MicroSight® MSI

For Use with BaseTyper™ real-time PCR Instruments

MicroSight® MSI PentaBase Panel for Detection of Microsatellite Instability in Cancer Patients
For Analysis of unpaired Tumor Samples

Instructions for use

Please read these instructions carefully before using MicroSight® MSI PentaBase Panel assay. It is recommended to save the "Instructions for use" for future use. Purchasers of MicroSight® MSI PentaBase Panel assay are only granted the right of use, but no general licensing or patent rights.

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1. Intended use

MicroSight® MSI PentaBase Panel assay is intended for *in vitro* diagnosis of an impaired mismatch repair (MMR) system affecting prognosis and selection of treatment in cancer patients. MMR system deficiency is normally manifested as replicative errors affecting the length (number of nucleotides) of repeated sequences in the genome commonly referred to as microsatellites. MicroSight® MSI PentaBase Panel assay offer an analysis of the stability/instability in five mononucleotide microsatellite loci, two loci (BAT25 and BAT26) being part of the Bethesda recommendations (Boland et al. 1998), two loci (NR22 and NR24) introduced by Suraweera and coworkers (Suraweera et al. 2002) and one locus (MONO27) introduced by Bacher and co-workers (Bacher et al. 2004).

MicroSight® MSI PentaBase Panel assay should be used on human DNA samples from fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tissue. MicroSight® MSI PentaBase Panel assay **is not intended for diagnosing disease**, but only as an aid to assist treatment planning. The tests are provided in one or more boxes, containing the necessary components for thermal amplification and High-Resolution Melt (HRM)-based length analysis of five microsatellite loci recommended for microsatellite instability (MSI) evaluation.

MicroSight® MSI PentaBase Panel assay has high requirements for precise block temperature regulation and performance of HRM module and can only be used with BaseTyper™ real-time PCR-instruments.
The *Instructions for Use* or *Quick guide* is also available for download on our webpage: www.pentabase.com.

2. Summary and explanation of the assay

2.1 Indications for use

Microsatellites are genetic motifs consisting of short (1-6 bases) nucleotide sequences repeated up to 100 times comprising approximately 3% of the human genome. During replication, these sequences are susceptible to errors, both deletions and insertions, normally corrected by the MMR system. Uncorrected microsatellite errors are commonly referred to as MSI and used to assist clinicians and genetic counsellors in the diagnosis of a type of neoplastic inherited syndrome (Vaksman and Garner, 2015).

Inherited deregulation by epigenetic and genetic changes in genes (like PMS2, MLH1, MSH2, or MSH6) and their gene products leading to compromised MMR system response is known as Lynch syndrome (LS) or hereditary nonpolyposis colorectal cancer. Apart from a high risk of developing colorectal cancer, people suffering from LS also have significantly increased risk of developing endometrial cancer, gastric cancer, ovarian cancer, small bowel (small intestinal) cancer, pancreatic cancer, urinary tract cancer, kidney cancer, bile duct cancer, and certain skin and brain tumors (Richman S, 2015). In patients with colorectal cancer, MSI is associated with a slightly better prognosis and low benefit of 5-flourouracil-based therapeutics.

Uniform evaluation of MSI status is in the “Bethesda Guidelines” (Boland et al., 1998) suggested to include two mononucleotide microsatellites (BAT25 and BAT26) and three dinucleotide microsatellites (D2S123, D5S346 and D17S250). With five microsatellites analyzed, patients can be categorized as microsatellite stable, MSS (0 markers exhibiting instability), microsatellite instable-low, MSI-L (1 marker being unstable) or microsatellite instable-high, MSI-H (2 or more loci being unstable). As individual dinucleotide markers of the Bethesda panel only exhibit instability in 60-80% of MSI-H tumors, it has been suggested to exchange these markers with an expanded set of mononucleotide markers. Thus, Suraweera and co-workers suggested to evaluate MSI status by five mononucleotide loci; BAT25, BAT26, NR21, NR22 and NR24 (Suraweera et al, 2002).

Apart from increasing sensitivity, the monomorphic nature of this panel has been suggested to eliminate the need for parallel investigation of paired samples from patients comparing un-involved (germline) tissue to tumor genomic DNA, (gDNA). Alternative mononucleotide loci such as MONO27 have revealed equally high specificity and sensitivity (Bacher et al. 2004).

2.2. Explanation of the assay

MicroSight® MSI PentaBase Panel assay is based on PentaBase’s highly sensitive DNA technology. MicroSight® MSI PentaBase Panel assay comprises of 5 (Su)Primer™ pairs (Table 1) and dual labelled EasyBeacon™ probes covering the indicated microsatellites.

Table 1. MicroSight® MSI PentaBase Panel primer sequences. Proprietary modifications not shown.

Marker	First primer	Second primer
BAT25	5'-ACTATGGCTCTAAAATGCTCT-3'	5'-TAAAGAGTTTTGTGTTTTGTT-3'
BAT26	5'-TCAGAGCCCTTAACCTTTTTC -3'	5'-ACATTTTTTAACCATTCAACATT-3'
NR22	5'-CCATCCAGTTTTGTTCTTACAA-3'	5'-CTGAGCACATCACATTTAGGA-3'
NR24	5'-TGGGTGACAGAGTGAGACTC-3'	5'-ACTCTTCTCTTCCCTGGGC-3'
MONO27	5'-CTGGGTGACATAATGAGACCC-3'	5'-TACTACTGTCCTACTGTGCC-3'

2.2.1 EasyBeacon™ Probes

EasyBeacon™ probes are PentaBase's alternative to molecular beacons labeled with a fluorophore at the 5'end, a quencher at the 3'end, and with the addition of pentabases thereby removing the need for adding a stem and giving the probe a significantly improved signal-to-noise ratio, higher specificity, and higher sensitivity compared to conventional molecular beacon probes. EasyBeacon™ probes in the MicroSight® MSI PentaBase Panel assay are labeled with PentaGreen™ (λ_{abs}. 495 nm and λ_{Em}. 516 nm, detected on the same channel as FAM™) in combination with a Green Quencher™.

2.2.2 SuPrimers™

SuPrimers™ are standard DNA primers modified with one or more pentabases. The pentabases provide increased specificity and sensitivity and reduce primer-dimer formation.

3. Reagents and materials

MicroSight® MSI PentaBase Panel is supplied as Ready-To-Use assays in pre-aliquoted 0.2 mL PCR strips. All reagents, needed for the PCR amplification and microsatellite length variation analysis of extracted genomic DNA using a BaseTyper™ real-time PCR instrument, are pre-loaded into the strips (Table 2).

Table 2. Contents of MicroSight® MSI PentaBase Panel ready-to-use assays for paired sample analysis.

Strip #	Tube #	Content	Template to be added
HR (black)	**A**	BAT25 assay + Universal reference DNA	Preloaded with universal reference DNA. **No DNA should be added**.
	B	BAT26 assay + Universal reference DNA	
	C	NR22 assay + Universal reference DNA	
	D	NR24 assay + Universal reference DNA	
	E	MONO27 assay + Universal reference DNA	
H1 (red)	**A**	BAT25 assay	Patient tumor gDNA
	B	BAT26 assay	
	C	NR22 assay	
	D	NR24 assay	
	E	MONO27 assay	

3.1 Mutation analysis

Reference and tumor tissue DNA are amplified using the MicroSight® MSI PentaBase Panel assay in separate strips. The melt curves of the tumor tissue DNA are compared to the melt curves of reference DNA to detect differences in the melt shape, which reflects the length variation of the microsatellite loci. See section "6. Data Analysis" for more details.

The materials provided for MicroSight® MSI PentaBase Panel for Detection of Microsatellite Instability Assays can be found in Table 2. Materials required, but not provided can be found in Table 3 and 4.

3.2 Reagent storage and handling

General laboratory precautions should be taken. MicroSight® MSI PentaBase Panel assays should only be used by personnel who have been trained in the appropriate techniques. All chemicals and biological material should be considered as potentially hazardous. When working with the assays, suitable personal protective equipment (lab-coat, disposable gloves, and safety glasses) should be used. It is recommended that all work is carried out in appropriate facilities. All waste should be disposed as clinical waste.

3.2.1 Precautions

The following precautions should be taken when working with MicroSight® MSI PentaBase Panel assay:

- The assay is only for *in vitro* diagnostic use.
- MicroSight® MSI PentaBase Panel assay is not intended for diagnosing any type of cancer, but only as a supplement for other prognostic factors for the selection of patients who might benefit from a specific treatment (companion diagnostics).
- Avoid several freeze/thaw cycles of the reagents as this might impair the performance of MicroSight® MSI PentaBase Panel assay. Use the reagents when thawed.
- Verify eligibility of the DNA samples as samples of poor quality might affect the analysis.
- The delivered reagents should not be diluted further. Further dilution can cause loss of performance.
- Use the specified volumes. It is not recommended to reduce the specified volumes as the results can be affected.
- No reagents should be substituted by others if the optimal performance should be maintained.
- To protect the included fluorescent probes, avoid exposure to light for extended periods. For more information, see section 4.3 ”Storage”.
- Use caution not to contaminate reagents and samples. It is recommended to separate preparation of PCR mixes and DNA addition. Dedicated pipettes should be used, and it is recommended to have separate areas for sample preparation and PCR running.
- All used instruments and equipment should be calibrated and perform in accordance with their original specifications.

3.2.2 Shipment

MicroSight® MSI PentaBase Panel Ready-To-Use assays are shipped on dry ice or -80˚C cooled gel packs and should be frozen on arrival. If the MicroSight® MSI PentaBase Panel packaging has been opened during transport or if the products are not cold upon arrival, please contact PentaBase A/S or your local. Please also contact PentaBase A/S if the shipment is missing a certificate of analysis (CoA), reagents or a ”Quick Guide”.

3.2.3 Storage

MicroSight® MSI PentaBase Panel assays should after arrival immediately be stored at -20°C or lower. Repeated freeze/thaw cycles should be avoided. If the assays are stored under the recommended conditions, they will be stable until the expiry date stated on the box and CoA.

3.3 Additional materials required

Table 3. Materials and consumables required but not provided.

Material
Pipette Tips
Centrifuge for spinning tubes or plate
Extraction Kit
DNA Extraction Kit

3.4 Instrumentation required

Table 4. Instrumentation.

Equipment
Nucleic Acid Extraction System
BaseTyper™ Real-Time PCR instrument

4. Sample collection, transport, and storage

Specimens should be human gDNA extracted from formalin fixed paraffin-embedded (FFPE), fresh, or fresh-frozen tumor sections. The samples should be collected and stored after standard pathology methodology to ensure optimal quality. Extracted gDNA should be stored at maximum -20˚C until use.

4.1 Recommended procedure for extraction of Genomic DNA

Any method designed, approved, and quality controlled for extraction of human genomic DNA from a suitable specimen of interest can be used with MicroSight® MSI PentaBase Panel including the BasePurifier™ Nucleic Acid Extraction Instrument (PentaBase, Cat. No.: 715) and QIAamp DNA FFPE Tissue Kit (Qiagen, Cat. No.: 56404). Thus, for extraction of genomic DNA from FFPE samples, it is recommended to use genomic DNA extraction kits and/or procedures specially designed for handling of FFPE samples including steps for deparaffinization and sample digestion.

4.2 Recommended concentration of Genomic DNA

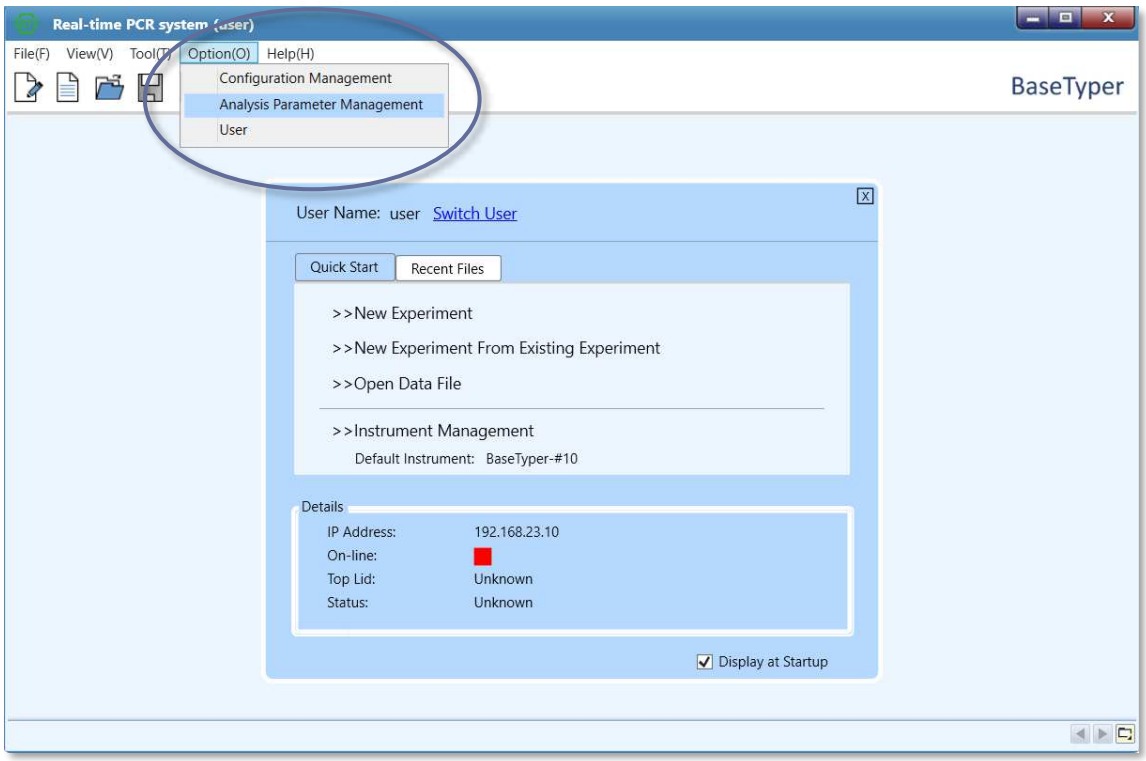
MicroSight® MSI PentaBase Panel assay is valid using a DNA concentration of 1-10 ng/µL. This concentration range is based on a real-time PCR quantification assay. We recommend using **2-5 ng/µL** if the concentration is determined by any other method than real-time PCR.

5. Instructions for use

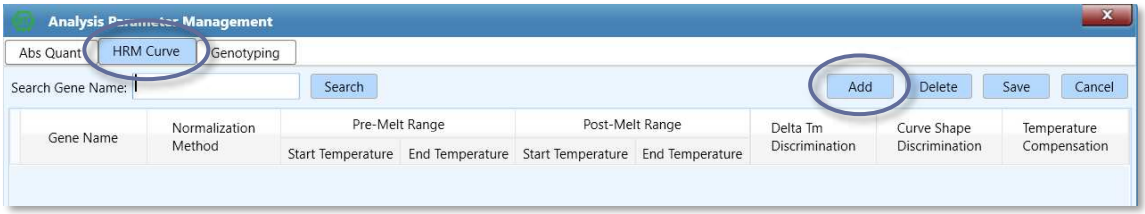
Important: When using MicroSight® MSI PentaBase Panel assay for **unpaired sample analysis**, a no patient-specific reference should be included for MSI analysis. A universal reference is provided with the kit. For each PCR run, one strip containing the universal reference (strip **HR**) must be included.

5.1 Set-up BaseTyper™ for MicroSight® MSI analysis

1. Click on **Option(O)** and go to **Analysis Parameter Management**.

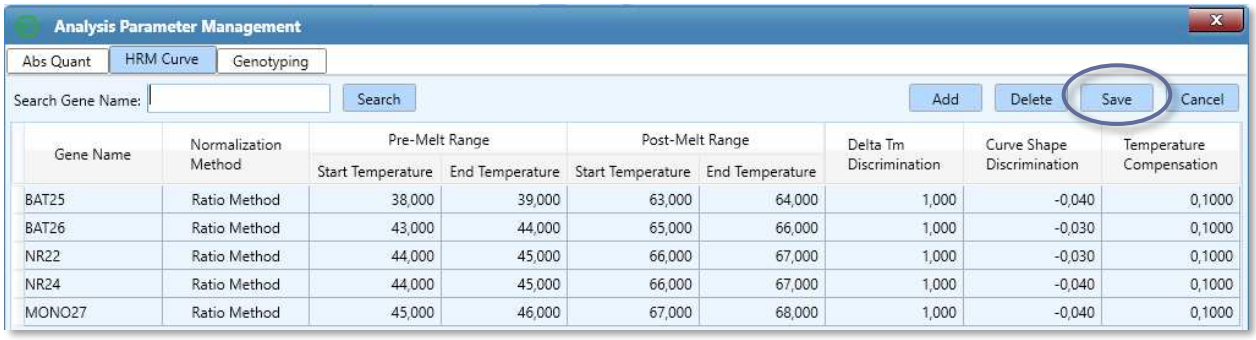


2. Click on the fan **HRM Curve** and press **Add**.



Now type “BAT25” under the column **Gene Name** and fill in the columns as shown in the figure below.

3. Repeat this for BAT26, NR22, NR24 and MONO27, by clicking on **Add** and type in the information shown below.
4. Remember to press **Save** after you finished typing in all the information.



Gene Name	Normalization Method	Pre-Melt Range		Post-Melt Range		Delta Tm Discrimination	Curve Shape Discrimination	Temperature Compensation
		Start Temperature	End Temperature	Start Temperature	End Temperature			
BAT25	Ratio Method	38,000	39,000	63,000	64,000	1,000	-0,040	0,1000
BAT26	Ratio Method	43,000	44,000	65,000	66,000	1,000	-0,030	0,1000
NR22	Ratio Method	44,000	45,000	66,000	67,000	1,000	-0,030	0,1000
NR24	Ratio Method	44,000	45,000	66,000	67,000	1,000	-0,040	0,1000
MONO27	Ratio Method	45,000	46,000	67,000	68,000	1,000	-0,040	0,1000

Note: It is only necessary to do this set-up once on a compute. However, if the software is re-installed, or if another computer is set up for the analysis, this procedure must be repeated.

5.2 Preparation of MicroSight® MSI PentaBase Panel assay

1. Thaw the Ready-To-Use tubes for approx. 5 minutes.
   a. Thaw a **H1** (red) strip for each tumor sample analyzed. You can maximum analyze 7 tumor samples in a PCR run.
   b. Thaw one universal reference **HR** (black) to analyze up till 7 tumor samples. The universal reference is pre-loaded with DNA and must NEVER be opened.
2. Do not leave thawed assays at room temperature for an extended period of time (>1 hour), alternatively keep on ice-bath/ice-block.
3. Centrifuge the tubes for approx. 30 seconds before opening the lids.

   ***Note**: 7 patients can be analyzed in one run. The BaseTyper™ real time PCR-instrument measures fluorescence from the side of the tubes which allows for marking of strips with e.g., patient or sample number on the lids of the tubes.*

5.3 Adding DNA to the tubes

The concentration of the DNA must be in the range of 1-10 ng/µL, but we recommend using **2-5 ng/µL.**

1. Vortex DNA samples briefly prior to use.
2. Open one lid at a time of strip **H1** (red). Load **5 µL** of DNA from **tumor** tissue of patient 1 to each of the 5 tubes. Close each lid after DNA addition.
3. Repeat for patient 2-7.

***Note**: Change the pipette tip between loading of each tube.*

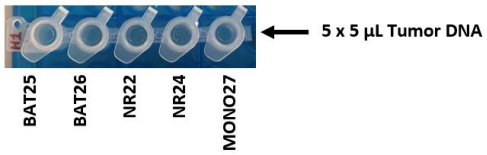


Figure 1. Orientation of the 5-tube PCR strips and assays when loading DNA. Add 5 µL of purified DNA from patient tumor tissue to strip **H1** (red).

5.4 Placing tubes in the PCR instrument

1. Spin the tubes for **2 minutes** before loading them into the BaseTyper™.
2. The universal reference strip is placed with the **HR (black)** mark in position A1, and patient 1 tumor sample is placed with the **H2 (red)** mark in position B1, see Figure 2.
3. Repeat for patient 2 (C1, D1), 3 (E1, H1), and 4 (F1, G1) ect.
4. Gently press on the top of the tubes to make sure the strips are placed correctly.
5. Close the lid of the instrument.

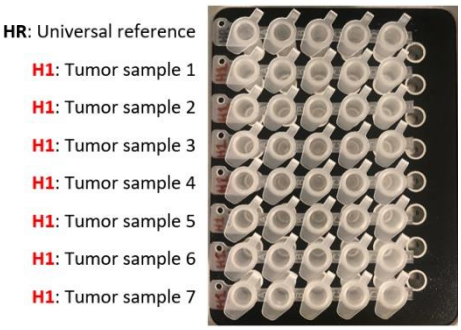
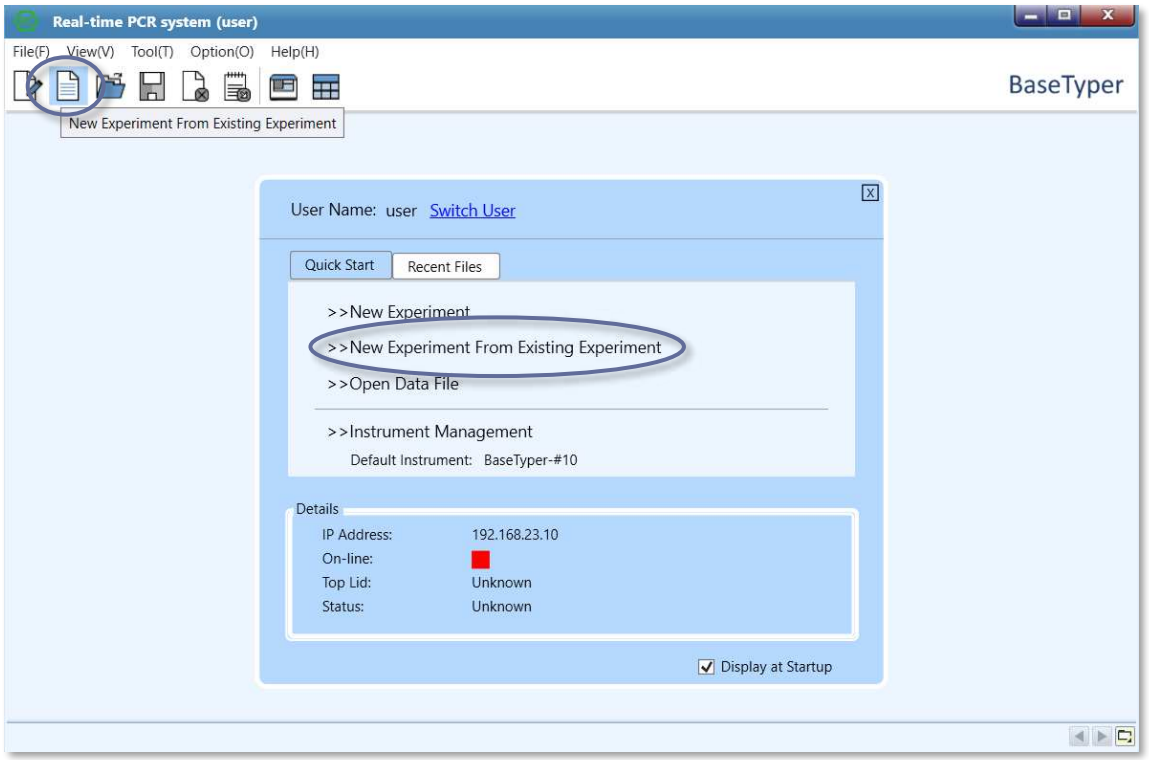


Figure 2. Placement of tubes in the BaseTyper™.

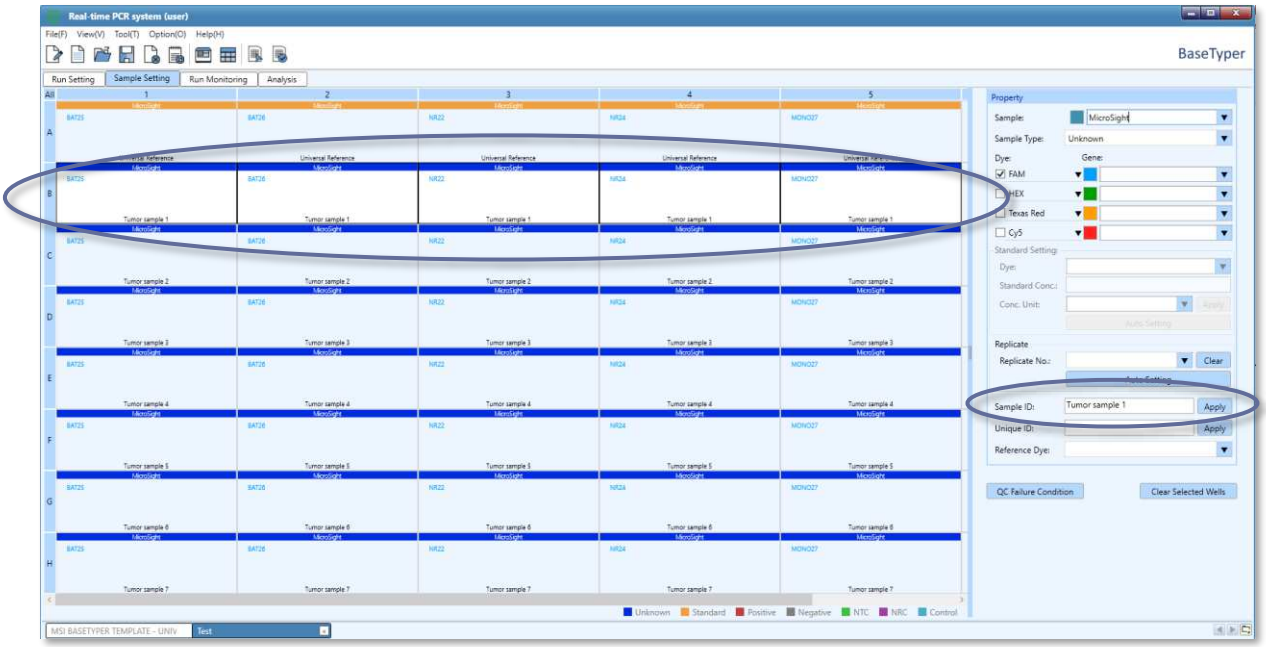
5.5 Set-up BaseTyper™ for Microsight® MSI PentaBase Panel analysis

1. In the BaseTyper software select **New Experiment From Existing Experiment** and open "MSI BASETYPER TEMPLATE – UNIVERSAL REFERENCE v2.1". Note you can select this in two tabs in the software.



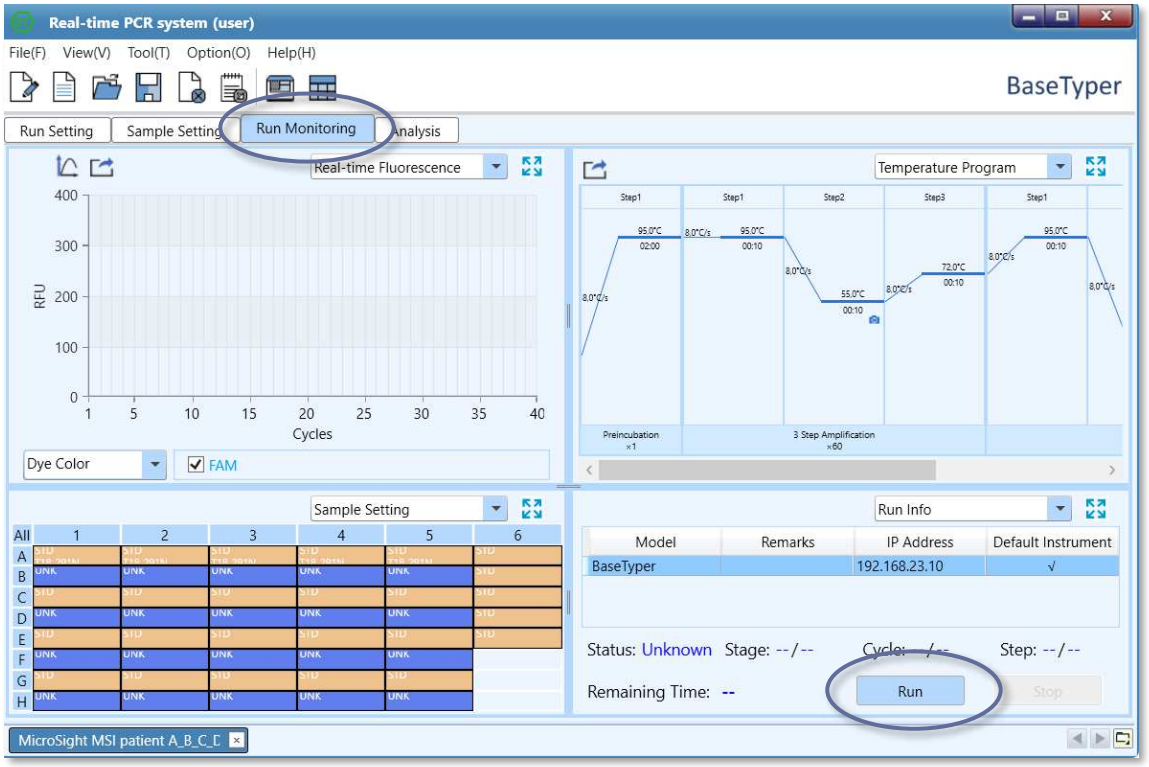
2. Give the experiment a name. It is recommended to name the experiment with the tumor sample ID numbers.
3. The temperature profile can be viewed under **Run Setting**, and it should look like this:

4. Go to the **Sample Setting** tab.
5. Name the samples by marking a row from 1-5. For row B1-B5: Delete the name “Tumor sample 1” and type the tumor ID in the **Sample ID** box. Press “enter” on the keyboard.
6. Repeat for all rows containing samples (the naming of samples can also be set after the run has finished, but not during the run!).
7. You should not change anything in the universal reference (position A1-A5).
8. Do not change any other property than the Sample ID. This can cause failure of analysis.



5.6 Starting the run

1. Go to the **Run Monitoring** tab.
2. Select the instrument under **Model** in the Run Info window and press **Run**.



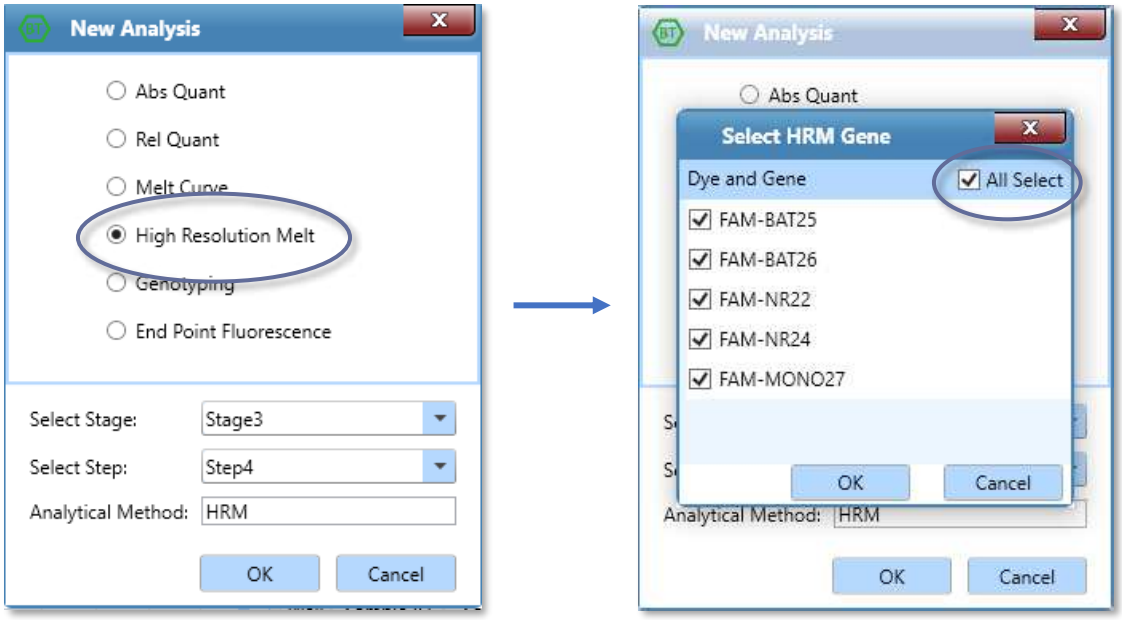
6. Data Analysis

Microsatellite loci are evaluated individually by comparing the melt properties of amplicons obtained with the patient's tumor DNA to those observed when applying the universal reference DNA. Both shorter and longer microsatellite amplicons are indicative of instability.

The run will automatically be saved in the folder you chose when setting up the run. If auto-saving was not selected, save the run manually before closing the program.

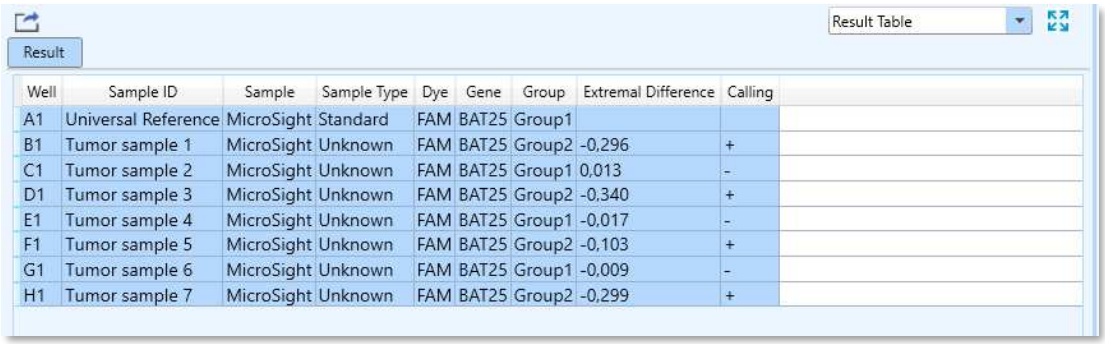
6.1 Evaluation of Microsatellite locus stability

1. Go to the **Analysis tab** and press  **New Analysis**.
2. Select **High Resolution Melt** in the **New Analysis** window.
3. Click the **All Select** checkbox in the **Select HRM Gene** window and press **OK**.



4. Five HRM tabs will then open – one for each locus:

5. Go to **HRM BAT25(Stage3)**. Under **Result Table** you find the stability result for that locus.
6. A "**+**" in the "**Calling**" column means that the sample is unstable in that locus. Repeat for BAT26, NR22, NR24 and MONO27.
7. In the example below, tumor sample 1, 3, 5 and 7 are unstable in that locus and sample 2, 4, and 6 are stable.



Well	Sample ID	Sample	Sample Type	Dye	Gene	Group	Extremal Difference	Calling
A1	Universal Reference	MicroSight	Standard	FAM	BAT25	Group1		
B1	Tumor sample 1	MicroSight	Unknown	FAM	BAT25	Group2	-0,296	+
C1	Tumor sample 2	MicroSight	Unknown	FAM	BAT25	Group1	0,013	-
D1	Tumor sample 3	MicroSight	Unknown	FAM	BAT25	Group2	-0,340	+
E1	Tumor sample 4	MicroSight	Unknown	FAM	BAT25	Group1	-0,017	-
F1	Tumor sample 5	MicroSight	Unknown	FAM	BAT25	Group2	-0,103	+
G1	Tumor sample 6	MicroSight	Unknown	FAM	BAT25	Group1	-0,009	-
H1	Tumor sample 7	MicroSight	Unknown	FAM	BAT25	Group2	-0,299	+

6.2 Sample DNA validation

If the Extremal difference value (found in the Result Table) is below -0.99, it is likely that no template has been added to the tube. Please go to "7.5 Special cases".

A qPCR analysis using the MONO27 assay amplification data should be made to verify that enough amplifiable DNA has been added if **1-5 loci are unstable**. If all loci are stable, the result should not be validated by qPCR.

Check the Ct values for MONO27 for each tumour sample under **Analysis** and **Abs Quant(Stage2_Step2)**.
If the Ct is higher than 35, it is recommended to make new dilutions of the DNA samples with a higher concentration.

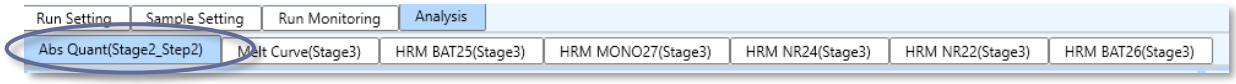
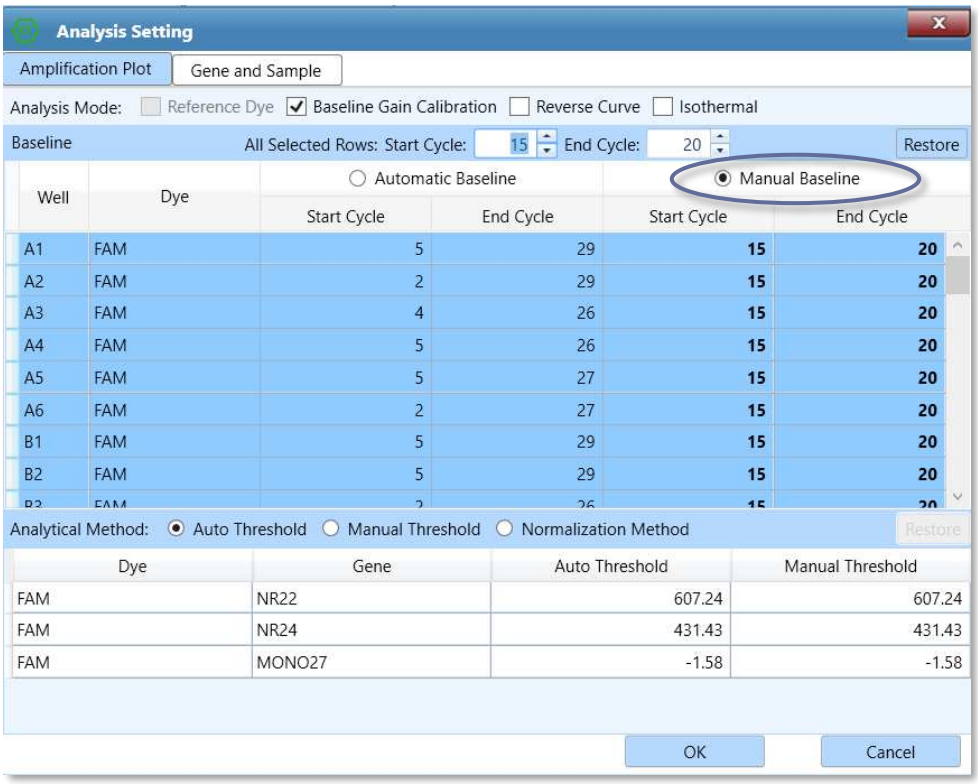


Table 5: Quality control criteria for the Ct value of MONO27.

Ct	Conclusion
>35	Invalid
<35	Valid

Note that the baseline can be adjusted in case of air bubbles in the PCR tubes. Press **Analysis Settings** and select **Manual Baseline.** To change the baselining for all wells, select one well and press **Ctrl + A**. Change the **Start Cycle** to a cycle after the air has disappeared (e.g. 15) and the **End Cycle** to a cycle number before the exponential phase of the PCR curves (e.g. 20). Press **OK**.



6.3 Overall evaluation of Microsatellite stability

With five microsatellites analyzed by MicroSight® MSI PentaBase Panel using **unpaired tumor samples,** patient samples are categorized as being either microsatellite stable (MSS, 0-2 loci being unstable) or microsatellite unstable-high, (MSI-H, 3 or more loci being unstable, see Table 6). **Note:** samples with only 2 unstable loci should be rerun until 2 runs produce the same result.

Table 6: Evaluation of microsatellite stability of unpaired tumor samples using MicroSight® MSI PentaBase Panel assay

Unstable loci (#)	Category
0-2	MSS
3-5	MSI-H

6.4 Data examples

Figure 3 and Figure 4 below illustrate a case of an unstable and a stable locus, respectively.

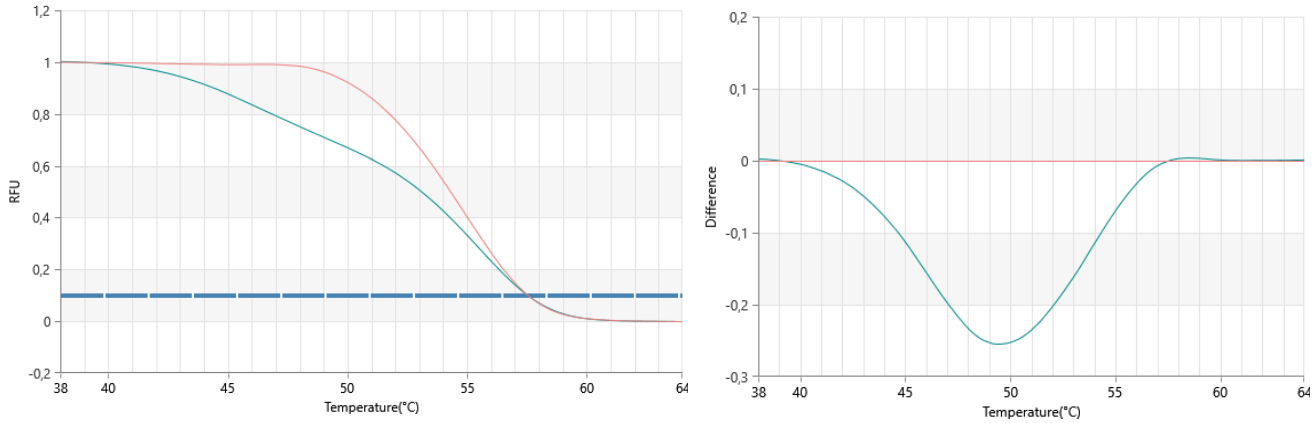


Figure 3. Microsatellite unstable locus. Left: The normalized melting curves. A difference is seen between the curve of the normal tissue and tumor tissue. Right: The difference graph.

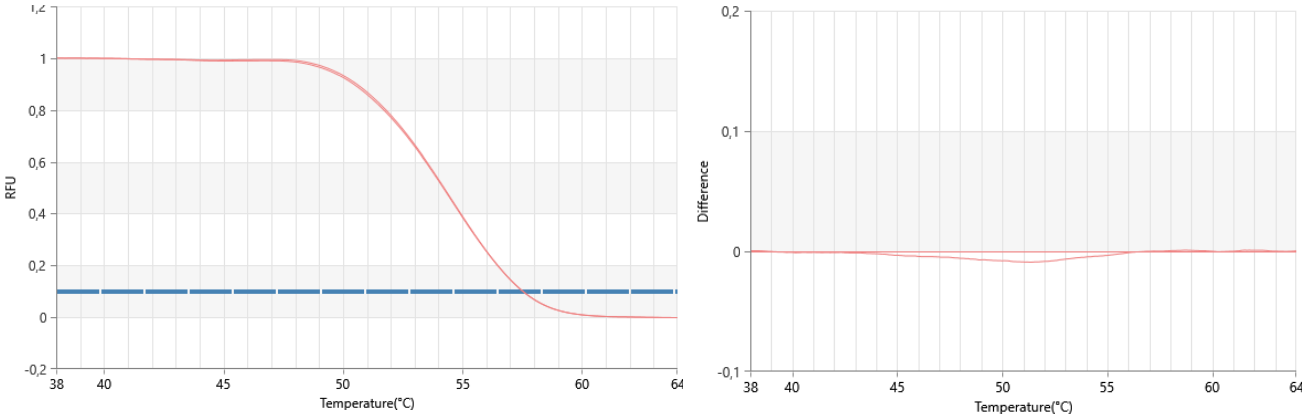


Figure 4. Microsatellite stable locus. Left: The normalized melting curves. No difference is seen between the curve of the normal and tumor tissue. Right: The difference graph.

6.5 Special cases

If no template DNA is added to the well, it might cause a false positive. Therefore, it is necessary to check the Ct values of the samples.

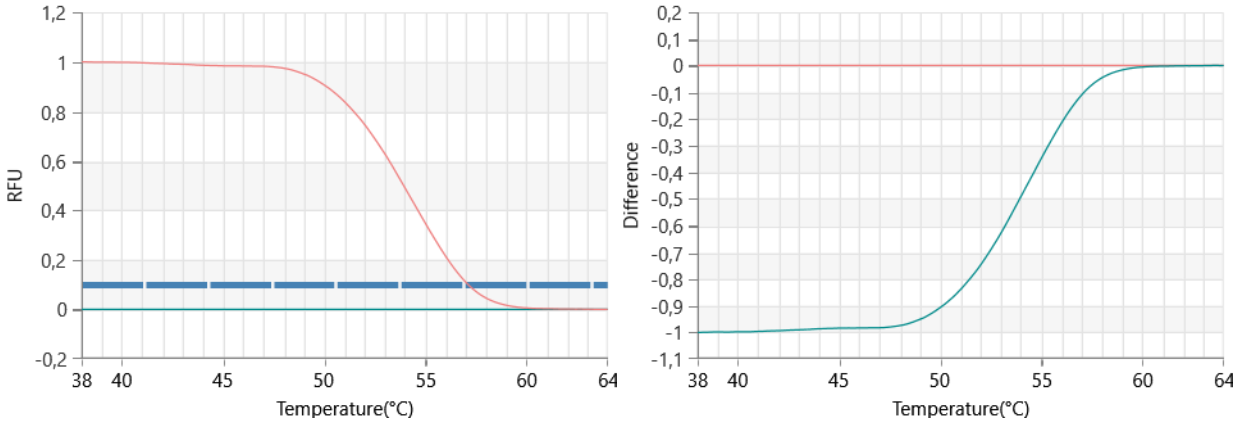


Figure 5. False positive caused by no template DNA added as tumor sample (green curve). Left: The normalized melting curves. Right: The difference graph.

7. Troubleshooting

If problems occur, which are not covered by the manual, please contact support@pentabase.com

8. Non-Clinical Performance

8.1 Analytical sensitivity – Limit of blank

The non-clinical specificity and performance of MicroSight® MSI PentaBase Panel assay in the absence of template has been established and evaluated during assay validation, and it is evaluated during quality control of produced lots using PCR grade water. The criterium for approval of assays is MONO27($Ct_{(NTC)}$) > 45.

8.2 Analytical sensitivity – Limit of detection

The non-clinical limit of detection (LOD) of MicroSight® MSI PentaBase Panel assay has been evaluated using serial dilutions of the human MSI cancer cell-line DLD-1 in Universal Reference DNA. All five MicroSight® MSI PentaBase Panel loci were found to be unstable at 50% and 25% dilution points while 3 out of 5 loci were unstable at the 12.5% dilution point. All five loci were found to be stable at the 6.25% dilution point (Table 5).

Table 7. Limit of detection of MicroSight® MSI PentaBase Panel using DLD-1 MSI cell-line DNA.

MicroSight® MSI PentaBase Panel Limit of detection (LOD)				
Locus	**DLD-1 cell line DNA - Fraction of total DNA**			
	50%	**25%**	**12.5%**	**6.25%**
BAT25	MSI	MSI	MSS	MSS
BAT26	MSI	MSI	MSI	MSS
NR22	MSI	MSI	MSS	MSS
NR24	MSI	MSI	MSI	MSS
MONO27	MSI	MSI	MSI	MSS
Conclusion	**MSI-H**	**MSI-H**	**MSI-H**	**MSS**

8.3 Stress tolerance

8.3.1 Freeze-thaw cycle and in-use stability

Assay performance has been evaluated during repeated freeze-thaw cycles in a 72-hour time period. The MicroSight® MSI PentaBase Panel assay was subjected to 8 freeze-thaw cycles. At each cycle, the assay was thawed at room temperature and exposed to light for at least 30 min. 3 reactions were subsequently transferred to new tubes and stored at 4°C until analysis. The stock mix was frozen in 2 h intervals or overnight. Assay performance was found to be unchanged after 8 freeze-thaw cycles.

8.3.2 Stability at elevated temperature

Examination of stability after prolonged exposure to high temperature and subsequent long-term storage at correct temperature (-20°C) has not been tested. Therefore, the contents of any shipment received that is no longer cold (temperature is clearly below room temperature) should be regarded as damaged and returned to PentaBase.

8.3.3 Long term stability

Assay performance has been evaluated after storage at -20°C for 6 months. Assay performance was found to be within specifications. Further studies are ongoing to investigate further shelf-life extension.

9. Clinical performance

Validation of MicroSight® MSI PentaBase Panel has been performed in four different laboratories in Italy and Switzerland. The MicroSight® MSI PentaBase Panel was used in a retrospective analysis of FFPE samples from 116 patients with histologically confirmed colorectal cancer previously analyzed with traditional capillary electrophoresis. Each of the four laboratories used different panels for the capillary electrophoresis. The samples were evaluated by MicroSight® MSI PentaBase Panel assay by comparing the melt properties of amplicons obtained when applying the patient's tumor-derived genomic gDNA with those observed when applying the patient's non-tumor DNA. The MSI-L samples were excluded from the cohort as MSI-L cannot be called by MicroSight® MSI PentaBase Panel for unpaired samples.

Table 8. Clinical performance of MicroSight® MSI PentaBase Panel for unpaired samples.

MicroSight® MSI PentaBase Panel Clinical performance			
	MSS (agreement %)	**MSI-L (agreement %)**	**MSI-H (agreement %)**
Unpaired samples	78/79 (98.7)	-	37/37 (100)

10. Disposal

The disposal of unused kit reagents, biological samples and post-amplified PCR tubes or plates according to local, state and federal regulations.

11. Symbols

The following symbols are used in labeling for MicroSight® MSI PentaBase Panel assay products.

Table 10. Symbols used in labelling for MicroSight® MSI PentaBase Panel assay products.

Date of manufacture	In vitro diagnostic medical device
Use-by date	Do not reuse
Contains sufficient for <*n*>	Manufacture
Temperature limit	CE marking of conformity; this device is in conformity with the applicable requirements for CRof an *in vitro* diagnostic medical device
Consult instructions for use	

12. Manufacturer and distributors

For technical assistance in Denmark please contact PentaBase A/S:
Petersmindevej 1A
DK-5000 Odense, Denmark

Telephone: (+45) 36 96 94 96
Email: support@pentabase.com
Webpage: www.pentabase.com

For technical assistance in all other countries, contact your local distributor. A complete list of distributors is available at www.pentabase.com.

13. MicroSight® Product overview

MicroSight® Ready-to-use Assay[1]	
MicroSight® Assay	**Cat. No.**
MicroSight™ MSI PentaBase Panel (H1) CE IVD For analysis of unpaired samples[2] Box of 16 reactions	7200
MSI PentaBase Panel[2] (H2) CE IVD For analysis of paired samples Box of 8 reactions	7205
MSI PentaBase Panel Universal Reference (HR) CE IVD Universal Reference (HR)[3] Box of 16 reactions	7210

[1]For use with BaseTyper™ real-time PCR Instruments.
[2]In combination with Universal Reference assay.
[3]For analysis of unpaired samples. One Universal Reference can be used for up to 7 patient samples.

14. Additional information

Products or parts of it must not be resold or transferred without PentaBase's acceptance. PentaBase A/S takes certain reservation for changes. PentaBase A/S disclaim all responsibility for any errors that may appear in this Instructions for Use. Furthermore, PentaBase A/S disclaim all responsibility for misinterpretation that can occur by using this product.

15. Date of revision

MicroSight® protocol was revised June 2021.

Changes from previous versions

PROCEDURE No.	Effective Date	Significant Changes	Previous PROCEDURE No.
2.1	15.01.2020	First release	NA
2.2	20.02.2020	Corrected locus correlation data (Table 7) Added PPV and NPV values to tables 6+7	2.1
3.0	07.06.2021	Data analysis and set-up adjusted to the BaseTyper™ real-time PCR instrument.	2.2
3.1	13.09.2021	Read through and visual update	3.0
3.2	27.09.2021	Correction of formatting error on page 4	3.1