


*TRUPCR*TM



TRUPCR[®] JAK2 Kit

Detection of *V617F* mutation



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TRUPCR® JAK2 Kit

Version 1.1

Detection of V617F mutation in the Human Janus Kinase 2 gene



Product No.: 3B1246/3B1247



48 tests/ 96 tests



Temperature limitation



Nov 2015

Manufactured By-

3B BlackBio Biotech India Ltd.

7-C Industrial Area, Govindpura, Bhopal-462023 (M.P)

EC

REP

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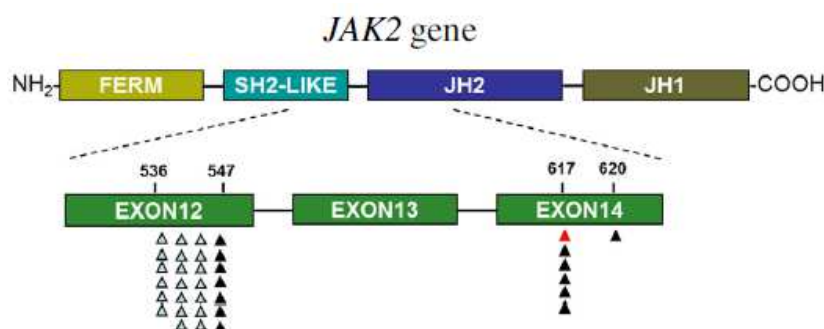
INTENDED USE

The TRUPCR® JAK2 V617F is an *in vitro* diagnostic kit designed for the qualitative detection of the V617F mutation in the Janus Kinase 2 gene by Real Time PCR on genomic DNA of patients suspected with myeloproliferative disorder. Absence of the V617F mutation does not exclude the presence of other JAK2 mutations.

JAK 2 AND ITS CLINICAL RELEVANCE

The Janus kinase 2 gene (JAK2) codes for a tyrosine kinase (JAK2) that is associated with the cytoplasmic portion of a variety of transmembrane cytokine and growth factor receptors important for signal transduction in hematopoietic cells. Signaling via JAK2 activation causes phosphorylation of downstream signal transducers and activators of transcription (STAT) proteins (eg, STAT5) ultimately leading to cell growth and differentiation.

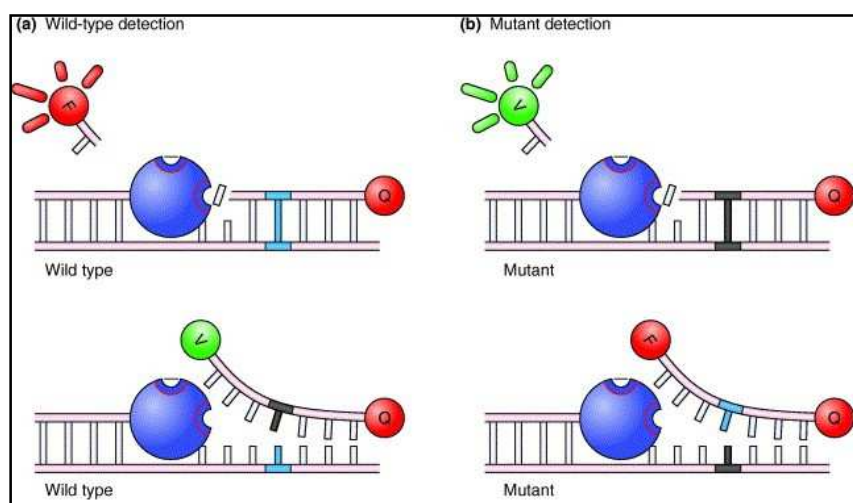
Chronic myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell malignancies characterized by excessive production of blood cells. BCR-ABL1-negative MPN frequently harbor an acquired single nucleotide mutation in JAK2 characterized as c.G1849T; p. Val617Phe (V617F) and it is a gain-of-function mutation that leads to clonal proliferation. The JAK2 V617F is present in 95% to 98% of polycythemia vera (PV), and 50% to 60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET). It has also been described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome. Diagnostic criteria for ET, MF, and PV adopted by the World Health Organization (WHO) include identification of a clonal marker, with a specific recommendation to test for the JAK2 V617F mutation in exon 14.¹ Detection of the JAK2 V617F is useful to help establish the diagnosis of MPN and The JAK2 allele burden decreases with successful therapy, disappears in some patients, and reappears during relapse.^{2,3}



PRINCIPLE

TRUPCR® JAK2 mutation kit is an allelic discrimination real-time polymerase chain reaction (PCR) assay for qualitative detection of V617F mutation in jak2 (Janus Kinase 2) gene against a background of wild-type genomic DNA. This mutation is a guanine to thymidine transversion in position 1849 of the JAK2 gene, which leads to a valine to phenylalanine substitution in position 617 of the protein (V617F). The test utilizes JAK2 wild type and V617F/G1849T targeted fluorescent probes to detect low-copy number (1%) JAK2 V617F-mutant DNA in sample.

In an allelic discrimination, two different probes specific for each allele are included in the PCR assay. Each probe is labeled with a different fluorescent dye (such as FAM or HEX/VIC) at its 5' end and contains a non fluorescent quencher at the 3' end. One Probe is a perfect match to the allele 2 sequence (eg., wild-type allele) and the other one is perfect match to the allele 1 sequence (eg., mutant allele). A completely hybridized probe remains bound during strand displacement, resulting in efficient probe cleavage by the 5'→3' exonuclease activity of Taq DNA Polymerase, separating the reporter dye from the quencher and thus releasing detectable fluorescence. A mismatch between probe and target greatly reduces the efficiency of probe hybridization and cleavage and no reporter dye is released. Thus, substantial increase in FAM or HEX/VIC dye fluorescence indicates homozygosity for the FAM- or HEX/VIC- specific allele. An increase in both signals indicates heterozygosity.



REAGENTS

The Kit contains amplification reagents for performance of 48/96 amplification reactions. Thaw and handle reagents on ice. Do not freeze/thaw Kit vials repeatedly. In case of frequent use, we recommend to aliquot the contents of the vials into 10 reactions each. This will also rule-out kit/ reagent contamination.

Reagent	Description	Volume in μL 48 reactions	Volume in μL 96 reactions
Multiplex Master Mix	<ul style="list-style-type: none"> • Reaction Buffer • MgCl_2 and stabilizers • Hot-start DNA polymerase • dNTPs (dATP, dCTP, dGTP, dTTP) 	240 μL X 2	240 μL X 4
JAK2 Primer Probe Mix	• Primer and probe mix for V617F Mutation detection	12 μL X 2	12 μL X 4
Wild Type Control	• Negative Control for V617F mutation	24 μL X 2	24 μL X 4
Mutant Control	• Positive Control for V617F mutation (100%)	24 μL X 2	24 μL X 4
Cut-Off Control	• Cut-off control for V617F Mutation (1%)	24 μL X 2	24 μL X 4
Negative Control	• Sterilized water	500 μL X 2	500 μL X 4

DNA EXTRACTION

Genomic DNA should be obtained either from whole blood, purified peripheral blood lymphocytes, polynuclear cells, or granulocytes. DNA Extraction kits are available from various manufacturers (Table below). Sample volumes for DNA Extraction procedure depend on the protocol used. The procedure for extraction should be carried out according to the manufacturer's instruction.

DNA quantity is determined by measuring optical density at 260 nm. DNA quality should be assessed by spectrophotometry.

The A260/A280 ratio should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals. The qPCR reaction is optimized for 200 ng of purified genomic DNA.

Sample Material	Nucleic Acid Isolation Kit	Cat No.
Whole blood, purified peripheral blood lymphocytes, polynuclear cells, or granulocytes	3B Speed Tools DNA Extraction Kit	3B205
	QIAamp DNeasy Blood & Tissue Kit	69581

REAL TIME PCR PROTOCOL

REACTION PREPARATION

- a) Prepare the PCR Mix as follows:

Name of the Reagent	For "1" rxn.
Multiplex Master Mix	10 µl
JAK2 Primer probe mix	0.5 µl
Sterile Water	4.5 µl

- b) Transfer **15 µl** of the above prepared PCR Master Mix in 0.2 ml PCR tubes and close the tubes.
- c) For **15 µl** of above reaction mix, add upto **5 µl** DNA (200ng) from the purified samples to each amplification vial.
- d) For Positive Controls use **5 µl** of 100% wild type, 100% mutant and cut-off control to each tube and for negative control add **5 µl** of sterilized water.

PROGRAM SET UP FOR APPLIED BIOSYSTEM 7500

1. ABSOLUTE QUANTITATION

- a) Define the following setting for Temperature Profile and Dye Acquisition

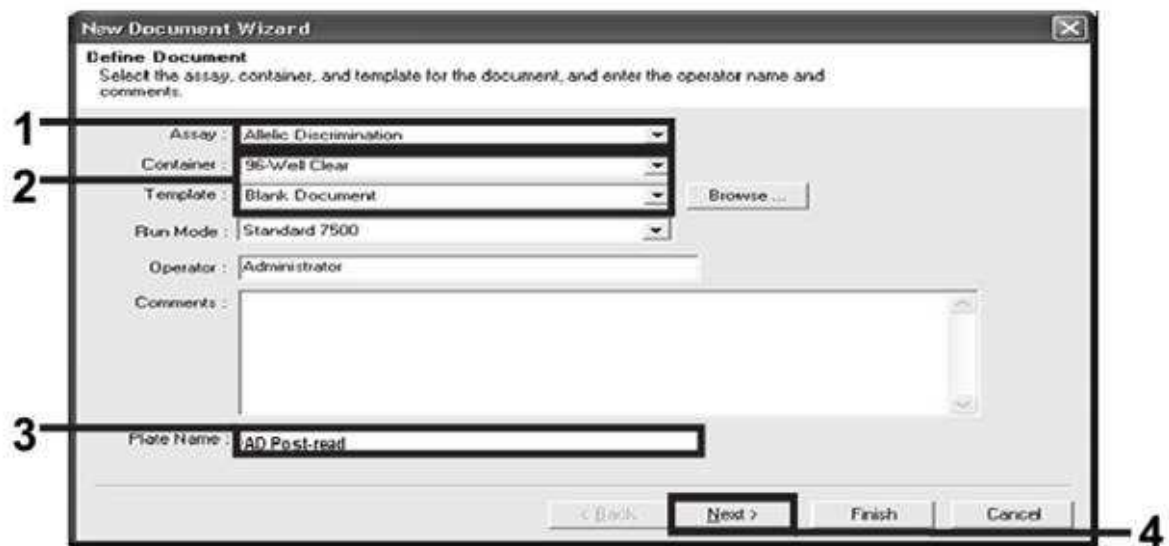
Step	Program name	Temperature, °C	Time	Dye Acquisition	Cycles
1	Initial Denaturation	94	10 min	-	1
2	Denaturation	94	15 sec	-	40
	Annealing/Extension	60	60 sec	YES	

- b) Define the following setting for channel selection

Detection	Detector Name	Reporter	Quencher	Gain Setup
Mutant	JAK2 Mutant	FAM	None	Auto
Wild Type	JAK2 Wild Type	HEX/VIC	None	Auto

2. POST READ (Allelic Discrimination)

In New document wizard select Allelic Discrimination (1), Blank Documents (2), Plate name (3) and proceed for Next (4)



The screenshot shows the 'New Document Wizard' window, 'Define Document' step. The window has a title bar with a close button. The main area contains several fields and buttons. A 'Browse...' button is next to the 'Template' field. The 'Plate Name' field is at the bottom. The 'Next >' button is highlighted with a black box. Numbered callouts 1 through 4 point to specific elements: 1 points to the 'Assay' dropdown, 2 points to the 'Template' dropdown, 3 points to the 'Plate Name' field, and 4 points to the 'Next >' button.

New Document Wizard

Define Document
Select the assay, container, and template for the document, and enter the operator name and comments.

1 Assay: Allelic Discrimination

2 Container: 96-Well Clear

Template: Blank Document

Run Mode: Standard 7500

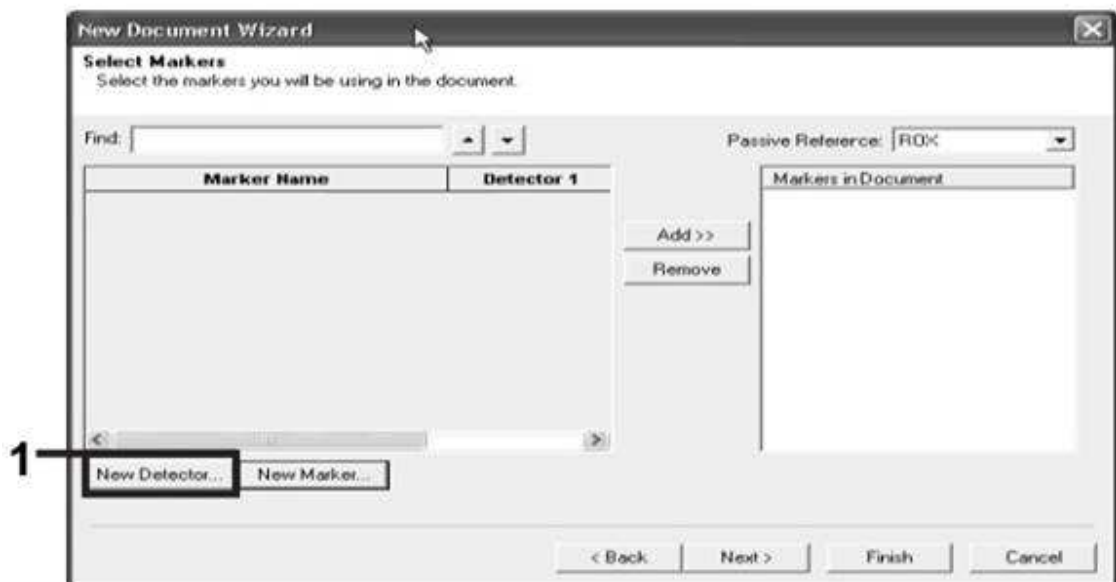
Operator: Administrator

Comments:

3 Plate Name: AD Post-read

< Back Next > Finish Cancel 4

1. In next screen click on "New Detector" (1)



The screenshot shows the 'New Document Wizard' window, 'Select Markers' step. The window has a title bar with a close button. The main area contains a 'Find:' field, a 'Passive Reference' dropdown, a table with 'Marker Name' and 'Detector 1' columns, and a 'Markers in Document' list. There are 'Add >>' and 'Remove' buttons. At the bottom, there are 'New Detector...' and 'New Marker...' buttons. A 'Next >' button is highlighted with a black box. Numbered callout 1 points to the 'New Detector...' button.

New Document Wizard

Select Markers
Select the markers you will be using in the document.

Find: []

Passive Reference: ROK

Marker Name	Detector 1
-------------	------------

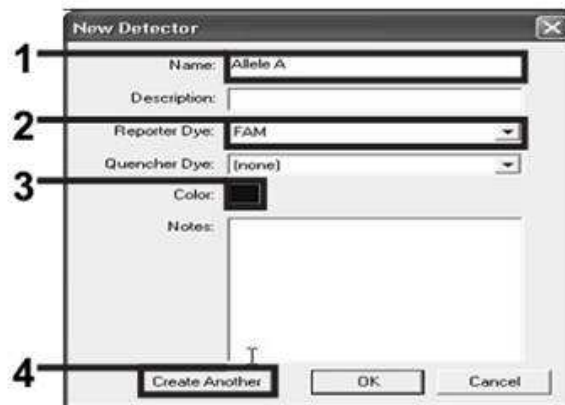
Add >> Remove

Markers in Document

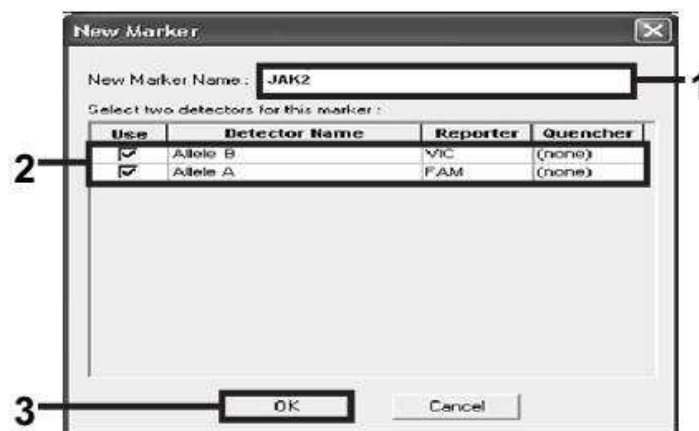
1 New Detector... New Marker...

< Back Next > Finish Cancel

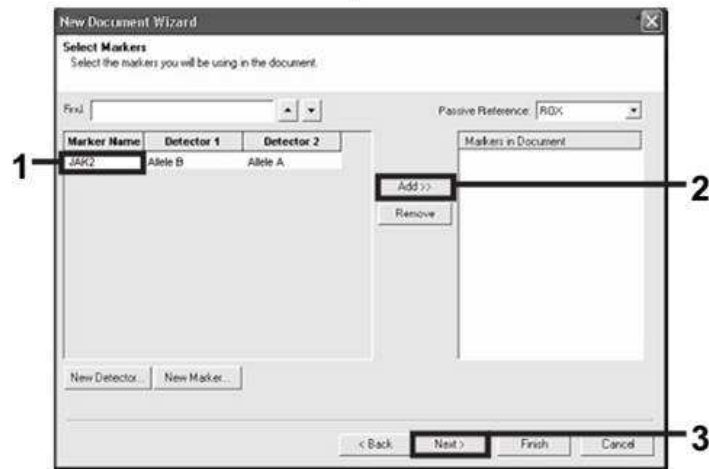
2. Create new detector, give name as Allele A (1), select reporter dye as FAM (2), Color as preferred (3) and move to create another detector (4). Give name to second detector as Allele B, select reporter dye as VIC/HEX , Color as preferred, then click on “OK”



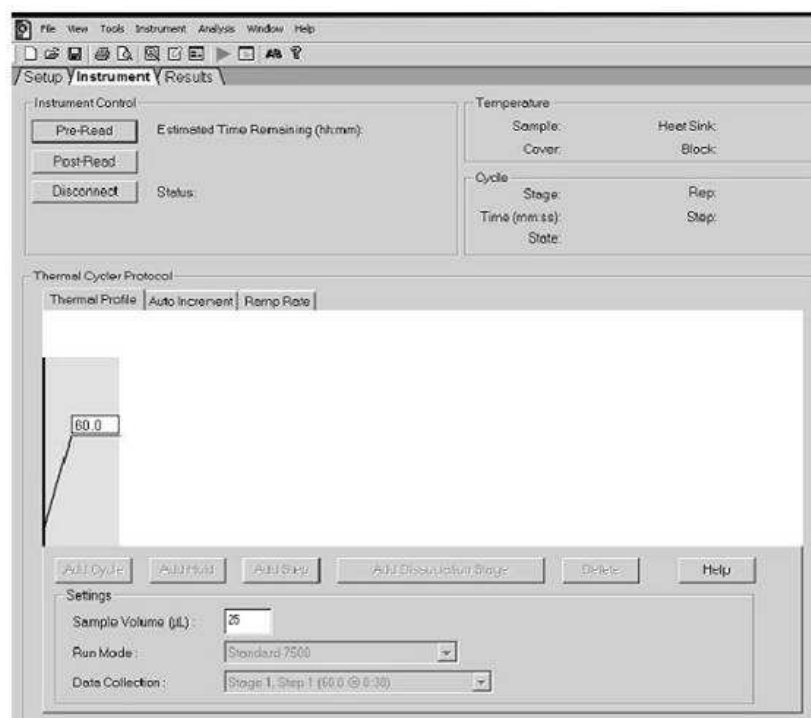
3. Now click on New Marker, Give name as JAK2 (1), Select detector Allele A and Allele B (2) and then click OK (3)



4. In the “Select Markers” dialog box, select “JAK2” (1), as created above, or a suitable predefined marker, and then click “Add>>”(2) and click on Next (3)

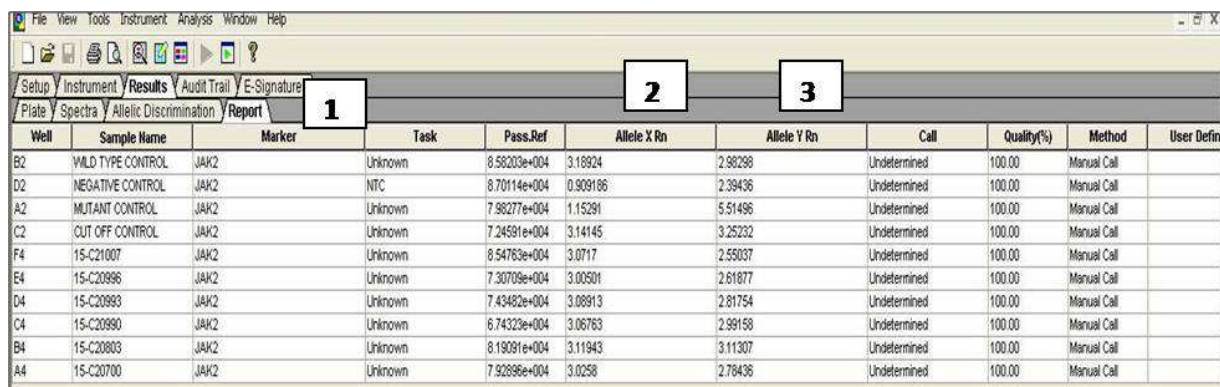


5. In the “Setup Sample Plate” dialog box, click and drag to select the marker for wells that contain samples. Click “Finish”.
6. Select the “Instrument” tab, and change the sample volume to 20 μ l
7. Start the post-read run. Click “Post-Read”. The instrument will perform a run of 1 cycle for 60 seconds at 60°C. During this run, the instrument collects FAM and VIC/HEX fluorescence in each well



RESULT ANALYSIS FOR APPLIED BIOSYSTEM 7500

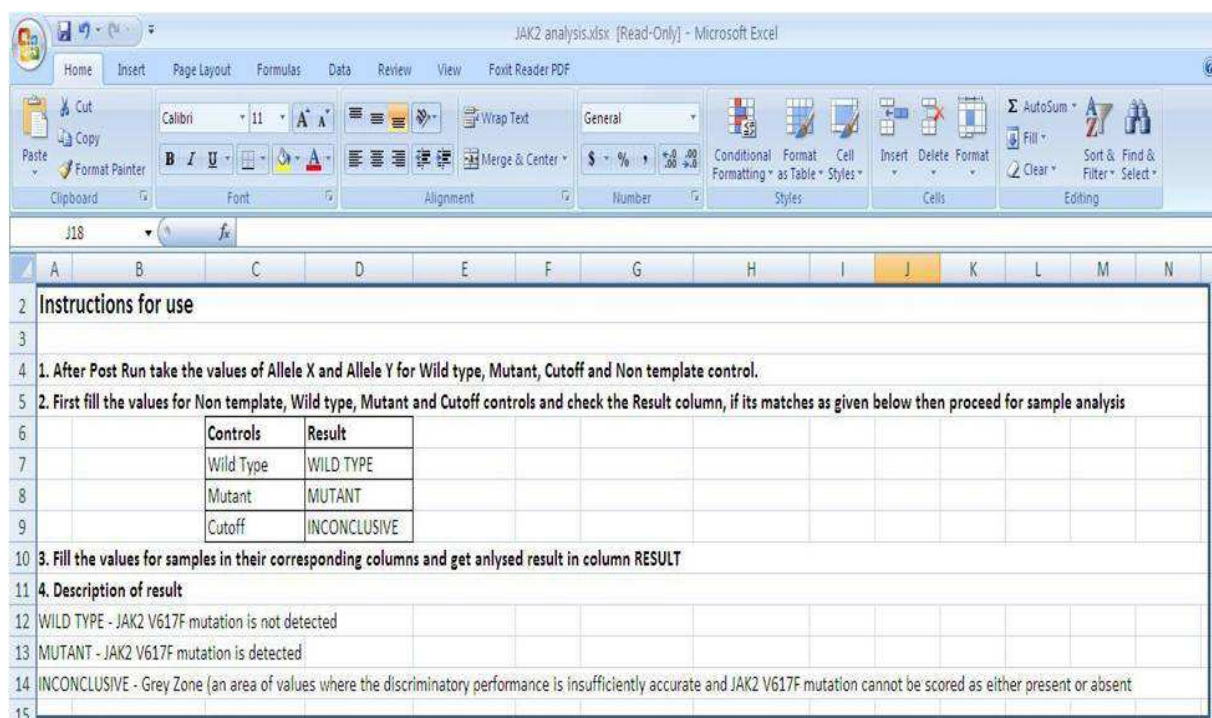
1. After completion of run, view Report (1) then note down the values of Allele X Rn (2) and Allele Y Rn (3) of each control and sample.



Well	Sample Name	Marker	Task	Pass, Ref	Allele X Rn	Allele Y Rn	Call	Quality(%)	Method	User Defn
B2	WILD TYPE CONTROL	JAK2	Unknown	8.56203e+004	3.16924	2.98298	Undetermined	100.00	Manual Call	
D2	NEGATIVE CONTROL	JAK2	NTC	8.70114e+004	0.909186	2.39436	Undetermined	100.00	Manual Call	
A2	MUTANT CONTROL	JAK2	Unknown	7.98277e+004	1.15291	5.51496	Undetermined	100.00	Manual Call	
C2	CUT OFF CONTROL	JAK2	Unknown	7.24591e+004	3.14145	3.25232	Undetermined	100.00	Manual Call	
F4	15-C21007	JAK2	Unknown	8.54783e+004	3.0717	2.55037	Undetermined	100.00	Manual Call	
E4	15-C20986	JAK2	Unknown	7.30709e+004	3.00501	2.61877	Undetermined	100.00	Manual Call	
D4	15-C20993	JAK2	Unknown	7.43482e+004	3.08913	2.81754	Undetermined	100.00	Manual Call	
C4	15-C20990	JAK2	Unknown	6.74323e+004	3.06763	2.99158	Undetermined	100.00	Manual Call	
B4	15-C20803	JAK2	Unknown	8.19091e+004	3.11943	3.11307	Undetermined	100.00	Manual Call	
A4	15-C20700	JAK2	Unknown	7.92896e+004	3.0258	2.78436	Undetermined	100.00	Manual Call	

2. Analysis using given analysis tool

Follow the instruction given in analysis tool



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
2	Instructions for use													
3														
4	1. After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.													
5	2. First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis													
6			Controls	Result										
7			Wild Type	WILD TYPE										
8			Mutant	MUTANT										
9			Cutoff	INCONCLUSIVE										
10	3. Fill the values for samples in their corresponding columns and get analysed result in column RESULT													
11	4. Description of result													
12	WILD TYPE - JAK2 V617F mutation is not detected													
13	MUTANT - JAK2 V617F mutation is detected													
14	INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)													
15														

Fill the sample ID and value of allele X and allele Y in given column for each sample in analysis tools, and find the result in result column

TRUPCR® JAK2 Kit

Instructions for use

- After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.
- First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis

Controls	Result
Wild Type	WILD TYPE
Mutant	MUTANT
Cutoff	INCONCLUSIVE

- Fill the values for samples in their corresponding columns and get analysed result in column RESULT

4. Description of result

WILD TYPE - JAK2 V617F mutation is not detected

MUTANT - JAK2 V617F mutation is detected

INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)

No	Sample	Sample ID	Allele Y (FAM)	Allele X (VIC)	Ratio	Normalized ratio	Result
1	NTC		1.394	0.179	7.787709	1	
2	Mutant Control		4.899	0.393	12.46565	1.600682314	MUTANT
3	Wild Type Control		1.701	1.552	1.096005	0.140735239	WILD TYPE
4	Cutoff Control		1.989	1.456	1.366071	0.175413763	INCONCLUSIVE
5	Sample 1	15-C20700	1.266	0.892178	1.419	0.114562259	WILD TYPE
6	Sample 2	16-C20700	2.706	0.774	3.496124	0.448928409	MUTANT
7	Sample 3				#DIV/0!	#DIV/0!	#DIV/0!
8	Sample 4				#DIV/0!	#DIV/0!	#DIV/0!

PROGRAM SET UP FOR QUANTSTUDIO® 3 AND 5

QuantStudio™ Design & Analysis Software v1.4

File Edit Analysis Tools Help

Properties Method Plate Run Results Export

Experiment Properties

Name: TRUPCR JAK2 kit

Barcode: Barcode - optional

User name: User name - optional

Instrument type: QuantStudio™ 5 System

Block type: 96-Well 0.2-mL Block

Experiment type: Genotyping

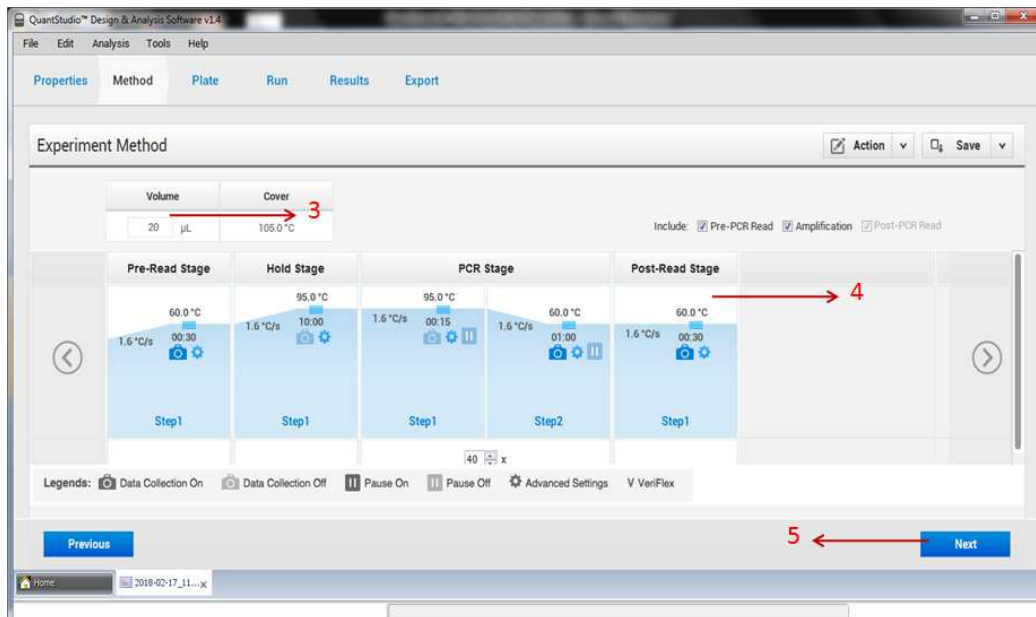
Chemistry: TaqMan® Probes

Run mode: Standard

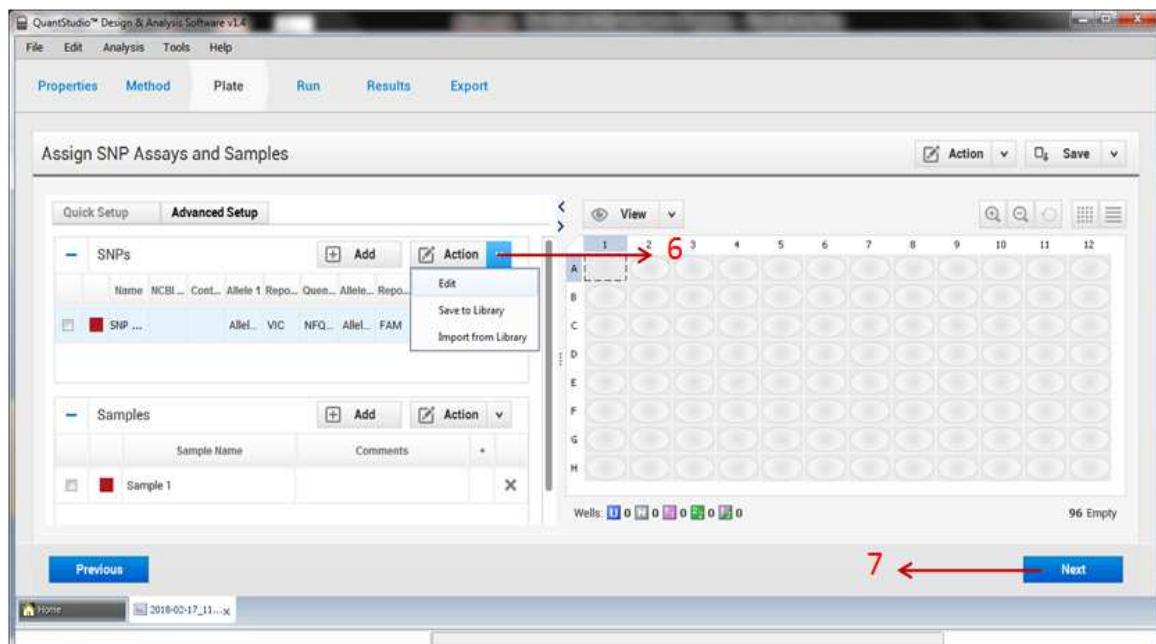
Manage chemistry files

Next

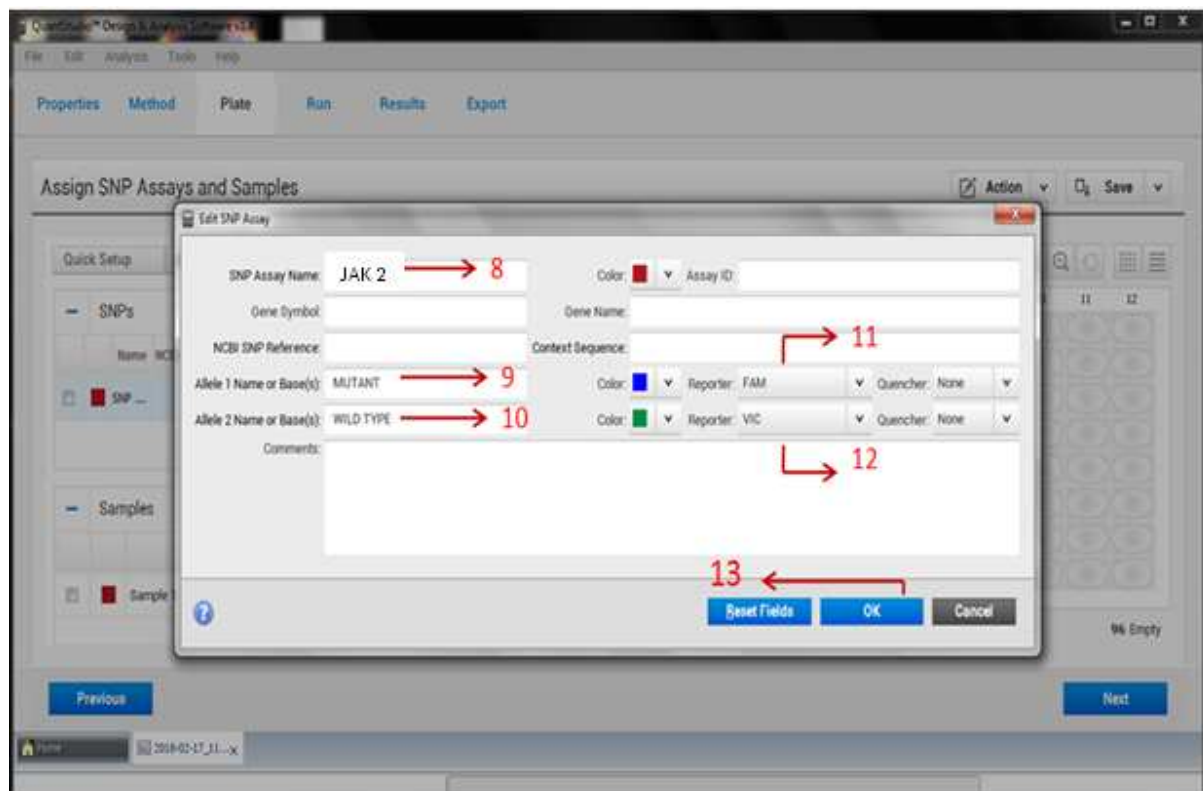
Open Experiment Properties and give name (1) and select experiment type as “Genotyping” (2) and click on NEXT



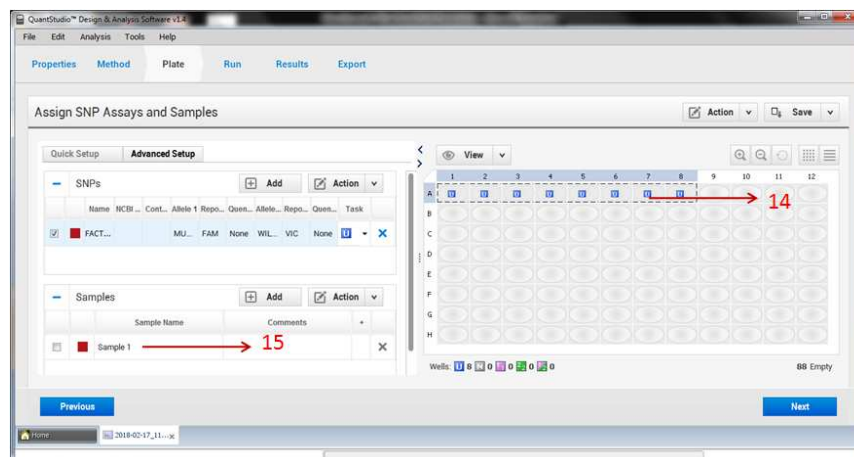
Set the volume to 20 µl (3) and fill experiment method as given and leave post read stage as default (4) then click “Next” (5)



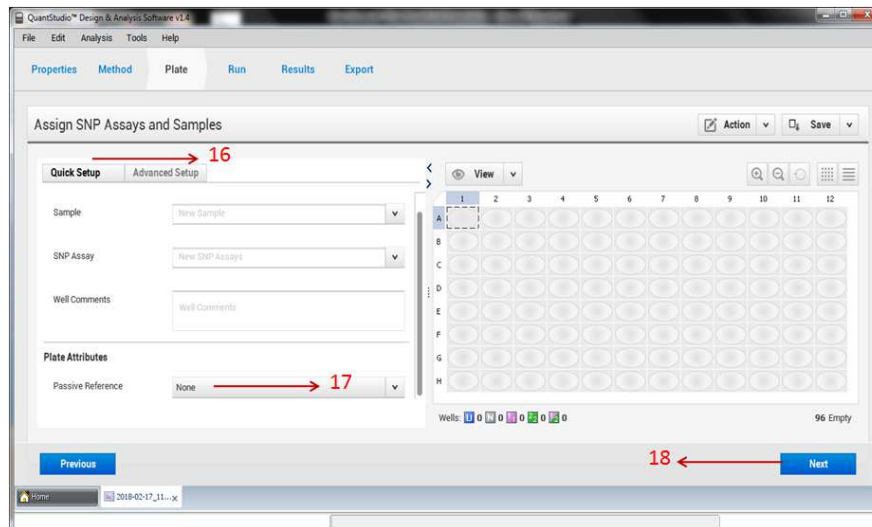
Click on Advanced setup and click on edit SNP (6) then click “Next” (7)



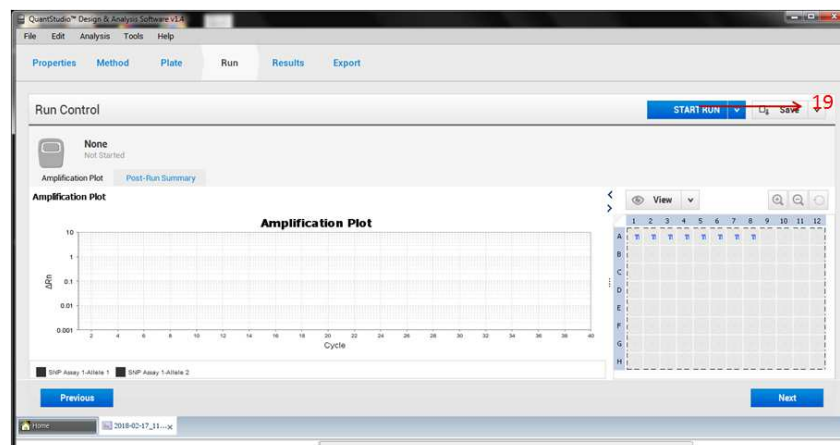
Give SNP name (8) and allele name (9, 10) then select dye for alleles (11, 12) then click “OK” (13)



Select the samples and tag with SNP (14) and give sample name (15)

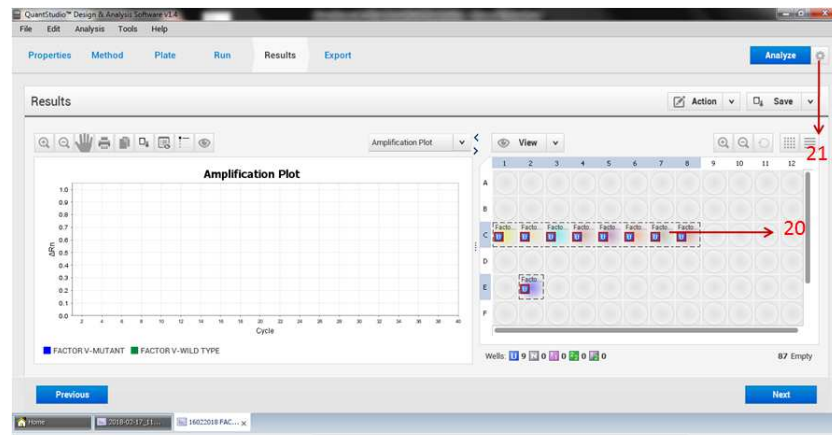


Click on Quick Setup (16), select passive reference dye as “NONE” (17) then click on “NEXT” (18)

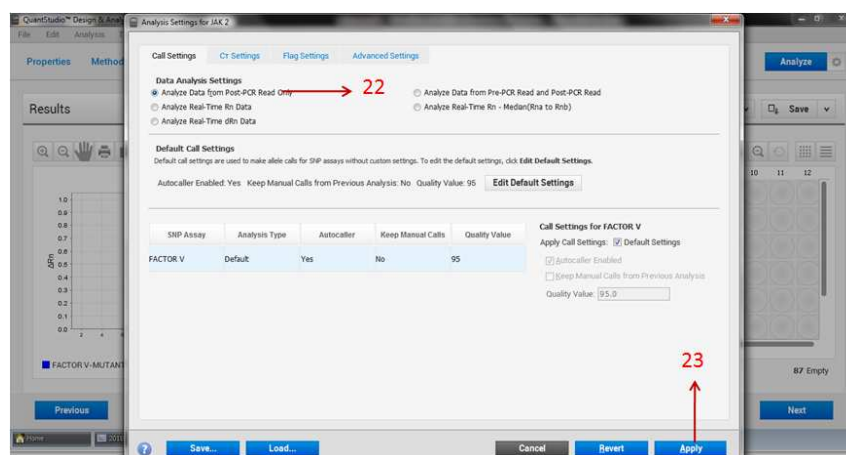


To start run click on Start Run (19)

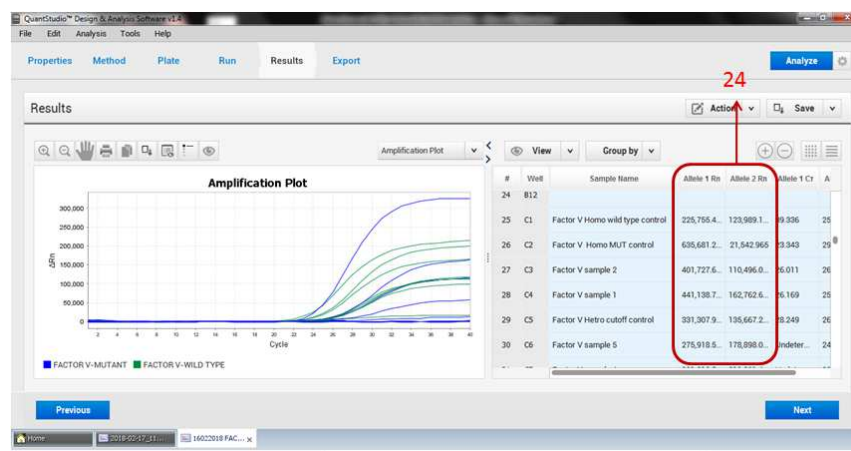
RESULT ANALYSIS FOR QUANTSTUDIO® 3 AND 5



After completion of run, select samples (20) click on screw near analysis (21)



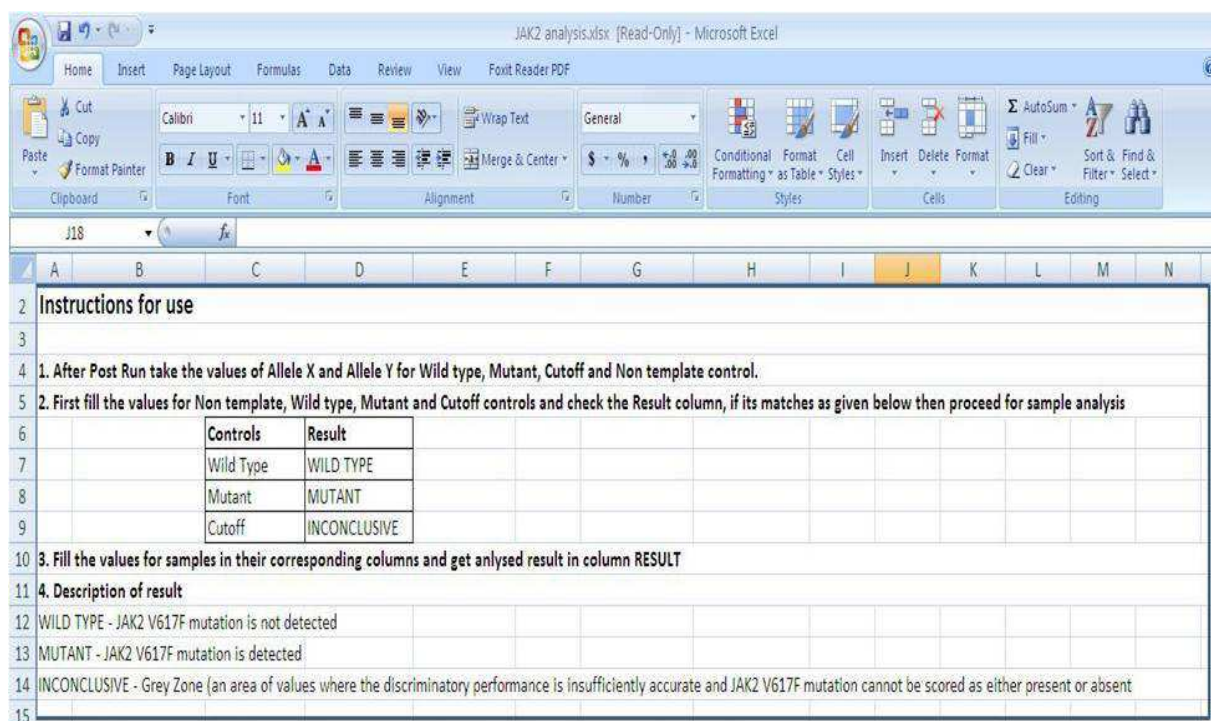
Click on Analysis data from post PCR (22) and then click on APPLY (23)



Note down data of Allele 1 Rn and Allele 2 Rn for all samples and control (24)

1. Analysis using given analysis tool

Follow the instruction given in analysis tool



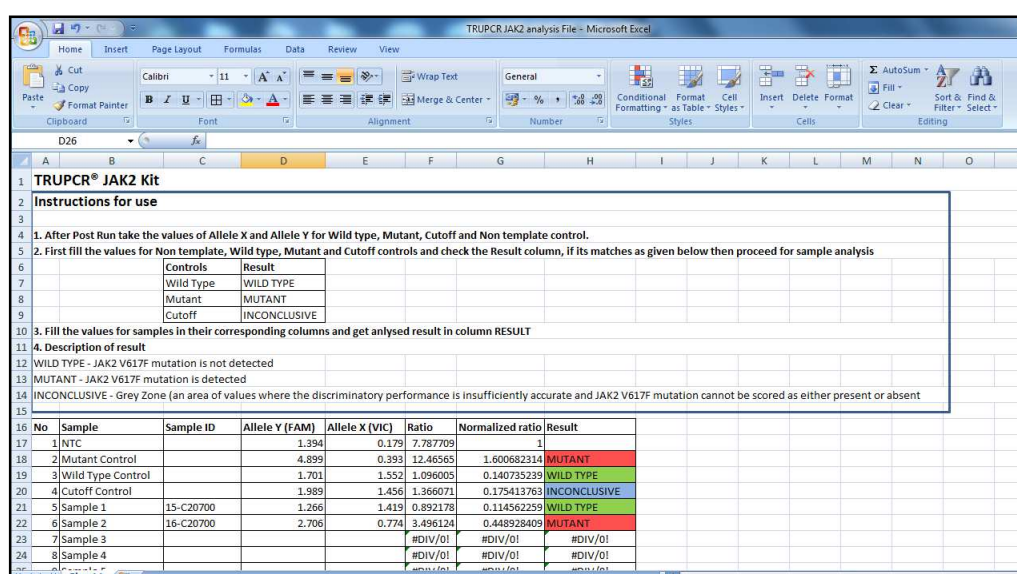
Instructions for use

- After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.
- First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis

Controls	Result
Wild Type	WILD TYPE
Mutant	MUTANT
Cutoff	INCONCLUSIVE

- Fill the values for samples in their corresponding columns and get analysed result in column RESULT
- Description of result**
 - WILD TYPE - JAK2 V617F mutation is not detected
 - MUTANT - JAK2 V617F mutation is detected
 - INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)

Fill the sample ID and value of allele X and allele Y in given column for each sample in analysis tools, and find the result in result column



TRUPCR® JAK2 Kit

Instructions for use

- After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.
- First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis

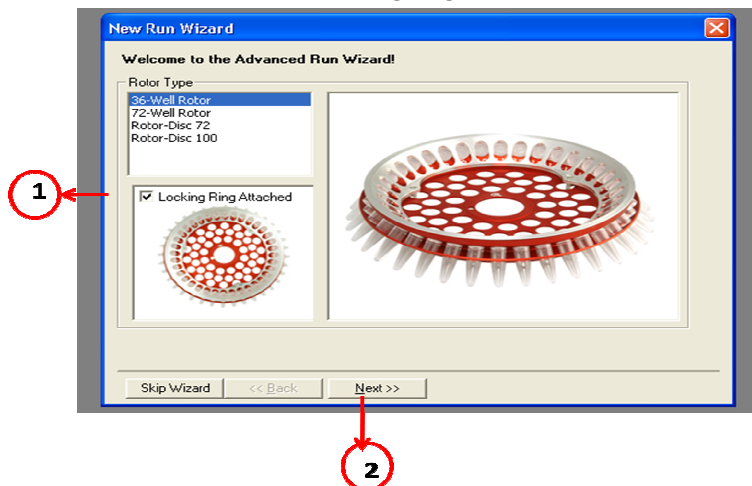
Controls	Result
Wild Type	WILD TYPE
Mutant	MUTANT
Cutoff	INCONCLUSIVE

- Fill the values for samples in their corresponding columns and get analysed result in column RESULT
- Description of result**
 - WILD TYPE - JAK2 V617F mutation is not detected
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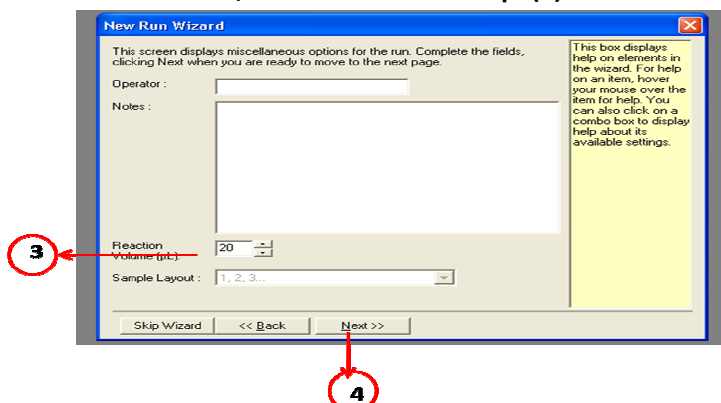
No	Sample	Sample ID	Allele Y (FAM)	Allele X (VIC)	Ratio	Normalized ratio	Result
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6	Sample 2	16-C20700	2.706	0.774	3.496124	0.448928409	MUTANT
7	Sample 3				#DIV/0!	#DIV/0!	#DIV/0!
8	Sample 4				#DIV/0!	#DIV/0!	#DIV/0!

PROGRAM SET UP IN ROTOR-GENE Q

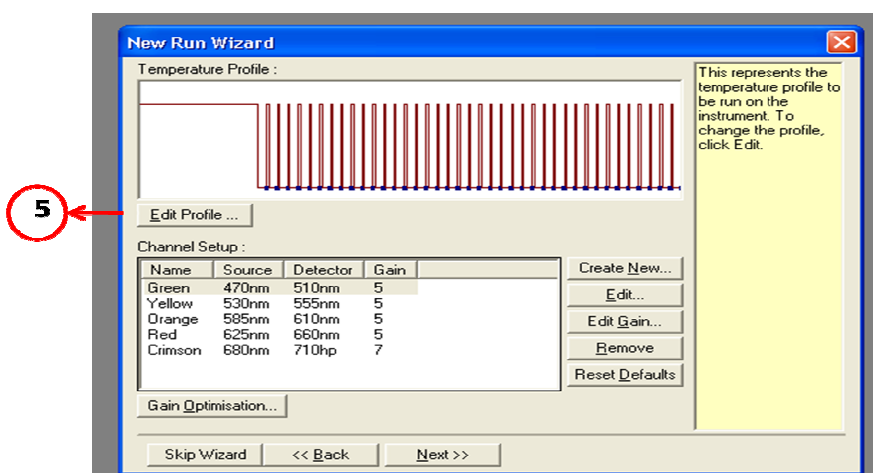
Open New RUN Wizard and select locking ring attached (1) and Click on Next (2)



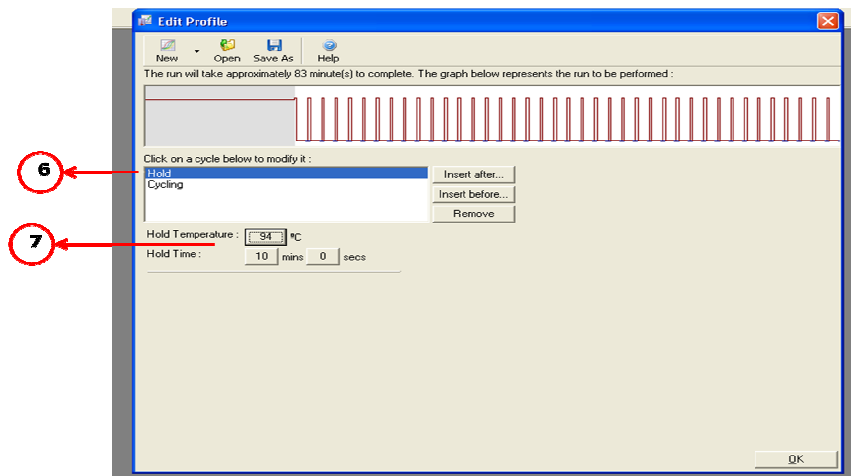
In the “New Run Wizard”, set the volume to 20 µl (3) and click “Next” (4)



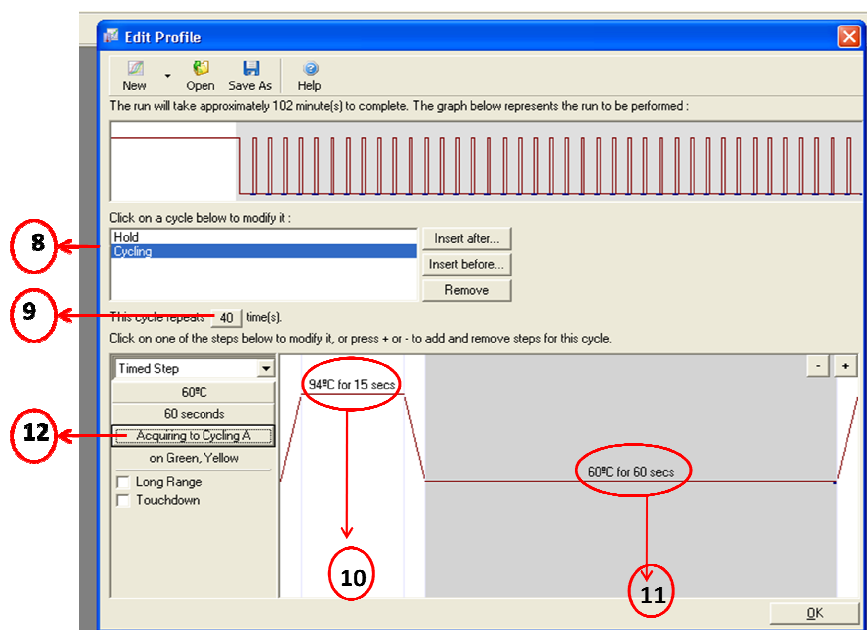
Click on Edit Profile (5) in New Run Wizard



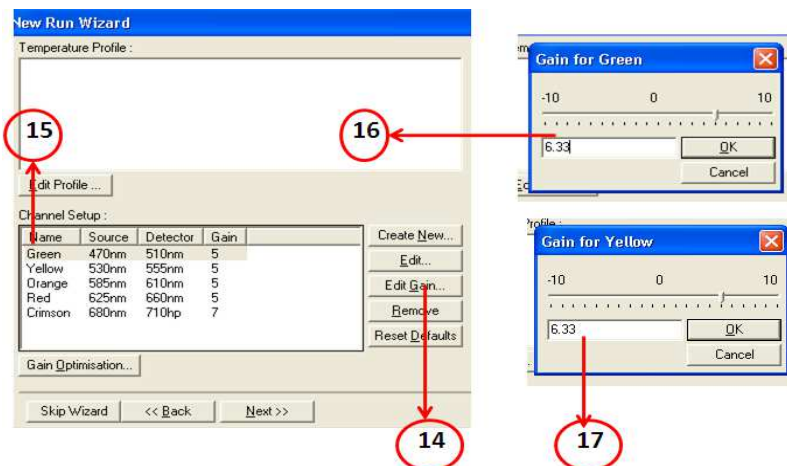
Click on Hold (6) and set temperature to 94 degree and time 10 min (7)



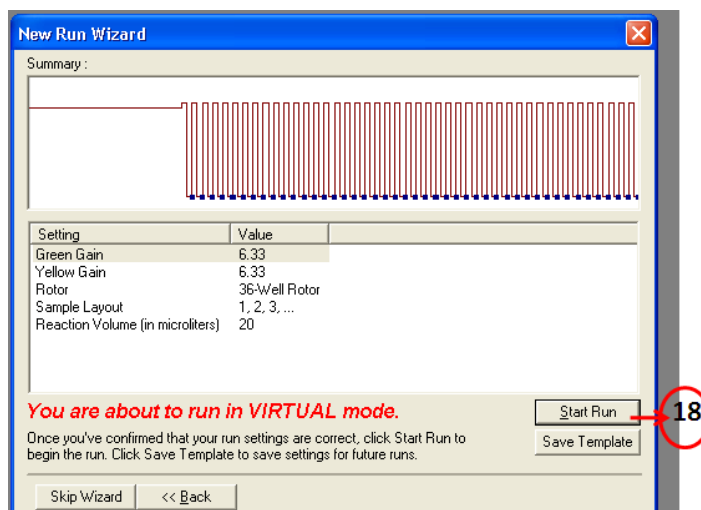
Click on Cycling (8), cycle repeat 40 (9), then cycling 94 degree for 15 sec (10), then enter 60 degree for 60 sec (11), then click on acquiring to cycling A (12) and select Green and Yellow Channel



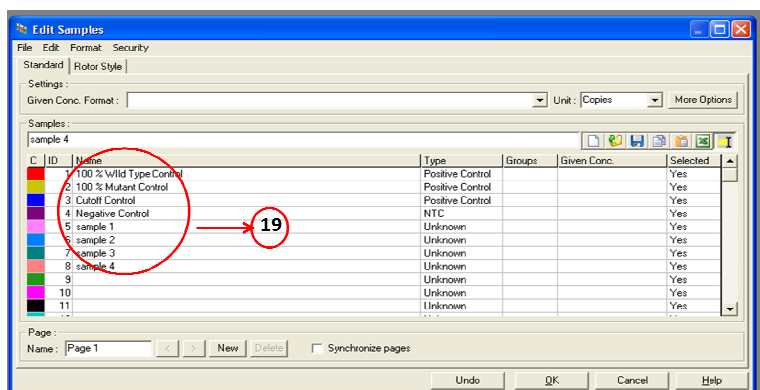
Click on Edit Gain (14), select Green and Yellow channel one by one (15), then keep Gain 6.33 for Yellow and Green (16) (17)



To start run click on Start Run (18)

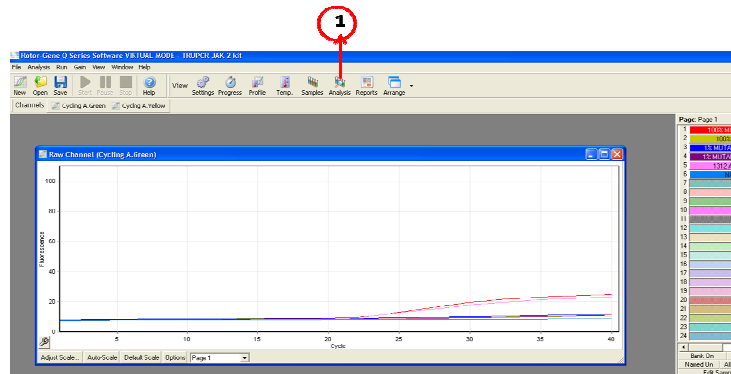


In Edit sample Fill sample details (19)

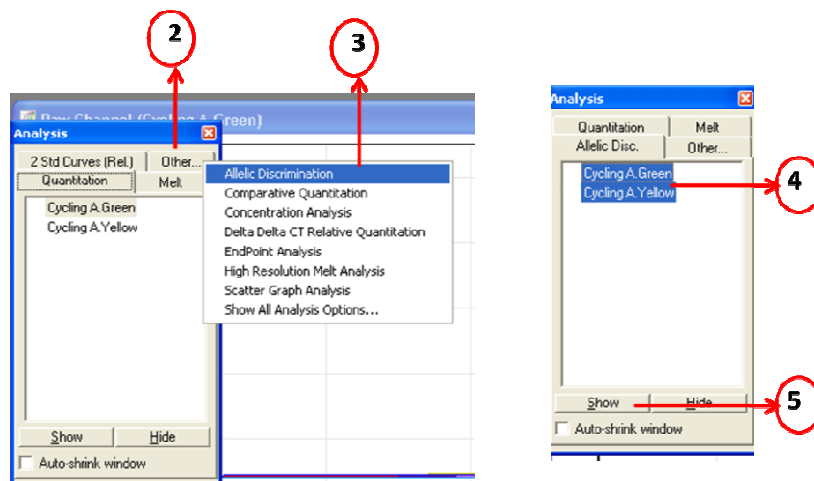


RESULT ANALYSIS FOR ROTOR-GENE Q

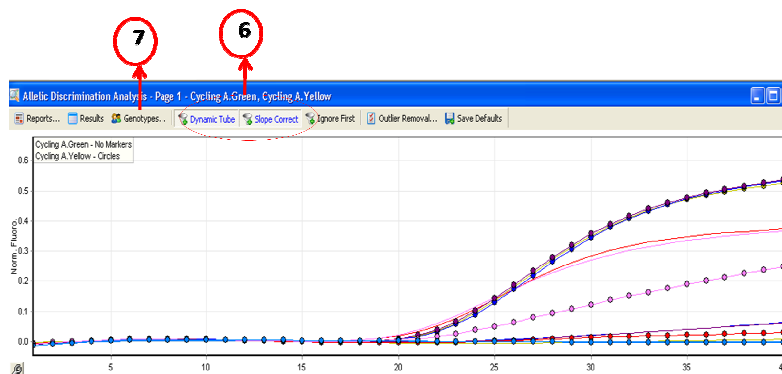
After completion of run, click on analysis (1)



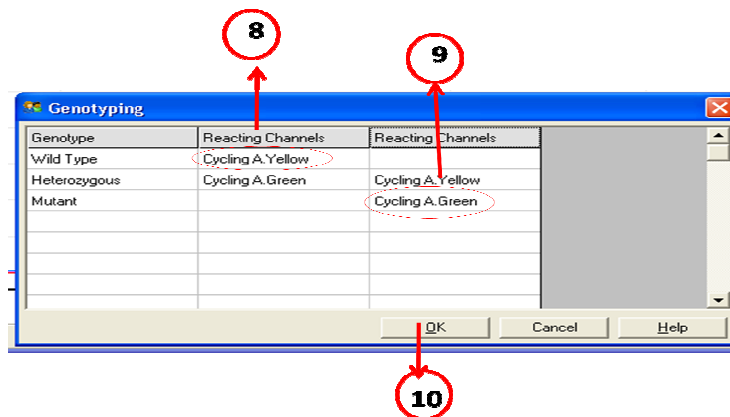
In Analysis window click on others (2), then click on Allelic discrimination (3), further select green and yellow channel (4) and click on Show (5)



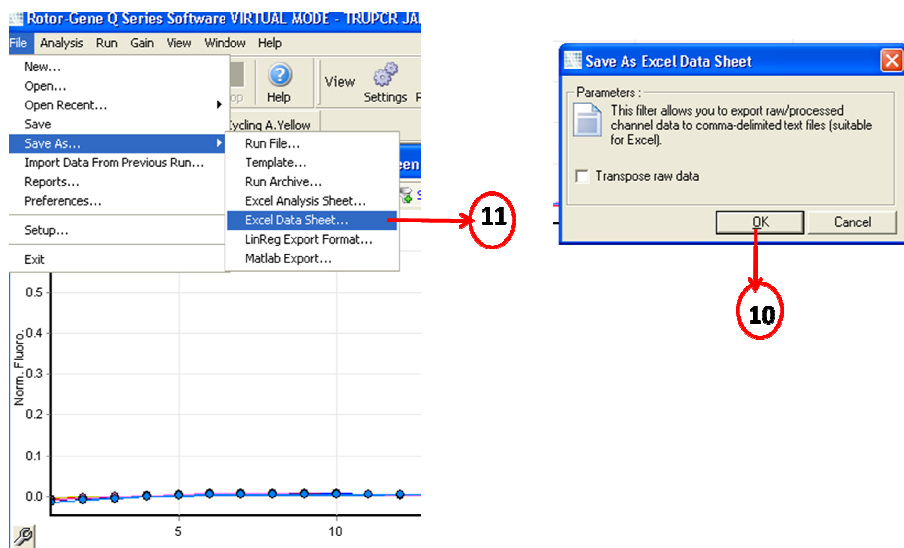
Click on Dynamic Tube and Slope correct (6) and then click on Genotype (7)



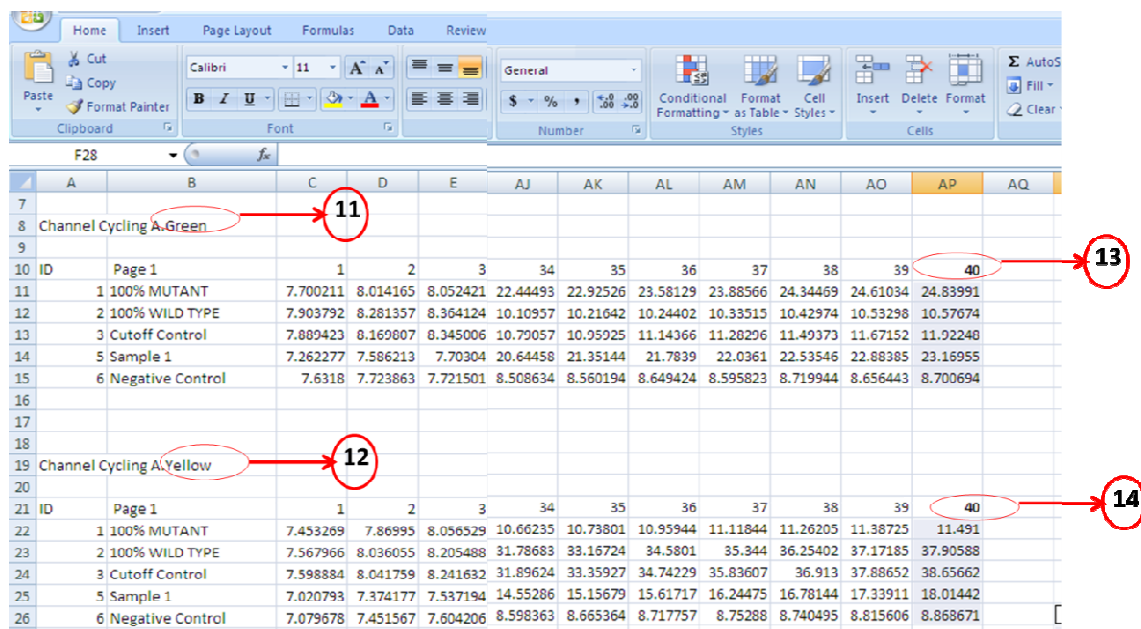
In Genotyping select Yellow for wild type (8) and Green for Mutant (9) and click on OK (10)



Save data by clicking on “File” then “Save as” then “Excel Data sheet” (11) then in popup window click OK



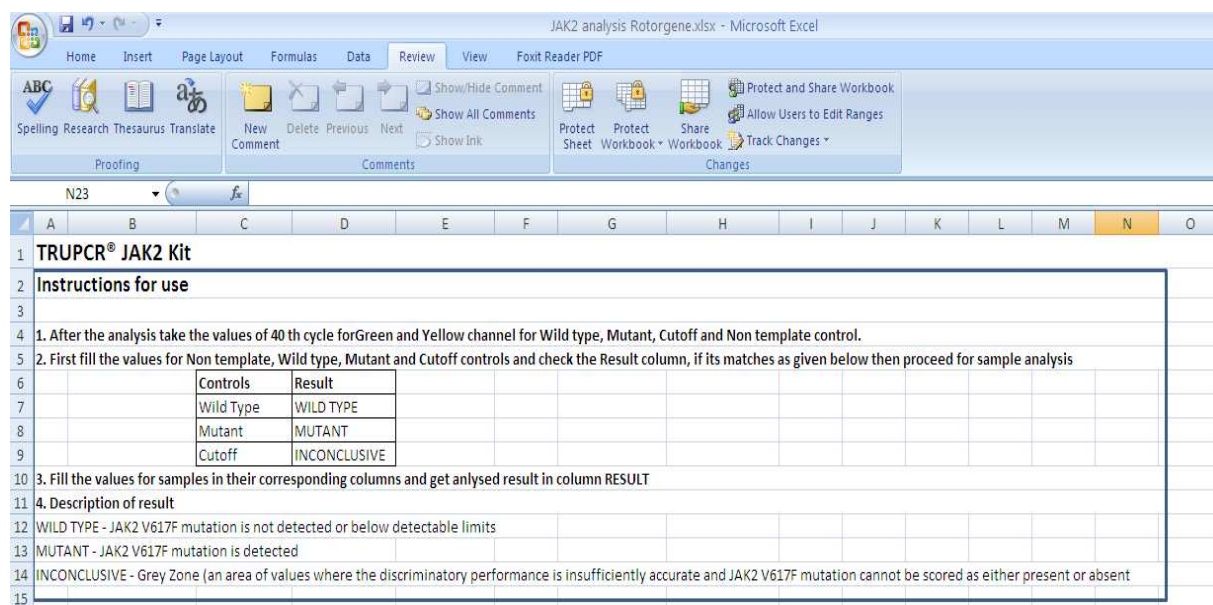
Open saved data and check for values for Green (11) and Yellow (12) and take the value of each channels in 40th Cycle



ID	Page 1	1	2	3	34	35	36	37	38	39	40
1	100% MUTANT	7.700211	8.014165	8.052421	22.44493	22.92526	23.58129	23.88566	24.34469	24.61034	24.83991
2	100% WILD TYPE	7.903792	8.281357	8.364124	10.10957	10.21642	10.24402	10.33515	10.42974	10.53298	10.57674
3	Cutoff Control	7.889423	8.169807	8.345006	10.79057	10.95925	11.14366	11.28296	11.49373	11.67152	11.92248
5	Sample 1	7.262277	7.586213	7.70304	20.64458	21.35144	21.7839	22.0361	22.53546	22.88385	23.16955
6	Negative Control	7.6318	7.723863	7.721501	8.508634	8.560194	8.649424	8.595823	8.719944	8.656443	8.700694

ID	Page 1	1	2	3	34	35	36	37	38	39	40
1	100% MUTANT	7.453269	7.86995	8.056529	10.66235	10.73801	10.95944	11.11844	11.26205	11.38725	11.491
2	100% WILD TYPE	7.567966	8.036055	8.205488	31.78683	33.16724	34.5801	35.344	36.25402	37.17185	37.90588
3	Cutoff Control	7.598884	8.041759	8.241632	31.89624	33.35927	34.74229	35.83607	36.913	37.88652	38.65662
5	Sample 1	7.020793	7.374177	7.537194	14.55286	15.15679	15.61717	16.24475	16.78144	17.33911	18.01442
6	Negative Control	7.079678	7.451567	7.604206	8.598363	8.665364	8.717757	8.75288	8.740495	8.815606	8.868671

Open analysis tool and Follow the instruction given for analysis



Controls	Result
Wild Type	WILD TYPE
Mutant	MUTANT
Cutoff	INCONCLUSIVE

3. Fill the values for samples in their corresponding columns and get analysed result in column RESULT

4. Description of result

WILD TYPE - JAK2 V617F mutation is not detected or below detectable limits

MUTANT - JAK2 V617F mutation is detected

INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)

Fill the values of Green and Yellow channel in given column for controls and samples in analysis tool (15), it will automatically give mutation status of sample in Result column

JAK2 analysis Rotorgene.xlsx - Microsoft Excel

Home Insert Page Layout Formulas Data Review View Foxit Reader PDF

Clipboard Font Alignment Number Styles

15

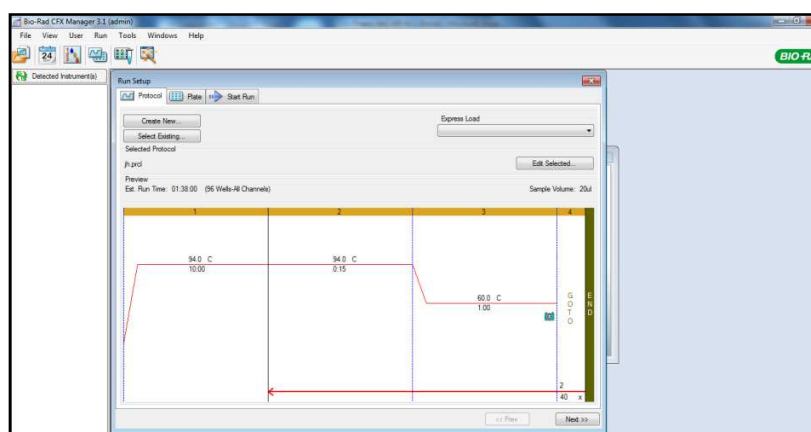
No	Sample	Sample ID	Green (FAM)	Yellow (HEX)	Ratio	Normalized ratio	Result
1	NTC		8.700694285	8.868670685	0.98106	1	
2	Mutant Control		24.83990743	11.49099718	2.16168	2.203417907	MUTANT
3	Wild Type Control		10.57674016	37.90587531	0.27903	0.284413316	WILD TYPE
4	Cutoff Control		11.92248417	38.65661919	0.30842	0.31437464	INCONCLUSIVE
5	Sample 1	15-C20700	23.16955316	18.01441978	1.28617	1.31099791	MUTANT
6	Sample 2		0	0	#DIV/0!	#DIV/0!	#DIV/0!
7	Sample 3		0	0	#DIV/0!	#DIV/0!	#DIV/0!
8	Sample 4		0	0	#DIV/0!	#DIV/0!	#DIV/0!
9	Sample 5		0	0	#DIV/0!	#DIV/0!	#DIV/0!
10	Sample 6		0	0	#DIV/0!	#DIV/0!	#DIV/0!
11	Sample 7		0	0	#DIV/0!	#DIV/0!	#DIV/0!

PROGRAM SET UP FOR BIORAD CFX 96

1. ABSOLUTE QUANTITATION

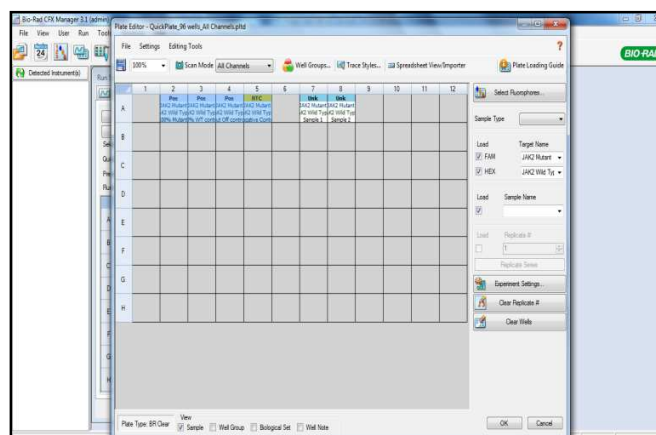
a) Define the following setting for Temperature Profile and Dye Acquisition

Step	Program name	Temperature, °C	Time	Dye Acquisition	Cycles
1	Initial Denaturation	94	10 min	-	1
2	Denaturation	94	15 sec	-	40
	Annealing/Extension	60	60 sec	YES	



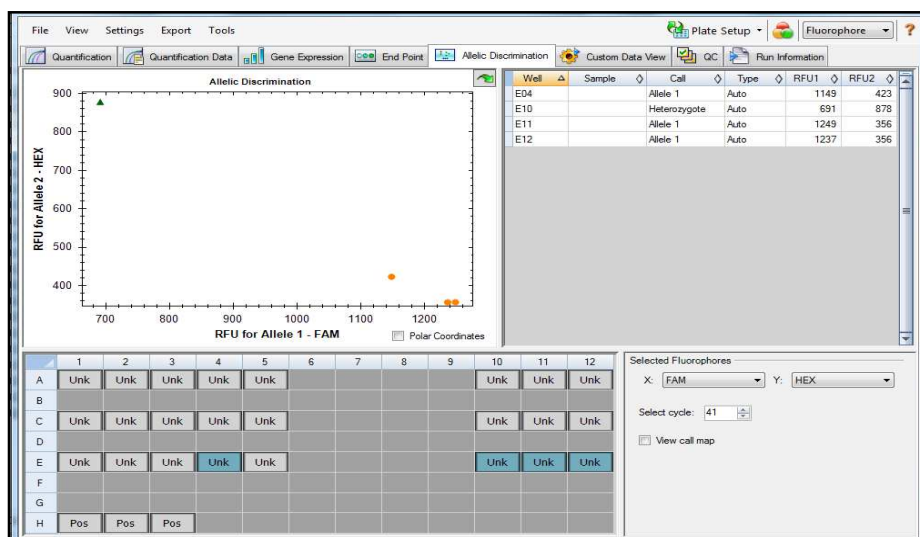
b) Define the following setting for channel selection

Detection	Detector Name	Reporter	Wavelength	Gain Setup
Mutant	JAK2 Mutant	FAM	465-510	Auto
Wild Type	JAK2 Wild Type	HEX/VIC	533-580	Auto



RESULT ANALYSIS FOR BIORAD CFX96

1. At the end of the amplification run go to “Allelic Discrimination” window and in subset select all the samples and controls. In allelic discrimination select Allele 1 (FAM) and Allele 2 (Hex). To view and analyse the results, export the value of RFU1 (FAM) and RFU2 (HEX) and enter in the provided software
2. Note down the values of Allele 1 RFU and Allele 2 RFU of each control and sample.

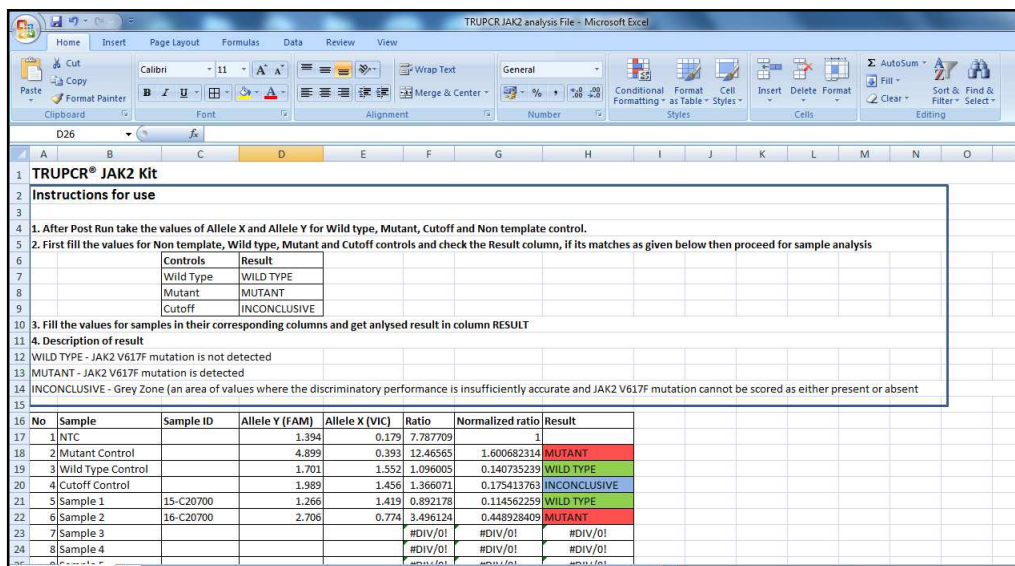


3. Analysis using given analysis tool

Follow the instruction given in analysis tool

JAK2 analysis.xlsx (Read-Only) - Microsoft Excel													
Instructions for use													
1. After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.													
2. First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis													
		Controls	Result										
		Wild Type	WILD TYPE										
		Mutant	MUTANT										
		Cutoff	INCONCLUSIVE										
3. Fill the values for samples in their corresponding columns and get analysed result in column RESULT													
4. Description of result													
WILD TYPE - JAK2 V617F mutation is not detected													
MUTANT - JAK2 V617F mutation is detected													
INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)													

Fill the sample ID and value of allele X and allele Y in given column for each sample in analysis tools, and find the result in result column



TRUPCR® JAK2 Kit

Instructions for use

- After Post Run take the values of Allele X and Allele Y for Wild type, Mutant, Cutoff and Non template control.
- First fill the values for Non template, Wild type, Mutant and Cutoff controls and check the Result column, if its matches as given below then proceed for sample analysis
- Fill the values for samples in their corresponding columns and get analysed result in column RESULT

Description of result

- WILD TYPE - JAK2 V617F mutation is not detected
- MUTANT - JAK2 V617F mutation is detected
- INCONCLUSIVE - Grey Zone (an area of values where the discriminatory performance is insufficiently accurate and JAK2 V617F mutation cannot be scored as either present or absent)

No	Sample	Sample ID	Allele Y (FAM)	Allele X (VIC)	Ratio	Normalized ratio	Result
1	NTC		1.394	0.179	7.787709	1	
2	Mutant Control		4.899	0.393	12.46565	1.600682314	MUTANT
3	Wild Type Control		1.701	1.552	1.096005	0.140735239	WILD TYPE
4	Cutoff Control		1.989	1.456	1.366071	0.175413763	INCONCLUSIVE
5	Sample 1	15-C20700	1.266	1.419	0.892178	0.114562259	WILD TYPE
6	Sample 2	16-C20700	2.706	0.774	3.496124	0.448928409	MUTANT
7	Sample 3				#DIV/0!	#DIV/0!	#DIV/0!
8	Sample 4				#DIV/0!	#DIV/0!	#DIV/0!

ANALYTICAL SENSITIVITY

The analytical sensitivity of the TRUPCR® JAK 2 Kit was determined by mixture of JAK2 Wild type and V617F Mutant cells in different ratio to get range of JAK 2 mutation percentage. The study was performed on DNA extracted from mixture of wild type and mutant JAK2 cell lines. Five different levels of were tested in triplicates. The analytical sensitivity of kit is determined as $\leq 1\%$ mutant alleles. The result obtained are summarized below

JAK 2 V617F Mutation %	Standard Deviation	Coefficient of Variation (%)
0.1	1.37	4.72
1	0.87	3.34
2	0.72	3.13
10	0.33	1.65
50	0.35	1.84

COMPARISON BETWEEN TRUPCR® JAK 2 KIT AND SEQUENCING

DNA samples from 77 patients with suspected MPN were tested in parallel with the TRUPCR® JAK 2 Kit and the gold standard reference technique direct sequencing. Comparisons of results obtained from the 77 interpretable samples are summarized in tables below

		SEQUENCING		
		JAK 2 V617F Mutation Positive	JAK 2 Wild Type	Total
TRUPCR JAK 2 KIT	JAK 2 V617F Mutation Positive	29	1	30
	JAK 2 Wild Type	2	45	47
	Total	31	46	77

	Results	Percentage (%)
Positive data Agreement with sequencing	29/31	93.54
Negative data Agreement with sequencing	45/46	97.82
Total agreement with sequencing		95.68

LIMITATIONS

1. The users must be trained and familiar with real time PCR technology prior to the use of this kit.
2. Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings.
3. It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the TRUPCR® JAK 2 Kit performance studies.
4. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
5. The kit has been designed according to the "Laboratory Practice Guidelines for Detecting and Reporting JAK2 and MPL Mutations in Myeloproliferative Neoplasms"⁵ with the updated international recommendations.

TROUBLESHOOTING REAL TIME PCR

No.	Observation	Probable causes	Comments
1	Amplification signal in negative control	Cross contamination during handling	Check for contamination of kit's component
2	No amplification signal with positive controls	Incorrect PCR mixture	Check whether all components are added.
		Missing control sample during DNA mixing	Be careful when pipetting
		Changing DNA during DNA mixing	Write down sample number on the 1.5 ml micro centrifuge tube and the PCR tube
		Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage condition and the expiration date(see the kit label) of the reagents and use a new kit, if necessary
		The PCR conditions do not comply with the protocol	Repeat the PCR with corrected settings
3	Weak or no signal of the Internal Control in Hex channel	Reagent has been thawed and frozen too often or exposed to inappropriate storage conditions	Please mind the storage conditions given in manual
		The PCR was inhibited	DNA of Poor quality may interfere with the PCR reaction, use a recommended isolation method



STORAGE AND HANDLING

All the components of kit should be stored at -20°C and stable until the date of expiry stated. The reagents can be aliquoted and stored at -20°C in-order to maintain the stability and sensitivity.

MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

- Adjustable pipettes with sterile filter or positive displacement tips
- Disposable powder-free gloves
- Sterile bidistilled water
- Sterile 1.5 ml and 2 ml microcentrifuge tubes
- 50 ml conical tubes
- Vortex mixer
- 3B Termi-DNA-Tor or equivalent, in order to remove DNA from working surfaces
- Real time PCR
- Laminar airflow cabinet
- PCR vials (0.2 ml, thin-walled)
- Personal protection equipment (lab coat, gloves, goggles)
- DNA isolation kit

KIT IS COMPATIBLE TO USE WITH FOLLOWING REAL TIME PCR INSTRUMENTS

- Applied Biosystems™ 7500
- StepOne and StepOnePlus
- QuantStudio® 3, 5 and 12
- Rotor-Gene Q
- Bio-Rad CFX96, CFX384
- AriaMx Real-Time PCR
- Roche - LightCycler® 480 -II
- Line gene K real time PCR



GENERAL PRECAUTIONS

The user should always pay attention to the following:

- The product should be delivered on dry ice. Check for presence of dry ice upon arrival.
- Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, pyrogen free micropipette tips with filters, and DNase, RNase, pyrogen free microcentrifuge tubes should be used.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices. Infected material and disposable plasticware that was in contact with infected material must be treated with chlorine-containing solutions.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be uni-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.



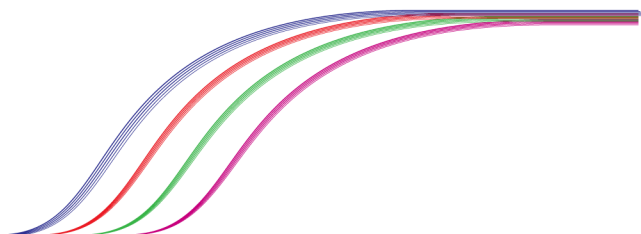
NOTICE

- The user should always pay attention to the following:
- **Store DNA samples at -20°C until ready for use and keep on ice during use.**
- Avoid microbial contamination of reagents when removing aliquots from reagent tubes. The use of sterile disposable pipette tips is recommended.
- Specimens should be handled as if infectious using safe laboratory procedures. Thoroughly clean and disinfect all work surfaces with 0.5% Sodium Hypochlorite in de-ionized or distilled water.
- This product is to be used by personnel specially trained to perform in vitro diagnostic procedures.

REFERENCES

1. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2008;22:14-22.
2. Kiladjian JJ, Cassinat B, Turlure P, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon α -2a. *Blood*. 2006;108:2037-2040.
3. Kröger N, Badbaran A, Holler E, et al. Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. *Blood*. 2007;109:1316-1321.
4. Jerald Z. Gong, et al. Laboratory Practice Guidelines for Detecting and Reporting JAK2 and MPL Mutations in Myeloproliferative Neoplasms. *J Mol Diagn* 2013, 15: 733e744.
5. GONG et al, 2013, Laboratory Practice Guidelines for Detecting and Reporting JAK2 and MPL Mutations in Myeloproliferative Neoplasms. A Report of the Association for Molecular Pathology. *The Journal of Molecular Diagnostics*, Vol. 15, No. 6.

Handwriting practice lines consisting of multiple sets of three horizontal dashed lines for tracing and writing practice.



TRUPCR® Molecular Diagnostic Kits

Oncology	TRUPCR® BCR-ABL Quantitative Kit - M m μ	Detection, differentiation and quantitation of BCR-ABL major (M), minor (m) and micro (μ) transcripts. Reporting of Major transcripts ratios on WHO IS.
	TRUPCR® JAK 2 QT Kit	Detection and quantitation of Jak2 V617F Allele burden on real-time PCR
	TRUPCR® PML/RARA Quantitative Kit	Differentiation and quantitation of BCR1, BCR2 and BCR3 transcripts
	TRUPCR® KRAS Qualitative Kit	Detection of 22 mutations across codons 12, 13, 59, 61, 117 & 146 of exons 2, 3 & 4
	TRUPCR® EGFR Mutation Kit	Detection of 32 different mutations in a single run
	TRUPCR® AML Panel Kit*	Qualitative detection of diagnostic markers (AML1-ETO, CBFB MYH11, BCR ABL1 and PML RARA) and prognostic markers (FLT3 ITD/TKD, CKIT and NPM1) of acute myelogenous leukaemia (AML) in peripheral blood samples using real time and conventional PCR system.
	TRUPCR® ALL Panel Kit*	Detection and differentiation of fusion genes (E2A/PBX1, TEL/AML1, MLL-AF4, MLL-ENL, MLL AF9 and BCR ABL1) associated with acute lymphoblastic leukaemia.
	TRUPCR® Leukemia Panel Kit*	Detection of E2A-PBX1, TEL-AML1, MLL-AF4, BCR-ABL1, CBFB MYH11, AML1-ETO, PML-RARA & ABL1 in single panel kit on real-time PCR
	TRUPCR® MPN Mutation Panel Kit*	Detection of BCR-ABL1, JAK-2, CALR & MPL in single panel kit on real-time PCR
* All Markers are also available as individual test.		
Genetics	TRUPCR® HLA B27 Kit	Detection of highest number of HLA B27 allelic subtypes
Infectious Disease	TRUPCR® MTB/NTM Nested Kit	Detection of Mycobacterium DNA from any sample type on real-time PCR
	TRUPCR® H1N1 Detection Kit	Based on CDC certified primers and probes for the detection of type A influenza virus, pandemic influenza A virus and pandemic H1N1 influenza virus
Drug Resistance	TRUPCR® Rifampicin Resistant MTB Detection Kit	Detection of MTBC & Rifampicin resistance from any sample type
Coagulation Factor	TRUPCR® Thrombophilia Panel Kit	Detection of 3 Markers: Factor V, Factor II, MTHFR in single panel kit on real-time PCR
Virology	TRUPCR® CMV QT Kit	Detection and quantitation of Cytomegalovirus on real-time PCR
	TRUPCR® HSV 1/2 Kit	Detection of Herpes Simplex virus 1 & 2 on real-time PCR
Tropical Diseases	TRUPCR® Dengue/Chikungunya/ Malaria Kit	Simultaneous detection of Dengue & Chikungunya and P.falciparum, P.Vivax & Mixed infection on real-time PCR
Women's Health	TRUPCR® HPV 16/18 Kit	Detection & differentiation of HPV 16 and HPV 18 genotypes on real-time PCR
	TRUPCR® HPV HR-16/18 Kit	Detection of 14 High risk HPV genotypes & differentiation of HPV 16 and HPV 18 on real-time PCR

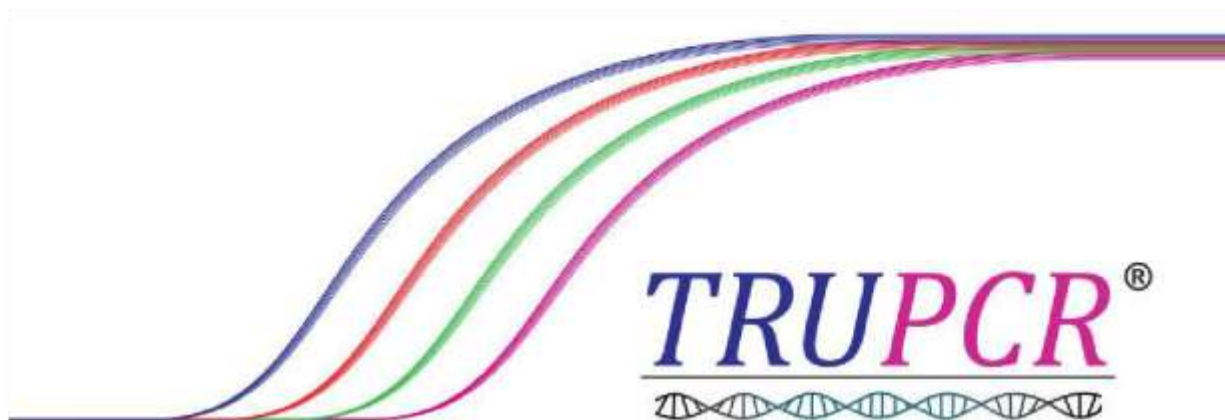
To know more about complete product range & technical details please visit our website

www.3bblackbio.com



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