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## Data in Brief

# Differential RNA-seq analysis comparing APC-defective and APC-restored SW480 colorectal cancer cells



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## ABSTRACT

The *adenomatous polyposis coli* (APC) tumour suppressor gene is mutated in about 80% of colorectal cancers (CRC) Brannon et al. (2014) [1]. APC is a large multifunctional protein that regulates many biological functions including Wnt signalling (through the regulation of beta-catenin stability) Reya and Clevers (2005) [2], cell migration Kroboth et al. (2007), Sansom et al. (2004) [3,4], mitosis Kaplan et al. (2001) [5], cell adhesion Faux et al. (2004), Carothers et al. (2001) [6,7] and differentiation Sansom et al. (2004) [4]. Although the role of APC in CRC is often described as the deregulation of Wnt signalling, its other biological functions suggest that there are other factors at play that contribute to the onset of adenomas and the progression of CRC upon the truncation of APC. To identify genes and pathways that are dysregulated as a consequence of loss of function of APC, we compared the gene expression profiles of the APC mutated human CRC cell line SW480 following reintroduction of wild-type APC (SW480 + APC) or empty control vector (SW480 + vector control) Faux et al. (2004). Here we describe the RNA-seq data derived for three biological replicates of parental SW480, SW480 + vector control and SW480 + APC cells, and present the bioinformatics pipeline used to test for differential gene expression and pathway enrichment analysis. A total of 1735 genes showed significant differential expression when APC was restored and were enriched for genes associated with cell polarity, Wnt signalling and the epithelial to mesenchymal transition. There was additional enrichment for genes involved in cell–cell adhesion, cell–matrix junctions, angiogenesis, axon morphogenesis and cell movement. The raw and analysed RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE76307. This dataset is useful for further investigations of the impact of APC mutation on the properties of colorectal cancer cells.

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Specification	
Organism/cell line/tissue	SW480 Human epithelial cell line
Sex	Male
Sequencer or array type	Illumina HiSeq RNASEQ
Data format	Raw and analysed
Experimental factors	Colorectal cancer cell line with/without functional APC
Experimental features	Determine gene expression alterations in the presence/absence of APC gene in the colorectal cancer context.
Consent	NA
Sample source location	Melbourne, Australia

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## 1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76307>.

## 2. Experimental design, material and methods

### 2.1. Cell culture and RNA-extraction

The SW480, SW480 + APC (SW480APC.15) and SW480 + control (SW480control.7) cells have been previously described [6]. The cells were thawed from liquid nitrogen and grown in RPMI supplemented with 0.001% thioglycerol, 1 µg/ml hydrocortisone, 0.025 U/ml insulin, 10% foetal calf serum and 1% penicillin/streptomycin (SW480 cells)

**Table 1**  
Summary sequencing statistics for RNA-seq data.

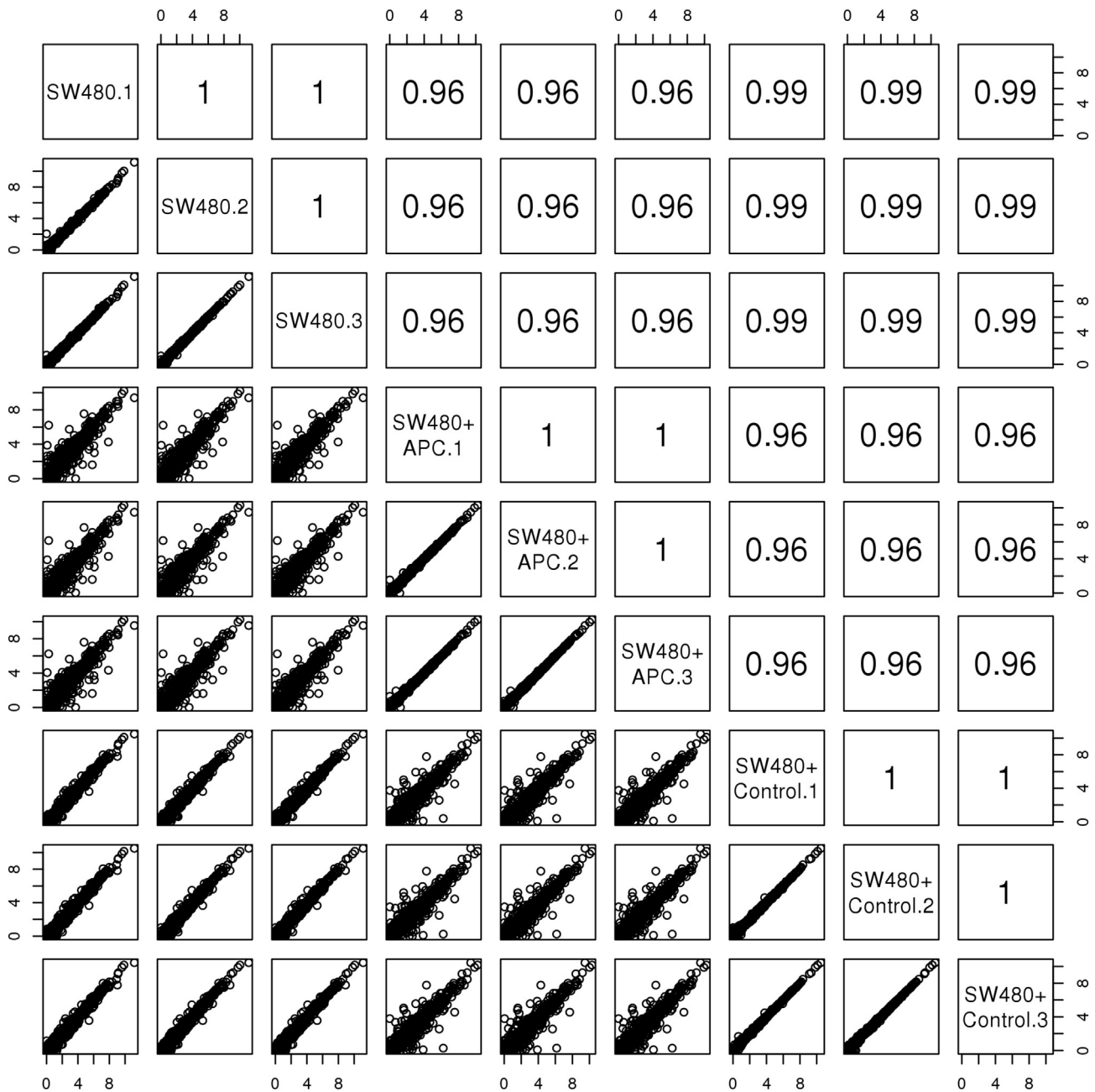
Sample	Replicate	Raw reads	Trimmed reads	Aligned reads
SW480 + APC.1	1	22,518,756	21,966,108	21,050,021
SW480 + APC.2	2	21,381,045	20,806,066	19,682,638
SW480 + APC.3	3	20,825,899	20,295,588	19,438,311
SW480 + Control.1	1	22,596,671	22,056,529	21,039,650
SW480 + Control.2	2	19,832,005	19,354,879	18,551,827
SW480 + Control.3	3	23,352,479	22,793,011	21,855,257
SW480.1	1	22,075,530	21,528,124	20,699,835
SW480.2	2	22,389,667	21,835,786	20,798,952
SW480.3	3	22,563,469	22,007,875	21,000,302

plus 1.5 mg/ml G418 (SW480 + APC and SW480 + control cells). Cells were passaged three times before being plated onto 100 mm tissue culture plates at a density of  $3.35 \times 10^5$  for SW480 and SW480 + control cells and  $2 \times 10^5$  for SW480 + APC cells, in triplicate. Seventy two

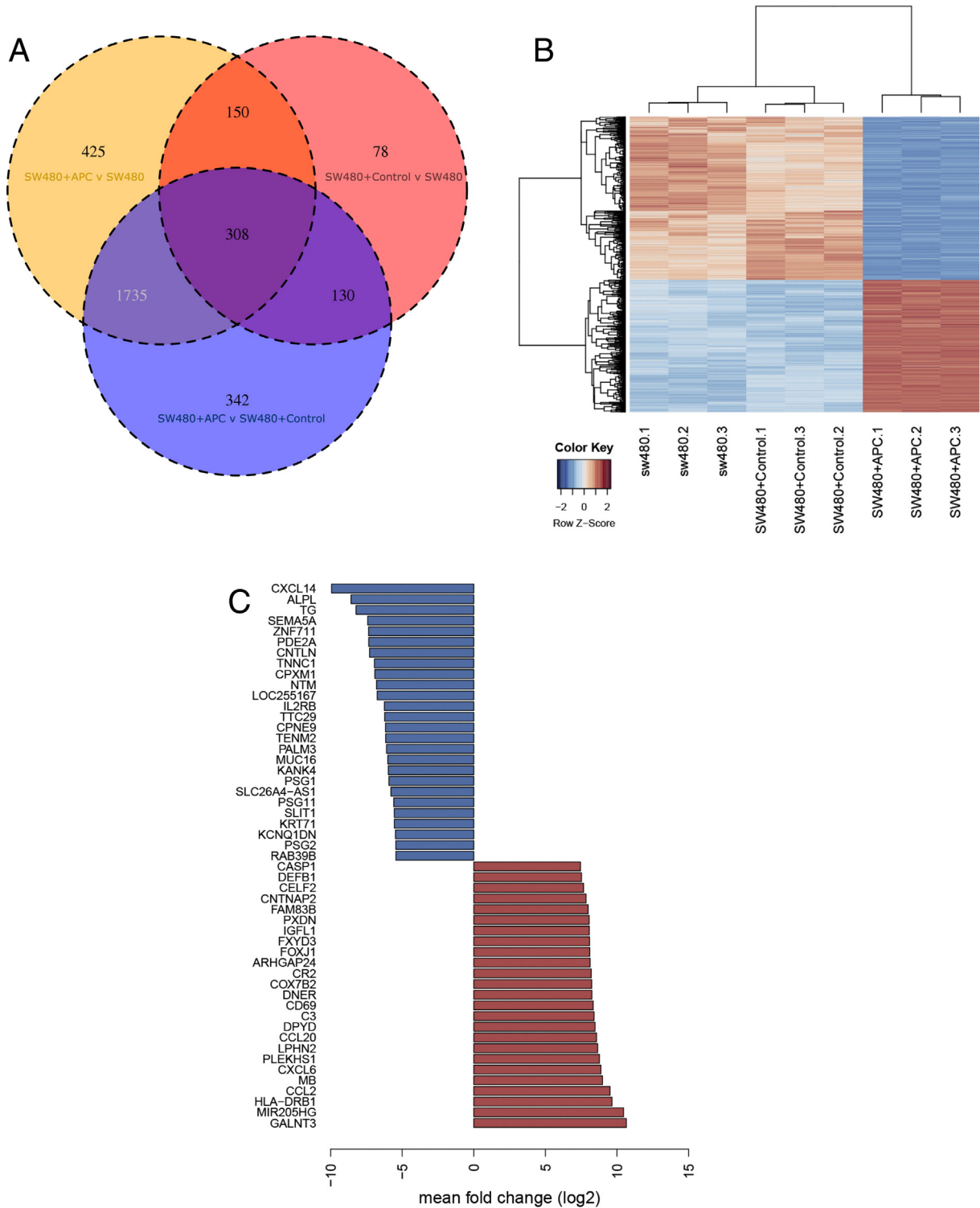
hours later RNA was extracted from the cells using the RNAspin Mini RNA isolation kit (Illustra 25-0500-70).

2.2. Sequencing, mapping and normalisation

RNA samples were prepared for sequencing using the Illumina TruSeq RNA Library Preparation Kit v2. Libraries were pooled (9 samples per pool) and clustered using the Illumina cBot system with TruSeq SR Cluster Kit v3 reagents, followed by sequencing on the Illumina HiSeq 2000 system with TruSeq SBS Kit v3 reagents (101 cycles) at the Australian Genome Research Facility. Each sample was sequenced to a depth of approximately 20 million reads (see Table 1). Sequencing reads were quality assessed and trimmed for any remaining sequencing adaptor using Trimmomatic (v0.22) [8]; reads smaller than 50 bp were removed. Reads were subsequently aligned to human genome build Hg19 using Tophat (v2.0.6.Linux\_x86\_64) [9] with parameters -g 1



**Fig. 1.** Scatter plots of log<sub>2</sub> expression values (RPKM) for 1000 randomly selected genes between cell line samples. Pearson correlation coefficients are indicated in the top half of quadrant.



**Fig. 2.** (a) Venn diagram indicating differentially expressed genes overlapping between the samples. (b) A heatmap displaying the differentially expressed genes in SW480 + APC compared to SW480 and SW480 + control (from the 1735 subset shown in the Venn diagram). The heatmap was drawn using  $\log_2$  (+1 offset) expression values, mean centred and scaled by gene. Gene and sample dendrograms were generated using divisive hierarchical clustering (DIANA). (c) Barplot of top 25 upregulated (red) and downregulated (blue) genes in SW480 + APC compared to SW480 and SW480 control cells. Values plotted are mean ( $\log_2$ ) fold change in gene expression of SW480 + APC vs SW480 and SW480 + APC vs SW480 + control.

and the corresponding transcript gtf file. Reads aligning to transcripts were counted and quantified by RPKM using the RNASEQC software [10]. The expression level of the canonical (longest) transcript was taken as representative of gene level expression. Sample expression levels between replicates showed a high correlation (Pearson correlation coefficient > 0.9) (Fig. 1).

### 2.3. Differential gene expression

Differential gene expression analysis was conducted using read counts with the Bioconductor edgeR package [11]. Genes represented with a frequency of > 1 read per million in at least one sample were considered as expressed, limiting the analysis to 13,965 genes. The edgeR

**Table 2**

Significantly enriched pathways from DAVID analysis of the SW480/SW480 + control and SW480 + APC cell lines

Cluster function	Enrichment score
Membrane proteins	7.621
Cell–matrix junctions	4.610
Cell–cell junctions	4.348
Angiogenesis	5.516
Axon/neuron morphogenesis	3.684
Cell movement	2.983
Organogenesis/development	2.566
Immune system development	2.404
Wnt signalling	2.388
Alkaloid responses	2.327
Carbohydrate binding	2.269
Muscle development	2.052
Epithelial cell development	2.136
Cytoskeleton	1.956
Epidermal cell development	1.939

GLM approach was subsequently applied to determine the differential expression between groups using TMM normalisation [12]. Three comparisons were performed:

1. SW480 + APC v SW480 (restored APC against defective APC).
2. SW480 + control v SW480 (control vector against defective APC).
3. SW480 + APC v SW480 + control (restored APC against control vector).

FDR adjustment was performed to account for multiple testing. Genes with an adjusted two-sided P-value of less than 0.05 and showing a greater than 2-fold change in expression were considered differentially expressed.

Gene expression changes associated with loss of the APC gene and unrelated to introduction of empty vector were identified by overlapping the differential gene sets from the three comparisons as shown in the Venn diagram in Fig. 2a. We identified a total of 1735 genes specific to APC loss, all of which showed concordant up- or down-regulation in SW480 + APC v SW480 and SW480 + APC v SW480 + control cells, represented as a heatmap in Fig. 2b. The top 25 upregulated and down-regulated genes comparing SW480 + APC cells to the average expression score of SW480 and SW480 + control cells are shown as a barplot in Fig. 2c. All differentially expressed genes with their associated log<sub>2</sub> fold change values cross-referenced to the Venn diagram are summarised in Supplementary Table 1. Upregulated genes in the SW480 + APC cells include the Rho GTPase-activating protein 24, ARHGAP24, a protein involved in cell polarity, cell morphology and cytoskeletal organisation [13] and the mir-205 host gene, MIR205HG, an established tumour suppressor [14]. Downregulated genes in the SW480 + APC cells include semaphorin 5A, SEMA5A, an axonal regulator molecule associated with tumour growth, invasion and metastasis [15].

#### 2.4. Pathway enrichment analysis

Functional category enrichment analysis was performed using DAVID [16] to test gene ontology (GO) categories. Enriched GO categories describing the same function were combined to within a single cluster to reduce redundancy in the results. The enrichment score was calculated as per the DAVID cluster enrichment score; by calculating the mean -log<sub>10</sub> GO category P-value within a cluster. A cluster

enrichment score threshold of 1.3 was applied, corresponding to a significant cluster enrichment cut-off of  $P < 0.05$ . The top 15 highest scoring clusters are shown in Table 2 and include functions important in cell–cell adhesion, cell–matrix junctions, angiogenesis, axon morphogenesis and cell movement. Gene details pertaining to all significant GO clusters are available in Supplementary Table 2.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2016.02.001>.

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