Techniques for detection of BCR-ABL mutations

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Sensitivity	Method	Authors	Reference
10-20%	Selection of clones/ DNA sequencing	Gorre et al. Shah et al.	Science 2001 Cancer Cell 2002
	Nested PCR/ DNA sequencing	Branford et al. ● Hochhaus et al.	Blood 2002 Leukemia 2002
1-5%	Denaturing high performance liquid chromatography (D-HPLC)	Soverini et al. Irving et al. Deininger et al. ● Ernst et al.	Clin Chem 2004 Clin Chem 2004 Leukemia 2004 Haematologica 2008
	Pyrosequencing	Khorashad et al.	Leukemia 2006
	High-resolution melting (HRM)	Poláková et al.	Leuk Res 2008
	Double gradient denaturing electrophoresis	Sorel et al.	Clin Chem 2005
	MALDI-TOF mass spectrometry	Vivante et al.	Leukemia 2007
	Nanofluidic array	Oehler et al.	Leukemia 2008
< 1%	Fluorescence PCR and PNA clamping	Kreuzer et al.	Ann Hematol 2003
	Allele-specific oligonucleotide (ASO) PCR	Roche-Lestienne et al. Willis et al. ● Gruber et al. ● Pelz-Ackermann et al. Preuner et al.	Blood 2002 Blood 2005 Leukemia 2005 Leukemia 2008 Leukemia 2008

Aims of the study

1. Comparison of two mutation screening methods for routine use:

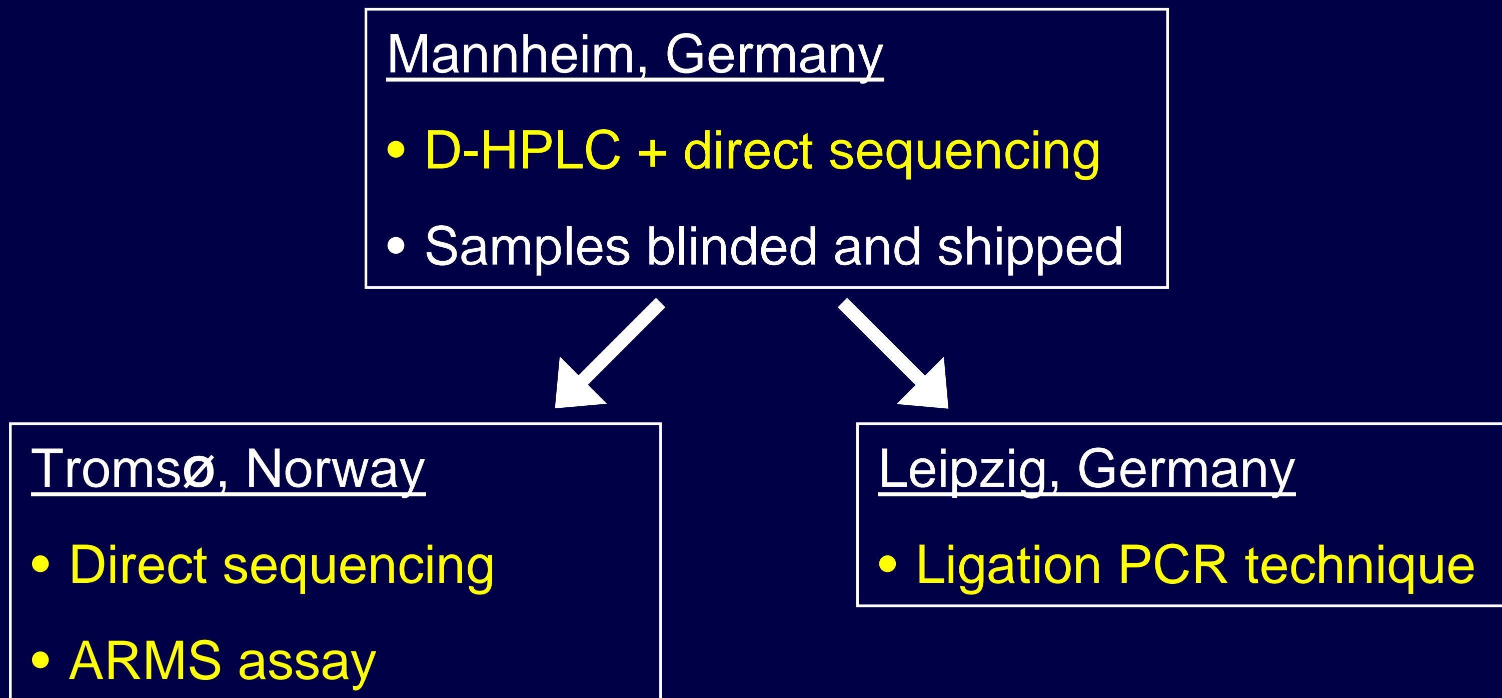
   direct sequencing vs. D-HPLC + sequencing

2. Comparison of D-HPLC and two allele-specific PCR techniques for detection of low-level mutations

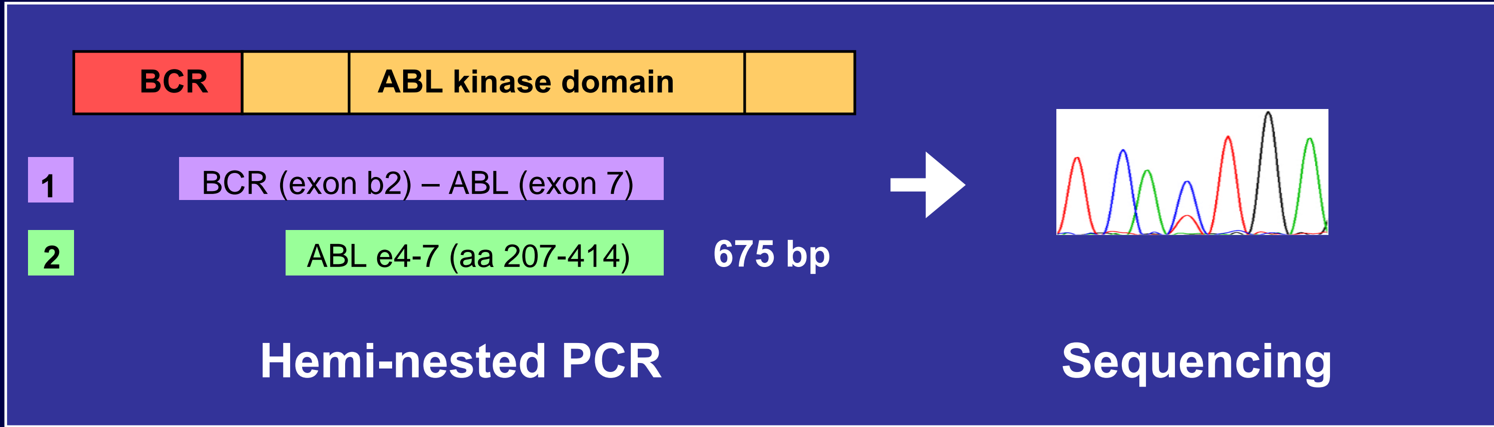
3. Kinetics of low-level mutations and their correlation to molecular response

Study design

- Mutation analysis of 40 imatinib-resistant patients after 0, 3, 6, 9, and 12 months on 2nd line Dasatinib or Nilotinib therapy
- N=200 samples



Direct sequencing of one large PCR product (sensitivity ~10-20%)



Hochhaus t al. Leukemia 2002

D-HPLC + direct sequencing of D-HPLC products (sensitivity ~1-5%)

BCR | **ABL kinase domain**

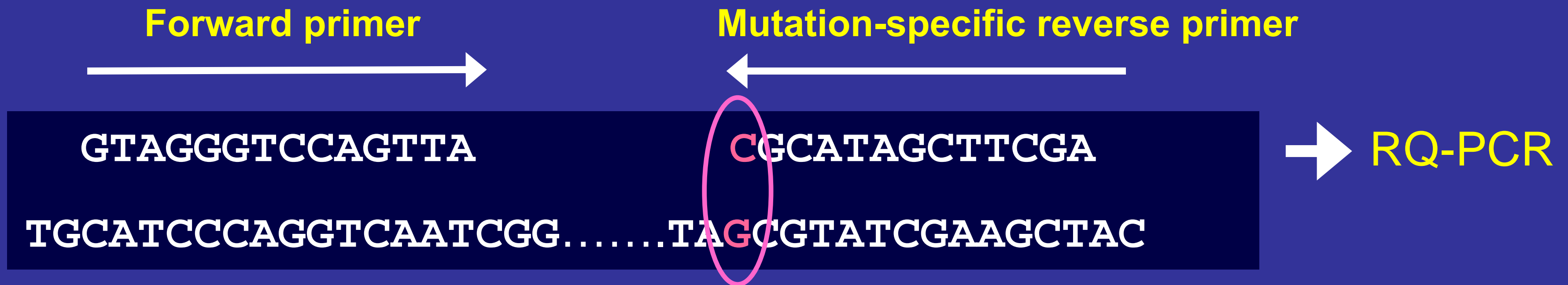
1 BCR (exon b2) – ABL (exon 10/11)

2 **A** (aa 207-324) · **B** (aa 279-414) · **C** (aa 382-517) — **~400 bp**

Nested PCR → **D-HPLC** → **Sequencing**

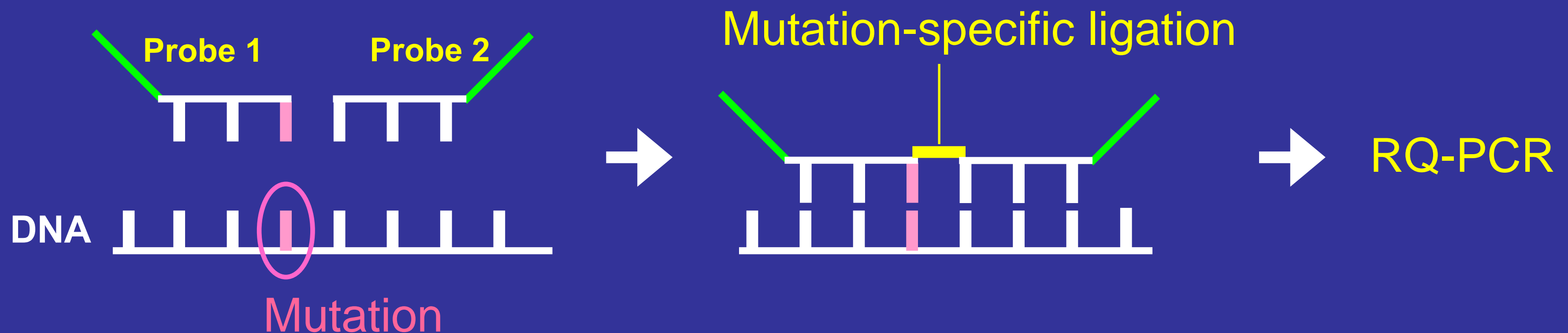
Ernst et al. Haematologica 2008

ARMS assay (sensitivity ~0.1-1%)



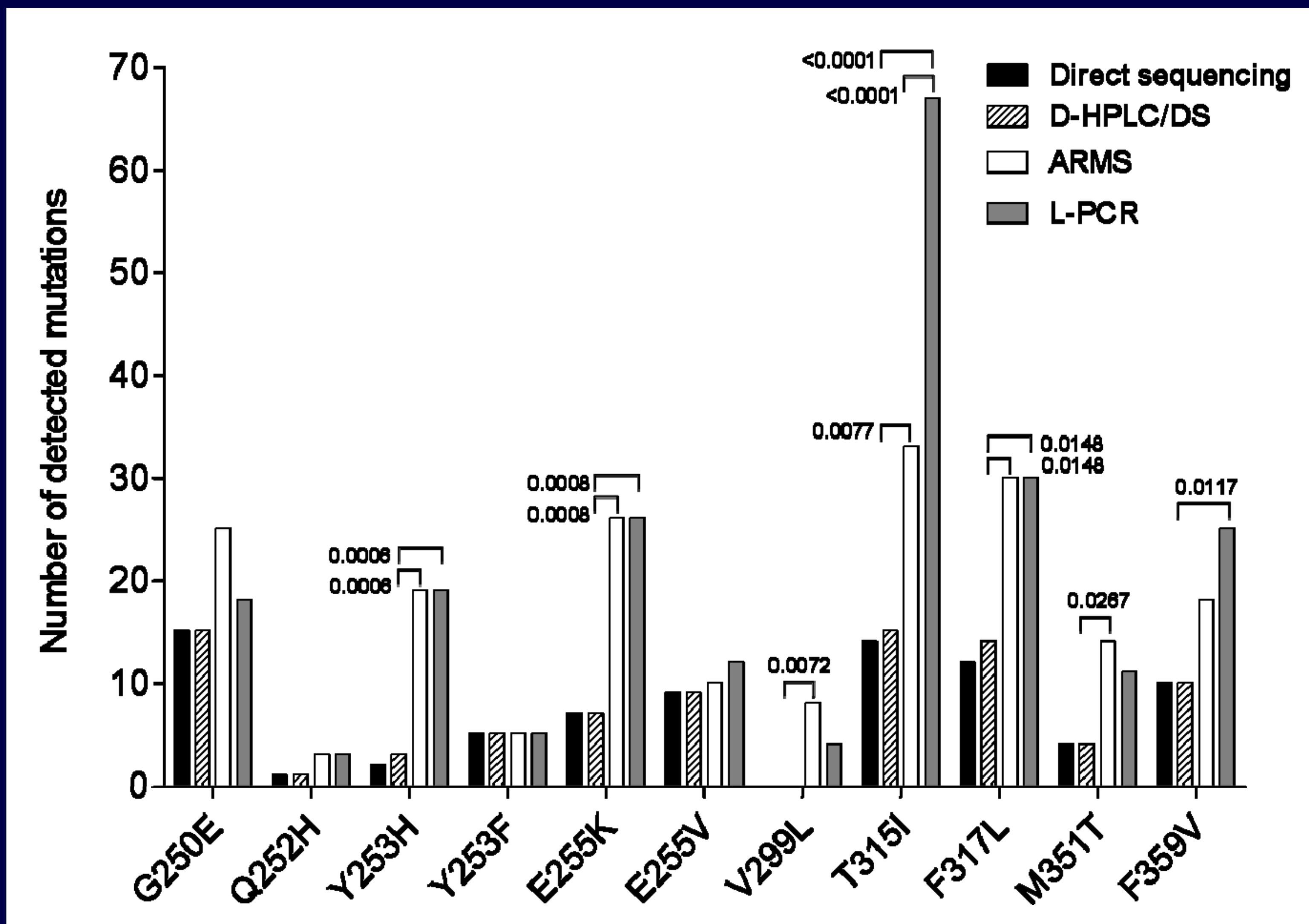
Gruber et al. Leukemia 2005

Ligation PCR technique (sensitivity ~0.05-0.1%)



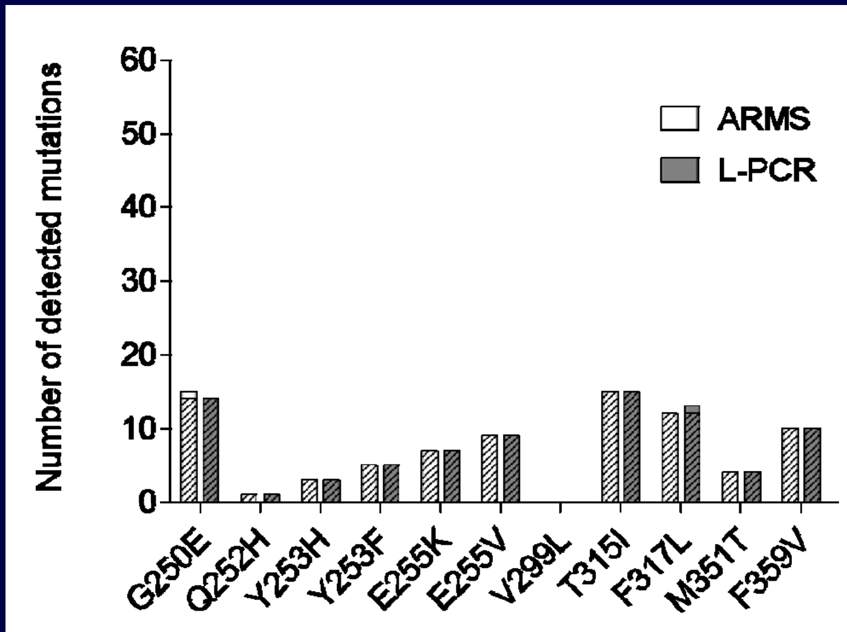
Pelz-Ackermann et al. Leukemia 2009

Comparison of DS, D-HPLC and ASO PCRs in N=200 blinded samples

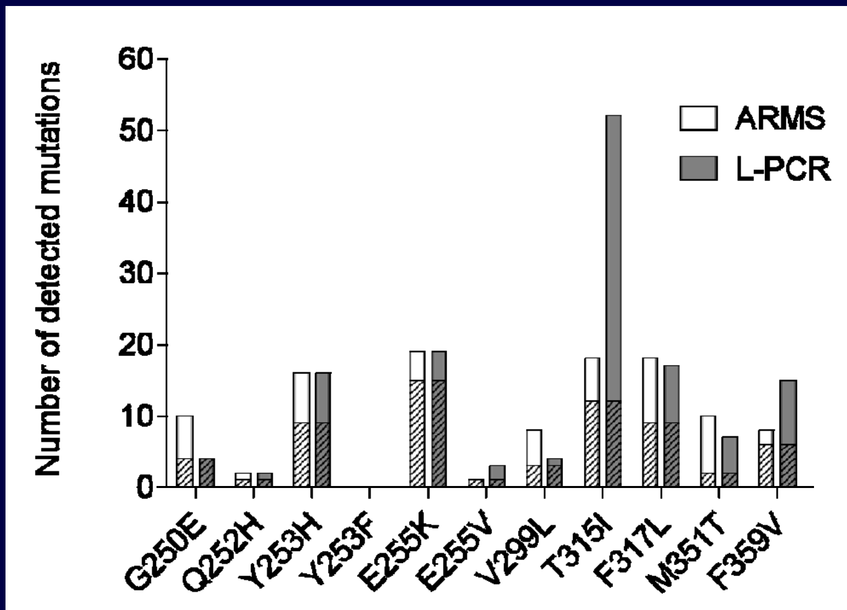


Comparison of DS, D-HPLC and ASO PCRs in N=200 blinded samples

	DS	D-HPLC/DS	ARMS	L-PCR
Total number of detected mutations	79	83	191	220
Additionally detected mutations to DS	--	4	112	142
Additionally detected mutations to D-HPLC/DS	0	--	110	139
Additionally detected mutations to ARMS	0	0	--	77
Additionally detected mutations to L-PCR	0	0	48	--



"High-level" mutations
(= mutations detected by DS and/or D-HPLC)



"Low-level" mutations
(= mutations not detected by DS and/or D-HPLC)

Summary and conclusions

1. DS and D-HPLC/DS were suitable and closely comparable for the detection of major resistant clones
2. ASO PCR further increases the number of detected mutations and indicates a high prevalence of mutations at low level
3. The clinical impact of high-sensitivity detection of low-level mutations is still unknown and needs further follow-up investigation

Harmonised testing for BCR-ABL mutations:

1st control round within the national reference laboratories

Objectives

1. Recording of mutation analysis methods and protocols routinely used in Europe
2. Evaluation of the respective technique by analysis (qualitative and quantitative) of blinded samples harbouring BCR-ABL kinase domain mutations
3. Technical recommendations based on these findings

Questionnaire

1. General questions
2. PCR method
3. Mutation analysis technique
4. Comments

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Please enter information about your PCR step.

21. Is your PCR step published?

- Yes
- No

If yes, please add the reference

22. Which primary material do you use?

- Peripheral blood (PB)
- Bone marrow (BM)
- Both PB and BM

23. Are you using genomic DNA (gDNA) or complementary DNA (cDNA) as template for your PCR?

- genomic DNA
- cDNA

24. PCR method

- Single step PCR
- Nested PCR

25. Please give the ABL exons range you analye (e.g. ABL exons 4-9)

26. Please give the range of amino acids you are analysing (e.g. AA240-490)

27. Please enter your primer sequences

Forward primer 1

Reverse primer 1

Internet

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HAS 2003 - Citrix Presentation Server Client [SpeedScreen On]
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Exit this survey >>

12. Which technique do you use for routine analysis?

- [ ] Direct sequencing
- [ ] Denaturing high performance liquid chromatography (D-HPLC)
- [ ] High resolution melting (HRM)
- [ ] Pyrosequencing
- [ ] Allele-specific oligonucleotide (ASO) PCR
- [ ] Selection of clones and sequencing

Other (please specify)

13. Is it a published method?

- ( ) Yes
- ( ) No

If yes, please give the reference

*** 14. Please specify the technical equipment you use for mutation analysis (e.g. Applied Biosystems ABI 3130, Transgenomic WAVE,...)**

15. In case you use direct sequencing, do you routinely sequence in

- [ ] both forward- and reverse directions
- [ ] only forward
- [ ] only reverse
- [ ] only in both directions when first direction was positive

16. Do you use POSITIVE controls (e.g. sample with a known mutation) in your runs?

- ( ) Yes
- ( ) No

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Samples

- Ba/F3 cell lines harbouring BCR-ABL kinase domain mutations
- Samples will be sent as cDNA (5-10 µl)
- N=20 blinded samples will be sent to each participating lab
  - 10 samples for part 1: qualitative study
  - 10 samples for part 2: quantitative study

Part 1: Qualitative Study

- Aim: To check whether laboratories are analysing the entire BCR-ABL kinase domain
- Method: Cell lines (medium-high proportion of mutant alleles) covering AA 244-486
- Result: Mutations **should** be identified by **all** laboratories

Part 2: Quantitative Study

- Aim: To check approximate sensitivity level of respective mutation analysis method
- Method: Cell line dilution series 50-20-10-5-1% of mutant Ba/F3$^{BCR\text{-}ABL}$ in non-mutant Ba/F3$^{BCR\text{-}ABL}$
- Result:
  - 1-5% will be missed by laboratories using direct sequencing only - but could be detected by pyrosequencing, D-HPLC, HRM, ARMS, etc.
  - 10% **could** be detected by direct sequencing
  - 20-50% **should** be detected by direct sequencing

Time schedule 2009

- March - April: Letter of invitation and registration
- June - July: Web-based questionnaire
- August - September: Culturing of Ba/F3 cell lines and making of dilution series
- October: Shipment of samples
- November - December: Waiting for results
- January 2010: Study analysis

Country	City	Contact person
Austria	Wien	Gerlinde Mitterbauer-Hohendanner
Belgium	Leuven	Peter Vandenberghe
Croatia	Zagreb	Renata Zadro
Czech Republic	Prague	Katerina Machova
Czech Republic	Olomouc	Radek Plachy
Denmark	Aarhus	Charlotte Guldborg Nyvold
Finland	Turku	Veli Kairisto
France	Paris	Jean-Michel Cayuela
Germany	Mannheim	Martin Müller
Germany	Leipzig	Thoralf Lange
Greece	Athens	Katerina Zoi
Hungary	Budapest	Hajnalka Andrikovics
Israel	Tel-Hashomer	Tali Tohami
Italy	Turin	Francesca Arruga
Netherlands	Rotterdam	Peter Valk
Norway	Oslo	Dag Andre Nymoen
Poland	Kraków	Magdalena Zawada
Portugal	Lisboa	Joana Diamond
Romania	Bucharest	Rodica Talmaci
Russia	St. Petersburg	Michael Dubina
Russia	Moscow	Andrey Misyurin
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