



DDL DIAGNOSTIC LABORATORY

Partner in advanced diagnostic testing



Overview of Hepatitis C virus assays

HCV-DRAG, Tuesday Nov 17, 2015

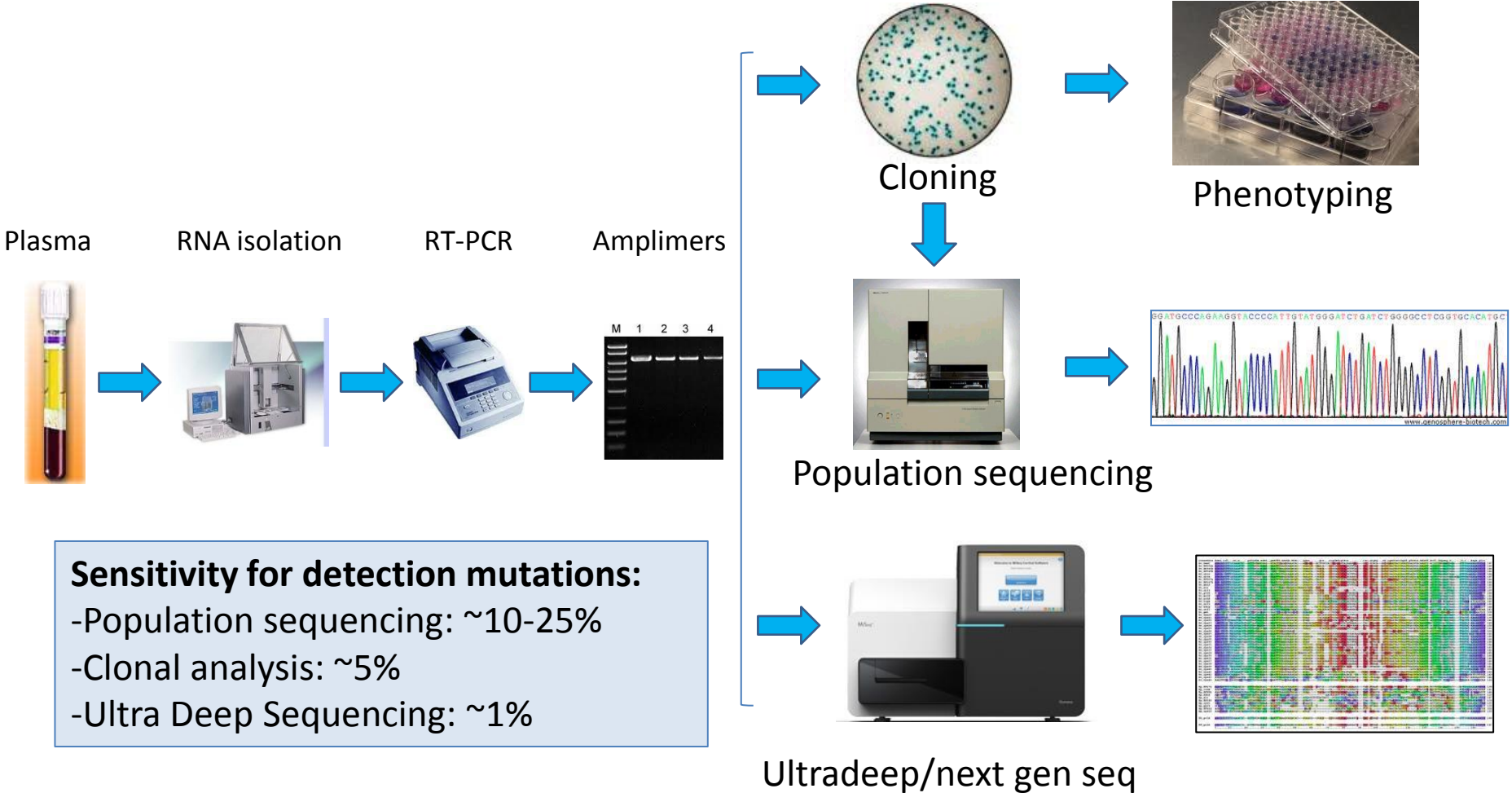


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Overview molecular HCV analyses sequence analysis

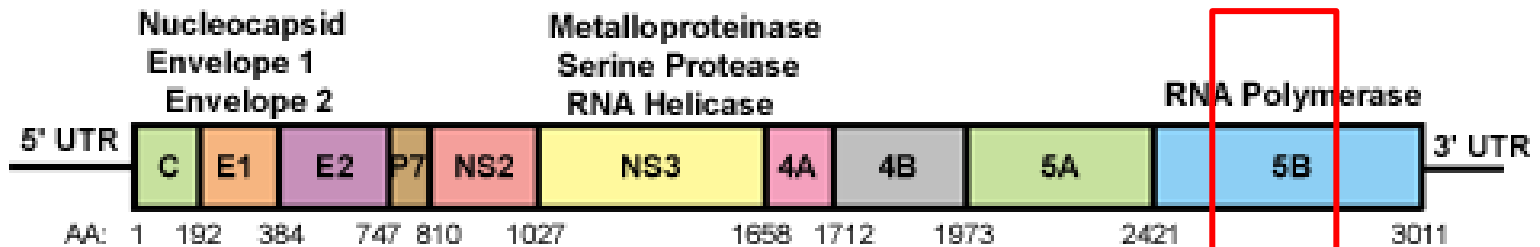


Sensitivity for detection mutations:

- Population sequencing: ~10-25%
- Clonal analysis: ~5%
- Ultra Deep Sequencing: ~1%

HCV genotyping

for confirmation of standard assays, e.g., HCV-LiPA



NS5B small (~325 bp)



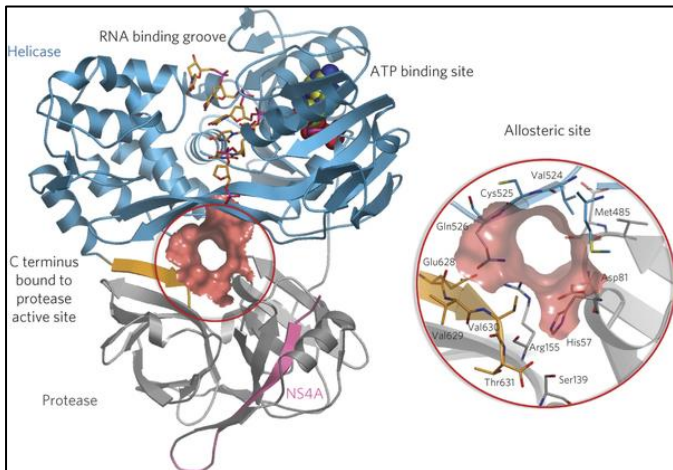
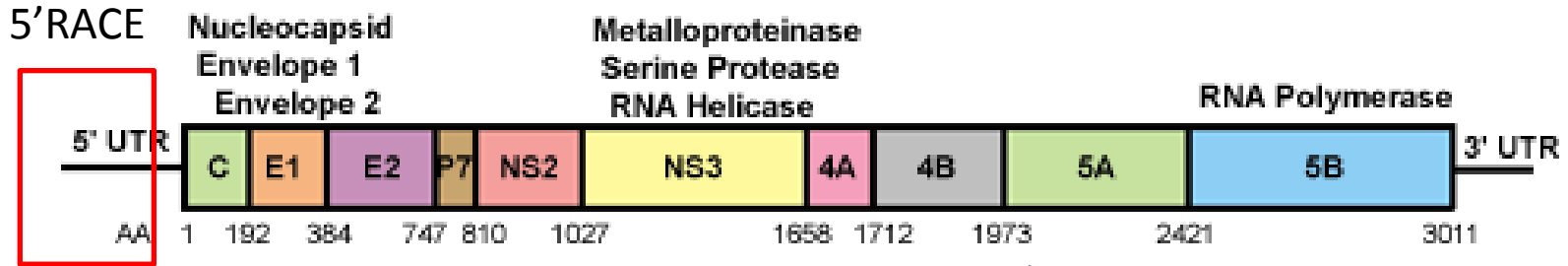
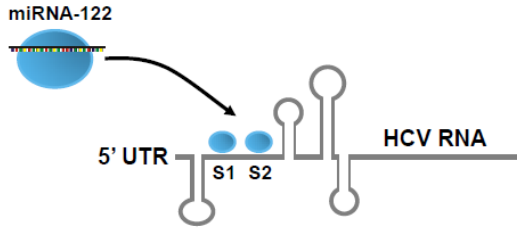
BLAST Search:
homology score

Phylogenetic analysis

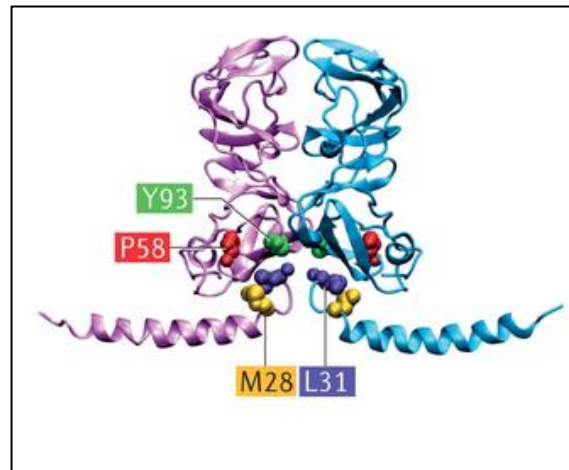


HCV (sub)type

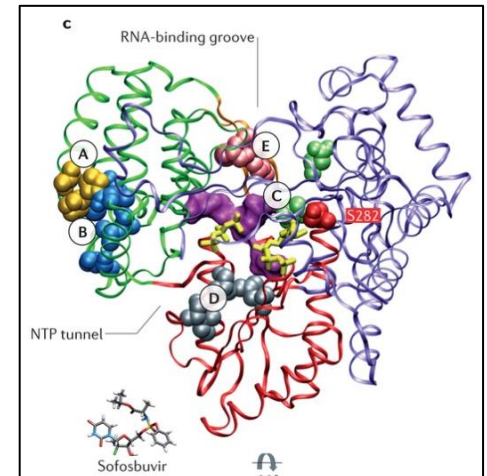
Antiviral treatments



NS3 protease/helicase

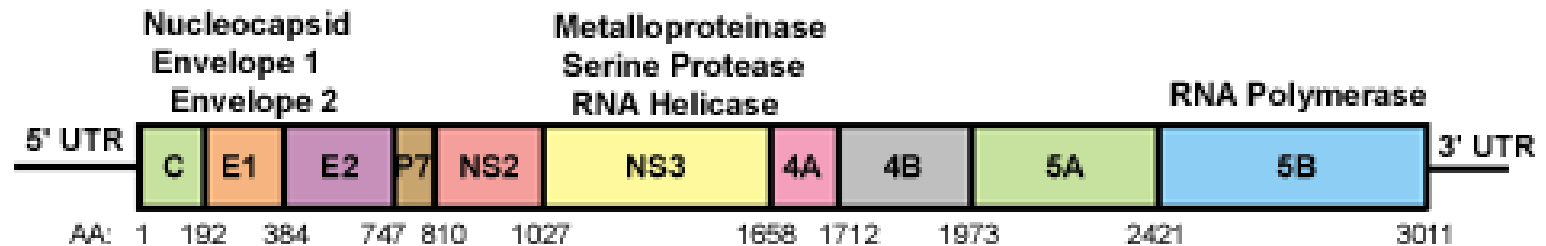


NS5A



NS5B RNA polymerase

Availability of PCR assays or Sanger and/or NGS



Target	GT1	GT2	GT3	GT4	GT5	GT6
5'UTR-core	+	+	+	+	+	-
NS3-4a	+	+	+	+	+	+
NS5a	+	+	+	+	+	+
NS5B	+	+	+	+	+	+
5'UTR RACE	+	-	-	+	-	-

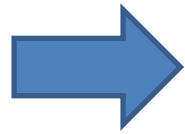
HCV Amplification & Sequencing

- No prior subtyping required, only HCV type (except for GT6)
 - minimal amplification bias
- Using high-fidelity enzymes (Roche Transcriptor & Expand)
 - minimal polymerase errors
- Detection limit: 1,000-10,000 IU/ml

Deep sequencing



Illumina MiSeq



Data analysis and reporting pipeline



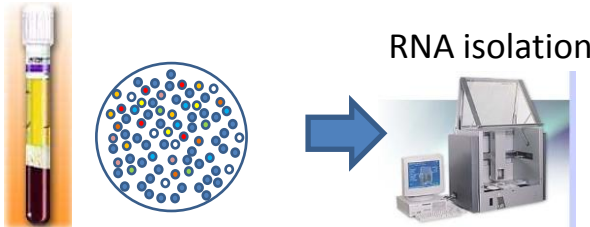
Lab report (extensive & complex)



Clinical site report (interpretation)

Total RNA sequencing HCV

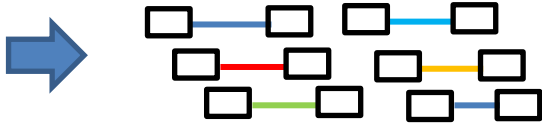
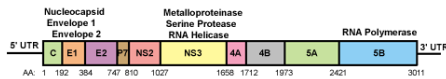
Plasma



Total RNA



First strand cDNA synthesis
Second strand cDNA synthesis
SPIA amplification



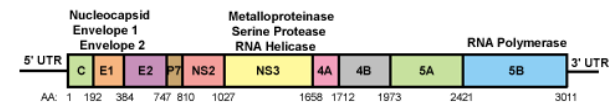
Ovation® RNA-Seq System v2
Library preparation



Illumina sequencing

Data analysis:
sequence annotation

Extract HCV-matching reads



Summary DDL HCV services

- Viral load testing
- Genotyping HCV
 - Siemens Versant HCV Genotyping LiPA 2.0
 - NS5B (samples failing standard typing methods)
- Sequencing
 - GT1 to 6, NS3-4A, NS5A, NS5B covered (type-specific)
 - Population
 - Deep sequencing (Illumina MiSeq)
 - Target-specific amplicons
 - Total RNA/full genome
 - Clonal
- HCV replicon-based phenotyping
- Data management and tailor-made reporting



Key issues

- Intended use of assays
- Quality assurance
 - Standardization, Validation & Proficiency testing
- Reporting complex data
 - Need for consensus (procedures & nomenclature)
 - FDA requirements (references)
- Clinical interpretation