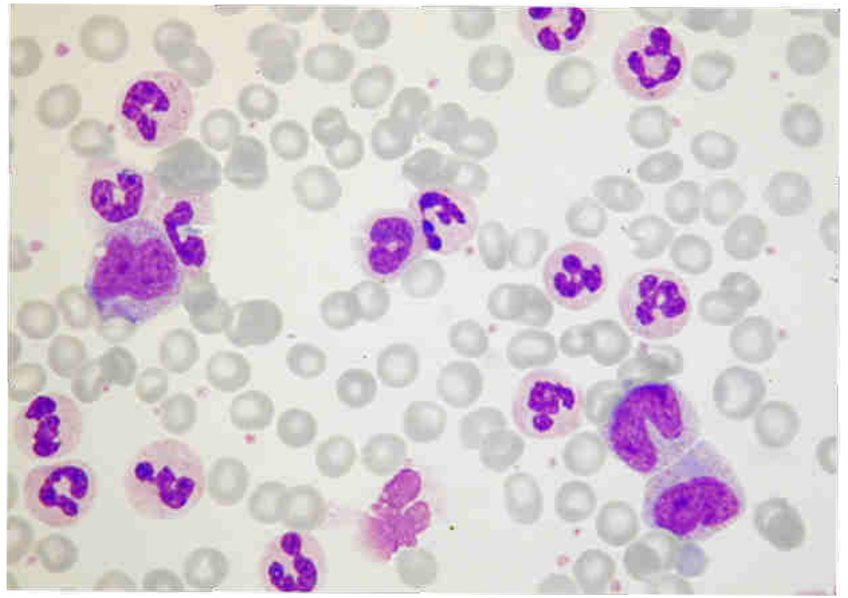
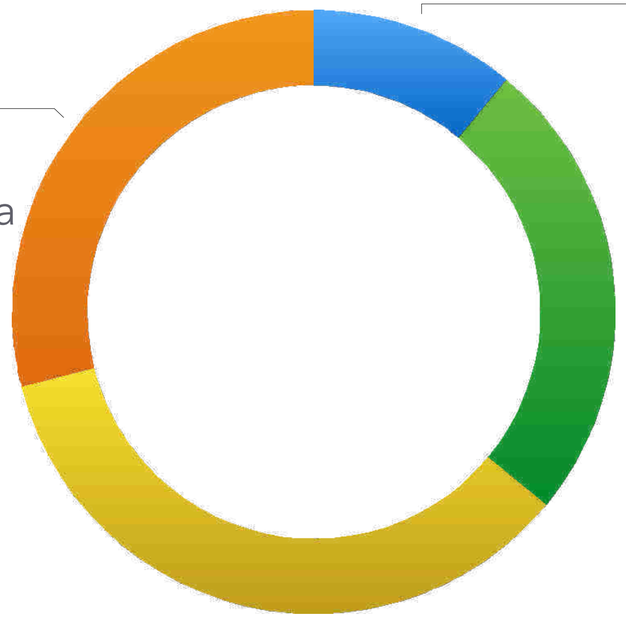
Understanding RT PCR for Diagnosis & Monitoring Leukemia

geneMAP



Leukemia Types



ALL(14,18)
%11
Acute Lymphoblastic Leukemia

MPN(14,15,18,19)
%25
Myeloproliferative Neoplasms

CLL(14,18)
%29
Chronic Lymphocytic Leukemia

AML(14,18)
%35
Acure Myeloid Leukemia

A

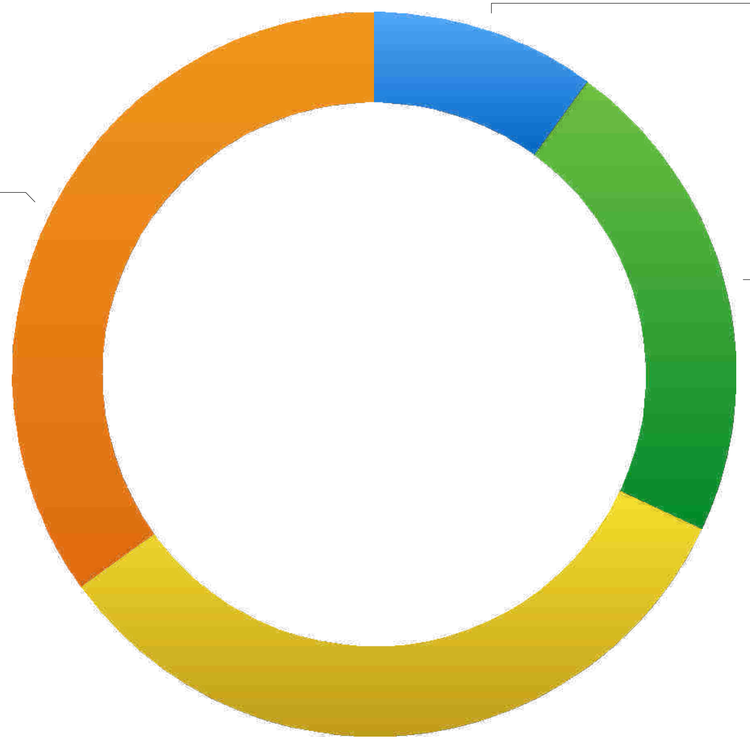
M

geneMAP
Multiplex. Accurate. Precise

Myeloid Types: MPN

Chronic myeloid Leukemia
%35

(9,22) BCR-ABL1, 95%



Primary myelofibrosis
%10

(JAK2, V617F, 55-65%
CALR, 20-25%
MPL, 5-10%)

Polycythemia vera
%25

(JAK2, V617F, 97%
JAK2 exon 12, 1-2%)

Essential thrombocythemia
%35

(JAK2, V617F, 50-60%
CALR, 20-25%,
MPL, 3-5%)

MPN

Diagnostic and prognostic biomarkers

- BCR-ABL1 mutations
- BCR-ABL1
- JAK2 mutations
- CALR mutations
- MPL mutations

Follow-up biomarkers

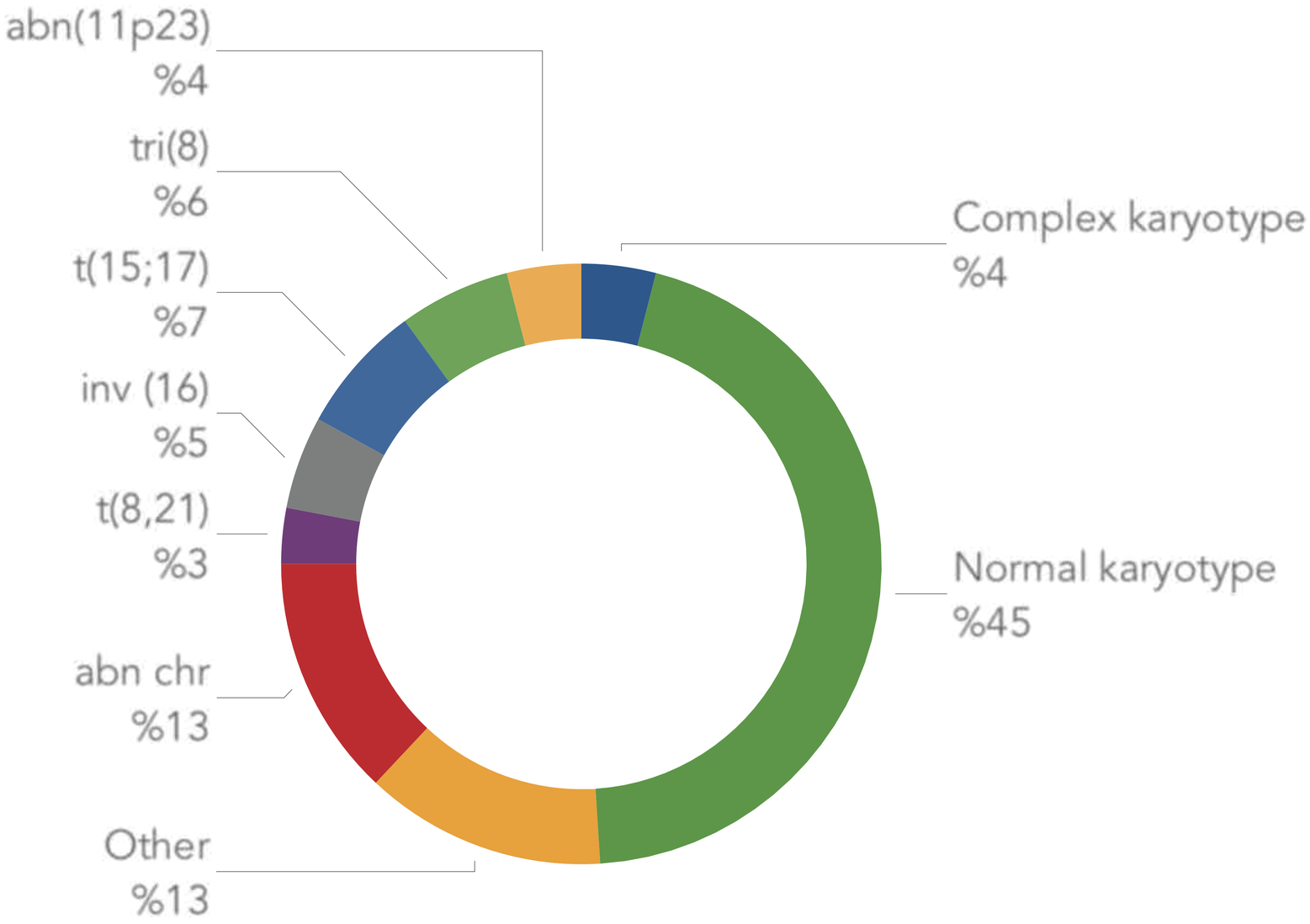
- BCR-ABL1 mutations
- JAK2 V617F

Targeted therapies and other treatments

- Imatinib mesylate (Gleevec)
- Dasatinib (Sprycel)
- Nilotinib (Tasigna)
- Basutinib (Bosulif)
- Ponatinib (ICLUSIG)
- Hydroxyurea(Hydrea)
- Ruxolitinib (Jakavi)
- Allogenic stem cell transplantation
- Alpha-interferon
- Other treatments

- Primary myelofibrosis
- Polycythemia vera
- Essential thrombocythemia
- Chronic myeloid Leukemia

MyeloidTypes: AML



AML

Diagnostic and prognostic biomarkers

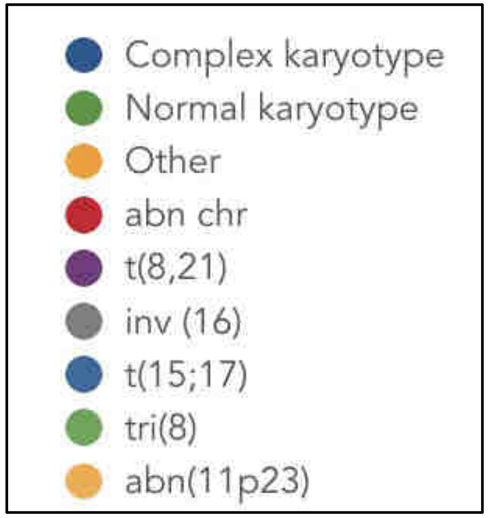
- PMK-RARa
- RAS
- EV11
- BAALC
- MN1
- NPM1 mutations
- WT1
- FLT3(ITDs or mutations)
- CEBPα
- MLL-partner genes
- RUNX1-RUNX1T1
- CMFβ- MYH11

Follow-up biomarkers

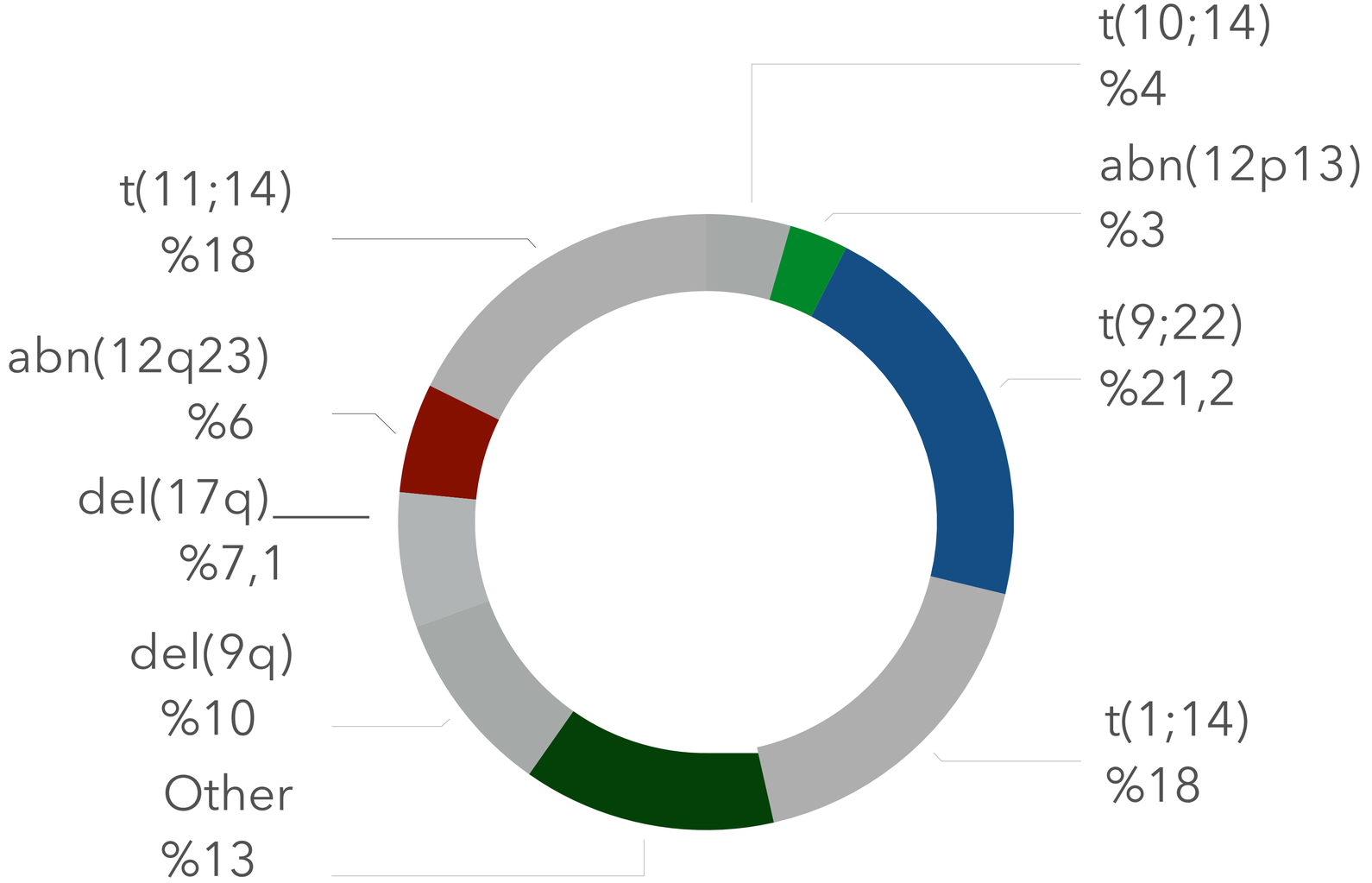
- PMK-RARa
- MLL-partner genes
- CMFβ- MYH11
- RUNX1-RUNX1T1
- WT1
- NPM1 mutation

Targeted therapies and other treatments

- ATRA/Arsenic trioxide
- Aurora/FLT3 kinase inhibitor
- Other kinase inhibitors
- Farnesyl transferase inhibitors
- Proteasome inhibitors
- Immunotargeting
- Other treatments



Lymphoid Types: ALL



ALL

Diagnostic and prognostic biomarkers

- BCR-ABL1
- MLL-partner genes
- ETV6-RUNX1
- TCF3-PBX1
  SIL-TAL1

Follow-up biomarkers

- BCR-ABL1
- MLL-partner genes
- ETV6-RUNX1
- TCF3-PBX1
  SIL-TAL1

Targeted therapies and other treatments

- Imatinib mesylate (Gleevec)
- Other kinase inhibitors
  Immunatargeting
  Other treatments

RT PCR for Minimal Resuduel Disease Testing (MRD)

- MRD is the name given to small numbers leukaemia cells (Cancer cells from the bone marrow) which remain in the body during treatment.
- RT PCR is a very sensitive method that can detect low amount of biomarkers (Fusion Transcripts or Point Mutations) for leukemia.
- Measuring MRD for;
    - RNA based
    - DNA based

RNA Based

- BCR ABL1 IS MMR p210
- BCR ABL1 Combi
- BCR ABL1 Minor p190
- BCR ABL1 Micro p230
- BCR ABL Screening p210 / p190 / p230

RNA Based

- PML RARA t(15;17) *bcr1, bcr2, bcr3*
- WT1 Expression Analysis
- FIP1L1 - PDGFRA Detection
- NPM1 (A, B, C,D)
- RUNX1 -RUNX1T1 t(8;21)
- TCF3 / PBX1 t(1;19)
- MLL - AF4 t(4;11)
- TEL - AML1 t(12;21)
- CBFB - MYH11 inv(16)

RNA Based RT PCR Tests for MRD

- **<u>BCR ABL1 (p210) IS MMR</u>**
  - Quantitative (Abs / Quant & LIVAK Method)
  - One Step PCR Technology
  - Multiplex
  - WHO International Scale
  - Validated on most common open system RT PCR instruments
  - LOG 5 (0,001% Sensitivity)
  - Chronic Myeloid Leukemia (CML), Acute Lymphoblastic Leukemia (ALL)

RNA Based RT PCR Tests for MRD

- **<u>BCR ABL1 p190 Minor</u>**
- **<u>BCR ABL1 p230 Micro</u>**
  - *Quantitative (LIVAK Method)*
  - *One step PCR Technology*
  - *Multiplex*
  - *Validated on most common RT PCR instruments*
  - *LOG4 (0,01 %) Sensitivity*
  - *CML, ALL*

RNA based RT PCR Tests for MRD

- **<u>BCR ABL1 Screening (p210, p190, p230)</u>**
  - *BCR ABL1 Screening in one tube*
  - *OneStep PCR Technology*
  - *Multiplex (4 targets)*
  - *Validated on BioRad CFX96, ABI 7500, Roche LC480*
  - *CML, ALL*

RNA based RT PCR tests for MRD

- **PML RARA t(15;17)**
- **RUNX1-RUNX1T1 t(8;21)**
- **CBFB-MYH11 inv 16**
- **MLL-AF4 t(4;11)**
- **TCF3-PBX1 t(1;19)**
    - *Quantitative (LIVAK Method)*
    - *OneStep PCR Technology*
    - *Multiplex*
    - *Validated on most common RT PCR System instruments*
    - *LOG4 (0,01%) Sensitivity*

DNA Based

- JAK2 Exon 14 V617F *(Somatic Mutation)*
- JAK2 Exon 12 Screening
- MPL A / L / K / R
- CALR *(37 Mutations)*
- c-Kit *(D617V)*
- FLT3 ITD *(Sequencer)* / D835Y *(Quantitative)*

DNA based RT PCR Tests for MRD

- **<u>JAK2 V617F</u>**
  - *Quantitative (LIVAK Method)*
  - *Multiplex*
  - *Validated on most common RT PCR instruments*
  - *0,1% Sensitivity*
  - *Polycthemia vera (97%), Primary myelofibrosis (50-60%), Essential thrombocythemia (3-5%)*

DNA based RT PCR Tests for MRD

- **<u>CALR Mutation Screening</u>**
  - 37 Mutations (Genotype Mutation I & II) in 5 wells
  - Multiplex
  - Validated on most common RT PCR instruments
  - (1%) Sensitivity
  - Primary Myelofibrosis (20-25%), Essential Thrombocythemia

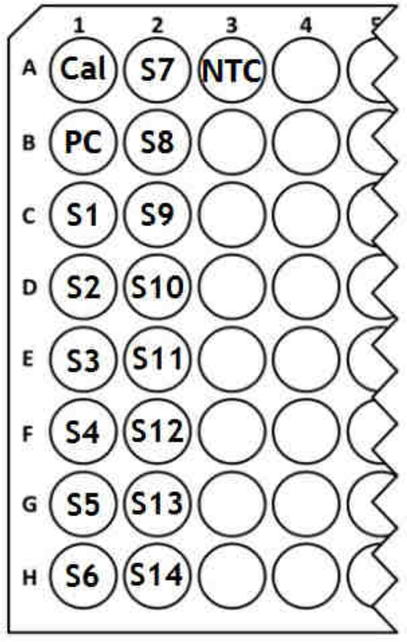
DNA based RT PCR Tests for MRD

- **<u>MPL (W515 A/L/K/R) Mutation Detection Kit</u>**
  - *5 mutations*
  - *Multiplex*
  - *Validated on most common RT PCR instruments*
  - *(1%) Sensitivity*
  - *Primary Myelofibrosis (3-5%), Essential Thrombocythemia (3-5%)*

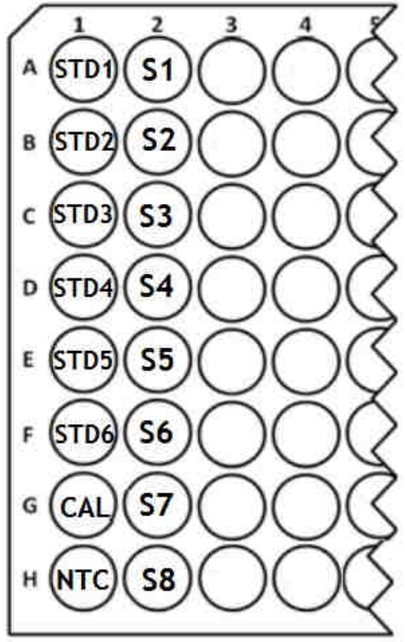
Key Advantages of geneMAP Kits

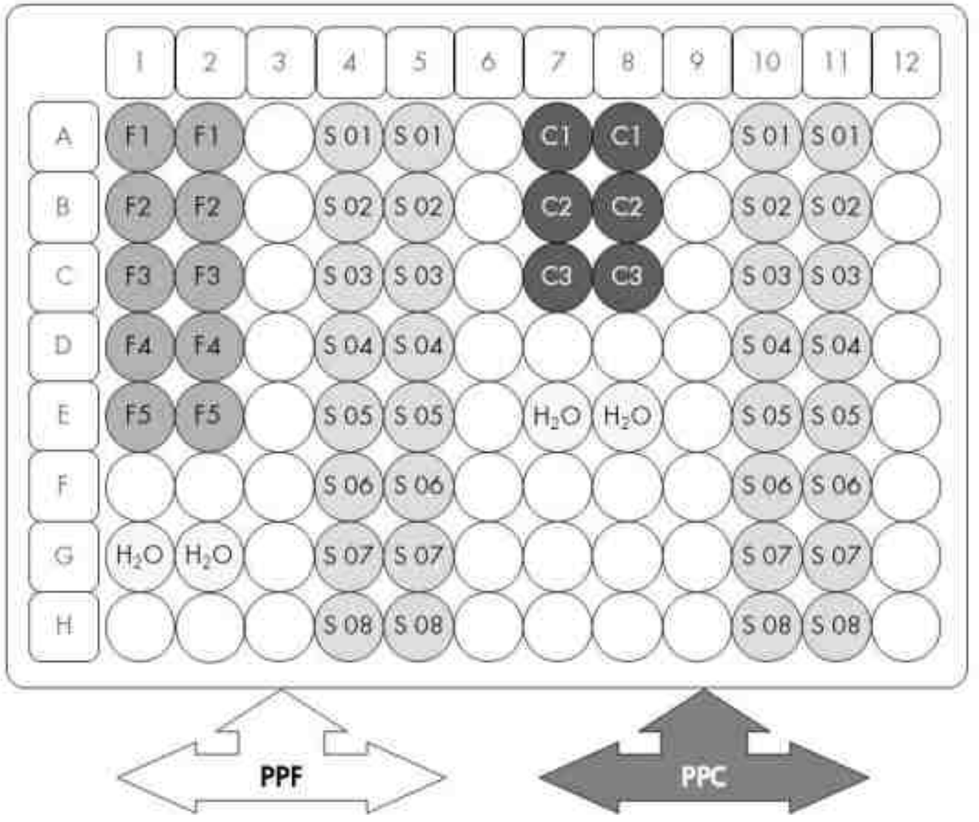
- OneStep enzyme system for RNA based tests.
- Minimized contamination risk.
- Lower hands on & turn around time.
- More specific. (Target Reverse Transcription)
- Multiplex RT PCR.
- More reliable results for quantification.
- Validated on most common used RT PCR instruments.
- Highest sensitivity (0,001%)

Livak/Compherative Ct Method Layout



Absolute Quantification Layout





Thank you