

**Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 10-09
Summary of results¹**

December 16, 2009

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from October 27, 2009, MCTM 10-09.

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) or **R** (Rearranged/positive) 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**. A consensus interpretation was assigned if the results from a minimum of 70% of all labs that performed a given test agreed with each other. **I** (Indeterminate) is shown if no consensus was reached, or if only one or two labs performed a test. 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 as 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 available) 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, 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

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. 1/2 would mean you analyzed only two samples or only two samples were evaluable, and you agreed with the consensus for only one 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.

NYS#L/L 1 (Table 1):

B-cell tests: For IGH, all 35 results were G(ermline) (PCR=30, SB=3, FISH=2). Likewise, all twelve results for IGK were G(ermline) (PCR=10, SB=2), as was IGL by both SB and PCR. All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the eleven laboratories that tested for the IGH/CCND1 translocation by any method found a rearrangement. Thus, the overall consensus was that this sample did not contain cells with any immunoglobulin gene rearrangements.

T-cell tests: 22 out of 29 laboratories (76%) that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V γ 1-8 region targeted by the Biomed-2 tube A and home brew V γ 1-8 primers (Table 6); six of the seven labs that reported no rearrangement used primers that may not have targeted this region (4 homebrew, 2 IVS), whereas one lab failed to detect this rearrangement despite using Biomed-2 tube A primers. Nine out of ten (90%) labs that tested for TcRBeta (TRB) reported a rearrangement by PCR and all used the Biomed-2 tube B primers that target the V β and J β regions (Table 7); the one lab that did not detect a TRB arrangement used home brew primers. Likewise, three out of four labs (75%) reported a TRB rearrangement by SB; the one lab that reported no rearrangement used the Dako probe. The one laboratory that also tested for TRD by SB and PCR found no rearrangement. These results suggest that this sample contained cells with T-cell receptor gamma and beta gene rearrangements.

IGHV mutation: Six labs reported no clonal band, whereas two labs reported G, indicating that they detected a clonal band without hypermutation. However, more likely this represents a false positive clonal band or a transcription error for a "no clonal band" result.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated an early T-cell population.

NYS#L/L 2 (Table 2):

B-cell tests: For IGH, 34 out of 35 results (97%) were G(ermline) (PCR=30, SB=2, FISH=2); only one lab reported a rearrangement by SB using a home brew probe, and suggested that this result could be due to lineage infidelity. All twelve results for IGK also were G(ermline) (PCR=10, SB=2), as was IGL by both SB and PCR. All laboratories that tested for IGH/BCL2 by various methods reported no translocation at any of the three breakpoint clusters. Similarly, none of the eleven laboratories that tested for the IGH/CCND1 translocation by any method found a rearrangement. Thus, the overall consensus was that this sample did not contain cells with any immunoglobulin gene rearrangements.

T-cell tests: all 29 laboratories that tested for TcRGamma (TRG) by PCR found a rearrangement, possibly in the V γ 1-8 region targeted by the Biomed-2 tube A and home brew V γ 1-8 primers (Table 6). All ten labs that tested for TcRBeta (TRB) reported a rearrangement by PCR that was detected by one or more of the three Biomed-2 tubes A, B, and C in various combinations (Table 7). Also, three out of four labs (75%) reported a rearrangement by SB; the one lab that did not detect a TRB arrangement used the Dako probe, and is the same lab that also did not detect a TRB rearrangement for L/L1. The one laboratory that tested for TRD by SB and PCR found no rearrangement. These results suggest that this sample contained cells with T-cell receptor gamma and beta gene rearrangements.

IGHV mutation: Six labs reported no clonal band, whereas two labs reported G, indicating that they detected a clonal band without hypermutation. However, more likely this represents a false positive clonal band or a transcription error for a “no clonal band” result. These two labs are the same labs that reported G for L/L 1.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal T-cell population. This conclusion is in agreement with the result from Flow Cytometry, which indicated an early T cell population.

NYS#L/L 3 (Table 3):

B-cell tests: 22 out of 30 labs (73%) that tested for IGH by PCR reported a rearrangement that was detected by all labs using the Biomed-2 tube B primers and the majority of labs that used either IVS (80%) or home brew (64%) FR2 primers (Table 4). Interestingly, a rearrangement was also detected by all ten labs that used the Biomed-2 tube C primers that target the framework 3, but not by the majority of labs that used the original IVS (80%) or homebrew FR3 primers (87%). Uniformly, however, none of the FR1 primers detected a rearrangement in this sample (Table 4). Two out of three labs (67%) reported a rearrangement by SB; the one lab that did not detect an IGH arrangement used the Dako probe, and is also the same lab that reported no rearrangement in TRB by SB for L/L1 and 2. Since all three SB results from this lab were against the consensus for that specific sample, this lab may want to reassess the performance of its SB procedure. Two labs that used FISH detected an IGH rearrangement. Also, all twelve results reported for IGK showed a rearrangement (SB=2, PCR=10), possibly in V κ and J κ regions targeted by the Biomed-2 tube A primers (Table 5). No lab reported a translocation involving IGH/BCL2 MBR, mcr, and MBR3', IGH/CCND1 (Bcl-1), or a rearrangement in IGL by SB and PCR. These results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: All 29 laboratories that tested for TRG by PCR found no rearrangement. Nine out of ten labs (90%) reported no TRB rearrangement by PCR, as did all four labs that tested for TRB by SB. The one laboratory that tested for TRD by SB and PCR also found no rearrangement. These results suggest that this sample did not contain cells with T-cell receptor gene rearrangements.

IGHV mutation: seven out of eight labs (88%) reported IGHV hypermutation (3=PCR, 4=RT-PCR) that was assigned to the IGHV4-34 family with mutation rates between 13.3-15.28%.

The results from all other tests performed were negative.

In aggregate, these results indicate that the sample contained a clonal B-cell population with IGH and IGK rearrangements, and IGVH hypermutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of an early 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 5, 1, and 5 µg per 5 ml specimen to a maximum of 11300, 14360, and 10220 µg, respectively, corresponding to a 2044- to 14360-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 14.8, 19, and 11 µg to 1871, 3252.5, and 1350 µg, respectively, corresponding to a 122- to 171-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. Presumably, the methods used for DNA and RNA isolation 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 still seems to be some confusion as to where to write your results. Please note: RT stands for reverse transcription, 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 or Biomed-2; they are not considered home brew. 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 dates for the Molecular and Cellular Tumor Marker PT mail-out in 2009 are:

Mail-out date

February 22

June 28

October 25

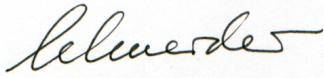
Due Date

March 23

July 27

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 -Summary of Results

Sample: NYS# L/L 1 (October 2009)

Interpretation: T-cell clone with TRB and TRG rearrangements																										
Assay	SB				PCR				RT-PCR				FISH			All methods					Method used					
	R	G	ind	Cons [#]	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)	FISH	
IGH		3		G	30		G						2		I		0	35	G	Dako (2), home brew (1)	Biomed (10), home brew (15), IVS (6)				Vysis (2)	
IGK		2		I	10		G										0	12	G	home brew (1), Dako (1)	Biomed (8), home brew (3)					
IGL		1		I	1		I										0	2	I	home brew (1)	Biomed (1)					
TRB		3	1	R	9	1	R										12	2	R	home brew (2), Dako (2)	Biomed (7), home brew (3)					
TRG					22	7	R										22	7	R		Biomed (7), home brew (19), IVS (3),					
TRD		1		I	1		I										0	2	I	home brew (1)	Biomed (1)					
IGH/BCL2	MBR		1	I	13		G										0	14	G	home brew (1)	Biomed (2), IVS (2), home brew (7)	home brew (2)				
	mcr				10		G										0	10	G		Biomed (2), IVS (1), home brew (5)	home brew (2)				
	MBR 3'				3		G										0	3	G		Biomed (2) IVS(1)					
	MBR/mcr												3		G		0	3	G							Vysis (3)
IGH/CCND1 (Bcl-1)		1		I	7		G						3		G		0	11	G	home brew (1)	home brew (3), Biomed (1), IVS (1)	home brew (2)				Vysis (3)
BCR/ABL1	p210		1	I				24		G							0	25	G	home brew (1)			home brew (8)	home brew (14), Ipsiogen(3), Cepheid(1),		
	p190							21		G							0	21	G				home brew (8)	home brew (12), Ipsiogen(4)		
	p210/190							9		G	4		G				0	13	G				home brew (2), Roche(2)	home brew (3), Roche(2), Ipsiogen(1)		Vysis (4)
Abl kinase domain mutation							2		I							0	2	I				home brew (2)				
PML/RARA	Long							12		G							0	12	G				home brew (6)	home brew (6)		
	Short							11		G							0	11	G				home brew (5)	home brew (6)		
Variable								3		G							0	3	G				home brew (2)	home brew (1)		
	Long/Short/Variable							1		I	4		G				0	5	G				home brew (1)			Vysis (4)
MYC t(8;14)													3		G		0	3	G							Vysis (3)
AML1/ETO t(8;21)								6		G			3		G		0	9	G				home brew (4)	home brew (2)		Vysis (3)
NPM/ALK t(2;5)													1		I		0	1	I							Vysis (1)
IGH/BCL-6													2		I		0	2	I							Vysis (2)
ETV6/RUNX1 (Tel-AML1)								2		I	2		I				0	4	G				home brew (1)	home brew (1)		Vysis (2)
EBV					3		G										0	3	G		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	3		G				0	6	G				home brew (2)	home brew (1)		Vysis (3)
E2A-PBX t(1;19) (4;11)								1		I							0	1	I				home brew (1)			
MLL(11q23)/ AF4 (4;11)								2		I	3		G				0	5	G				home brew (2)			Vysis (3)
JAK 2 (V617F)					25		G	2		I							0	27	G		home brew (17), IVS(1), Ipsiogen(4)	home brew(2) Invader(1) Ipsiogen(1)	home brew (2)	home brew (1)		
JAK 2 (Exon 12)					2		I	2		I							0	4	G		home brew (2)		home brew (2)	home brew (1)		
MPL W 515					2		I	2		I							0	4	G		home brew (1), Invader (1)		home brew (2)	home brew (1)		
MPL S 505								2		I							0	2	I				home brew (2)	home brew (1)		
FLT 3 ITD					14		G										0	14	G		home brew (11), IVS(2), Seegene(1)					
FLT 3 D835					12		G										0	12	G		home brew (9), IVS(2), Seegene(1)					
NPM1 mutation					12		G										0	12	G		home brew (12)					
P53					2		I				4		G				0	6	G		home brew(2)					Vysis (4)
IgVH mutation					*1,3		N	*1,3		N							0	2,6	N		home brew(2), IVS(1)	IVS(1)	home brew (3), IVS (1)			
c-kit					3		G										0	3	G		home brew (3)					
Other:(MYC,RARA,ALK,Bc I-6), (HFE, RB1, etc)					1	1	I				1	1	I				2	2	I		home brew					Vysis

* 1,3: 1G, 3N (No clonal band detected)

R,G: based on ≥70% consensus; I: <70% consensus or <3 results

Table 2: New York State Molecular Oncology Proficiency Test -Summary of Results

Sample: NYS# L/L 2 (October 2009)

Interpretation: T-cell clone with TRB and TRG rearrangements																									
Assay	SB				PCR				RT-PCR				FISH				All methods				Method used				
	R	G	ind	Cons*	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)	FISH
IGH	1	2		I	30		G						2		I	1	34	G	Dako (2), home brew (1)	Biomed (10), home brew (15), IVS (6)					Vysis (2)
IGK		2		I	10		G									0	12	G	home brew (1), Dako (1)	Biomed (8), home brew (3)					
IGL		1		I	1		I									0	2	I	home brew (1)	Biomed (1)					
TRB	3	1		R	10		R									13	1	R	home brew (2), Dako (2)	Biomed (7), home brew (3)					
TRG					29		R									29	0	R		Biomed (7), home brew (19), IVS (3)					
TRD		1		I	1		I									0	2	I	home brew (1)	Biomed (1)					
IGH/BCL2	MBR	1		I	13		G									0	14	G	home brew (1)	Biomed (2), IVS (2), home brew (7)	home brew (2)				
	mcr				10		G									0	10	G		Biomed (2), IVS (1), home brew (5)	home brew (2)				
	MBR 3'				3		G									0	3	G		Biomed (2), IVS(1)					
	MBR/mcr												3		G	0	3	G							Vysis (3)
IGH/CCND1 (Bcl-1)		1		I	7		G						3		G	0	11	G	home brew (1)	home brew (3), Biomed (1), IVS(1)	home brew (2)				Vysis (3)
BCR/ABL1	p210	1		I					25		G					0	26	G	home brew (1)			home brew (8)	home brew (15), Ipsogen(4), Cepheid(1)		
	p190								21		G					0	21	G				home brew (8)	home brew (12), Ipsogen (4)		
	p210/190								8		G	4		G	0	12	G				home brew (2), Roche(2)	home brew (2), Roche(2), Ipsogen (1)			Vysis (4)
Abl kinase domain mutation								2		I					0	2	I				home brew (2)				
PML/RARA	Long							12		G					0	12	G				home brew (6)	home brew (6)			
	Short							11		G					0	11	G				home brew (5)	home brew (6)			
	Variable							3		G					0	3	G				home brew (2)	home brew (1)			
	Long/Short/Variable							1		I	4		G	0	5	G						home brew (1)			
MYC t(8;14)												3		G	0	3	G								Vysis (3)
AML1/ETO t(8;21)								6		G		3		G	0	9	G				home brew (4)	home brew (2)			Vysis (3)
NPM/ALK t(2;5)												1		I	0	1	I								Vysis (1)
IGH/BCL-6												2		I	0	2	I								Vysis (2)
ETV6/RUNX1 (Tel-AML1)								2		I	2		I	0	4	G						home brew (1)	home brew (1)		Vysis (2)
EBV					3		G								0	3	G		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	3		G	0	6	G						home brew (2)	home brew (1)		Vysis (3)
E2A-PBX t(1;19) (4;11)					1		I								0	1	I					home brew (1)			
MLL(11q23)/ AF4 (4;11)					2		I				3		G	0	5	G						home brew (2)			Vysis (3)
JAK 2 (V617F)					25		G	2		I					0	27	G		home brew (17), IVS(1), Ipsogen(4)	home brew(2) Invader(1) Ipsogen(1)	home brew (2)	home brew (1)			
JAK 2 (Exon 12)					2		I	2		I					0	4	G		home brew (2)		home brew (2)	home brew (1)			
MPL W 515					2		I	2		I					0	4	G		home brew (1), Invader(1)		home brew (2)	home brew (1)			
MPL S 505								2		I					0	2	I					home brew (2)	home brew (1)		
FLT 3 ITD					14		G								0	14	G		home brew (11), IVS(2), Seegene(1)						
FLT 3 D835					12		G								0	12	G		home brew (9), IVS(2), Seegene(1)						
NPM1 mutation					12		G								0	12	G		home brew (12)						
P53					1		I					4		G	1	4	I/G		home brew(1)						Vysis (4)
IgVH mutation					*1,3		N	*1,3		N					0	2,6	N		home brew(2), IVS(1)	IVS(1)	home brew (3), IVS (1)				
c-kit					3		G								0	3	G		home brew(3)						
Other:(MYC,RARA,ALK,Bcl 6), (HFE, RB1, etc)					1	1	I				1	1	I	2	2	I			home brew						Vysis

* 1,3: 1G, 3N (No clonal band detected)

R,G: based on ≥70% consensus; I: <70% consensus or <3 results

Table 3: New York State Molecular Oncology Proficiency Test -Summary of Results

Sample: NYS# L/L 3 (October 2009)

Interpretation: B-cell clone with IGH and IGK rearrangements, and IgVH mutation																										
Assay	SB				PCR				RT-PCR				FISH				All methods				Method used					
	R	G	ind	Cons*	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)	FISH	
IGH	2	1		I	22	8		R				2			I	26	9	R	Dako (2), home brew (1)	Biomed (10), home brew (15), IVS (6)					Vysis (2)	
IGK	2			I	10			R								12	0	R	home brew (1), Dako (1)	Biomed (8), home brew (3)						
IGL		1		I	1			I								0	2	I	home brew (1)	Biomed (1)						
TRB		4		G	1	9		G								1	13	G	home brew (2), Dako (2)	Biomed (7), home brew (3)						
TRG					29			G								0	29	G		Biomed (7), home brew (19), IVS (3)						
TRD		1		I	1			I								0	2	I	home brew (1)	Biomed (1)						
IGH/BCL2	MBR	1		I	13			G								0	14	G	home brew (1)	Biomed (2), IVS (2), home brew (7)	home brew (2)					
	mcr				10			G								0	10	G		Biomed (2), IVS (1), home brew (5)	home brew (2)					
	MBR 3'				3			G								0	3	G		Biomed (2), IVS(1)						
	MBR/mcr											3		G	0	3	G								Vysis (3)	
IGH/CCND1 (Bcl-1)		1		I	7			G				3		G	0	11	G	home brew (1)	home brew (3), Biomed (1), IVS (1)	home brew (2)					Vysis (3)	
BCR/ABL1	p210	1		I					25		G					0	26	G	home brew (1)			home brew (8)	home brew (15), Ipsiogen(4), Cepheid(1)			
	p190								21		G					0	21	G				home brew (8)	home brew (12), Ipsiogen(4)			
	p210/190								8		G	4		G	0	12	G				home brew (2), Roche(2)	home brew (2), Roche(2), Ipsiogen(1)			Vysis (4)	
Abl kinase domain mutation									2		I				0	2	I				home brew (2)					
PML/RARA	Long								12		G					0	12	G				home brew (6)	home brew (6)			
	Short								11		G					0	11	G				home brew (5)	home brew (6)			
	Variable								3		G					0	3	G				home brew (2)	home brew (1)			
	Long/Short/Variable								1		I	4		G	0	5	G						home brew (1)			Vysis (4)
MYC t(8;14)												3		G	0	3	G								Vysis (3)	
AML1/ETO t(8;21)									6		G					0	9	G				home brew (4)	home brew (2)		Vysis (3)	
NPM/ALK t(2;5)												1		I	0	1	I								Vysis (1)	
IGH/BCL-6												2		I	0	2	I								Vysis (2)	
ETV6/RUNX1 (Tel-AML1)									2		I	2		I	0	4	G					home brew (1)	home brew (1)		Vysis (2)	
EBV					3			G								0	3	G		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	3		G	0	6	G					home brew (2)	home brew (1)		Vysis (3)	
E2A-PBX t(1;19) (4;11)					1			I								0	1	I				home brew (1)				
MLL(11q23)/ AF4 (4;11)					2			I				3		G	0	5	G					home brew (2)			Vysis (3)	
JAK 2 (V617F)					1	24		G	2		I					1	26	G		home brew (17), IVS(1), Ipsiogen(4)	home brew(2) Invader(1) Ipsiogen(1)	home brew (2)	home brew (1)			
JAK 2 (Exon 12)					2			I	2		I					0	4	G		home brew (2)		home brew (2)	home brew (1)			
MPL W 515					2			I	2		I					0	4	G		home brew (1) Invader(1)		home brew (2)	home brew (1)			
MPL S 505									2		I					0	2	I				home brew (2)	home brew (1)			
FLT 3 ITD					14			G								0	14	G		home brew (11), IVS(2), Seegene(1)						
FLT 3 D835					12			G								0	12	G		home brew (9), IVS(2), Seegene(1)						
NPM1 mutation					12			G								0	12	G		home brew (12)						
P53					2			I				4		G	0	6	G		home brew(2)						Vysis (4)	
IgVH mutation					3	*1		R	4		R					7	1*	R		home brew(2), IVS(1)	IVS(1)	home brew (3), IVS (1)				
c-kit					3			G								0	3	G		home brew(3)						
Other:(MYC,RARA,ALK,Bcl-6), (HFE, RB1, etc)					1	1		I				1	1	I	2	2	I		home brew						Vysis	

* No clonal band detected

R,G: based on ≥70% consensus; I: <70% consensus or <3 results

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		10	G		10	G		10	G
	B		11	G		11	G	11		R
	C		10	G		10	G	10		R
	D		3	G		3	G		3	G
	E		4	G		4	G		4	G
IVS	FR 1		4	G		4	G		4	G
	FR 2		5	G		5	G	4	1	R
	FR 3		5	G		5	G	1	4	G
HOMEBREW	FR 1		2	I		2	I		2	I
	FR 2		11	G		11	G	7	4	I
	FR 3		15	G		15	G	2	13	G

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		10	G		10	G	10		R
	B		10	G		10	G		10	G

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	8	1	R	9		R		9	G
	B		9	G	1	8	G		9	G
IVS	Mix 1	4	2	I	5	1	R		6	G
	Mix 2	1	4	G	4	1	R		6	G
HOMEBREW	Vy1-8	7	3	R	9	1	R		10	G
	Vy9	1	7	G	1	7	G		8	G
	Vy10		5	G		5	G		5	G
	Vy11		5	G	1	5	G		6	G
	Vy10,11		1	I		1	I		1	I
	Vg11+Jg11	1		I		1	I		1	I
	Vg11+JP11	1		I		1	I		1	I
	not defined	2	2	I	4		R		4	R

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	5	4	I	7	2	R		9	G
	B	9		R	5	4	I		9	G
	C		6	G	6	1	R	1	6	G
HOMEBREW			1	I	1		I		1	G

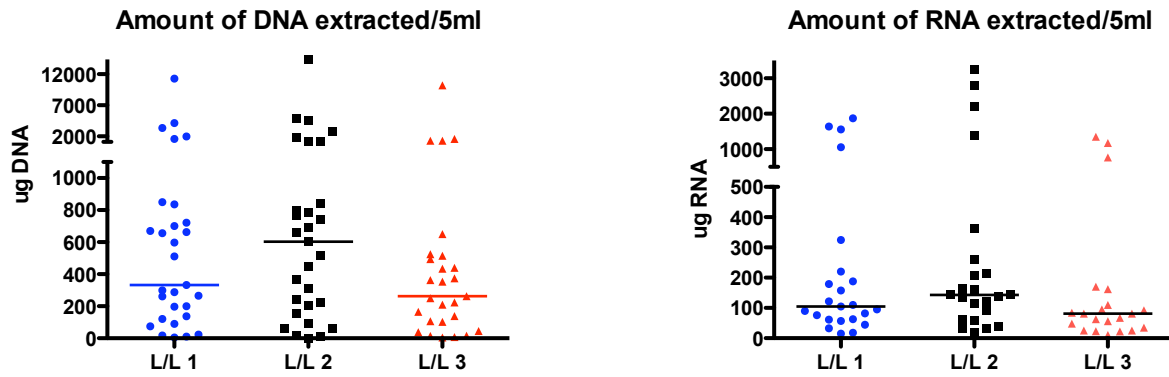


Figure 1. NYS PT MCTM 10-09 DNA and RNA yields

	LL1	LL2	LL3		LL1	LL2	LL3
Yield (ug)	DNA	DNA	DNA		RNA	RNA	RNA
Median	332	603.7	263.0		105.0	143	81
Max	11300	14360	10220		1871	3252.5	1350
Min	5.0	1.0	5.0		14.8	19	11