STATE OF NEW YORK
DEPARTMENT OF HEALTH

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Commissioner

James W. Clyne, Jr.
Executive Deputy Commissioner

Molecular and Cellular Tumor Marker Proficiency Test Program
MCTM 2-10
Summary of results[1]

April 19, 2010

Dear Laboratory Director,

Below is a summary and discussion of the New York State proficiency test for Molecular and Cellular Tumor Markers from February 22, 2010, MCTM 2-10.

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 36 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, 27 out of 29 laboratories (93%) that used PCR reported no rearrangement. Similarly, three labs reported no rearrangement by other methods (2=SB, 1=FISH). In contrast, seven out of nine labs (78%) that tested for IgKappa (IGK) by PCR found a rearrangement with both Biomed-2 A and B tubes (Table 5). Likewise, the two laboratories that tested for IGK by SB also reported a rearrangement, thus corroborating the PCR result. One lab tested for IgLambda (IGL) by SB, and found a rearrangement. None of the laboratories that tested for IGH/BCL2 by various methods reported a translocation at any of the three breakpoint clusters. Similarly, none of the twelve laboratories that tested for the IGH/CCND1 translocation (also known as Bcl-1) by any method found a rearrangement. Thus, the consensus was that this sample contained a clonal population of cells with immunoglobulin kappa and possible lambda gene rearrangements.

T-cell tests: All 28 laboratories that tested for TcRGamma (TRG) by PCR found no rearrangement. Likewise, all thirteen labs that tested for TcRBeta (TRB) reported no rearrangement (2=SB, 11=PCR). The one laboratory that tested for TcRDelta (TRD) by both SB and PCR also found no rearrangement. These results indicate that this sample did not contain a clonal population of cells with T-cell receptor gene rearrangements.

P53: Two laboratories found a mutation, E285K, and the FISH results indicate that this region of chromosome 17p13.1 was not deleted.

RAS: One lab tested for and detected a mutation in codon 12, G12A, of K-ras, whereas no other mutations were found in codons 13 and 61, or codons 12, 13, and 61 of H-ras and N-ras.

The results from all other tests performed were negative, including test for PDGFRa, B-raf, and HFE mutations.

In aggregate, these results indicate that the sample contained a clonal B-cell population, possibly with a P53 and K-ras mutation. This conclusion is in agreement with the result from Flow Cytometry, which indicated the presence of an aberrant B cell population.

NYS#L/L 2 (Table 2):
B-cell tests: For IGH, all 30 laboratories that used PCR reported a rearrangement, which was confirmed by the two laboratories that used the Dako probe for SB. In contrast, the one lab that used a home brew probe in their SB did not detect a rearrangement. IGH rearrangements were detected with the Biomed-2 tubes A, B, and C, but not D and E, as well as with the IVS and home brew primers that target all three framework regions (Table 4). One exception, however, were the IVS FR2 primers with which three out of five labs did not detect a rearrangement. Interestingly, the one lab that used FISH reported no large scale chromosome 14q32 alteration. Similarly, eight out of ten labs (80%) that tested for IGK by PCR reported a rearrangement with both or either of the Biomed-2 tube A and/or B primers (Table 5). The two labs that did not detect a rearrangement are the same two labs that did not detect the IGK rearrangement for L/L1. They may want to reevaluate their assay design and performance. In contrast to the PCR consensus, the two labs that also used SB reported no rearrangement, although they found a rearrangement by PCR. The one lab that also tested for IGL by SB and PCR found no rearrangement. No translocations involving IGH/BCL2, or IGH/CCND1 (Bcl-1) were detected by any method. Thus, these results suggest that this sample contained a B-cell clone with IGH and IGK gene rearrangements.

T-cell tests: 23 out of 28 (82%) laboratories that tested for TRG by PCR found no rearrangement. Likewise, nine out of eleven (82%) labs that tested for TRB by PCR also indicated no rearrangement, and two out of three labs detected no rearrangement by SB. The one lab that tested for TRD found no rearrangement by both SB and PCR. These results suggest that this sample did not contain a clonal population of cells with T-cell receptor gene rearrangements.

EBV: All three labs that tested for also detected the presence of EBV virus sequences by PCR, which is consistent with a possible EBV-mediated transformation of the clonal cell population.

IGHV mutation: Eight out of nine (89%) labs reported IGHV hypermutation. Of those, five labs assigned the sequence to the IGHV3-23 family, and four reported mutation rates between 8.4 to 31.6%, whereas one lab did not report the mutation rate. The other four labs assigned the sequence to the IGHV3-9 family, but only three reported hypermutation. Of those, only two reported the mutation rate as 2.7 and 97.57%, respectively. The one lab that reported no hypermutation presumably found less than 2% of the analyzed sequence mutated, but did not give a number. Furthermore, it is possible that the 97.57% mutation rate given by one lab really reflects the degree of identity, which would result in a mutation rate of 2.43, in close agreement with the other lab's 2.7%.

The results from all other tests performed were negative, including tests for PDGFRa, B-raf, HFE, and K-, H-, and N-ras mutations.

In aggregate, these results indicate that the sample contained a B-cell clone with IGH and IGK rearrangements and IGHV hypermutation. This overall conclusion is consistent with the results from Flow Cytometry, which indicated an aberrant B cell population.

NYS#L/L 3 (Table 3):
B-cell tests: For IGH, there was perfect agreement across all labs and methods (2=SB, 30=PCR, 1=FISH) that this sample did not contain a clonal cell population with an IGH rearrangement. Similarly, nine out of ten labs that tested for IgKappa (IGK) by PCR found no rearrangement, as did the two laboratories that tested for IGK by SB. One lab also tested for IgLambda (IGL) by SB and PCR, and found no rearrangement. All laboratories that tested for IGH/BCL2 and/or IGH/CCND1 by various methods reported no translocation. Thus, the consensus was that this sample did not contain a clonal cell population with immunoglobulin gene rearrangements.

T-cell tests: All 28 and all 13 laboratories that tested for TcRGamma (TRG) and TcRBeta (TRB), respectively, found a rearrangement. For TRG the rearrangement possibly occurred in the Vγ1-8 regions detected by the Biomed-2 tube A, the IVS mix 1 or home brew Vγ1-8 primers (Table 6), and for TRB the rearrangement possibly occurred in the Jβ1 region detected by the Biomed-2 tube A primers (Table 7). The one laboratory that tested for TcRDelta (TRD) by both SB and PCR found no rearrangement. These results suggest that this sample contained a clonal cell population with T-cell receptor beta and gamma gene rearrangements.

P53: Only one lab tested for and detected three mutations (R175H, R248Q, R282W), whereas no chromosomal abnormality was detected by three out of four labs (75%) using FISH.

RAS: One lab tested for and detected a mutation in codon 12, G12D, of K-ras, whereas no other mutations were found in codons 13 and 61, or codons 12, 13, and 61 of H-ras and N-ras.

The results from all other tests performed were negative, including tests for PDGFRa, B-raf, and HFE mutations.

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 aberrant T-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 1, 0.7, and 0.4 µg per 5 ml specimen to a maximum of 173,400, 173,400, and 313,250 µg, respectively, corresponding to a 173,400- to 783,125-fold difference between lowest and highest yield for each sample. RNA yields for samples L/L1, 2 and 3 also ranged broadly from 1.2, 0.7, and 1.1 µg to 41,275, 19,142, and 35,029 µg, respectively, corresponding to a 27,346- to 34,396-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, the methods used for DNA and RNA isolation contributed also 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 **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 dates for the Molecular and Cellular Tumor Marker PT mail-out in 2010 are:

Mail-out date	**Due Date**
June 28	July 27
October 25	November 23

Sincerely,

Erasmus Schneider, Ph.D.
Director, Oncology Section
Clinical Laboratory Evaluation Program
Wadsworth Center, Room E604
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Table 1: New York State Molecular Oncology Proficiency Test -Summary of Results

Sample: NYS# L/L 1 (February 2010)

Interpretation: B-cell clone with IGK rearrangement

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		I	2	27		G						1		I	2	30	G	Dako (1), home brew (1)	Biomed (11), home brew (15), IVS (4)				Vysis (1)
IGK	2			I	7	2		R									9	2	R	home brew (1), unk (1)	Biomed (7), home brew (2)				
IGL	1			I													1	0	I	unk (1)					
TRB		2		I		11		G									0	13	G	home brew (1), Dako (1)	Biomed (8), home brew (3),				
TRG						28		G									0	28	G		Biomed (8), home brew (17), IVS (3),				
TRD		1		I		1		I									0	2	I	home brew (1)	Biomed (1)				
IGH/BCL2 MBR		1		I		14		G									0	15	G	home brew (1)	Biomed (3), 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														2		I	0	2	I						Vysis (2)
IGH/CCND1 (Bcl-1)		1		I		8		G						3		G	0	12	G	home brew (1)	home brew (3), Biomed (2), IVS (1)	home brew (2)			Vysis (3)
BCR/ABL1 p210		1		I						25		G					0	26	G	home brew (1)			home brew (7)	home brew (16), Ipsogen(4), Cepheid(1)	
p190										20		G					0	20	G				home brew (7)	home brew (12), Ipsogen(3)	
p210/190										7		G		2		I	0	9	G				home brew (2), Roche(2)	home brew (2), Roche(1), Ipsogen(1)	Vysis (2)
Abl kinase domain mutation										2		I					0	2	I				home brew (2)		
PML/RARA Long										12		G					0	12	G				home brew (7)	home brew (6)	
Short										11		G					0	11	G				home brew (6)	home brew (6)	
Variable										3		G					0	3	G				home brew (3)	home brew (1)	
Long/Short/Variable										1		I		3		G	0	4	G						Vysis (3)
MYC t(8;14)														2		I	0	2	I						Vysis (2)
AML1/ETO t(8;21)										6		G		2		I	0	8	G				home brew (4)	home brew (2)	Vysis (2)
IGH/BCL-6														1		I	0	1	I						Vysis (1)
ETV6/RUNX1 (Tel-AML1)										2		I		1		I	0	3	G				home brew (1)	home brew (1)	Vysis (1)
EBV						3		G									0	3	G		home brew (2)	home brew (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		2		I	0	5	G				home brew (2)	home brew (1)	Vysis (2)
E2A-PBX t(1;19) (4;11)										1		I					0	1	I				home brew (1)		
MLL(11q23)/ AF4 (4;11)										2		I		2		I	0	4	G				home brew (2)		Vysis (2)
JAK 2 (V617F)						25		G		2		I					0	27	G		home brew (16), IVS(4), Ipsogen(1)	home brew(1) Invader(1) Ipsogen(2)	home brew (2)	home brew (1)	
JAK 2 (Exon 12)						6		G		3		G					0	9	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						14		G									0	14	G		home brew (11), IVS(2), Seegene(1)				
FLT 3 D835						12		G									0	12	G		home brew (10), IVS(1), Seegene(1)				
NPM1 mutation						13		G									0	13	G		home brew (13)				
P53					2			I						4		G	2	4	I/G		home brew(2)				Vysis (4)
IgVH mutation						4N/1G		N*		3		N*					0	0	N*		home brew(3), IVS(1)	IVS(1)	home brew (2), IVS (1)		
c-kit						4		G									0	4	G		home brew(4)				
Other ‡																									

N*: No clonal band detected

Cons #: R or G based on ≥70% consensus; I if <70% consensus or <3 results

Other ‡ : See critique for details.

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

Sample: NYS# L/L 2 (February 2010)

Interpretation: B-cell clone with IGH and IGK rearrangements, and IgVH hypermutation.

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	30			R						1		I	32	2	I/R/I	Dako (2), home brew (1)	Biomed (11), home brew (14), IVS (5)				Vysis (1)
IGK		2		I	8	2		R									8	4	I/R	home brew (1), unk (1)	Biomed (8), home brew (2)				
IGL		1		I		1		I									0	2	I	unk (1)	Biomed (1)				
TRB	1	2		I	2	9		G									3	11	I/G	home brew (1), Dako (2)	Biomed (8), home brew (3),				
TRG					5	23		G									5	23	G		Biomed (8), home brew (17), IVS (3),				
TRD		1		I		1		I									0	2	I	home brew (1)	Biomed (1)				
IGH/BCL2 MBR		1		I		14		G									0	15	G	home brew (1)	Biomed (3), 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'		1		I		3		G									0	4	G		Biomed (2), IVS(1)				
MBR/mcr														2		I	0	2	I						Vysis (2)
IGH/CCND1 (Bcl-1)		1		I		7		G						3		G	0	11	G	home brew (1)	home brew (2), Biomed (2), IVS (1)	home brew (2)			Vysis (3)
BCR/ABL1 p210		1		I						25		G					0	26	G	home brew (1)			home brew (7)	home brew (16), Ipsogen(4), Cepheid(1)	
p190										20		G					0	20	G				home brew (7)	home brew (12), Ipsogen(3)	
p210/190										7		G		3		G	0	10	G				home brew (2), Roche(2)	home brew (2), Roche(1), Ipsogen(1)	Vysis (3)
Abl kinase domain mutation										2		I					0	2	I				home brew (2)		
PML/RARA Long										12		G					0	12	G				home brew (7)	home brew (6)	
Short										11		G					0	11	G				home brew (6)	home brew (6)	
Variable										3		G					0	3	G				home brew (3)	home brew (1)	
Long/Short/Variable										1		I		3		G	0	4	G						Vysis (3)
MYC t(8;14)														2		I	0	2	I						Vysis (2)
AML1/ETO t(8;21)										6		G		2		I	0	8	G				home brew (4)	home brew (2)	Vysis (2)
IGH/BCL-6														1		I	0	1	I						Vysis (1)
ETV6/RUNX1 (Tel-AML1)										2		I		1		I	0	3	G				home brew (1)	home brew (1)	Vysis (1)
EBV					3			R									3	0	R		home brew (2)	home brew (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		2		I	0	5	G				home brew (2)	home brew (1)	Vysis (2)
E2A-PBX t(1;19) (4;11)										1		I					0	1	I				home brew (1)		
MLL(11q23)/ AF4 (4;11)										2		I		2		I	0	4	G				home brew (2)		Vysis (2)
JAK 2 (V617F)						25		G		2		I					0	27	G		home brew (16), IVS(4), Ipsogen(1)	home brew(1) Invader(1) Ipsogen(2)	home brew (2)	home brew (1)	
JAK 2 (Exon 12)						6		G		3		G					0	9	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						14		G									0	14	G		home brew (11), IVS(2), Seegene(1)				
FLT 3 D835						12		G									0	12	G		home brew (10), IVS(1), Seegene(1)				
NPM1 mutation						13		G									0	13	G		home brew (13)				
P53						2		I						4		G	0	6	G		home brew(2)				Vysis (4)
IgVH mutation					5			R	3	1		R					8	0	R		home brew(3), IVS(1)	IVS(1)	home brew (2), IVS (1)	home brew (1)	
c-kit						4		G									0	4	G		home brew(4)				
Other ‡																									

N*: No clonal band detected

Cons #: R or G based on ≥70% consensus; I if <70% consensus or <3 results

Other ‡ : See critique for details.

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

Sample: NYS# L/L 3 (February 2010)

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		2		I		30		G						1		I	0	33	G	Dako (1), home brew (1)	Biomed (11), home brew (16), IVS (5)				Vysis (1)
IGK		2		I	1	9		G									1	11	G	home brew (1), unk (1)	Biomed (7), home brew (2)				
IGL		1		I		1		I									0	2	I	unk (1)	Biomed (1)				
TRB	2			I	11			R									13	0	R	home brew (1), Dako (1)	Biomed (8), home brew (3),				
TRG					28			R									28	0	R		Biomed (8), home brew (17), IVS (3),				
TRD		1		I		1		I									0	2	I	home brew (1)	Biomed (1)				
IGH/BCL2 MBR		1		I		14		G									0	15	G	home brew (1)	Biomed (3), 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														1		I	0	1	I						Vysis (1)
IGH/CCND1 (Bcl-1)		1		I		7		G						3		G	0	11	G	home brew (1)	home brew (2), Biomed (2), IVS (1)	home brew (2)			Vysis (3)
BCR/ABL1 p210		1		I						25		G					0	26	G	home brew (1)			home brew (7)	home brew (16), Ipsogen(4), Cepheid(1)	
p190										20		G					0	20	G				home brew (7)	home brew (12), Ipsogen(3)	
p210/190										7		G		3		G	0	10	G				home brew (2), Roche(2)	home brew (2), Roche(1), Ipsogen(1)	Vysis (3)
Abl kinase domain mutation										2		I					0	2	I				home brew (2)		
PML/RARA Long										12		G					0	12	G				home brew (7)	home brew (6)	
Short										11		G					0	11	G				home brew (6)	home brew (6)	
Variable										3		G					0	3	G				home brew (3)	home brew (1)	
Long/Short/Variable										1		I		3		G	0	4	G						Vysis (3)
MYC t(8;14)														2		I	0	2	I						Vysis (2)
AML1/ETO t(8;21)										6		G		2		I	0	8	G				home brew (4)	home brew (2)	Vysis (2)
IGH/BCL-6														1		I	0	1	I						Vysis (1)
ETV6/RUNX1 (Tel-AML1)										2		I		1		I	0	3	G				home brew (1)	home brew (1)	Vysis (1)
EBV						3		G									0	3	G		home brew (2)	home brew (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		2		I	0	5	G				home brew (2)	home brew (1)	Vysis (2)
E2A-PBX t(1;19) (4;11)										1		I					0	1	I				home brew (1)		
MLL(11q23)/ AF4 (4;11)										2		I		2		I	0	4	G				home brew (2)		Vysis (2)
JAK 2 (V617F)						25		G		2		I					0	27	G		home brew (16), IVS(4), Ipsogen(1)	home brew(1) Invader(1) Ipsogen(2)	home brew (2)	home brew (1)	
JAK 2 (Exon 12)						6		G		3		G					0	9	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						14		G									0	14	G		home brew (11), IVS(2), Seegene(1)				
FLT 3 D835						12		G									0	12	G		home brew (10), IVS(1), Seegene(1)				
NPM1 mutation						13		G									0	13	G		home brew (13)				
P53					1			I					1	3		G	2	3	I/G		home brew(1)				Vysis (4)
IgVH mutation						4N/1G		N*		3		N*					0	0	N*		home brew(3), IVS(1)	IVS(1)	home brew (2), IVS (1)		
c-kit						4		G									0	4	G		home brew(4)				
Other ‡																	0	0							

N*: No clonal band detected

Cons #: R or G based on ≥70% consensus; I if <70% 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		10	G	9	1	R		10	G
	B	1	10	G	10	1	R		11	G
	C		11	G	8	3	R		11	G
	D		3	G		3	G		3	G
	E		4	G		4	G		4	G
IVS	FR 1		4	G	4		R		4	G
	FR 2	1	4	G	2	3	I		5	G
	FR 3		5	G	5		R		5	G
HOMEBREW	FR 1		2	I	2		I		2	I
	FR 2		12	G	11	1	R		11	G
	FR 3	1	13	G	13	1	R		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	8	2	R	5	5	I	1	9	G
	B	8	2	R	8	2	R		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		11	G		11	G	11		R
	B		11	G	2	9	G	1	10	G
IVS	Mix 1		6	G		6	G	6		R
	Mix 2		6	G		6	G	2	4	I
HOMEBREW	Vγ1-8		7	G		7	G	6	1	R
	Vγ9		6	G	2	4	I		6	G
	Vγ10		5	G		5	G		5	G
	Vγ11		5	G	3	2	I	1	4	G
	Vg11+Jg11		1	I		1	I			I
	Vg11+JP11		1	I		1	I			I
	not defined		3	G		3	G	3		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		10	G		10	G	9	1	R
	B		10	G		10	G	5	5	I
	C		8	G	2	6	G	5	3	I
HOMEBREW	not defined		1	I		1	I	1		I

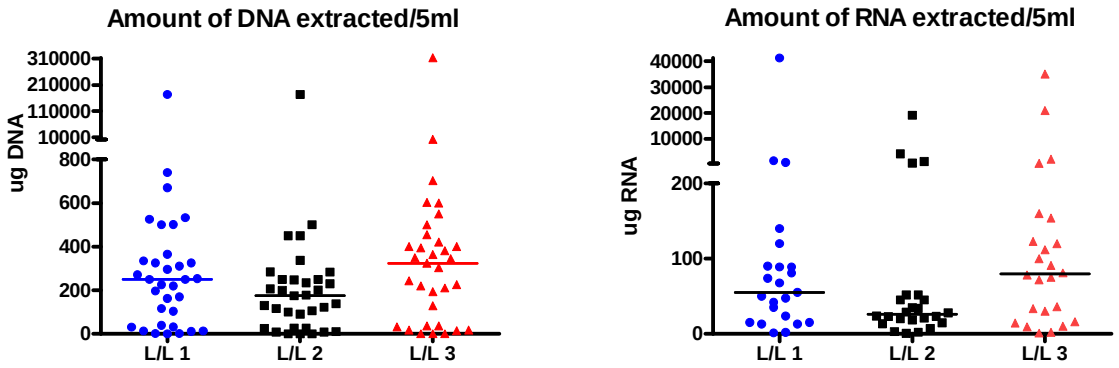

Figure 1. NYS PT MCTM 2-10 DNA and RNA yields

	LL1	LL2	LL3		LL1	LL2	LL3
Yield (ug)	DNA	DNA	DNA		RNA	RNA	RNA
Median	250.15	176.5	334.0		67.9	28	78
Max	173400	173400	313250		41275	19142	35029
Min	1.0	0.7	0.4		1.205	0.675	1.149