified as most significantly overrepresented in the two datasets, Figure 4.

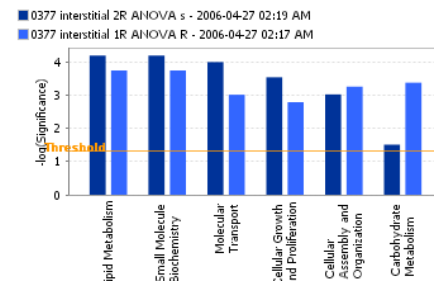


Figure 4. Histogram showing a comparison of biological functions assigned to genes that were overrepresented in the 1R and 2R datasets. Fishers Exact Test was used to calculate a p-value determining the probability that each biological function assigned to that data set is due to chance alone.

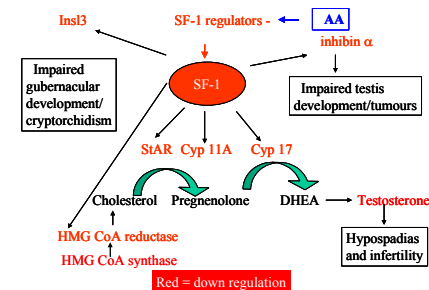


Figure 5. Hypothesis surrounding the mechanism of AA-induced testicular dysgenesis.

Summary and Conclusions

- The 2R labelling protocol identified ~60% more signature genes than the 1R protocol
- ~25% of signature genes were common to the 1R and 2R signature lists
- Biological interpretation of the data generated by both methods reached similar conclusions.
- Due to differences in the numbers of signature genes identified by 1R and 2R methods, it would not be advisable to 'mix' the two methods in a single study.