SUPPLEMENTAL MATERIAL

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I. Supplemental Methods

For AlloMap Kidney, an RNA-seq platform incorporating unique molecular tags was used to enable improved detection of low-expression genes, higher reproducibility, and accurate measurement of gene expression changes that can be readily expanded to additional gene sets and classifiers. The training data were generated on the AlloMap Heart qPCR platform. As both platforms measure the level of RNA in the same starting material, a conversion equation was defined based on the principles of the two methods. The conversion assumes that for the qPCR platform there is 98% PCR amplification efficiency and that a C_T of 39 is equivalent to one starting molecule (internal data at CareDx). For RNA-seq, the molecule counts were converted to C_T, then used in the AlloMap Kidney classifier as trained on qRT-PCR results. This conversion of the raw RNA-seq data before application to the classifier enabled the use of the locked classifier algorithm without any modification. To ensure reliable results, the conversion equations were tested using DART samples analyzed on both platforms that were not members of the sets used for training or testing the classifier but covered the critical range of the test results. The results generated from the original method (qRT-PCR) and the final test method (RNA-seq) on the same samples showed 92% correlation, demonstrating the validity of the conversion (**Supplemental Figure S1**).

The single-center sample set from Albert Einstein Medical Center was processed by extracting the RNA from the PAXgene tubes using manual spin columns. Due to the authors' experience with varying RNA transcript levels across extraction methods, the manual methods and the automated methods

were first compared on an independent sample set. Forty samples with paired PAXgene tubes (same venipuncture) from DART and two other studies in the biobanks at CareDx were extracted by the two methods and a conversion equation defined for the small overall difference **(Supplemental Figure S2**). After this conversion was determined and locked, the single-center validation set samples were subsequently run and the conversion was then applied as part of the data analysis to generate AlloMap Kidney results.

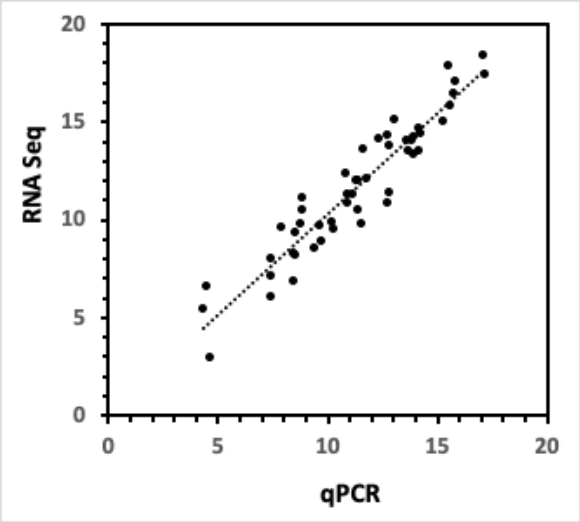

Figure S1: Conversion of AlloMap Kidney classifier trained on qPCR data to produce the same results from RNA-seq data.

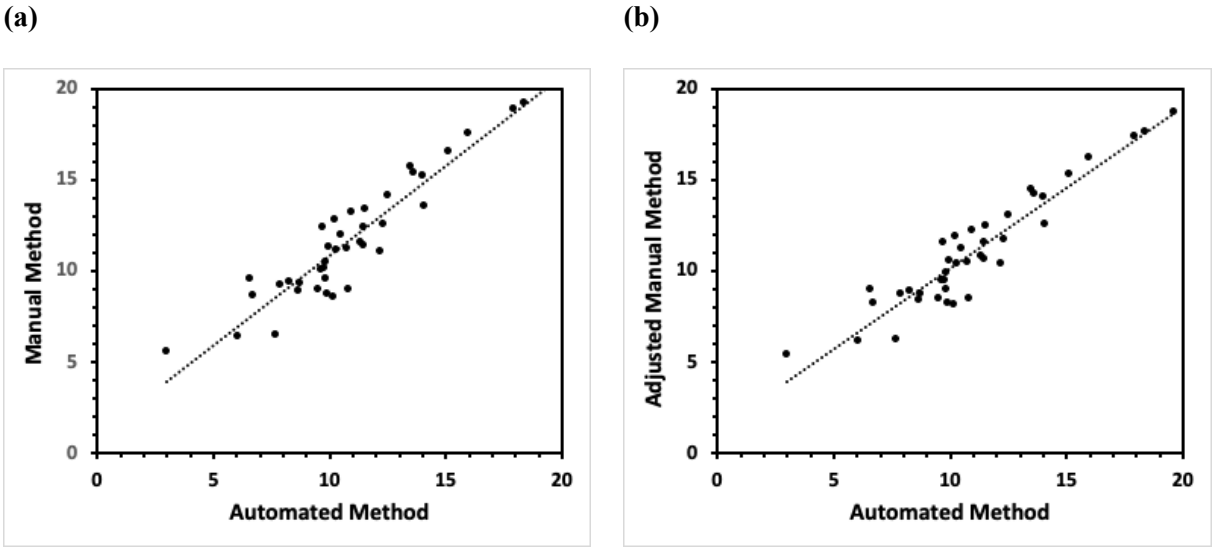

Figure S2: Comparison of AlloMap Kidney data from manual and automated extraction methods on the same 40 samples. **(a)** before adjustment, mean difference 0.7951, intercept = 0.422, slope – 0.897. **(b)** after adjustment, mean difference -0.0015, intercept -0.001, slope = 1.000.

II. Supplemental Table S1

Table S1. Overview of the AlloMap genes. Utility is from Dedrick et al., 2007. Gene names and function from GeneCards (https://www.genecards.org/). Significance in training is the p-value of t-test for individual genes in the training sample set.

Gene		Associated Pathway	Gene Name	Alternative Symbol(s)	Function	Significance in Training
AlloMap Kidney	AlloMap Heart					
PDCD1	PDCD1	T-cell priming	Programmed cell death 1	PD-1, SLEB2	Immune inhibitory receptor. Regulates T cell functions.	0.002
MARCH8	MARCH8	Proliferation and mobilization of blood cells	Membrane Associated Ring-CH-Type Finger 8	MARCHF8, MIR	Ubiquitin ligase, induces internalization of membrane glycoprotens	0.005
DCAF12	WDR40A	Proliferation and mobilization of blood cells	DDB1 and CUL4 Associated Factor 12	WDR40A, KIAA1892	Regulates activity of culling RING E3 ligases	<0.001
IL1R2	IL1R2	Steroid Responsive	Interleukin 1 receptor type 2	IL1RB, CD121b	IL-1a, IL1B and IL1R1 decoy receptor inihibits signaling	0.019
FLT3	FLT3	Steroid Responsive	FMS Related Receptor Tyrosine Kinase 3	FLT3	Regulates hematopoiesis by activating pathways involved in apoptosis, proliferation, and differentiation	0.017
	ITGA4	T-cell priming	Integrin alpha-4	ITGA4	T cell motility and adhesion	0.176
	ITGAM	T-cell priming	Integrin alpha-M	MAC-1, CD11b, CR3A	Alpha subunit of MAC-1 involved in cell trafficking	0.662
	PF4	Platelet Activation	Platelet factor 4	CXCL4	Platelet aggregation, chemotactic for numerous cell types	0.017
	C6orf25	Platelet Activation	G6b inhibitory receptor	G6b, MPIG6B	MHC class III Inhibitory receptor of the Ig superfamily	0.420
	SEM7A	Lymphocyte activation	Semaphorin 7A	SEMAL	Marker of activated lymphocytes.	0.169
	RHOU	Leukocytes migrating into tissues	Ras homolog gene family member U	ARHU	Rho GPT-ase amiliy involved in cytoskeleton ogranization	0.435

III. Supplemental Table S2

Table S2. List of the AlloMap reference genes. In both cases, reference genes were chosen as invariant in a set of NR or Q samples. Gene names from GeneCards (https://www.genecards.org/).

Gene		Gene Name
AlloMap Kidney Training	AlloMap Kidney Validation	
ERCC5		ERCC Excision Repair 5, Endonuclease cell death 1
GABPB2	GABPB2	GA Binding Protein Transcription Factor Subunit Beta 2
CCDC159		Coiled-Coil Domain Containing Protein 159
GPI		Glucose-6-Phosphate Isomerase
RPLP1		Ribosomal Protein Lateral Stalk Subunit P1
GUSB	GUSB	Glucuronidase Beta
	DECR1	2,4-Dienoyl-CoA Reductase 1
	EWSR1	EWS RNA Binding Protein 1
	GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
	HSP90AB1	Heat Shock Protein 90 Alpha Family Class B Member 1
	MAP3K3	Mitogen-Activated Protein Kinase Kinase Kinase 3
	MAPK9	Mitogen-Activated Protein Kinase 9
	NONO	Non-POU Domain Containing Octamer Binding
	RXRB	Retinoid X Receptor Beta
	SDHA	Succinate Dehydrogenase Complex Flavoprotein Subunit A
	SRRM1	Serine And Arginine Repetitive Matrix 1
	TBC1D10B	TBC1 Domain Family Member 10B
	TBP	TATA-Box Binding Protein
	TOP2B	DNA Topoisomerase II Beta