STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Richard F. Daines, M.D.
Commissioner

James W. Clyne, Jr.
Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 6-10
Summary of results[1]

August 12, 2010

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from June 28, 2010, MCTM 6-10.

Samples: All laboratories received three (3) different specimens prepared by Wadsworth Center personnel.

Evaluation: Laboratories were asked to perform those molecular assays for which they hold or have applied for a NYS permit. A total of 37 laboratories participated, performing various numbers and combinations of tests. The attached tables summarize the results and methods that were used by participating laboratories. A consensus interpretation of **G** (Germline/normal/wild type) or **R** (Rearranged/mutated/translocated) is also indicated where possible. Please note that **R** includes anything that is not normal, i.e. that is rearranged, translocated, contains a fusion gene or viral sequences, or contains a mutation. Only truly normal samples, i.e. those without any evidence of a disease-related process of any nature, should be called **G** (but see discussion below for TRB for L/L 1). **I** (Indeterminate) is shown if no consensus was reached because less than three labs performed a test, or if the difference between the number of labs reporting R or G is not sufficient to derive a clear consensus, defined as ≥75% agreement between all responses. However, in cases where there was a clear difference in the results obtained with one method vs. another, e.g. southern blot (SB) vs. PCR, then the "consensus" was expressed for each method separately, e.g. **R/G**, where the first letter belongs to the first method, and the second belongs to the second method.

In addition, you also receive a personalized result sheet that gives your lab's result in comparison to the all lab consensus (if any) derived from all methods combined. Two scores were calculated, one for each assay (**assay score**) across all three samples, and one for each sample (**sample score**) across all assays performed by your lab. From the latter we also calculated an overall score. Your **assay** score is expressed as a fraction, whereby the denominator is the number of samples you analyzed with a given assay and that were evaluable, i.e. produce a consensus, and the numerator is the number of samples for which you agree with the consensus. For example, 3/3 means you analyzed all 3 samples and agreed with the consensus for all 3 of them. 1/2 would mean you analyzed only two samples, and agreed with the consensus for only one

[1] The use of brand and/or trade names in this document does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health

of them. If you reported results from two different methods, each method was scored independently, and the results added together. This score is indicated in the 'score' column to the right of each assay you performed. The **sample** score was calculated as the percentage of 'correct' answers per sample (i.e. that agree with the consensus), based on the number of assays performed per sample that were evaluable. Assays for which no clear consensus was obtained, as indicated by "I", were not included in either the assay or sample score calculation. At the bottom of each sample column on your result sheet you will find the number of assays performed by your lab for the sample, the number of results that were evaluable and used to calculate the score, and the number of 'correct' answers. The actual sample score as % 'correct' answers was calculated by dividing the number of 'correct' answers by the number of evaluable answers times 100. Finally, we also calculated an overall sample score as the average of the three individual sample scores. At this time we did not assign a grade, but may do so in the future. If any of your results are different from the corresponding consensus we ask that you take a careful look at your analysis and investigate why you may have reported a discrepant result. While this may be because of your assay's design and/or sensitivity and thus does not represent an error *per se*, it could also be a true error, indicating suboptimal performance of your assay, or be due to a contamination in case of apparent false positives.

NYS#L/L 1 (Table 1):
B-cell tests: For IGH, 22 out of 31 labs (71%) reported that this locus was rearranged by PCR, just below the 75% agreement required for a consensus call. Six labs that reported G used home brew primers for the framework (FR) 2 and/or FR3 only, and thus possibly missed a rearrangement in FR1, whereas all five labs using the original IVS FR1 primers and eight out of ten (80%) labs using the Biomed-2 tube A detected a rearrangement (Table 4). Interestingly, seven out of eleven labs (64%) using the Biomed-2 tube B that detects the FR2 rearrangement reported a rearrangement; in contrast, only one out of five labs (20%) using the IVS FR2 and none of the labs using home brew FR2 primers reported a rearrangement. One of the possible reasons for this discrepancy could be that the Biomed-2 tube B contains additional primer pairs that detect this rearrangement in FR2. Another interesting observation was that the majority (90%) of Biomed-2 tube C results were germline, whereas all six of the original IVS (100%) and six out of twelve home brew primers (50%) for FR3 did detect a rearrangement. Thus, the results for individual primer mixes were inconsistent, and overall there was less than the required agreement to declare a consensus for this sample to contain cells with an IGH rearrangement. However, the results from Southern blot, with all three labs reporting a rearrangement, suggest that this sample indeed contained a B-cell population with an IGH rearrangement; however, even when combining the results from the two methods the overall agreement was less than 75%. All eleven labs (100%) that tested for IGK reported a rearrangement with the Biomed-2 tube A, whereas only one lab reported a rearrangement with tube B (Table 5). Since the two labs that used SB also detected a rearrangement there was overall consensus that this sample contained a clonal population with an IGK rearrangement. The one lab that tested for IGL by SB and PCR detected no arrangement. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGK and possible IGH gene rearrangements. It is unclear at the moment why no clear consensus for IGH rearrangement by PCR was obtained. However, similar results were obtained in two previous PT events with this sample. We are currently investigating this question further.

T-cell tests: All 29 laboratories (100%) that tested for TRG by PCR found no rearrangement. In contrast five out of eleven (45%) labs that tested for TRB by PCR reported a rearrangement based on the results with the Biomed-tube C; however, the two other labs that also detected a rearrangement with the Biomed-2 tube C reported an overall result of G since the tube C possibly detects an incomplete (D-J) TCR beta gene rearrangement that usually has a poor correlation with the neoplastic process. Thus, the clinical significance of a rearrangement detected with tube C is unclear. By SB, one out of three labs detected a

rearrangement by SB using the Dako TRB probe. The one laboratory that tested for TRD by SB and PCR found no rearrangement. Thus, while a majority of labs detected a possible TRB gene rearrangement analytically, its clinical significance is unclear.

EBV: All three labs that tested for EBV detected the presence of EBV virus sequences by PCR.

IGHV mutation: Of the nine labs that tested for IGHV hypermutation, two reported mutation rates of 5.2 and 6.3%, respectively, and assigned the sequence to the IGHV3-9 family. In contrast, six labs (PCR=3, RT-PCR=3) did not even detect a clonal band, and one lab reported an indeterminate result.

RAS: One lab tested for and detected a mutation in codon 61, CAA to AAA or Q61K, of N-ras, whereas no mutations were found in H-ras and K-ras.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, and HFE.

In aggregate, these results indicate that the sample contained a clonal B-cell population, possibly with an N-ras mutation. This conclusion is in agreement with the result from Flow Cytometry, which also indicated the presence of an aberrant B-cell population.

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, all 31 laboratories detected a rearrangement by PCR, which was confirmed by all three results from SB. A rearrangement was detected by all primer mixes used except for the Biomed-2 tube E (Table 4). All thirteen labs that tested for IGK (PCR=11, SB=2) reported a rearrangement, as did the one lab that also tested for IGL by SB and PCR. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: all 29 laboratories (100%) that tested for TRG by PCR found no rearrangement. Likewise, all fourteen labs that tested for TRB (SB=3, PCR=11) reported no rearrangement, as did the one lab that tested for TRD by SB and PCR. These results suggest that this sample did not contain cells with a T-cell receptor gene rearrangement.

EBV: Two out of three labs that tested for EBV detected the presence of EBV virus sequences by PCR.

IGHV mutation: Of the nine labs that tested for IGHV hypermutation, four reported mutation rates between 4.59-20.8%, and assigned the sequence to the IGHV3-23 family, whereas three labs found mutation rates less than 2% and did not classify this sample as hypermutated; however, they too assigned the sequence to the IGHV3-23 family. In contrast, one lab did not detect a clonal band and one lab reported an indeterminate result.

The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, HFE, K-, H-, and N-ras.

In aggregate, these results indicate that the sample contained a B-cell clone possibly with IGHV hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which also indicated the presence of an aberrant B-cell population.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, all 31 laboratories detected a rearrangement by PCR, which was confirmed by all three results from SB. A rearrangement was detected by the majority of primer mixes used with the exception of the original IVS FR2 primers and the Biomed-2 tube E (Table 4). All thirteen labs that tested for IGK (PCR=11, SB=2) reported a rearrangement, as did the one lab that also tested for IGL by SB and PCR. No translocations involving IGH/BCL2 MBR, mcr, and MBR3', or IGH/CCND1 (Bcl-1) were detected by any methods. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: all 29 laboratories (100%) that tested for TRG by PCR found no rearrangement. Likewise, all fourteen labs that tested for TRB (SB=3, PCR=11) reported no rearrangement, as did the one lab that tested for TRD by SB and PCR. These results suggest that this sample did not contain cells with a T-cell receptor gene rearrangement.

EBV: All three labs that tested for EBV detected the presence of EBV virus sequences by PCR.

IGHV mutation: Of the nine labs that tested for IGHV hypermutation six (PCR=4, RT-PCR=2) detected mutation rates between 6.4-18.3%, and assigned the sequence to the IGHV4-31 family. In contrast, two labs did not even detect a clonal band and one lab reported an indeterminate result. Since this lab also reported indeterminate for the other two samples, it should check and verify that its method performs as expected.
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The results from all other tests performed were negative, including tests for mutations in BRAF, PDGFRA, PIK3CA, WT1, CEBPA, HFE, K-, H-, and N-ras.

In aggregate, these results indicate that the sample contained a B-cell clone possibly with IGHV hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which also indicated the presence of an aberrant B-cell population.

The attached tables show a summary of the results both in aggregate (Tables 1-3) as well as by individual primer mixes for the B- and T-cell tests (Tables 4-7). Figure 1 shows the DNA and RNA yield distributions for the three samples. DNA yields from samples L/L1, 2, and 3 ranged from a minimum of 2, 2, and 5 µg per 5 ml specimen to a maximum of 10,688, 4,070, and 5,083 µg, respectively, corresponding to a 1,016- to 5,344-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 4.87, 2, and 2.5 µg to 4,590, 1,770, and 3,330 µg, respectively, corresponding to a 942- to 1332-fold difference between lowest and highest yield for each sample. Please make sure that you report the DNA/RNA yields calculated for the entire 5 ml sample even if you only extract it from a smaller aliquot, and your units are in microgram (µg), not nanogram (ng) or milligram (mg). Presumably, difference in the methods used for DNA and RNA isolation also contributed to this wide range. However, it also raises the question of how accurate some of the measurements are. We realize that shipping the samples at room temperature is suboptimal for subsequent RNA analysis. However, because of the combined shipping with the malignant immunophenotyping samples we cannot change that.

Finally, some general comments. There is sometimes confusion as to where to write the results. Please note: RT stands for **r**everse **t**ranscription, not real time, and thus should only be used for assays whose starting material is RNA. If your starting material is DNA you must record your result in the PCR column. Vice versa, if your starting material is RNA, you must report your results in the RT-PCR column. Please make sure that your results are written in the correct column that corresponds to the starting material you used. A few labs did not indicate the methods and/or reagents that they used for their assays. We cannot properly evaluate your results without this information. In particular, we ask that if you obtain your primers from InVivoScribe you correctly identify the source as IVS (identified as gene rearrangement assays in their catalog) or Biomed-2 (identified as gene clonality assays in their catalog); for the purpose of this PT evaluation they are not considered home brew even if you obtain the individual primer tubes separately as ASR reagents instead of as part of a RUO kit. This will make it easier to compare the performance of individual primer mixes. Finally, we ask that you analyze the samples by all molecular tests performed in your lab for which you hold or have applied for a NYS permit.

If you have any questions, comments or suggestions, you may contact me by phone or email at 518-474-2088 or schneid@wadsworth.org. For specific questions about your lab's report or the evaluation please contact Ms. Susanne McHale at (518) 486-5775 or smchale@wadsworth.org, or Dr. Rong Yao at (518) 474-1744 or yaor@wadsworth.org.

The date for the Molecular and Cellular Tumor Marker PT mail-out in 2010 is:

Mail-out date	**Due Date**
October 25	November 23

Sincerely,

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center, Room E604
Empire State Plaza
Albany, NY 12201-0509

Table 1: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L1 Consensus Summary June 2010.xls

Interpretation:	B-cell clone with IGH and IGK gene rearrangements, and EBV positive.																			
Assay	**SB**				**PCR**				**RT-PCR**				**All methods**			**Method used**				
	R	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**Cons#**	**SB**	**PCR (qualitative)**	**PCR (quantitative)**	**RT-PCR (qualitative)**	**RT-PCR (quantitative)**
IGH	3			R	22	9		I					25	9	R/I	Dako (2), home brew (1)	Biomed (11), home brew (15), IVS (6)			
IGK	2			I	11			R					13	0	R	home brew (1), Dako (1)	Biomed (9), home brew (2)			
IGL		1		I		1		I					0	2	I	home brew (1)	Biomed (1)			
TRB	1	2		I	5	6		I					6	8	I	home brew (1), Dako (2)	Biomed (8), home brew (3),			
TRG						29		G					0	29	G		Biomed (9), home brew (17), IVS (4),			
TRD		1		I		1		I					0	2	I	home brew (1)	Biomed (1)			
IGH/BCL2 MBR						14		G					0	14	G		Biomed (2), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (2), IVS (3), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (2), IVS(1)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)		1		I		8		G					0	9	G	home brew (1)	home brew (3), Biomed (2), IVS (1)	home brew (2)		
BCR/ABL1 p210										25		G	0	25	G				home brew (9)	home brew (14), Ipsogen(5), Cepheid(1)
p190										21		G	0	21	G				home brew (10)	home brew (10), Ipsogen(5)
p210/190										9		G	0	9	G				home brew (2), Roche(1)	home brew (3), Roche(3), Ipsogen(1)
Abl kinase domain mutation										2		I	0	2	I				home brew (2)	
PML/RARA Long										12		G	0	12	G				home brew (5)	home brew (7)
Short										11		G	0	11	G				home brew (4)	home brew (7)
Variable										3		G	0	3	G				home brew (2)	home brew (1)
Long/Short/Variable										1		I	0	1	I					Ipsogen(1)
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										6		G	0	6	G				home brew (4)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV					3			R					3	0	R		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										3		G	0	3	G				home brew (2)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I				home brew (2)	
JAK 2 (V617F)						23		G		2		I	0	25	G		home brew (18), Ipsogen(2)	home brew(1) Invader(1) Ipsogen(2)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						5		G		3		G	0	8	G		home brew (5)	Ipsogen (1)	home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4) Invader(1)		home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		home brew (2)	
FLT 3 ITD						10		G					0	10	G		home brew (8), IVS(1), Seegene(1)			
FLT 3 D835						8		G					0	8	G		home brew (6), IVS(1), Seegene(1)			
NPM1 mutation						11		G					0	11	G		home brew (10)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation				I	2	3N*		I		3N*	1	N*	2	6N*	I/N*		home brew(3) IVS (1)	IVS(1)	home brew (3) IVS (1)	
c-kit						5		G					0	5	G		home brew(5)			
Other ‡																				

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 2: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L2 Consensus Summary June 2010.xls

Interpretation:	B-cell clone with IGH and IGK gene rearrangements with IGHV mutation.																		
Assay	**SB**				**PCR**				**RT-PCR**				**All methods**			**Method used**			
	R	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**Cons#**	**SB**	**PCR (qualitative)**	**PCR (quantitative)**	**RT-PCR (qualitative)**	**RT-PCR (quantitative)**
IGH	3			R	31			R					34	0	R	Dako (2), home brew (1)	Biomed (11), home brew (15), IVS (6)			
IGK	2			I	11			R					13	0	R	home brew (1), Dako (1)	Biomed (9), home brew (2)			
IGL	1			I	1			I					2	0	I	home brew (1)	Biomed (1)			
TRB		3		G		11		G					0	14	G	home brew (1), Dako (2)	Biomed (8), home brew (3),			
TRG						29		G					0	29	G		Biomed (9), home brew (17), IVS (4),			
TRD		1		I		1		I					0	2	I	home brew (1)	Biomed (1)			
IGH/BCL2 MBR						14		G					0	14	G		Biomed (2), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (2), IVS (3), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (2), IVS(1)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)		1		I		8		G					0	9	G	home brew (1)	home brew (3) Biomed (2) IVS (1)	home brew (2)		
BCR/ABL1 p210										25		G	0	25	G				home brew (9)	home brew (14), Ipsogen(5), Cepheid(1)
p190										21		G	0	21	G				home brew (10)	home brew (10), Ipsogen(5)
p210/190										9		G	0	9	G				home brew (2), Roche(1)	home brew (3), Roche(3), Ipsogen(1)
Abl kinase domain mutation										2		I	0	2	I				home brew (2)	
PML/RARA Long										12		G	0	12	G				home brew (5)	home brew (7)
Short										11		G	0	11	G				home brew (4)	home brew (7)
Variable										3		G	0	3	G				home brew (2)	home brew (1)
Long/Short/Variable										1		I	0	1	I					Ipsogen(1)
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										6		G	0	6	G				home brew (4)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV					2	1		I					2	1	I		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										3		G	0	3	G				home brew (2)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I				home brew (2)	
JAK 2 (V617F)						24		G		2		I	0	26	G		home brew (18), Ipsogen(2)	home brew(2) Invader(1) Ipsogen(3)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						4		G		3		G	0	7	G		home brew (4)		home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4) Invader(1)		home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		home brew (2)	
FLT 3 ITD						10		G					0	10	G		home brew (8), IVS(1), Seegene(1)			
FLT 3 D835						8		G					0	8	G		home brew (6), IVS(1), Seegene(1)			
NPM1 mutation						11		G					0	11	G		home brew (10)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation				I	4	1		R		2G 1N*	1	I	4	4	R/I		home brew(3) IVS(1)	IVS(1)	home brew (3), IVS (1)	
c-kit						5		G					0	5	G		home brew(5)			
Other ‡																				

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 3: New York State Molecular Oncology Proficiency Test

Sample: NYS# L/L3 Consensus Summary June 2010.xls

Interpretation:	B-cell clone with IGH and IGK gene rearrangements with IGHV mutation, and EBV positive.																			
Assay	**SB**				**PCR**				**RT-PCR**				**All methods**			**Method used**				
	R	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**ind**	**Cons#**	**R**	**G**	**Cons#**	**SB**	**PCR (qualitative)**	**PCR (quantitative)**	**RT-PCR (qualitative)**	**RT-PCR (quantitative)**
IGH	3			R	31			R					34	0	R	Dako (2), home brew (1)	Biomed (11), home brew (15), IVS (6)			
IGK	2			I	11			R					13	0	R	home brew (1), Dako (1)	Biomed (9), home brew (2)			
IGL		1		I		1		I					0	2	I	home brew (1)	Biomed (1)			
TRB		3		G		11		G					0	14	G	home brew (1), Dako (2)	Biomed (8), home brew (3),			
TRG						29		G					0	29	G		Biomed (9), home brew (17), IVS (4),			
TRD		1		I		1		I					0	2	I	home brew (1)	Biomed (1)			
IGH/BCL2 MBR						14		G					0	14	G		Biomed (2), IVS (3), home brew (7)	home brew (2)		
mcr						12		G					0	12	G		Biomed (2), IVS (3), home brew (5)	home brew (2)		
MBR 3'						3		G					0	3	G		Biomed (2), IVS(1)			
MBR/mcr													0	0						
IGH/CCND1 (Bcl-1)		1		I		8		G					0	9	G	home brew (1)	home brew (3), Biomed (2), IVS (1)	home brew (2)		
BCR/ABL1 p210										24		G	0	24	G				home brew (9)	home brew (14), Ipsogen(5), Cepheid(1)
p190										20		G	0	20	G				home brew (10)	home brew (10), Ipsogen(4)
p210/190										8		G	0	8	G				home brew (2), Roche(1)	home brew (3), Roche(3)
Abl kinase domain mutation										2		I	0	2	I				home brew (2)	
PML/RARA Long										11		G	0	11	G				home brew (5)	home brew (6)
Short										10		G	0	10	G				home brew (4)	home brew (6)
Variable										2		I	0	2	I				home brew (2)	
Long/Short/Variable										1		I	0	1	I					Ipsogen(1)
MYC t(8;14)													0	0						
AML1/ETO t(8;21)										6		G	0	6	G				home brew (4)	home brew (2)
NPM/ALK t(2;5)													0	0						
IGH/BCL-6													0	0						
ETV6/RUNX1 (Tel-AML1)										2		I	0	2	I				home brew (1)	home brew (1)
EBV					3			R					3	0	R		home brew (2)	Roche (1)		
KSHV/HHV8						3		G					0	3	G		home brew (3)			
HTLV1						2		I					0	2	I		home brew (2)			
CBFB INV(16)/MYH11										3		G	0	3	G				home brew (2)	home brew (1)
E2A-PBX t(1;19) (4;11)										1		I	0	1	I				home brew (1)	
MLL(11q23)/ AF4 (4;11)										2		I	0	2	I				home brew (2)	
JAK 2 (V617F)						24		G		2		I	0	26	G		home brew (18), Ipsogen(2)	home brew(2) Invader(1) Ipsogen(3)	home brew (2)	home brew (1)
JAK 2 (Exon 12)						5		G		3		G	0	8	G		home brew (5)	Ipsogen (1)	home brew (3)	home brew (1)
MPL W 515						5		G		2		I	0	7	G		home brew (4) Invader(1)		home brew (2)	
MPL S 505						3		G		2		I	0	5	G		home brew (3)		home brew (2)	
FLT 3 ITD						10		G					0	10	G		home brew (8), IVS(1), Seegene(1)			
FLT 3 D835						8		G					0	8	G		home brew (6), IVS(1), Seegene(1)			
NPM1 mutation						11		G					0	11	G		home brew (10)			
P53						2		I					0	2	I		home brew(2)			
IGHV mutation				I	4	1N*		R	2	1N*	1	I	6	2	R/I		home brew(3) IVS(1)	IVS(1)	home brew (3) IVS (1)	
c-kit						5		G					0	5	G		home brew(5)			
Other ‡																				

N*: No clonal band detected

Cons #: R or G based on ≥75% consensus; I if <75% consensus or <3 results

Other ‡ : See critique for details.

Table 4: Summary for IGH primer mix

Reagent Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
BIOMED-2	A	8	2	R	10		R	7	3	R
	B	7	4	I	11		R	11		R
	C	1	9	G	10		R	10		R
	D	3		R	3		R	2	1	I
	E		4	G		4	G		4	G
IVS	FR 1	5		R	5		R	5		R
	FR 2	1	4	G	6		R	1	4	G
	FR 3	6		R	6		R	6		R
HOMEBREW	FR 1	1	1	I	2		I	1	1	I
	FR 2		9	G	9		R	3		R
	FR 3	6	6	I	12		R	12		R

Table 5: Summary for IGK primer mix

Reagent Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
BIOMED-2	A	11		R	10	1	R	11		R
	B	1	10	G	1	10	G	10	1	R

Table 6: Summary for TRG primer mix

Primer Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
BIOMED-2	A		9	G		9	G		9	G
	B		9	G		9	G		9	G
IVS	Mix 1		10	G		10	G		10	G
	Mix 2		9	G		9	G		9	G
HOMEBREW	Vγ1-8		7	G		7	G		7	G
	Vγ9		7	G		7	G		7	G
	Vγ10		5	G		5	G		5	G
	Vγ11		6	G		6	G		6	G

Table 7: Summary for TRB primer mix

Primer Source	Mix	L/L1		CONSENSUS	L/L2		CONSENSUS	L/L3		CONSENSUS
		R	G		R	G		R	G	
BIOMED-2	A		8	G		8	G		8	G
	B		8	G		8	G		8	G
	C	7	1	R		8	G		8	G
HOME BREW	A		2	I		2	I		2	I
	B		2	I		2	I		2	I

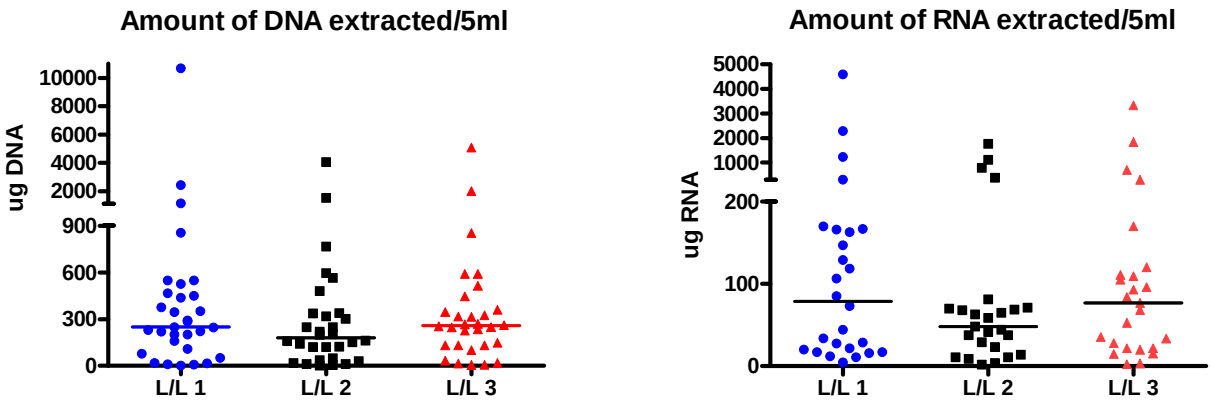

Figure 1. NYS MCTM PT 6-10 DNA and RNA yields

	LL1	LL2	LL3		LL1	LL2	LL3
Yield (ug)	DNA	DNA	DNA		RNA	RNA	RNA
Median	250	181.0	258.3		79.0	48	77
Max	10688	4070	5083		4590	1770	3330
Min	2.0	2.0	5.0		4.87	2	2.5