3Bseq: A Cost-Efficient 3′ Bulk RNA-seq Method for Rapid Transcriptome Profiling

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Incorporating sample barcodes into poly(A)-captured mRNA during first-strand cDNA synthesis enables the pooling of dozens – potentially hundreds – of cDNA libraries into a single reaction tube for subsequent NGS library preparation. When combined with 3′-end sequencing, this workflow provides a rapid and highly cost-efficient approach for bulk RNA-seq, allowing robust detection of major transcriptional signatures even at low sequencing depth. Here, we demonstrate the sensitivity and performance of the 3Bseq method using the human THP-1 monocyte cell line stimulated with lipopolysaccharide (LPS) derived from gram-negative bacteria, a well-established activator of innate immune responses.

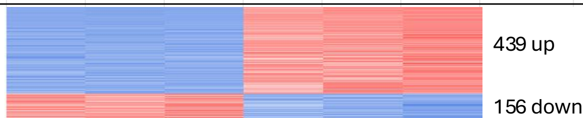
Methods

- THP-1 cells were cultured in 12-well plates using RPMI 1640 medium supplemented with 10 % Foetal bovine serum (FBS), 1% Penicillin/Streptomycin and 2% Gibco GlutaMAX. Cells were stimulated with 100ng/ml lipopolysaccharide (LPS) for 4 hours.
- Cells were lyzed with Qiagen RLT buffer and total RNA was extracted from three unstimulated controls and three LPS-stimulated wells using the Qiagen RNeasy Mini kit. RNA quantity and integrity were assessed with a Qubit fluorometer and Agilent TapeStation (RIN 8.4-8.8).
- Reverse transcription was performed using 10 ng of total RNA and a poly(T) capture oligo containing a PCR handle, well-specific sample barcode, and unique molecular identifier (UMI), together with a template-switching oligo.
- Full-length cDNAs were treated with Exonuclease I, amplified, and subsequently pooled into a single tube.
- One microgram of the purified, quality-controlled pooled cDNA was tagmented using the Illumina Nextera XT kit.
- 3′-terminal fragments were selectively enriched by index PCR using Nextera-compatible Illumina P7 index primers and custom P5 primers complementary to the PCR handle.
- Purified libraries, with a median fragment size of approximately 630 bp, were sequenced on an Element Biosciences AVITI instrument using a 2 × 75-cycle kit. Read 1 covered the sample barcode and UMI, and Read 2 captured the transcript sequence.
- Raw sequencing data were quality-filtered, demultiplexed, aligned to the genome, and UMI-collapsed using a modified Drop-seq pipeline (1). The resulting transcript count matrix was further analyzed using CSC Chipster, DESeq2, and Enrichr tools.

Results

Sequencing yielded an average of **2.5M raw reads**, **1.7M aligned reads** and **949,662 transcript-level UMIs** per sample, representing **15,199 detected genes**.

Metrics	CTRL1	CTRL2	CTRL3	LPS1	LPS2	LPS3	Pool	Average/sample
Raw reads							15205100	2534183
Aligned reads	1310941	1545825	1852524	2341441	1485680	1803736	10340147	1723358
Transcripts	768711	883779	1008551	1227939	826212	982780	5697972	949662
Genes detected	14515	15142	15765	15967	14592	15213		15199



Differential expression analysis using DESeq2 identified **595 genes** with significant expression changes following LPS stimulation. Of these, **439 genes were upregulated** and **156 were downregulated** in response to LPS.

NAME	CTRL1	CTRL2	CTRL3	LPS1	LPS2	LPS3	log2FC	pvalue
SOD2	51	73	66	2110	2617	2589	5,2	6,60E-251
CCL3L3	33	37	37	1738	2458	2072	5,8	7,50E-208
CCL4	12	20	18	2249	3021	2646	7,2	2,20E-200
NFKBIA	106	95	79	1237	1479	1457	3,9	4,30E-162
CCL3	18	23	15	1305	1714	1497	6,3	3,90E-161
CCL4L2	22	10	20	2086	2864	2527	7,1	2,90E-118
TNFAIP6	6	9	6	1335	1597	1597	7,6	8,70E-113
TNIP1	103	94	121	890	804	869	3,0	9,30E-107
SLC2A6	38	31	28	599	541	567	4,1	4,20E-101
WTAP	96	86	101	699	762	866	3,0	1,00E-91
ATP2B1	41	33	48	480	564	589	3,7	3,80E-85
TNF	40	20	34	489	487	458	3,9	8,00E-84
BCL2A1	10	13	7	469	655	666	5,8	6,50E-79
TNFAIP2	14	15	20	304	343	371	4,3	1,50E-62
CXCL8	6	1	4	653	910	807	7,6	1,20E-59
TREM2	325	299	287	171	160	175	-0,8	2,20E-09
STING1	164	150	154	81	48	59	-1,2	6,60E-10
TSC22D3	174	157	164	85	56	66	-1,2	3,60E-10
DUSP7	141	129	147	55	62	50	-1,3	2,50E-10
ANKRD13I	101	98	107	41	24	32	-1,5	1,80E-10
RGS19	140	110	134	50	49	43	-1,4	1,10E-10
CDC20	590	525	536	366	257	287	-0,8	8,90E-11
ERVH-3	522	540	515	323	295	326	-0,7	8,10E-11
CCR1	90	90	96	33	19	20	-1,8	1,30E-11
MCEMP1	314	332	326	169	179	152	-0,9	4,00E-12
CITED2	344	267	330	148	118	161	-1,1	1,20E-12
DHRS9	130	154	175	42	53	37	-1,7	7,60E-16
CCR2	118	124	140	10	15	11	-3,5	3,60E-27
CEBPA	967	907	1000	392	459	346	-1,3	2,20E-28
ZFP36L2	666	523	582	234	210	203	-1,4	1,50E-28

Top 15 upregulated and top 15 downregulated genes following LPS stimulation, shown with normalized read counts.

Top 10 enriched pathways derived from the set of 595 differentially expressed genes.

Reactome Pathways 2024	p-value
Cytokine Signaling in Immune System	3,55E-27
Signaling by Interleukins	4,25E-24
Immune System	6,76E-22
Interleukin-10 Signaling	1,47E-17
Innate Immune System	1,12E-10
Neutrophil Degranulation	4,11E-10
Chemokine Receptors Bind Chemokines	8,65E-10
Toll Like Receptor 3 (TLR3) Cascade	2,19E-09
Toll Like Receptor 4 (TLR4) Cascade	3,03E-09
MyD88-independent TLR4 Cascade	4,04E-09

Conclusions

Even at shallow sequencing depth, the 3Bseq workflow reliably captured the expected gene signatures and immune-related pathways induced by LPS stimulation. The results closely matched findings reported in previous studies examining LPS responses in monocytes (2), underscoring both the accuracy and robustness of the approach.

3Bseq has been refined and successfully applied across a wide range of RNA-seq projects since 2018 (3–13), demonstrating its maturity, versatility, and proven performance. Together, these results highlight 3Bseq as a sensitive, efficient, and cost-effective solution for high-throughput transcriptome profiling – ideal for customers seeking affordable, rapid, and reliable RNA-seq data generation.

References (PMID numbers):
1) 26000488, 2) 41601641, 3) 30519244, 4) 30787919, 5) 31843201, 6) 32032584, 7) 33863907, 8) 35685923, 9) 36824489, 10) 37059703, 11) 37085458, 12) 37155564, 13) 39891027

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