TECHNICAL NOTE

Targeted Gene Expression Custom Panel Performance Metrics and Best Practices

Introduction

The Targeted Gene Expression product is a modular enrichment kit designed to enrich libraries for relevant genes, while decreasing sequencing requirements by up to 90%. With this product, panels of baits are used to target a selection of genes. When used to enrich whole transcriptome libraries, these panels enable significantly reduced sequencing costs while accurately reflecting gene expression information from the corresponding whole transcriptome analysis (WTA) parent library for targeted genes. 10x Genomics provides pre-designed panels of baits. For customers interested in creating custom panels, the 10x Genomics Custom Panel Designer enables two customization options: adding genes to pre-designed panels (add-on panels) and designing fully custom panels. Panels can be a combination of human genes and exogenous sequences. This Technical Note describes the technical performance for each customization option and outlines best practices for panel design and sequencing.

Method

Each customization option was tested by choosing sets of genes arbitrarily to use as either add-on panels or as fully custom panels. Each custom bait was designed using the Custom Panel Designer available on the 10x Genomics website. Pre-designed panels used in this Technical Note were the Human Pan-Cancer Panel (PN-1000247), the Human Immunology Panel (PN-1000246), and the Human Gene Signature Panel (PN-1000245).

Targeted libraries were generated using the reagents and protocol described in the Targeted Gene Expression Reagent Kits User Guide (Document CG000293) using single cell gene expression libraries previously sequenced for WTA as shown in Figure 1. Add-on panels were tested with Chromium Single Cell 3' Gene Expression (v3.1) and 5' Gene Expression (v1.1, v2). Fully custom panels were tested with Chromium Single Cell 3' Gene Expression (v3, v3.1) and 5' Gene Expression (v1, v1.1, v2) libraries. Panels were tested across 53 human samples. A list of sample types is provided in the Appendix.

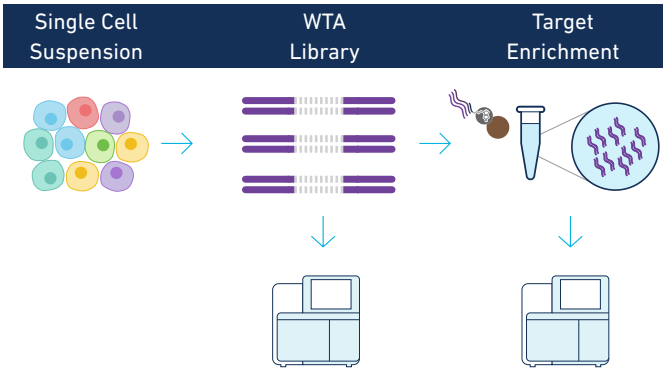

Figure 1. Targeted Gene Expression workflow overview. Whole Transcriptome Analysis (WTA) libraries are generated from single cell suspensions. WTA libraries are incubated with biotinylated baits from Targeted Gene Expression panels to generate targeted libraries. WTA and targeted libraries are both sequenced. These data are used for metrics comparisons.

Add-on Panel Experiment

Pre-designed panels customized with 10 or 200 additional genes (add-on panel) were generated to test the number of genes in the add-on panel as well as relative expression level as shown in Table 1. Bait sequences for each add-on panel were obtained from the Custom Panel Designer and ordered from Integrated DNA Technologies (IDT) as NGS Discovery Pools. A list of genes for each add-on panel is provided in the Appendix.

Add-on Panel	Number of Genes	Expected Relative Expression Level	Number of Samples
Cancer-High	10	High	10
Cancer-Low	10	Low	9
Immune-High	10	High	4
Immune-Low	10	Low	9
Immune-200	200	Even Distribution	24

Table 1. Summary of add-on panels tested with pre-designed panels. Genes in Cancer and Immune add-on panels appear in the Human Pan-Cancer Panel and Human Immunology Panel respectively. Immune-200 genes span a range of expected expression levels. Samples were run in pulldown reactions with 1, 4, or 8 input libraries.

Fully Custom Panel Experiment

Fully custom panels were created by selecting a 10, 25, 50, 100, or 200 gene subset from the Human Immunology Panel. Genes were chosen at random; however, genes with very high or very low levels of expression were omitted. Fully custom panels were ordered from IDT as NGS Discovery Pools. A list of genes for fully custom panels is provided in the Appendix.

Fully Custom Panel	Expected Panel Expression Level	Number of Samples
Immune-10	0.10%	12
Immune-25	0.10%	16
Immune-25	0.25 - 1%	4
Immune-50	0.25 - 1%	11
Immune-50	2 - 4%	4
Immune-100	0.25 - 1%	18
Immune-200	0.25 - 1%	17
Immune-200	2 - 4%	9

Table 2. Summary of conditions tested for fully custom panels. Samples were run in pulldown reactions with 1, 2, 4, or 8 input libraries.

Different combinations of samples and fully custom panels were chosen to create reactions that represented panels with a range of expression levels (Table 2). The targeted-depth subcommand in Cell Ranger was used to calculate the "Fraction of Reads from Targeted Genes" in WTA libraries, and this value is used as a proxy for the expression level of the targeted genes in that sample (Expected Panel Expression Level). For more information, consult the Targeted Depth section of the 10x Genomics Support website.

Sequencing Depth

To calculate parent-targeted comparison metrics, both libraries were computationally downsampled and compared at the following depths:

Parent WTA Libraries: 20,000 or 50,000 read pairs per cell.

Targeted Gene Expression Libraries: two-fold the number of on target panel reads in the parent WTA library.

Example: Parent WTA library with 6% on target reads=6,000 read pairs per cell (0.06 x 50,000 x 2) for the associated target library. Sequencing libraries were computationally downsampled and compared at the read numbers described in the calculation above.

Data Analysis

Data from Targeted Gene Expression libraries were analyzed by comparing parent and targeted data on a matched (parent derived) barcode set, and metrics related to UMI recovery that compare parent WTA and targeted libraries were calculated over the cell-associated barcodes. In cases where add-on gene panels were used, metrics related to UMI recovery (targeted total UMI recovery, fraction targeted genes with ≥80% UMI recovery, and pseudo-bulk targeted UMI R^2) can be calculated over the add-on genes or pre-designed panel genes separately.

Metrics Analyzed

Targeting Metrics:

- **Reads mapped confidently to the targeted transcriptome**: Fraction of reads that mapped to a unique and targeted gene in the transcriptome. The read must be consistent with annotated splice junctions. These reads are considered for UMI counting. This metric can be calculated for the parent WTA library, the targeted library, or both, and does not require a matched WTA library control.
- **Targeted total UMI recovery**: Fraction of cell-associated, UMIs for all targeted genes recovered at the sequencing depth described above, calculated over cell-associated barcodes. Requires a matched WTA library control.
- **Fraction targeted genes with ≥80% UMI recovery**: Fraction of observed (defined as ≥ 10 UMI in WTA library) panel genes in a WTA library control for which ≥80% of UMIs are recovered in the matched targeted library at the sequencing depth described above. Requires a matched WTA library control.
- **Pseudo-bulk targeted UMI R^2**: Bulk UMI correlation R^2 value for observed genes (defined as ≥10 UMI in a WTA library). Requires a matched WTA library control. Pearson correlation coefficient of $\log_{10}$(1+ UMI counts) on cell-associated, targeted UMIs.

Cell calling concordance: A comparison of the number of cells detected, calculated as the number of cells detected in the targeted library divided by the number detected in the WTA library. Requires a matched WTA library control.

Results

Targeting Metrics - Add-on Panels:

Targeting metrics were assessed using similar criteria and thresholds for optimal performance used to demonstrate the performance of pre-designed panels as shown in Tables 3 and 4. Consult the Targeted Gene Expression Pre-designed Panel Performance Metric Technical Note (Document CG000345) for more information. All Single Cell 3' Gene Expression libraries passed the reads mapped confidently to the targeted transcriptome metric, and most (23/26) Single Cell 5' Gene Expression libraries passed the same metric as shown in Figure 2. Single Cell 5' Gene Expression libraries typically have a decreased fraction of reads mapped to the targeted transcriptome due to slightly higher levels of antisense reads present in these libraries compared to Single Cell 3' Gene Expression libraries.

Targeted total UMI recovery is a measure of how many mRNA molecules (i.e. UMIs for genes of interest) in the parent WTA library are recovered in the targeted library. For all libraries tested, this ratio is above 0.8 for entire panel (Figure 3A) and when analyzing pre-designed panel genes alone (Figure 3B) or add-on panel genes alone (Figure 3C).

UMI correlations between parent and targeted libraries typically exceeded the threshold of R^2 >0.9 for the entire panel, pre-designed panel genes alone, or add-on panel genes alone (Figure 4A-C) for all samples tested. In a few instances, this correlation for the add-on panel genes is lower than 0.9. This is often due to calculating the correlation coefficient over very few genes.

Examining UMI recovery on a gene-by-gene basis is another way to assess the evenness of enrichment for targeted genes. The distribution of UMI recovery values of a representative example is shown for each of the pre-designed/add-on panel combinations (Figure 5). The majority of targeted genes, whether from the pre-designed or add-on panel genes, have greater than 80% UMI recovery (dashed line). The narrow distribution of UMI recovery values across genes in the pre-designed and add-on panels demonstrates the even enrichment of the targeted genes. Genes with low expression show additional variability, primarily because 20,000 or 50,000 read pairs per cell is not sufficient to read all UMIs for these genes in the parent WTA library. The UMI recovery metric is less variable for genes with higher expression because the number of UMIs detected in the parent WTA library with a set number of read pairs is a better reflection of the true number of UMIs for those genes in the parent WTA library, resulting in UMI recovery values near 1. For genes with low expression, the high degree of enrichment provided by the targeted approach results in UMI recovery >1.

Pre-designed Panels Customized with Add-on Panels

Targeting Metric	Performance Threshold	Cancer-High % Pass	Cancer-Low % Pass	Immune-High % Pass	Immune-Low % Pass	Immune-200 % Pass
Reads Mapped Confidently to the Targeted Transcriptome	80% 70%	100%	100%	100%	89%	92%
Fraction Targeted Genes with ≥80% UMI Recovery	0.9	100%	100%	100%	100%	88%
Targeted Total UMI Recovery	0.8	100%	100%	100%	100%	100%
Pseudo-bulk Targeted UMI R^2	0.9	100%	100%	100%	100%	100%

Table 3. Percentage of pre-designed panels with add-on panels that meet optimal performance thresholds. The 80% performance threshold (red) applies to Single Cell 3' Gene Expression libraries, while the 70% performance threshold (blue) applies to Single Cell 5' Gene Expression libraries. Some Single Cell 5' Gene Expression samples are marginally below threshold due to elevated antisense reads. Pre-designed panels customized with add-on panels may require more sequencing depth for full UMI recovery.

Add-on Panels Alone

Targeting Metric	Performance Threshold	Cancer-High % Pass	Cancer-Low % Pass	Immune-High % Pass	Immune-Low % Pass	Immune-200 % Pass
Fraction Targeted Genes with ≥80% UMI Recovery	0.9	100%	67%	100%	100%	100%
Targeted Total UMI Recovery	0.8	100%	100%	100%	100%	100%
Pseudo-bulk Targeted UMI R^2	0.9	90%	78%	100%	100%	100%

Table 4. Percentage of samples that meet optimal performance thresholds based on add-on panels alone. For add-on panels with few genes, suboptimal performance of a single gene can prevent the sample from meeting performance thresholds. R^2 noise in small panels is due to the lower number of genes.

Targeting Metrics for Add-on Panels

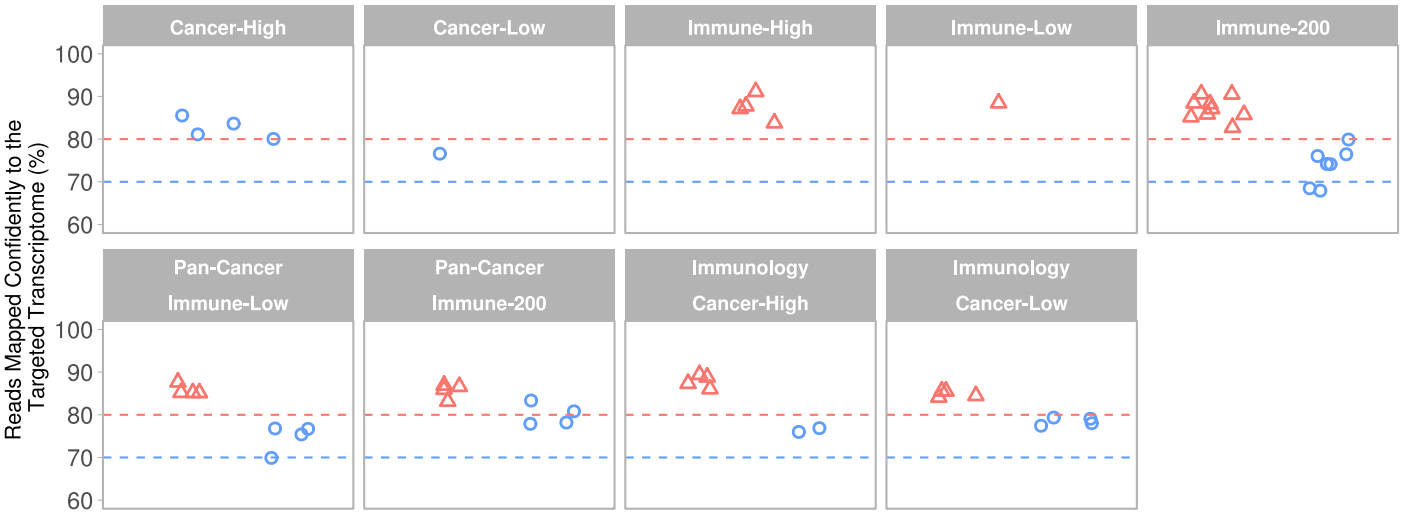

Figure 2. Reads mapped confidently to the targeted transcriptome for add-on panels. The names of the pre-designed panel and add-on panel used are indicated above each plot. Dashed line indicates threshold for optimal performance. Red triangles indicate Single Cell 3' Gene Expression libraries, blue circles indicate Single Cell 5' Gene Expression libraries. Thresholds differ for Single Cell 3' and 5' libraries for reasons described in the Results.

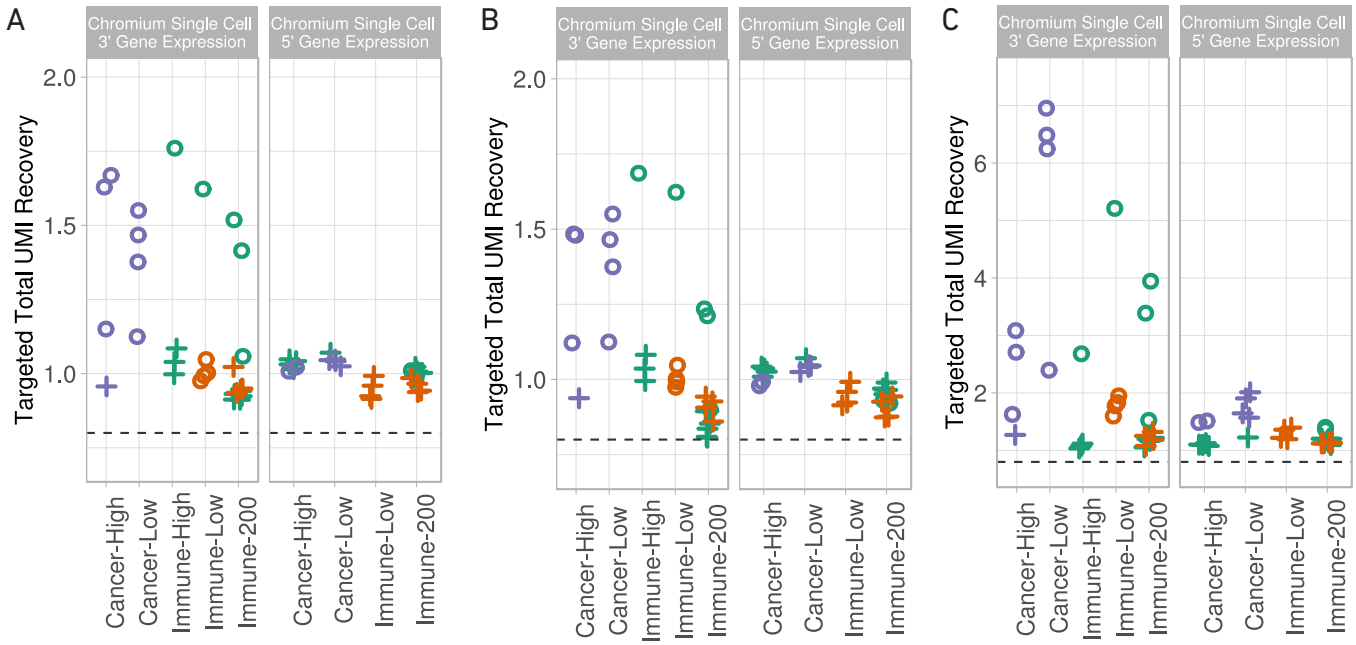

Figure 3. Targeted total UMI recovery for add-on panels, pre-designed panels or both. Each data point represents a single Targeted Gene Expression library for pre-designed panels customized with an add-on panel (A). The same data were calculated for the pre-designed panel alone (B) or the add-on panel alone (C). Data points are colored according to the pre-designed panel used: Human Gene Signature Panel (green), Human Pan-Cancer Panel (orange), Human Immunology Panel (purple). The targeted parent comparison was made using either 20,000 (circle) or 50,000 (plus sign) read pairs per cell from the parent WTA library. Dashed line indicates threshold for optimal performance.

Targeting Metrics for Add-on Panels

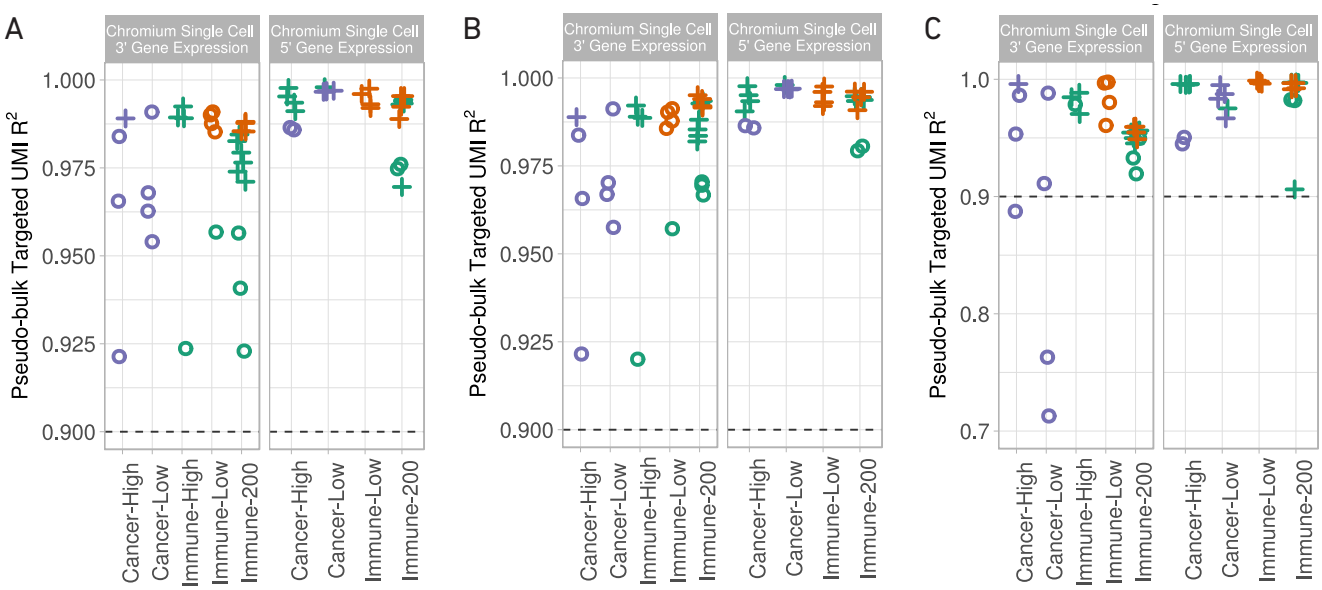

Figure 4. Pseudo-bulk targeted UMI R². Each data point represents a single Targeted Gene Expression library for pre-designed panels customized with an add-on panel (A). The same data were calculated for the pre-designed panel alone (B) or the add-on panel alone (C). Data points are colored according to the pre-designed panel used: Human Gene Signature Panel (green), Human Pan-Cancer Panel (orange), Human Immunology Panel (purple). The targeted parent comparison was made using either 20,000 (circle) or 50,000 (plus sign) read pairs per cell from the parent WTA library. In (C), the data point for one sample (A375 cell line sample with Gene Signature and Immune-Low panels) was omitted because only one gene of the add-on panel was observed in the parent WTA library; thus, pseudo-bulk targeted UMI R² cannot be calculated. Dashed line indicates threshold for optimal performance.

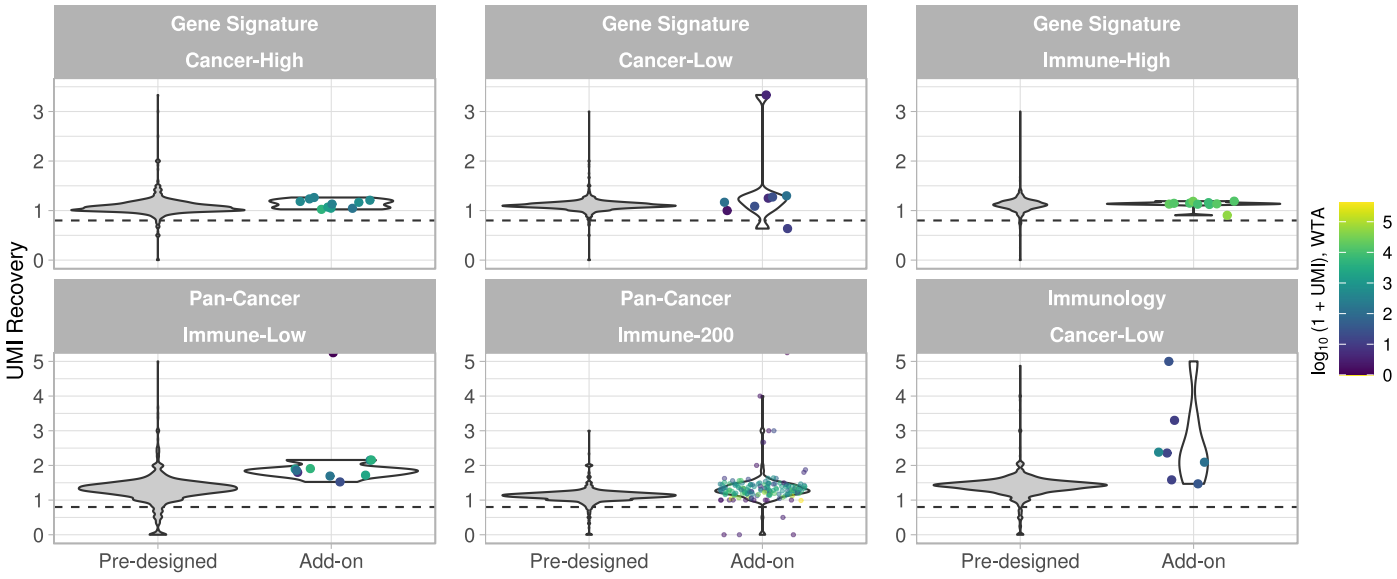

Figure 5. UMI recovery for 10 and 200 add-on panels. Representative samples for add-on panels tested. The names of the pre-designed panel and add-on panel used are indicated above each plot. UMI recovery distributions for pre-designed panel genes are shown separately from the add-on genes. Each data point represents the UMI recovery value for an individual gene and data points are color coded by expression level (i.e. the number of UMIs in the parent WTA library). Data points are omitted when no UMIs are detected for a targeted gene in both the WTA and the targeted library. Data points appear clipped at the y-axis when no UMIs are detected for a targeted gene in the WTA library, but have a non-zero UMI count in the targeted library. Dashed line indicates threshold for optimal performance.

Targeting Metrics - Fully Custom Panels

Targeting metrics were assessed using similar criteria and thresholds for optimal performance used to demonstrate the performance of pre-designed panels. Consult the Targeted Gene Expression Pre-designed Panel Performance Metric Technical Note (Document CG000345) for more information. Fully custom panels perform well based on these metrics (Table 5). The expression level of fully custom panels is determined by both the sample and the genes on the panel. Figure 6 demonstrates that the same panel of genes will have different expression levels in different samples; thus, the fraction of reads in a WTA library for that panel of genes will vary and is only weakly correlated with the number of genes on the panel.

Reads confidently mapped to the targeted transcriptome values are generally on par with the pre-designed panels, but can be variable when the expression level of panel genes is low (Figure 7A). This can result from the enrichment of related, but non-targeted, genes and is related to the selection of genes in the fully custom panel (refer to Discussion). Despite a higher percentage of reads mapped to non-targeted genes, fully custom panels perform well as shown by targeting metrics that measure how accurately the targeted library reflects gene expression information from the corresponding WTA library (Figure 7B-D).

Samples that did not meet the optimal performance threshold for fraction targeted genes with ≥80% UMI recovery (Figure 7D) often had low expression of panel genes. However, these samples were able to achieve the optimal performance threshold with additional sequencing of the targeted sample. Figure 7E also shows fraction targeted genes with ≥80% UMI recovery, but after discarding non-panel reads from the targeted library and downsampling the targeted library at two-fold the number of on target panel reads in the parent WTA library. By considering only the on target panel reads in the targeted library, the fraction targeted genes with ≥80% UMI recovery metric is decoupled from reads mapped confidently to the targeted transcriptome.

The amount of additional sequencing used to make the comparison shown in Figure 7E often represents, at most, a few hundred additional reads per cell. For example, the values in Figure 7D assess the targeted library at two-fold the number of on target panel reads in the parent WTA library as described in the Methods. This equates to 100 read pairs per cell for a fully custom panel where expression of panel genes in a WTA library at 50,000 read pairs per cell is 0.1% (0.001 x 50,000 x 2 = 100). For the sample with the lowest reads mapped to the targeted transcriptome in this data set (36%), the parent-targeted comparison is made considering only reads that map to panel genes from the targeted library (Figure 7E).

The targeted library is assessed at 278 read pairs per cell (100/0.36 = 277.7) and now achieves expected performance.

Targeting Metric	Performance Threshold	% Pass	Mean ± stdev
Reads Mapped Confidently to the Targeted Transcriptome	80% 70%	77%	79±12%
Fraction Targeted Genes with ≥80% UMI Recovery	0.9	91%	0.95±0.05
Targeted Total UMI Recovery	0.8	100%	1.12±0.19
Pseudo-bulk targeted UMI R^2	0.9	100%	0.98±0.03
Cell calling concordance	± 25% of parent WTA library detected	88%	0.91±0.14

Table 5. Percentage of fully custom panels that meet optimal performance thresholds. Sample statistics are computed over all sample-panel combinations (N=91). Samples were combined into pulldown reactions to maintain similar expression levels for targeted genes based on the percentage of library reads in the parent WTA library mapped to the respective panel. The 80% performance threshold (red) applies to Single Cell 3' Gene Expression libraries, while the 70% performance threshold (blue) applies to Single Cell 5' Gene Expression libraries.

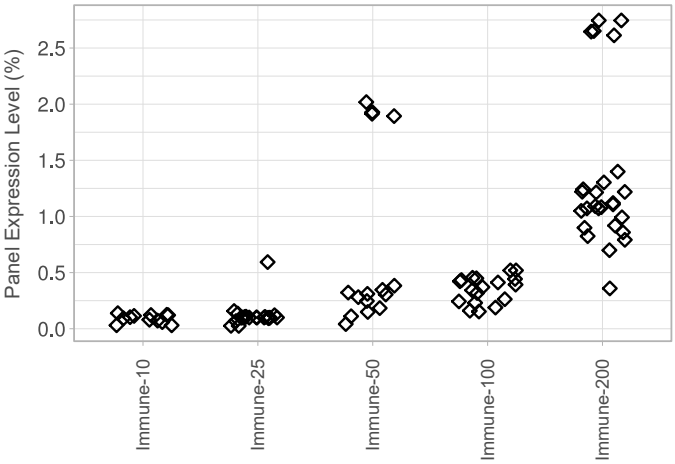

Figure 6. Expression level of a fully custom panel. Each data point represents a single WTA library. Panel Expression Level is the sum of the fraction of reads in the parent WTA library across targeted genes in a given panel.

Targeting Metrics for Fully Custom Panels

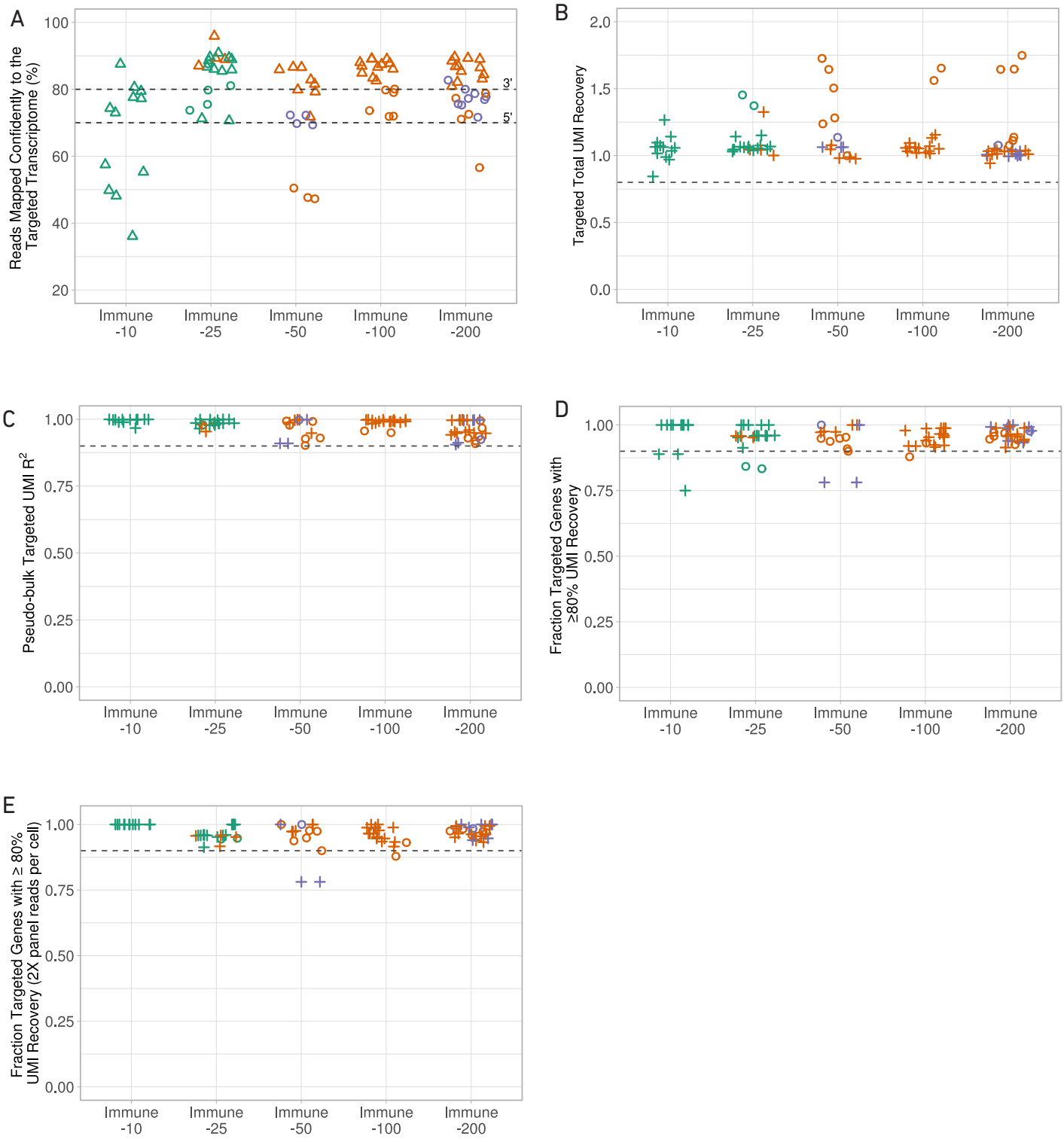

Figure 7. Targeting metrics for fully custom panels. Each data point represents a single Targeted Gene Expression library that is colored for expression level: 0.10% (green), 0.25-1% (orange), and 2-4% (purple). For panels B-E, the targeted parent comparison was made using either 20,000 (circle) or 50,000 (plus sign) read pairs per cell from the parent WTA library. A. Reads mapped confidently to the targeted transcriptome. Sample data are grouped by the fully custom panel used. Dotted lines indicate threshold for optimal performance. Triangles indicate Chromium Single Cell 3' Gene Expression libraries and circles indicate Chromium Single Cell 5' Gene Expression libraries. B. Targeted Total UMI recovery. C. Pseudo-bulk targeted UMI R^2. D. Fraction of genes with UMI recovery ≥80%. E. Same as (D) but assessed with a greater sequencing depth in the targeted sample to compensate for lower Reads Mapped Confidently to the Targeted Transcriptome. Refer to Results text for more information.

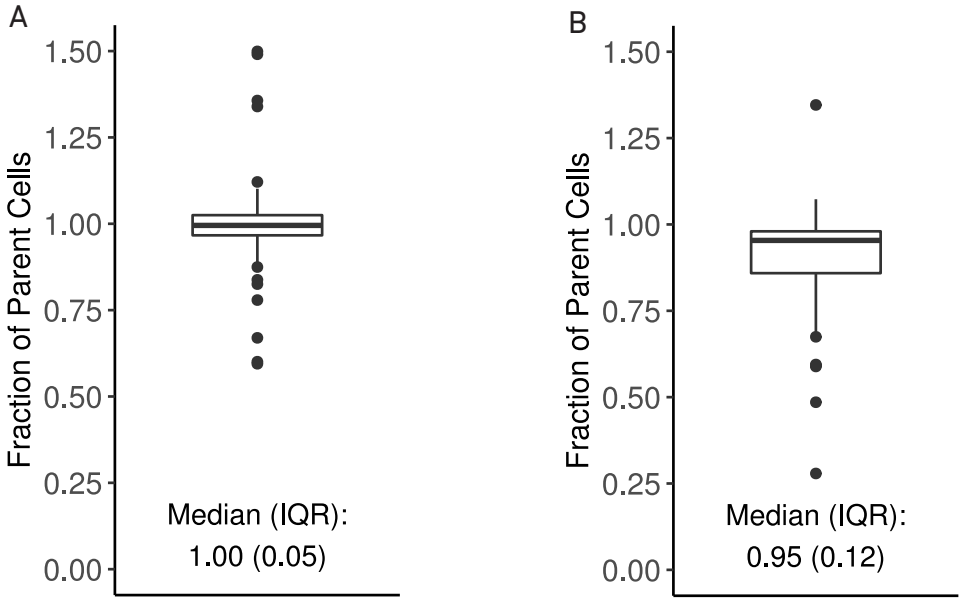

Figure 8. Cell calling concordance for add-on panels (A) and fully custom panels (B). Median and interquartile range (IQR) are indicated.

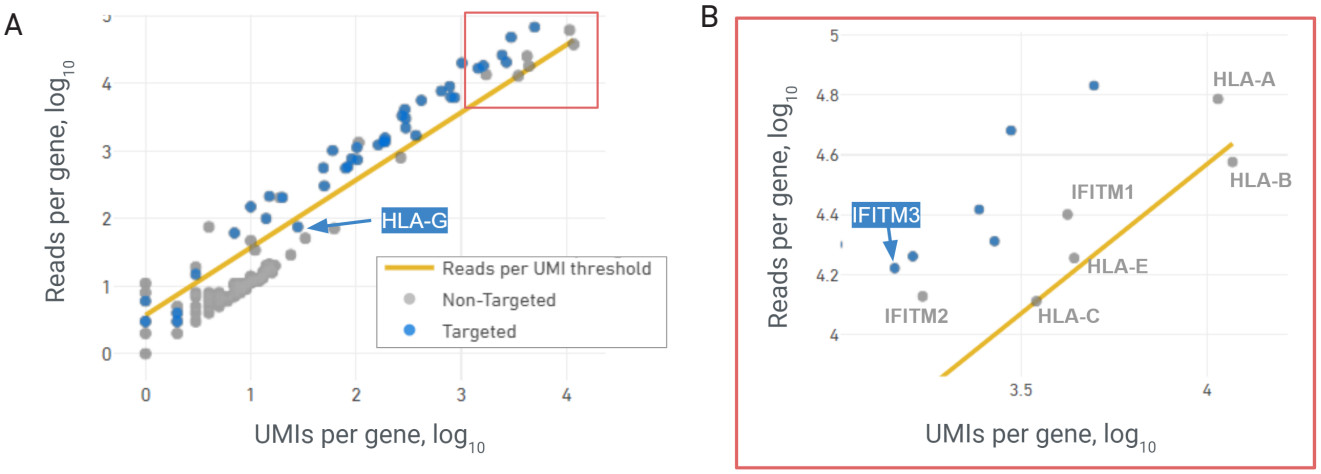

Figure 9. Target Enrichment plot from the Cell Ranger Web Summary for the Immune-50 custom panel. Panel (B) is a zoomed in view of the red highlighted area in (A).

Discussion

10x Genomics pre-designed panels supply a comprehensive set of genes relevant to several research areas. If additional genes are required, the Custom Panel Designer allows the design of add-on panels to supplement pre-designed content. Supplementing a pre-designed panel with add-on genes is a robust and cost-effective way to perform a targeted experiment.

Fully custom panels may vary in their number of genes, fraction of reads from targeted genes in the parent WTA library, and the expression of panel genes in cell subpopulations in a sample. Fully custom panels may be required when there is minimal overlap between the desired gene set and the genes in a pre-designed panel, or when a smaller panel (less than 1,000 genes) is desired.

Whether designing add-on panels or fully custom panels, consider the following:

Expression level of genes: Inclusion of highly expressed genes that are present in many cells in the sample will result in greater sequencing read pair requirements for the targeted sample. The Cell Ranger targeted-depth subcommand can help estimate the sequencing requirement for a custom panel. For instructions on running this script, refer to the Targeted Depth section of the 10x Genomics Support website. 10x Genomics recommends that the panel genes account for at least 0.1% of the WTA reads for optimal performance.

Differentially expressed genes that define a cell type: Choose differentially expressed genes that define cell types of interest to ensure that these cell types can be detected by the cell calling algorithm and differentiated during analysis. Cell calling should be robust, as shown in Figure 8 for this data set.

Presence of high-expression homologs: The Targeted Gene Expression workflow is sensitive to the sequence similarity of gene families. The inclusion of one gene in a homologous family can result in high non-targeted gene enrichment due to bait hybridization to family members. High expression homologs can consume baits and result in a lower relative enrichment of the desired gene. Conversely, the inclusion of baits for related members of a gene family that are not expressed may result in higher than expected UMI recovery for the gene family. Figure 9 shows an example of this phenomenon occurring in the Immune-50 fully custom panel. The panel gene IFITM3 shows good enrichment, but the related genes IFITM1 and IFITM2 that are also expressed in this cell are enriched despite not being included on the panel. This issue is also observed with the panel gene HLA-G and its related (but non-panel) genes HLA-A, HLA-B, HLA-C, and HLA-E. Presence of high-expression homologs decreases the fraction of reads mapping to the targeted transcriptome, and competition for baits may result in a lower relative enrichment of the desired gene.
Panel bait format (applicable to both fully custom and add-on panels): Bait sequences obtained from the 10x Genomics Custom Panel Designer are synthesized through compatible partners. Selecting the appropriate panel for the application is important for best results and for minimizing cost. One compatible partner, IDT, offers two different panel formats that have been tested by 10x Genomics: xGen Lockdown Probes and NGS Discovery Pools. xGen Lockdown Probes are individually synthesized and undergo per-bait quality control, while NGS Discovery Pools do not undergo the same level of quality control but are offered at a reduced cost.

Although NGS Discovery Pools are appropriate for many applications, some applications require panels that have undergone per-bait quality control. In this data set, NGS Discovery Pools were compatible with both add-on panels and fully custom panels, but exhibited some bait dropout that was not detected when using xGen Lockdown Probes. Variability in the actual concentration of NGS Discovery Pool baits ocassionally resulted in over-representation of reads mapping to the add-on genes in the targeted library. This is illustrated in Figure 5, where a small concentration difference between add-on baits and pre-designed panel baits for the Pan-Cancer panel with Immune-Low add-on panel resulted in a higher proportion of reads mapping to add-on genes. This also led to an increased recovery of UMIs from add-on genes as compared to pre-designed panel genes.

Conclusion

As shown by the metrics evaluated in this Technical Note, the 10x Genomics Custom Panel Designer may be used to generate add-on panels to supplement pre-designed panels or generate fully custom panels. These custom panels deliver a high recovery of unique molecules and efficient targeting of panel genes over a variety of sample and tissue types.

References

- Targeted Gene Expression Reagent Kits User Guide (CG000293)
- Targeted Gene Expression Pre-designed Panel Performance Metrics (CG000345)

Appendix

Panel	Genes											
Cancer-High	CALR	EIF4A2	H3F3A	HNRNPK	MARCKSL1	MCL1	RPL5	SF1	ZFP36L1	ZFP36L2		
Cancer-Low	AR	CACNA2D1	ERBB4	FZD9	HIST2H3D	LAMC3	LEFTY2	MPO	NKX2-1	RPS6KA6		
Immune-High	ANP32B	ANXA1	CD63	CD99	DUSP1	HMGB1	LGALS1	NAMPT	S100A10	SLC25A3		
Immune-Low	CES1	CLEC4D	CXCR6	IGHD	KIR2DL3	KLRF1	LCN2	MASP2	S100A7	ZNF683		
Immune-10	BIN2	CCL3	CXCL5	GNLY	HLA-DMB	IGHG2	NIBAN3	RORA	TOLLIP	TREM2		
Immune-25	BCL11B	BTLA	CD160	CD33	CD36	CD37	CD46	CD5	CD55	CHI3L1	CLEC4E	CX3CR1	DOCK8
	FCGR2B	IFIT2	INPP5D	ITGA1	ITGA5	LAMP3	MZB1	PBK	SH2D1B	SLAMF6	SLC25A37	WDHD1	
Immune-50	ABCG2	ADGRG3	ADORA2A	AIRE	C1QA	C4B	CCL27	CCL4	CD180	CD2	CD28	CD69	CD9
	CFP	CLEC6A	CR2	CX3CR1	CXCL10	CYBB	ENTPD1	FCN1	FCRL2	GNLY	HLA-DMB	HLA-G	IFITM3
	IGHM	IL12RB1	IL32	JAM3	LAMP2	LAMP3	MAF	MAGEA1	MASP2	MME	MSH4	NCF4	PI3
	PYCARD	RUNX3	S100A12	SELL	SLC7A7	SMPD3	TAL1	TNFSF11	TNFSF18	VMO1	ZBED2		
Immune-100	AICDA	ALCAM	ANP32B	AOC3	AQP9	ATF2	ATG5	ATG7	AZU1	BIN2	BLK	BORA	C1S
	CAMP	CASP5	CCL18	CCL20	CCL23	CCL26	CD1A	CD1B	CD247	CD46	CD47	CD52	CD55
	CD72	CD9	CFI	CFP	CHST10	CLEC10A	CLEC4C	CNOT2	CR2	CTRC	CXCL14	CXCL16	CXCL3
	DOCK8	EGR2	ENG	ENTPD1	ERAL1	FAM30A	FCGR3B	FUT7	GIMAP5	HLA-DQA1	IGBP1	IGHM	IL17RB
	IL18	IL1RL2	IL27	IRGM	ISG15	ITCH	ITGAX	ITGB1	JAM3	KLRC1	KLRD1	LCN2	LILRA1
	LILRB2	LRRC32	LY9	MAF	MAPK11	MS4A2	MSH5	NCAM1	NCR1	PNOC	PPBP	PRF1	PSEN1
	PSEN2	RIPOR2	RPN2	S100A8	S100B	SBNO2	SIGIRR	SPANXB1	SPN	SPOCK2	TFEB	TICAM2	TIRAP
	TLR10	TLR5	TLR7	TLR9	TNFRSF11B	USP9Y	VEGFB	VMO1	YTHDF2				
Immune-200	A2M	ABCB1	ABCB11	ABCC2	ADA	ADGRG3	ANP32B	APOBEC3G	ARG2	ATF6B	ATG16L1	ATG5	ATG7
	B3GAT1	BCL11B	BTN3A1	C1QA	C1QB	C7	CAMP	CASP1	CASP5	CCL17	CCL25	CCL26	CCL3L1
	CCL4	CCR10	CCR4	CCR9	CD160	CD180	CD1B	CD200	CD207	CD27	CD33	CD34	CD36
	CD37	CD3E	CD3G	CD40LG	CD46	CD5	CD52	CD53	CD8B	CD9	CEACAM1	CFP	CHIT1
	CLEC10A	CMTM2	CSF1	CSF2RB	CTSH	CXCL1	CXCL10	CXCL14	CXCL9	CYBB	DDX58	DOCK8	DUSP1
	EBF1	ECSIT	EGR2	F13A1	F2RL1	F5	FCAR	FCER1A	FCER1G	FCER2	FCGR3B	FCN1	FCRL2
	FICD	FRYL	FUT4	GNLY	GTF3C1	HAMP	HDC	HLA-DPB1	HLA-DQA1	HLA-DQB1	ICAM4	IFIH1	IFIT2
	IFITM1	IFNL1	IGHA1	IGHG3	IGHG4	IGHM	IGKC	IKZF2	IL17B	IL18R1	IL1RL1	IL1RL2	IL2
	IL21R	IL2RG	IL9R	IRF7	ITGA4	ITGB1	JAM3	JAML	KIR2DL3	KIR3DL1	KIR3DL2	KLRC3	KLRC4
	KLRG1	LILRA1	LILRA4	LILRB1	LILRB2	LRBA	LTB	LTBR	LTK	LY9	MAGEA12	MAP3K7	MAPK14
	MARCO	MCAM	MGST1	MNX1	MSH4	MZB1	NAMPT	NCF4	NCR3	NOD1	NOD2	NUP107	PDIA6
	PIK3AP1	PNOC	POU2AF1	PPBP	PRAME	PSMB7	REPS1	RGS1	RIPK2	RNASE2	RORA	RRAD	RUNX3
	S100A10	S100A8	S1PR1	SBNO2	SELL	SEMA7A	SH2B2	SH2D1B	SIGIRR	SIGLEC1	SKAP1	SLAMF1	SLAMF6
	SLC25A3	SPA17	SPINK5	SPOCK2	ST6GAL1	STAT2	STAT6	SYT17	TAP1	TFEB	TGFBI	THBD	TICAM2
	TLR10	TLR5	TMEFF2	TNFRSF11A	TNFRSF12A	TNFRSF13B	TNFRSF13C	TNFRSF1A	TNFRSF1B	TNFRSF8	TNFSF18	TOLLIP	TRDC
	USP9Y	VAV1	VSIG4	VSIR	ZAP70								

Table 6. Genes used in add-on panels and fully custom panels.

	Sample
Cell Lines	A375
	Jurkat/Raji Mixture
	Peripheral Blood Mononuclear Cells/Jurkat/GM12878 Mixture
Primary Cells	Gastroesophageal
	Glioblastoma
	Hodgkin's Lymphoma
	Lymphadenopathy
	Mucosa-associated Lymphoid Tissue
	Lymphoma
	Multiple Myeloma
	Ovarian Cancer
	Peripheral Blood Mononuclear Cells
	Peripheral Blood Mononuclear Cells (Lupus)
	Peripheral Blood Mononuclear Cells (Multiple Myeloma)
	Primary Bone Marrow Mononuclear Cells

Table 7. Human samples used for add-on and fully custom panel experiments.

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