



Location Twenteborg, ALMELO



Location SMT, HENGELO

# Hospital Group Twente The Netherlands

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1st Dutch Corbett Research user meeting  
May 13 2005

# Molecular Diagnostics with the Rotor-Gene;

# Laboratory set-up and integration with LIMS

Jan Danneberg



# Set-up of automated real-time PCR analysis and integration with LIMS



Automated  
extraction of  
Nucleic Acids



CAS-1200

Liquid Handling  
System for  
setting up of  
PCR reactions



Rotor-Gene 3000

Real Time DNA  
Amplification  
System



# Virtual Workspace



CAS-1200

PCR-mix

Primers / Probes  
DNA-controls

The screenshot displays the Corbett Robotics v4.7.93 software interface. The workspace is divided into several sections:

- Tip racks:** Two racks at the top, labeled 'A1: Tip (200uL Tecan tip)' and 'A2: Tip (50uL Tecan tip)'. Each rack has a 12x8 grid of wells. The A1 rack contains red tips, and the A2 rack contains blue tips.
- PCR-reactions:** A large oval-shaped area in the center labeled 'B1: Multiple Roles' containing several grey circles representing reaction wells.
- DNA-samples:** A red-bordered area at the bottom right labeled 'C2: Sample (1.5mL tapered tube)' containing a 12x4 grid of grey circles representing sample wells.
- Multiple Roles:** Two vertical panels on the left side, each labeled 'Multiple Roles'. The top panel shows four colored circles (blue, yellow, orange, yellow) and a grey circle. The bottom panel shows a vertical list of 12 roles, each with a colored circle and a grey circle.

At the bottom of the interface, the text 'Corbett Robotics v4.7.93' and 'OK' is visible on the left, and 'CAS' is visible on the right.





## Gene-Disk [72]

When automatic  
pipetting completed,  
heat sealed with  
plastic foil

Loading Plate

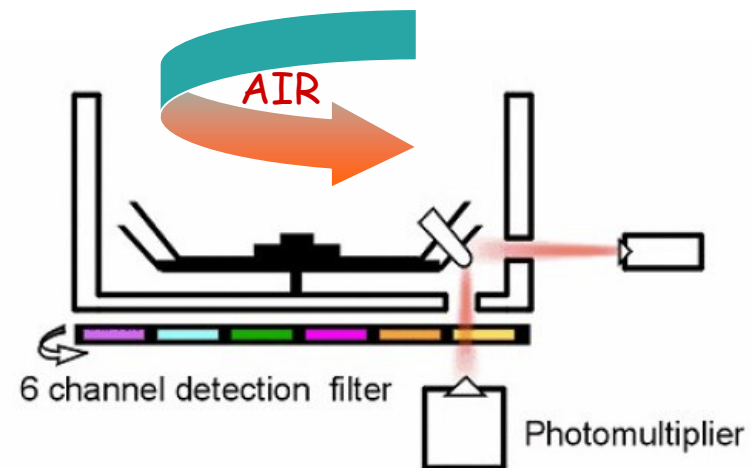
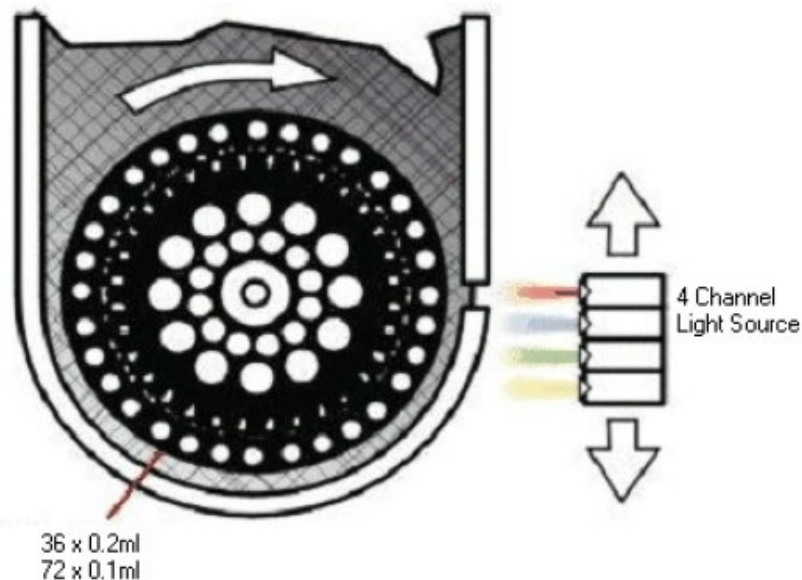


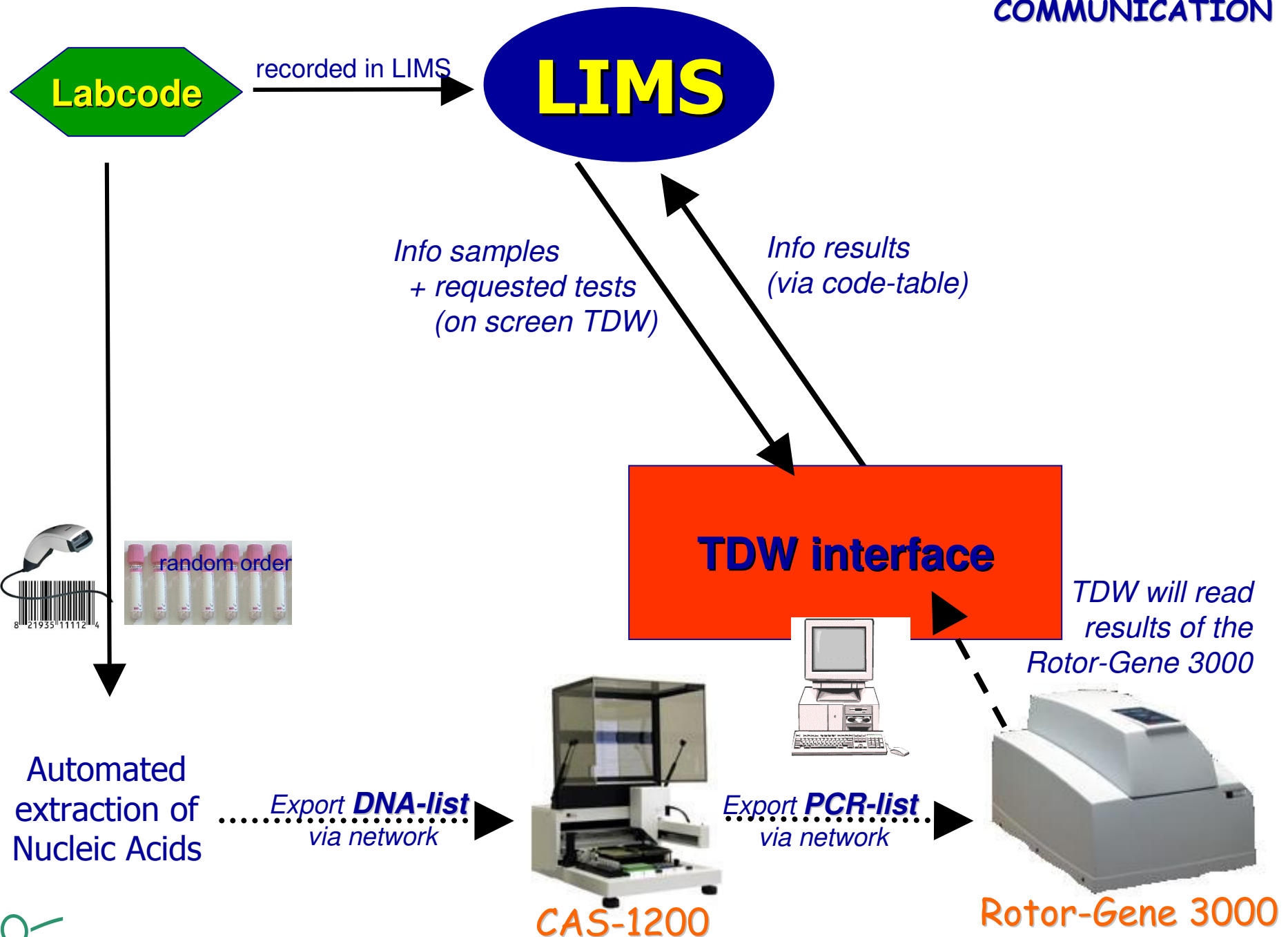


## Rotor-Gene 3000

- **4 excitation LEDs**
- 20 nm bandwidth 470 nm filter
- 10 nm bandwidth 530, 585 and 625 nm filters

- **6 detection filters**
- 3x 10nm band-pass (510 nm, 555 nm and 610 nm)
- 3 high pass filters (570 hp, 610 hp and 665 hp)







Automated extraction of Nucleic Acids

CAS-1200



Rotor-Gene 3000



# 56895	DNA 1
# 56888	DNA 2
# 58965	DNA 3
# 85969	DNA 4
# 96586	DNA 5
# 69698	DNA 6
# 98966	DNA 7
...	
...	

*DNA-list*



NTC	
F5 WT	
F5 Het	
# 56895	DNA 1
# 58965	DNA 3
# 85969	DNA 4
# 69698	DNA 6

NTC	
F2 WT	
F2 Het	
# 56895	DNA 1
# 56888	DNA 2
# 85969	DNA 4

*PCR-list*

Factor 5

LIMS

Factor 2





# Reaction setup on the CAS-1200

The screenshot shows the Corbett Robotics software interface in virtual mode. The main workspace is divided into four tip configurations:

- A1: Tip (200uL Tecan tip)**: A 12x8 grid of wells with red and blue dots.
- A2: Tip (50uL Tecan tip)**: A 12x8 grid of wells with blue dots.
- B1: Multiple Roles**: A circular arrangement of wells with various colored dots.
- B2: Tip (50uL Tecan tip)**: A 12x8 grid of wells with blue dots.

On the right side, the **Sample Banks** panel lists:

- Water
- Contr-DNA's F5 Leiden (Sample, 3) 3 samples
- Contr-DNA's F2 (G20210A) (Sample, 3) 3 samples
- F5+2 (Sample, 1 @ C2) 24 samples

The **Sample Data** table for the selected plate (48 well Qiagen Biorobot @ C2) is as follows:

Well	Sample Name	Sample ID	Conc.
1	54879		0
2	54789		0
3	54878		0
4	56551		0
5	55211		0
6	54265		0
7	54896		0
8	56584		0
9	54954		0
10	54785		0
11	58545		0
12	54555		0
13	55545		0
14	54971		0
15	54213		0
16	89562		0
17	85474		0
18	14568		0
19	25852		0
20	23658		0
21	36563		0
22	36963		0
23	32584		0
24	98587		0
25	Sample 25 @ C2		0
26	Sample 26 @ C2		0
27	Sample 27 @ C2		0

Annotations in the image include:

- F5 mastermix**: A red box highlighting the F5 mastermix role in the A1 tip configuration.
- Primers** and **Probes**: Labels pointing to the B1 tip configuration.
- Automated extraction of Nucleic Acids**: A grey box with a red arrow pointing to the B2 tip configuration.
- DNA-samples**: A red box with a red arrow pointing to the sample data table.
- Import sample names...**: A red box highlighting the button in the Sample Data panel.

The status bar at the bottom shows: Corbett Robotics v4.7.93, OK, CAS1200 (simulated), Level Sense: On, Tip re-use: 8 times.



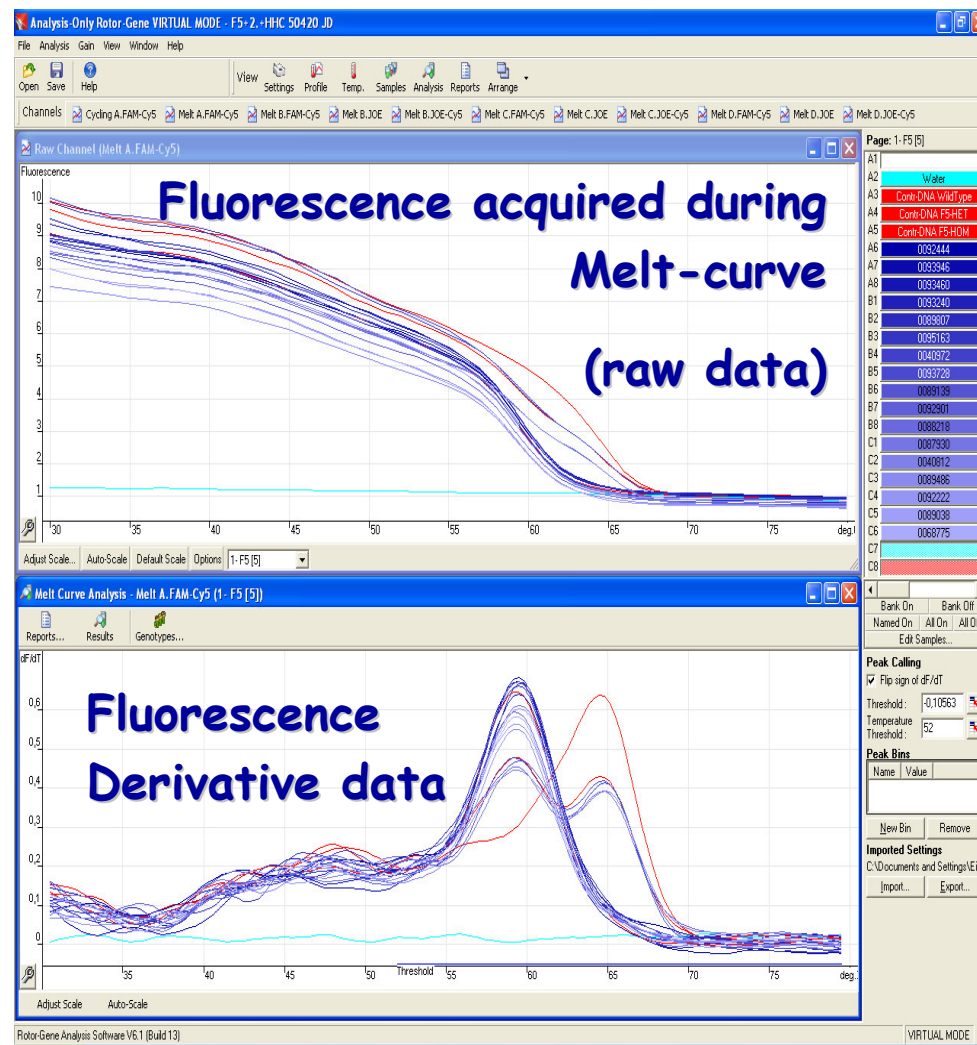
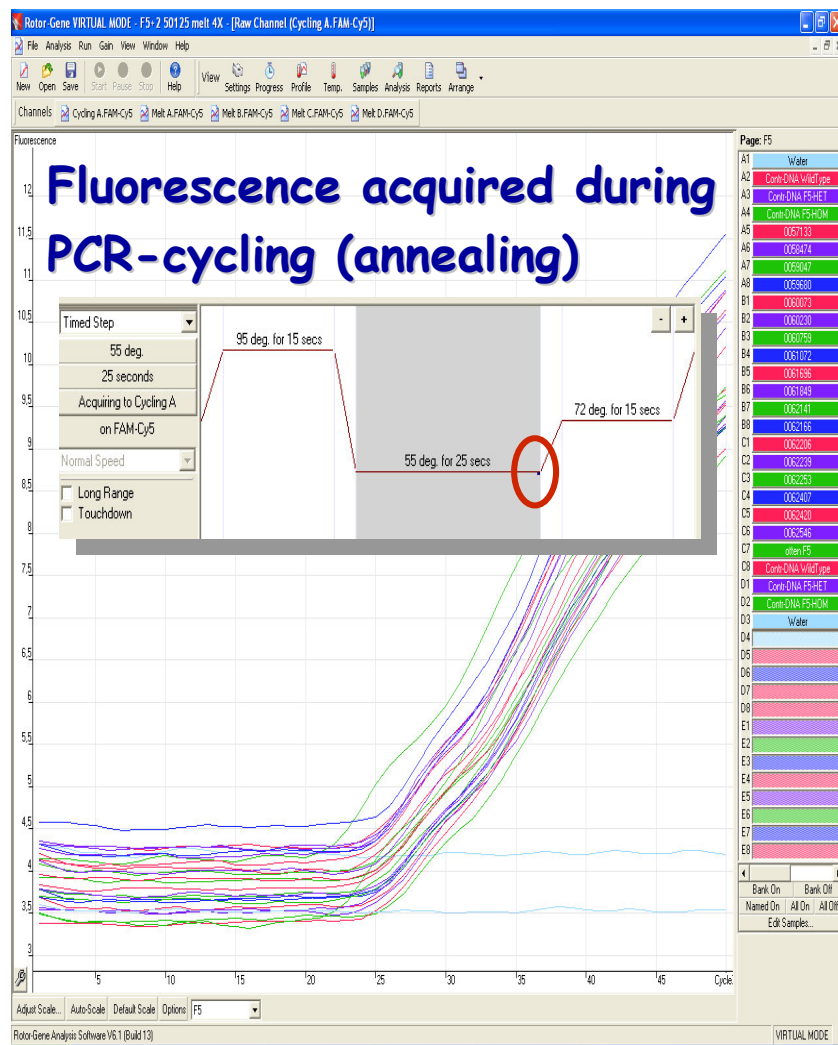
# Import reaction list from CAS-1200 into the Rotor-Gene software

The screenshot shows the 'Edit Samples' window in the Rotor-Gene software. The window title is 'Edit Samples' and it has a menu bar with 'File', 'Edit', and 'Security'. Below the menu bar is a 'Settings' section with 'Given Conc. Format' and 'Unit: Copies'. The main area contains a table of samples with the following columns: ID, Name, Type, Groups, Given Conc., and Selected. The table lists 28 samples, with the first 22 having 'Yes' in the 'Selected' column and the last 6 having 'No'. A red circle highlights the 'Import' icon in the toolbar. A blue arrow points from the 'Import' icon to a photograph of the Rotor-Gene 3000 machine, which is labeled 'Rotor-Gene 3000' in orange text.

ID	Name	Type	Groups	Given Conc.	Selected
A1	Water	NTC			Yes
A2	Contr-DNA WildType	Unknown			Yes
A3	Contr-DNA F5-HET	Unknown			Yes
A4	Contr-DNA F5-HOM	Unknown			Yes
A5	54879	Unknown			Yes
A6	54789	Unknown			Yes
A7	54878	Unknown			Yes
A8	56981	Unknown			Yes
B1	54211	Unknown			Yes
B2	54265	Unknown			Yes
B3	54896	Unknown			Yes
B4	56584	Unknown			Yes
B5	54954	Unknown			Yes
B6	54785	Unknown			Yes
B7	58545	Unknown			Yes
B8	54555	Unknown			Yes
C1	32584	Unknown			Yes
C2	98587	Unknown			Yes
C3		None			No
C4		None			No
C5		None			No
C6		None			No
C7		None			No
C8		None			No
D1		None			No
D2		None			No
D3		None			No
D4		None			No
D5		None			No
D6		None			No
D7		None			No
D8		None			No
E1		None			No
E2		None			No
E3		None			No
E4		None			No
E5		None			No
E6		None			No
E7		None			No
E8		None			No
F1		None			No
F2		None			No
F3		None			No
F4		None			No
F5		None			No
F6		None			No
F7		None			No



# Analysis & Genotyping



# Automatic Genotyping using Analysis Templates

The screenshot displays the Gene Analysis Software V6.1 interface. The main window shows a 'Melt Curve Analysis - Melt A.FAM-Cy5 (1- F5 [5])' plot with a dF/dT vs. Threshold (deg.) graph. Below the plot is a 'Melt Curve Results' table. A 'Load Melt Analysis Template' dialog box is open, showing a file list with 'F5ML analyse.met' selected. A red box highlights the 'Import...' and 'Export...' buttons in the 'Imported Settings' section of the software interface.

**Melt Curve analysis F5**

- Automatic GENOTYPING using Import / Export function of Analysis Templates

No.	C	Name	Genotype	Peak 1	Peak 2	Peak 3
A2		Water		52.2	55.7	62.3
A3		Contr-DNA WildType		59.3	72.5	76.8
A4		Contr-DNA F5-HET		59.3	64.5	76.7
A5		Contr-DNA F5-HOM		64.5	76.7	
A6		0092444		59.5	74	
A7		0093946		59.5	74.5	
A8		0093460		59.5	71.3	75.3
B1		0093240		59.5	71.5	75.7
B2		0089807		53	59.5	70.5
B3		0095163		59.7	71.7	75.7
B4		0040972		59.3	64.8	76.3
B5		0093728		59.5	64.7	75.8
B6		0089139		59.5	72.2	77.5
B7		0092901		59.3	72.2	76.5
B8		0088218		59.3	64.7	75.7
C1		0087930		59.5	71.7	
C2		0040812		53	59.3	64.8
C3		0089486		59.3	73.5	77
C4		0092222		59.5	76.3	
C5		0089038		59.5	73	76
C6		0068775		59.5	75.3	





Analysis-Only Rotor-Gene VIRTUAL MODE - F5+2.+HHC 50420 JD

File Analysis Gain View Window Help

Open Save Help View Settings Profile Temp. Samples Analysis Reports

Channels Melt A.FAM-Cy5 Melt A.JOE Melt A.JOE-Cy5 Melt B.FAM-Cy5 Melt B.JOE Melt B.JOE-Cy5

### Melt Curve Analysis - Melt A.FAM-Cy5 (1- F5 [5])

Reports... Results Genotypes...

Click to select the threshold height. Cancel

Adjust Scale Auto-Scale

### Melt Curve Results - Melt A.FAM-Cy5 (1- F5 [5])

No.	C	Name	Genotype	Peak 1	Peak 2
A2	Water				
A3	Contr-DNA WildType	F5-WT		59,3 (Bin A)	
A4	Contr-DNA F5-HET	F5-HET		59,3 (Bin A)	64,5 (Bin B)
A5	Contr-DNA F5-HOM	F5-HOM		64,5 (Bin B)	
A6	0092444	F5-WT		59,5 (Bin A)	
A7	0093946	F5-WT		59,5 (Bin A)	
A8	0093460	F5-WT		59,5 (Bin A)	
B1	0093240	F5-WT		59,5 (Bin A)	
B2	0089807	F5-WT		59,5 (Bin A)	
B3	0095163	F5-WT		59,7 (Bin A)	
B4	0040972	F5-HET		59,3 (Bin A)	64,8 (Bin B)
B5	0093728	F5-HET		59,5 (Bin A)	64,7 (Bin B)
B6	0089139	F5-WT		59,5 (Bin A)	
B7	0092901	F5-WT		59,3 (Bin A)	
B8	0088218	F5-HET		59,3 (Bin A)	64,7 (Bin B)
C1	0087930	F5-WT		59,5 (Bin A)	
C2	0040812	F5-HET		59,3 (Bin A)	64,8 (Bin B)
C3	0089486	F5-WT		59,3 (Bin A)	
C4	0092222	F5-WT		59,5 (Bin A)	
C5	0089038	F5-WT		59,5 (Bin A)	
C6	0068775	F5-WT		59,5 (Bin A)	

### Load Melt Analysis Template

Zoeken in: rg, Analyse Templates

- rg, Analyse Allelic Discr
- F2M analyse.met
- F5ML analyse.met**
- HHC63 analyse.met
- HHC282 analyse.met

Bestandsnaam: F5ML analyse

Bestandstypen: Rotor-Gene 6 Melt Analysis Template

Buttons: Openen, Annuleren

### Peak Calling

Flip sign of dF/dT

Threshold: -0,10563

Temperature Threshold: 52

### Peak Bins

Name	Value

Buttons: New Bin, Remove

### Imported Settings

C:\Documents and Settings\Fin...

Buttons: Import..., Export...

## Melt Curve analysis F5

- Automatic GENOTYPING using Import / Export function of Analysis Templates





# Export Results to LIMS

**Melt Curve Results - Melt A: FAM-Cy5 (1- F5 [5])**

No.	C	Name	Genotype	Peak 1	Peak 2
A2		Water	No Template		
A3		Contr-DNA WildType	F5 WT	59.3 (Bin A)	
A4		Contr-DNA F5-HET	F5 HET	59.3 (Bin A)	64.5 (Bin B)
A5		Contr-DNA F5-HOM	F5 HOM	64.5 (Bin B)	
A6		0092444	F5 WT	59.5 (Bin A)	
A7		0093946	F5 WT	59.5 (Bin A)	
A8		0093460	F5 WT	59.5 (Bin A)	
B1		0093240	F5 WT	59.5 (Bin A)	
B2		0089807	F5 WT	59.5 (Bin A)	
B3		0095163	F5 WT	59.7 (Bin A)	
B4		0040972	F5 HET	59.3 (Bin A)	64.8 (Bin B)
B5		0093728	F5 HET	59.5 (Bin A)	64.7 (Bin B)
B6		0089139	F5 WT	59.5 (Bin A)	
B7		0092901	F5 WT	59.3 (Bin A)	
B8		0088218	F5 HET	59.3 (Bin A)	64.7 (Bin B)
C1		0087930	F5 WT	59.5 (Bin A)	
C2		0040812	F5 HET	59.3 (Bin A)	64.8 (Bin B)
C3		0089486	F5 WT	59.3 (Bin A)	
C4		0092222	F5 WT	59.5 (Bin A)	
C5		0089038	F5 WT	59.5 (Bin A)	
C6		0068775	F5 WT	59.5 (Bin A)	

Name	Value
Bin A	59.11
Bin B	64.66



# LIMS

Txt-file is generated and will be send to TDW

```
F-Results 20-1-2005 21.17.35 - Kladblok
Bestand Bewerken Opmaak Beeld Help
Sid=water,Seq=A1,05/01/2005,15:27:01,F5ML analyse=No Template
Sid=Contr-DNA wildType,Seq=A2,05/01/2005,15:27:01,F5ML analyse=F5 WT
Sid=Contr-DNA wildType,Seq=A3,05/01/2005,15:27:01,F5ML analyse=F5 WT
Sid=Contr-DNA F5-HET,Seq=A4,05/01/2005,15:27:01,F5ML analyse=F5 HET
Sid=Contr-DNA F5-HET,Seq=A5,05/01/2005,15:27:01,F5ML analyse=F5 HET
Sid=Contr-DNA F5-HOM,Seq=A6,05/01/2005,15:27:01,F5ML analyse=F5 HOM
Sid=Contr-DNA F5-HOM,Seq=A7,05/01/2005,15:27:01,F5ML analyse=F5 HOM
Sid="0091168",Seq=A8,05/01/2005,15:27:01,F5ML analyse=F5 HET
Sid="0091939",Seq=B1,05/01/2005,15:27:01,F5ML analyse=F5 HET
Sid="0092151",Seq=B2,05/01/2005,15:27:01,F5ML analyse=F5 WT
Sid="0092714",Seq=B3,05/01/2005,15:27:01,F5ML analyse=F5 WT
```

**TDW interface**



*TDW will read results of the Rotor-Gene 3000*



**Rotor-Gene 3000**



# Validation of the Results

**Sample Control Panel**

TECHNIDA  
MEDICAL SOFTWARE EM

LIS BEP3 Prt

F5M	F2M	H63	H82	H27
*				

ASP.D/T Inc. - Incomplete - Review And Edit

Access Parameter Patient Sample View Customize Help

Name: TESTPATIENT-VAN DER TEST, /T  
Pat#: 12345678 Age: 4M Sex: U  
Pcom:

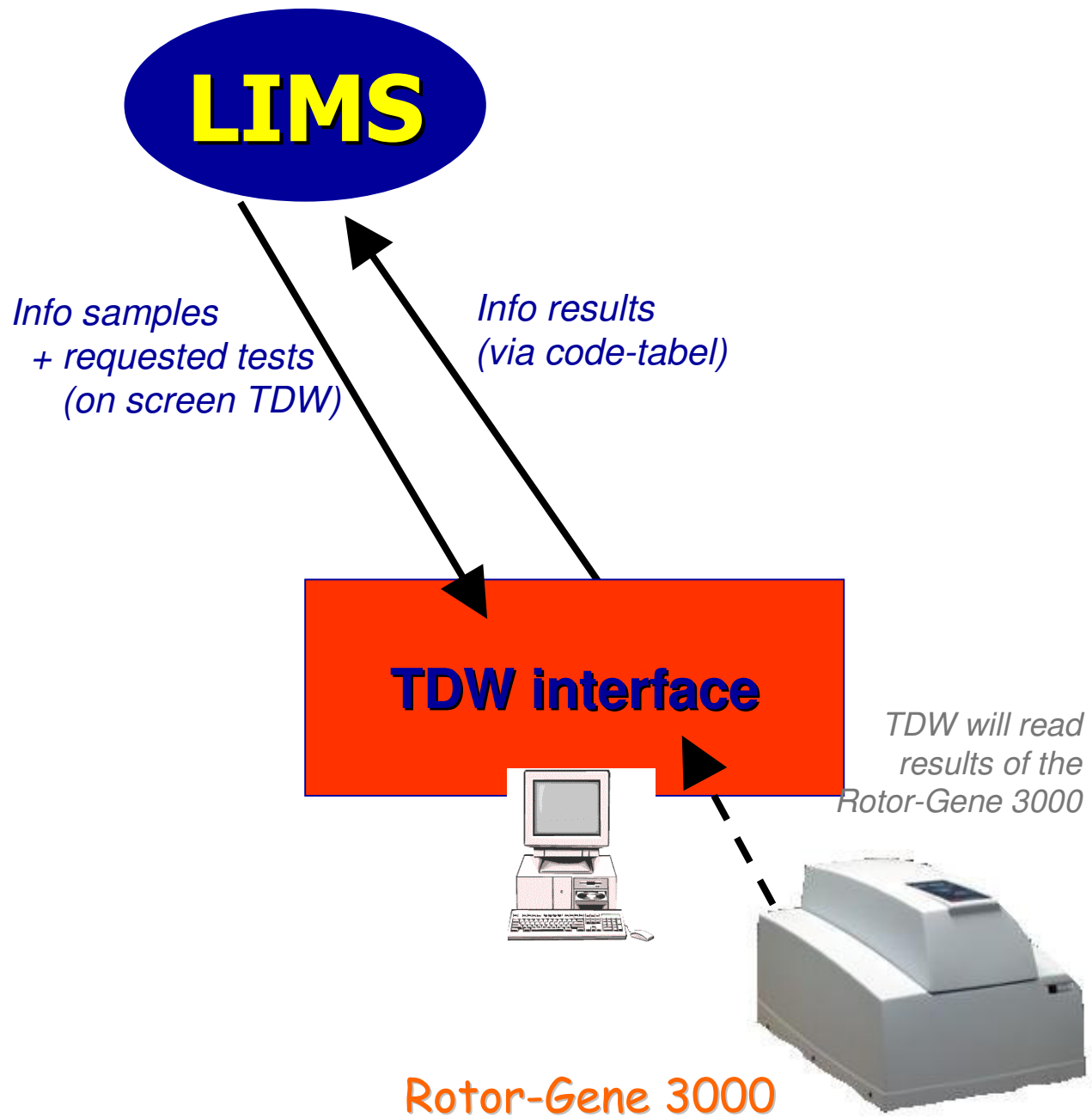
Sid#: 61086 R&P#: 000012  
Date: 20-04-05 Time: 13:19  
Doc: LLM Loc: CONTH  
Ocom:  
Dil: Type: EDT Stat: N Al:

QC	Test	Units	D	F	C.Res	Prev.Run	Time	Prev.Res
	F5ML				F5HT1			
	F2M				F2WTM			
	H63				63HT			
	H282				82HT			

TEST RESULT

Pending: 21  
Hold: 0  
Incomplete: 1  
C.Complete: 0  
Complete: 345  
All Complete: 49





## Temporary result-1

Advise to send in a second, independent, bloodsample for re-analysing

## Final result-2

The screenshot shows a software window titled "Sid# - Complete - Review And Edit". The window contains a menu bar (Access, Parameter, Patient, Sample, View, Customize, Help) and a toolbar with navigation arrows. Below the toolbar, patient information is displayed: Name, Pat#, Pcom, Age (66Y), Sex (M), Sid#, Date (10-02-05), Doc (SOOSR), R&P# (0000C3), Time (11:09), and Loc (REUMA). A table of test results is shown below, with columns for QC, Test, Units, D, F, C.Res, Prev.Run, Time, and Prev.Res. Two red boxes highlight the "C.Res" column and the "Prev.Res" column. The status bar at the bottom shows "Ready" and "NUM".

QC	Test	Units	D	F	C.Res	Prev.Run	Time	Prev.Res
	H63							63WT 13-01-05
	H282		E		82HM2	82HM1	11:09	82HM1 13-01-05





# Multiple Assays running simultaneously

**Reaction List**

- Pipette 2uL of Water + Mix Factor 5 Leiden [5] to Gene-Disc [72] loading p...
- Pipette 2uL of Contr-DNA WildType + Mix Factor 5 Leiden [5] to Gene-Di...
- Pipette 2uL of Contr-DNA's F5 Leiden + Mix Factor 5 Leiden [5] to Gene-D...
- Pipette 2uL of F5 + Mix Factor 5 Leiden [5] to Gene-Disc [72] loading plate
- Pipette 2uL of Water + Mix Factor 2 (G20210A) [2] to Gene-Disc [72] loadi...
- Pipette 2uL of Contr-DNA WildType + Mix Factor 2 (G20210A) [2] to Gene...
- Pipette 2uL of Contr-DNA's F2 (G20210A) + Mix Factor 2 (G20210A) [2] to
- Pipette 2uL of F2 + Mix Factor 2 (G20210A) [2] to Gene-Disc [72] loading p...
- Pipette 2uL of Water + Mix HHC (C282Y) LNA [2] to Gene-Disc [72] loadin...
- Pipette 2uL of Contr-DNA WildType + Mix HHC (C282Y) LNA [2] to Gene-D...

**Reaction Data**

Reaction Plate: Gene-Disc [72] loading plate @ B1

Well	Contents
A1	2uL Water + 23uL Mix Factor 5 Leiden [5]
A2	2uL Contr-DNA WildType + 23uL Mix Factor 5 Leiden [5]
A3	2uL Contr-DNA F5-HET + 23uL Mix Factor 5 Leiden [5]
A4	2uL Contr-DNA F5-HOM + 23uL Mix Factor 5 Leiden [5]
A5	2uL 54879 + 23uL Mix Factor 5 Leiden [5]
A6	2uL 54789 + 23uL Mix Factor 5 Leiden [5]
A7	2uL 54878 + 23uL Mix Factor 5 Leiden [5]
A8	2uL 56981 + 23uL Mix Factor 5 Leiden [5]

**Export reaction list...**

**Rotor-Gene 3000**



Corbett Robotics v4.7.93

Exceeded well count on plate!

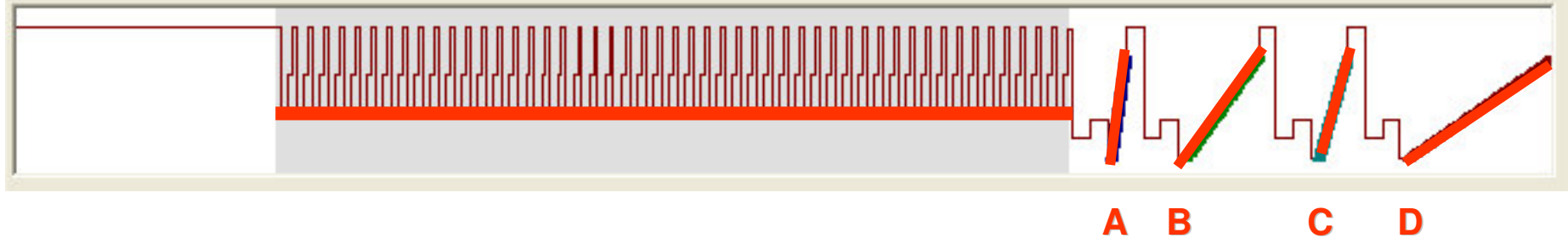
CAS1200 (simulated)

Level Sense: On

Tip re-use: 8 times

## Profile for quenched FRET assay with 4 different melt curves

The run will take approximately 282 minute(s) to complete. The graph below represents the run to be performed :

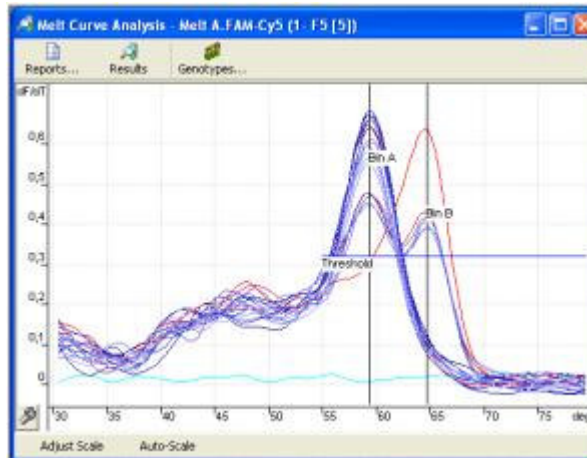


- **Combining 4 different cycling profiles (F5 / F2 / C282Y / H63D)**
- **Maybe sub-optimal cycling conditions for one ore more applications**  
Only specific amplicons will generate fluorescence signal (*target-specific FRET-probes*)
- **Melt-curve analysis can be repeated several times**
- **Each application can be analysed in his own optimal melt-condition**

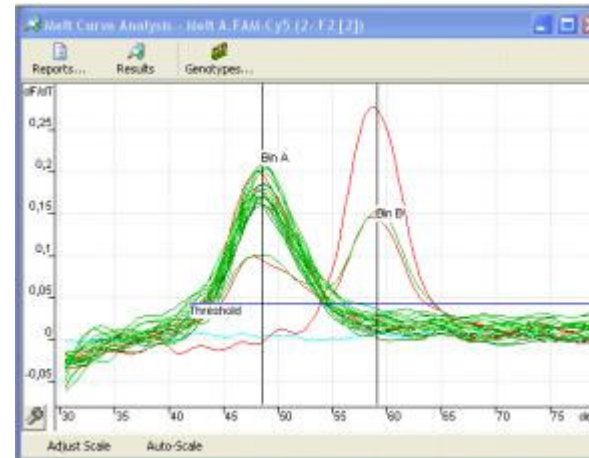


# Analysis & Genotyping

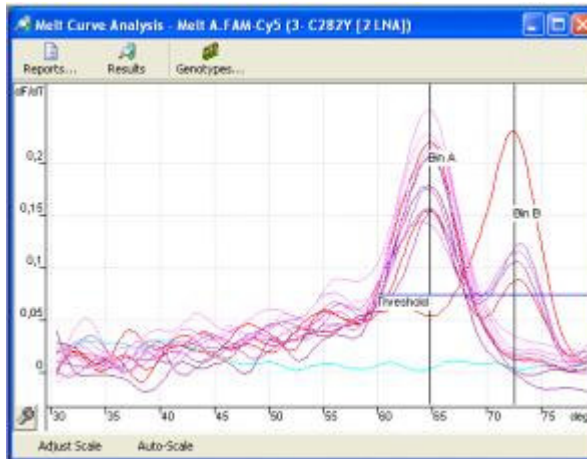
F5



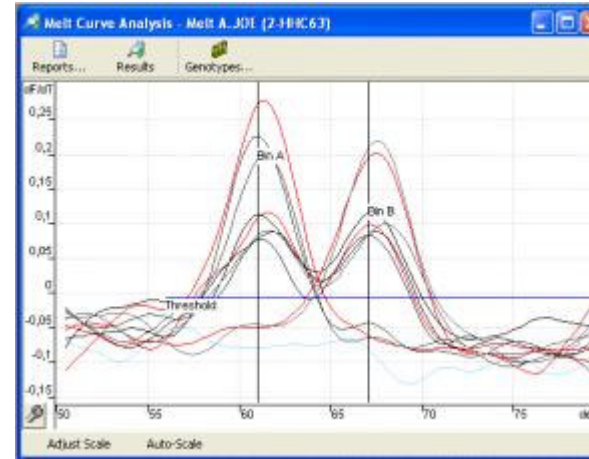
F2



C282Y



H63D



# CONCLUSIONS

## RFLP vs automated QPCR with CAS-1200 and Rotor-Gene

- **Drastic reduction of hands-on time** and manual handling steps
- **Complete sample tracking** (from DNA extraction to result-transmission) covered by import/export functions
- **No post-amplification steps** required, contamination-risk minimized
- **Flexible programming** allows customized set-up of tests
- **Different FRET-applications** assayed simultaneously in one run

## Acknowledgement

- Westburg Benelux Leusden The Netherlands
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